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Early postnatal immunisation against gonadotrophin-releasing hormone induces a high but differential immune response in heifer calves

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ABSTRACT

The aim of this study was to evaluate endocrinological and immunological effects of early postnatal immunisation against gonadotrophin-releasing hormone (GnRH) in heifer calves, as similar treatment in sheep provokes long-term immunocastration. Heifer calves were injected with either a construct of GnRH – bovine herpes virus 1 glycoprotein D (BHV1 gD; $n = 9$) or saline ($n = 9$) at 2, 6 and 13.5 weeks of age. Antibody (GnRH and carrier) and endocrine responses to immunisation were measured twice monthly (FSH and progesterone) or during intensive sampling regimes (LH). Early postnatal immunisation against GnRH induced a high, but variable, antibody response against both GnRH and carrier. Based on antibody responses, animals were divided into high-titre (HT, $n = 5$) and low-titre (LT, $n = 4$). Occurring mainly in HT, a further peak in anti-GnRH antibodies, stimulated independently of the carrier, was observed at 23 weeks of age, with antibody titres $\geqslant$10% binding for $\sim$9 weeks post-peak. Conversely immunisation had only temporary, reversible effects on reproductive function, not affecting age at puberty. We hypothesise that the newly generated antibody measured 10 weeks after the final immunisation resulted from antigenic stimulation and immunological memory cell activation to an endogenous GnRH release. This outcome offers an opportunity for further manipulation of reproductive function based on modulation of GnRH secretion and activity where long-term immunological memory may contribute to durable endocrine effects.

1. Introduction

Livestock production in extensive and intensive systems require the possibility of controlling reproductive function in an inexpensive and effective manner to avoid unwanted pregnancies and requiring minimum animal handling and management (D'Occhio, 1993). These requirements are met by immunocastration, which involves active or passive immunisation against the hormones controlling reproduction such as gonadotrophin-releasing hormone (GnRH).

After being produced in the medio-basal hypothalamus and stored in the median eminence (ME), GnRH is released into the local portal system and transported to the pituitary gland, where it binds to its specific receptors stimulating the synthesis and release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) (McNeilly et al., 2003). Therefore, immunisation against GnRH (i.e., immunocastration) in adult cows provokes a hypogonadotrophic state, characterised by a suppression of normal oestrous behaviour, arrest of ovarian folliculogenesis (Adams and Adams, 1990; Crowe et al., 2001; Prendiville et al., 1995), ovarian and uterine atrophy (Adams and Adams, 1990; Crowe et al., 2001; Johnson et al., 1988; Prendiville et al., 1995) and a reduction in pregnancy rates (Bell et al., 1997; Hoskinson et al., 1990; Sosa et al., 2000; Vizcarra and Wettemann, 1994); while pre-pubertal GnRH immunisation has been reported to delay puberty (D'Occhio, 1993; Prendiville et al., 1995; Sejrsen et al., 1994; Wettemann and Castree, 1994). However, these effects are only temporary, lasting approximately 6 to 8 months (D'Occhio, 1993). Even though reversibility may be advantageous in some circumstances, it may not be desirable when female animals have to be kept for long periods in mixed herds before reaching adequate slaughter weight (D'Occhio, 1993; Hoskinson et al., 1990). On the contrary, a

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permanent impairment of reproduction has been reported in sheep immunised during early postnatal life (Brown et al., 1995; Clarke et al., 1998).

Ewe lambs immunised against GnRH at 3–4 weeks of age (prepubertal stage) and at 20–21 weeks of age (peripubertal stage), with booster injections 10 weeks later, showed low gonadotrophin concentrations and failed to respond to an exogenous GnRH challenge 18–21 months after the last immunisation (Brown et al., 1995). Furthermore, 3 years after the last immunisation (~4 years of age), 80% of the immunised animals showed signs of impaired reproductive function such as absence of LH pulses, reduced pituitary LH content, reduced FSH concentrations and no follicles greater than 3 mm in diameter (Clarke et al., 1998). The authors hypothesised that exposure of the immature median eminence-pituitary gland to GnRH antibodies impaired normal maturation of the hypothalamus-pituitary unit during a critical period of development (Clarke et al., 1998). Whether this is also true in other important domestic species, such as cattle, has not been investigated.

The immune system of the neonatal calf is naïve but fully developed and reactive when properly stimulated (Chappuis, 1998; Morein et al., 2007; Siegrist, 2007). After birth, immediate protection from common local pathogens is obtained by passive transfer of colostral antibodies. Evidence supporting immunological maturity during the early postnatal period was observed when young bull calves (6 weeks of age) were immunised against GnRH with half of them responding with GnRH suppressive antibody titres (Adams et al., 1996). This implies that if an appropriate GnRH construct and/or formulation are used as an immunogen, it is possible to stimulate the immune system of postnatal calves to generate a specific immune response to this peptide hormone. Because of its small size and its recognition by the immune system as "self", GnRH is unable to function as an immunogen under normal conditions (Ferro et al., 2004; Thompson, 2000). However, it is possible to induce an adaptive immune response to GnRH by coupling this molecule to a protein carrier molecule in admixture with an appropriate adjuvant preparation (Ferro et al., 2004; Thompson, 2000).

