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Immunocastration of horses: A tool for behavioural modification?

Tom A.E. Stout

Introduction

Elsewhere in this issue, Meloen describes the techniques used by his group for the development of new vaccines. Over the last three years, the University of Utrecht's Faculty of Veterinary Medicine has been involved in collaborative experiments with Dr. Meloen to test the efficacy in horses of a vaccine against gonadotrophin releasing hormone (GnRH), the hypothalamic hormone that controls the reproductive endocrine system. In general, vaccination involves the injection of GnRH (or a modified form of the hormone), often conjugated to a non-native protein, to induce the formation of anti-GnRH antibodies. The antibodies bind to endogenous GnRH within the hypothalamic-pituitary portal vessels and prevent it from binding to receptors on pituitary gonadotrophes, thus removing the stimulus for gonadotrophin secretion (particularly luteinising hormone: LH). The follow-on effect is the loss of stimulation to the gonads and a subsequent reduction of gonadal steroid hormone secretion to baseline levels; in effect, an immune-mediated endocrine castration. In farm animals, anti-GnRH vaccination is seen as a potentially cost-effective and welfare-friendly way of reducing testosterone production in male animals to prevent androgen-induced odours that can affect the meat (e.g. 'boar taint' in pigs [6]). In addition, it is hoped that immunocastration will improve the feed conversion efficiency of male animals and reduce aggression directed towards other animals or towards handlers. In man, anti-GnRH vaccination is being developed as a treatment for hormone-induced neoplasia (e.g. prostate cancer) and in cats, dogs and wild animals it has been proposed as a means of contraception. In horses, the main desire is to develop a way of controlling inappropriate sexual or aggressive behaviour without resorting to surgical castration or long-term hormone administration.

Rationale for immunocastration of stallions

The expression of sexual behaviour by intact male horses in training or competition is undesirable because it can negatively affect their level of performance and may endanger their rider, handler or others. Fortunately, horses are relatively conducive to training and most can be taught not to exhibit certain behaviour in given circumstances. This can be more difficult in older, sexually mature stallions and especially those that combine a career at stud with competition or those that have been retired from breeding. In any case, mature stallions are generally more difficult to handle than geldings and may present problems because of their aggressive responses to other animals. When training fails as a means of behavioural control, the major alternatives used have been surgical castration or long-term treatment with exogenous progestagens. However, while surgical castration will certainly reduce the testosterone levels it also involves the irreversible loss of reproductive potential. Thus, even if the animal subsequently proves to be extremely good at its sport it can no longer be used for breeding; this situation is common in three-day eventing where many of the top horses are geldings. Furthermore, in sexually mature animals, surgical castration carries greater operative risks because the blood vessels supplying the testicles are larger and, since the stallion's behaviour may to some extent have become 'hardwired', castration may not fully resolve the problem. The major current alternative to surgical castration is long-term administration of progestagens. In theory, administering progestagenic hormones has a negative feedback effect on the

hypothalamic-pituitary axis that inhibits secretion of both GnRH and LH and thereby reduces testicular testosterone secretion. Exogenous progestagen administration also appears to have direct 'calming' or sedating effects at a higher (CNS) level, as witnessed by the fact that it can be used for ameliorating behavioural problems in geldings. The drawbacks of progestagen therapy include daily administration (for some preparations) and the fact that the reproductive side effects of long-term administration, particularly to peripubertal colts, are not clear. Furthermore, in many sports and countries the practice constitutes a doping-offence (progestagens are weakly anabolic) and, within the EU, horses are considered to be 'food-producing' animals and therefore subject to further rules against the long-term administration of steroid hormones.

A further situation in which reducing the circulating testosterone levels in stallions may be beneficial is in the case of stallions that shed equine arteritis virus (EAV) in their semen. Infection of a naïve horse with EAV generally causes a transient vasculitis with signs including respiratory disease and oedema of the head and extremities. Equine viral arteritis (EVA) can also cause abortion in pregnant mares. Although many stallions that are infected with EVA clear the virus after the acute stage, some 30 to 60% become asymptomatic carriers. In these carriers, the virus localises in the accessory sex glands and is shed into the semen at ejaculation. Because EVA can be transmitted to mares via the semen, in most countries stallions have to be certified as sero-negative or as non-shedders before they can be registered for breeding and certainly before their semen is approved for export. Stallions may eventually clear the EVA from their accessory glands, however they can keep shedding for a considerable period of time. On the other hand, it appears that the persistence of the virus in the accessory sex glands is testosterone-dependent and it has been suggested that reducing testosterone concentrations to baseline levels for a period of time may clear the carrier state [4].

In summary, in stallions immunocastration is seen as a means of controlling testosterone-dependent behaviour (libido and aggression) in a way that does not result in a permanent loss of breeding potential, does not carry the risks of surgery and does not infringe regulations with respect to pharmacological treatment.