In this study a new GnRH conjugate was used as immunogen, which comprised recombinant glycoprotein D (gD) of bovine herpes virus 1 (BHV1) as a carrier. This viral glycoprotein is essential for virus entry to susceptible cells and is required for direct cell-to-cell spread. Hence gD is important for early viral infection (Hughes et al., 1988) and a main target for virus-neutralising antibodies and cellular immunity (van Drunen Littel-van den Hurk et al., 1990; van Drunen Littel-van den Hurk et al., 1997). Hence, vaccination of calves with preparations containing gD followed by challenge with BHV1 virus showed that the gD specific immune response was necessary for protection against infection and reduction of viral shedding (van Drunen Littel-van den Hurk et al., 1990). Moreover, serum antibody to BHV1 gD is virus neutralising and is a significant component of the humoral immune response to both infection and vaccination with inactivated whole virus vaccines. Therefore, it was hypothesised that immunisation of <4 week-old female calves with GnRH-BHV1 gD construct would induce a specific antibody response to GnRH, possibly leading to a long-term effect on reproductive function such as delaying the onset of puberty and changing ovarian follicular dynamics.

2. Materials and methods

2.1. Experimental animals

This experiment was conducted in accordance with the Animal (Scientific Procedures) Act of 1986 (United Kingdom) and approved by the University of Nottingham School of Biosciences Ethical Review Committee. Eighteen cross-breed heifer calves (*Bos taurus* X *Bos taurus*), obtained from a local cattle market, were used in this study and monitored from week 2 to week 65 of age. During the experimental period, calves were group-penned and fed milk substitute (Volac International Ltd., Hertfordshire, United Kingdom). After weaning, calves received a post-weaning diet (19% crude protein) twice daily and hay *ad libitum*. At 6 months of age, diet was changed to protein cake (14% crude protein) and hay twice daily with *ad libitum* barley straw. Water was available as free access at all times.

2.2. Immunisation protocol

After a one-week adaptation period, animals were randomly divided into groups as described in Hernandez-Medrano et al. (2012). Briefly, treated calves ($n = 9$) received a primary subcutaneous immunisation (2 ml, 2.3 ± 0.1 weeks of age) and 2 boosters (2 ml at 6.3 ± 0.1 and 1.7 ml at 13.6 ± 0.1 weeks of age) with a recombinant protein subunit vaccine comprising a fusion protein containing components of GnRH and BHV1gD (GnRH-BHV1gD; Pfizer Central Research, Groton, CT, U.S.A.; (Campos et al., 2005). Control animals ($n = 9$) received saline solution (0.9% w/v NaCl) following the same immunisation protocol.

2.3. Blood sampling

Jugular blood samples were collected every 10–14 days into vacutainer tubes (Precision Glide™, Becton Dickinson, Plymouth, U.K.) in order to determine serum GnRH antibody titres and FSH concentrations. Additionally, weekly blood samples were taken from 35 to 55 weeks of age to measure progesterone concentrations and monitor the onset of oestrous cycles (puberty). Samples were left overnight at +4 °C to allow clot retraction and centrifuged at 2500*g* for 15 min. Serum was collected and stored at −20 °C until assayed.

In addition, to characterise circulatory LH pulsatile concentrations, serial blood sampling was carried out at 3, 16, 29 and 48 weeks of age, with two further sampling periods during synchronised follicular and luteal phases at 63 and 65 weeks of age. Synchronisation of oestrous cycles was performed using two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 11 days apart, with the follicular and luteal phase sampling periods carried out 1 (63 weeks of age) and 15 days (65 weeks of age) after the 2nd $PGF_{2\alpha}$ injection, respectively. These latter samples were also used to determine oestradiol concentrations during both the luteal and follicular phases of the oestrous cycle. During intensive sampling periods, calves were cannulated and blood samples collected every 15 min for 8 h. Samples were then centrifuged for 15 min at 2500*g*, plasma harvested and stored at −20 °C until assayed.

2.4. Anti-GnRH antibody titres

Serum antibody titres against GnRH were determined by radioimmunoassay (RIA) measuring the binding to ^{125}I-GnRH as described by Hernandez-Medrano et al. (2012). Antibody titres were calculated by expressing binding of labelled GnRH as the percentage of total counts after subtracting non-specific binding. Antibody titres presented as percentage (%) binding in the text refer to percentage binding at a 1:1000 dilution of serum.

2.5. Anti-BHV1-gD antibody titres

In order to determine the specificity of the immune response against the BHV1 gD glycoprotein used as carrier, enzyme-linked immunosorbent assays (ELISA) were performed as previously described (Ioannou et al., 2002) on serum samples taken before and