Potential uses of immunocastration in mares

As in stallions, the primary reason for wanting to ablate the reproductive endocrine system in mares is to control the expression of sexual behaviour during sporting competition; mares in oestrus often race or compete below their expected level. Some mares present a more extreme problem and, at certain stages of the oestrous cycle (most commonly oestrus but occasionally dioestrus), they become difficult to handle or ride. In some cases, the mare becomes so aggressive or unpredictable that it becomes a danger to itself, other horses and its owner/rider. If the problems occur when the mare is in oestrus, treatment can take the form of progestagen therapy, given the caveats outlined for stallions with regard to doping/food-safety regulations. Of course, pregnancy is an endogenous means of long-term progestagen administration, but is unlikely to be a suitable answer for a competing mare. When progestagen therapy cannot be used, or when the problem behaviour occurs when the mare is in dioestrus, the possible approaches include further training or ovariectomy. However, further training does not always work and the suggestion is not always well received by the owner/trainer. On the other hand, ovariectomy involves considerable expense, surgical risk and the irreversible loss of breeding potential. Furthermore, invasive surgery as a means of behavioural control is ethically questionable, except in the extreme circumstances of the animal presenting a clear danger to itself or those that it contacts.

There are other situations when it is desirable to have a mare that does not have reproductive cycles. The most common is the 'teaser' mare for semen collection. If normally cycling mares are used as teasers, even with careful pharmacological manipulation of their oestrous cycles, a minimum of three are needed to ensure that at

least one is in heat at any given time. If the mare is ovariectomised or anoestrus then she can be induced to show oestrous behaviour by exogenous administration of an oestrogen preparation (e.g. oestradiol benzoate). There are also reports of the use of ovariectomised or seasonally anoestrous mares as embryo transfer recipients [2]. Horse embryos do not survive freeze-thawing well and thus need to be transferred freshly into a synchronised recipient mare. However, because of the long and variable duration of oestrus in mares, synchronisation can be difficult and, to offer a good chance of success, it is generally recommended to have two to three recipient mares available for synchronisation with each donor. If, on the other hand, anoestrous or ovariectomised mares are used as recipients, they need only be treated with progesterone starting one to two days after the donor ovulates, very much simplifying the process. In both cases, immunocastration would be a safer, easier, cheaper and ethically less controversial way of producing an acyclic mare.

Thus, in mares immunocastration offers a reversible means of controlling undesirable sex hormone-related behaviour and a means of producing acyclic mares as teasers for semen collection or as user-friendly recipients for embryo transfer.

Immunocastration in horses: state of the art

The first report of anti-GnRH immunisation in a horse was by Schanbacher and Pratt [8] who described the use of vaccination to lower testosterone levels in a stallion with a cryptorchid testicle. Studies in young colts have demonstrated that vaccination against GnRH does reduce systemic testosterone concentrations along with testicle size, sperm numbers and even libido and aggressive behaviour [1, 5]. Furthermore, the effects on semen quality appear to be reversible. And while early vaccines, or at least their adjuvants, often caused extreme local reactions, newer milder adjuvants seem to have eliminated many of the local and systemic reactions to injection, although at some cost to efficacy [6]. Vaccination has also been shown to reduce testosterone concentrations, testicle size and sperm numbers and quality in mature stallions, although the effects on sexual and aggressive behaviour are less convincing [3]. However, the more frequent injections thought to be necessary in mature animals did cause injection site problems [3] and, in all studies to date, response to vaccination, in terms of anti-GnRH antibody titres, has varied greatly between animals. Since anti-GnRH antibody titres correlate with efficacy, relative failure to respond is a cause for concern that, along with injection-site reactions, reversibility and unwanted effects after long-term treatment, needs to be analysed further before a vaccine can be made ready for commercial use.

In mares, the ability of anti-GnRH vaccination to suppress LH release and prevent ovulation and cyclicity has also been demonstrated, and treated mares returned to normal patterns of cyclicity and fertility after the antibody titres dropped [7, 9]. Again, however, the level of the antibody response was very variable between animals and clearly dictated efficacy.

Conclusions

There are a number of situations in which the ability to shut down the reproductive endocrine system in horses would be clinically useful. The most common of these is the control of inappropriate sex steroid-induced sexual or aggressive behaviour. As a means of behavioural control, vaccination has advantages over surgical gonadectomy (safety, reversibility and less open to ethical challenge) and progestagen therapy (infrequent administration and avoiding the censorship of competition - it is not anabolic - and food-safety regulatory bodies - no long-term administration of steroids). The major challenges still to overcome are ensuring that local or systemic reactions to injection are insignificant, even after repeated injection, and to ensure a consistently good antibody response. Thereafter, studies are needed to establish how frequently booster doses are needed to maintain antibody titres (at present it appears that boosters are required three to six

weeks after the initial injection and at three to four month intervals thereafter) and to what extent long-term suppression is damaging to the gonads. Additionally, the appropriate legislative bodies will need to rule whether such treatment is legal for a supposed food-producing animal and whether it contravenes the spirit, if not the letter, of doping regulations.

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