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RESEARCH ARTICLE

Endocrine and ovarian responses in water buffalo cows immunized against inhibin and subjected to the Ovsynch protocol

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Abstract

The aim of this study was to investigate the feasibility of stimulating ovarian follicle development in order to improve fertility in water buffalo cows by immunization against inhibin. The experiment was carried out in early summer (May) and included 24 multi-parity crossbred Murrah-Swamp buffaloes that were divided into immunized (*n*=11) and control (*n*=13) groups. Each immunized cow was administered with a 2-mL immunogen of mineral oil adjuvant containing 2 mg of recombinant inhibin α-subunit fusion protein. The controls were treated with the adjuvant only. All animals received Ovsynch protocol treatment, starting on the day of the antigen administration, and they were artificially inseminated upon behavioral estrus. As a result, all of the immunized buffaloes generated antibodies against inhibin during the experimental period and had higher plasma concentrations of follicle-stimulating hormone (FSH), activin, and estradiol (E2) related to estrous expression. A higher proportion of immunized animals expressed estrus behavior than did the controls (72% *vs*. 30%, *P*<0.05). On average, inhibin-immunized buffaloes had significantly more large follicles (≥9 mm in diameter) than the controls (mean±SEM; 1.2±0.1 *vs*. 0.84±0.1, respectively; *P*<0.05) and a slightly higher mean total number of follicles (≥2 mm; 11.4±0.7 *vs*. 9.0±1.1, respectively; *P*=0.09) and small (2–4 mm) follicles (8.81±0.6 *vs*. 6.84±1.0, respectively; *P*=0.12). A higher percentage of cows ovulated in the immunized group than in the control group (91% (10/11) *vs*. 54% (7/13), respectively; *P*<0.05). Moreover, inhibin-immunized cows had slightly larger corpus luteum (CL) than the controls 9 days after ovulation and significantly higher (*P*<0.01) post-ovulation peak plasma progesterone (P4) concentrations. Immunization against inhibin also marginally increased the conception rate 42 days after insemination (45.8% *vs*. 15.4%; *P*>0.05). These results demonstrate that immunization against inhibin, coupled with the treatment with the Ovsynch protocol, can constitute a new technique to increase fertility in water buffalo cows.

Keywords: inhibin immunization, ovarian responses, luteal function, hormonal patterns, conception rate, water buffalo cows

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1. Introduction

The domestic water buffalo (*Bubalus bubalis*) is important to the rural economies of many tropical and subtropical

countries around the world, by providing draught power and producing milk and meat (Campanile *et al.* 2010; Perera 2011). In both river-type and swamp-type water buffaloes, reproductive performances are lower than those in cattle because of delayed puberty, longer post-partum anestrus, less intense estrus expression, and a low conception rate (Carvalho *et al.* 2002; Zicarelli 2010; Perera 2011). As poor reproductive efficiency could result in considerable economic losses to buffalo breeders (Perera 2011), various estrus synchronization protocols using gonadotropin-releasing hormone (GnRH), prostaglandin (PG), progesterone (P4), and even pregnant mare's serum gonadotropin (PMSG) have been invented or adopted to induce estrous expression and ovulation, and to improve conception rate following insemination (Presicce *et al.* 2005a, b). While some studies have reported conception rates as high as 50–60% following the Ovsynch protocol and timed artificial insemination (de Araujo *et al.* 2002; Baruselli *et al.* 2007, 2013), many other studies still report low conceptions rates in cycling buffalo cows, varying between 18 and 35% (Paul and Prakash 2005; De Rensis *et al.* 2005; Warriach *et al.* 2008; Karen and Darwish 2010; Derar *et al.* 2012). The low conception rate in water buffaloes could result from poorly developed ovarian follicles that are unable to ovulate (Baruselli *et al.* 2013), or they ovulate poor quality eggs (Nandi *et al.* 2002; Li *et al.* 2011), or form poorly developed corpus luteum (CL) that secrete insufficient P4 to establish pregnancy (Kanai and Shimizu 1986; Singh *et al.* 2000). Studies on cattle have indicated that they have better developed, larger follicles after ovulation, and form CL that can secrete high amounts of P4, resulting in a higher pregnancy rate after insemination (Perry *et al.* 2005). However, the currently used Ovsynch protocol only synchronizes follicle development and the timing of ovulation, but is unable to control follicle size, the quality of the ovulated ova, or the quality of the subsequent CL, which are the most important factors in the successful establishment of pregnancy. In order to improve animal reproductive performance, a new kind of follicle-regulating technique must be developed that can not only synchronize the timing of ovulation, but also enhance follicular development and the quality of the oocyte and the CL.

Recent studies in both cattle and water buffalo have demonstrated that immunization against the follicle-secreted hormone, inhibin, could enhance follicular, oocyte, and embryonic development, and improve the function of the CL in terms of P4 secretion (Medan *et al.* 2004; Li *et al.* 2009, 2011; Mei *et al.* 2009; Liu *et al.* 2013). These findings suggest that immunization against inhibin should facilitate the successful establishment of pregnancy, by enhancing ovarian follicle development that results in improved embryo development and quality, and enhanced luteal function. Therefore, the aim of this study was to investigate the feasibility of improving conception in water buffalo cows that have been immunized against inhibin and subjected to an Ovsynch fixed-time breeding protocol, and to evaluate the effects of both treatments on endocrine and ovarian responses.

2. Results

2.1. Anti-inhibin antibody titers

Antigen administration in the immunized group rapidly stimulated the production of anti-inhibin antibodies. The titers, represented by the $OD_{450\ nm}$ values in the ELISA test, were already significantly higher (P<0.01) in the immunized group than a base set of non-specific binding levels in the controls by day 9 after the immunization (Fig. 1). Thereafter, the titers continued to rise until day 21, the last day that blood samples were taken. Throughout the entire experimental period, there was no change in the anti-inhibin antibody titer in the control cows, which remained at the base set of non-specific binding levels.

2.2. Hormone concentrations

FSH Plasma follicle-stimulating hormone (FSH) concentrations on day 6 of the experiment were similar between the two experimental groups. Thereafter, in the immunized buffaloes, plasma FSH concentrations increased to above those in the control cows by day 8 after inhibin immunization, and 1 day after cloprostenol administration (Fig. 2-A). FSH

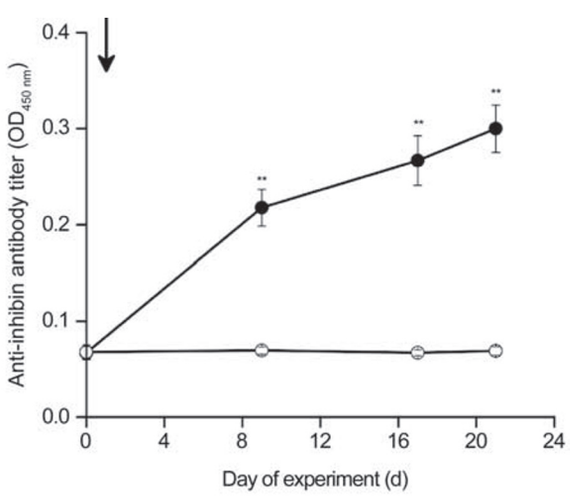

Fig. 1 Anti-inhibin antibody titers in immunized (●, n=11) and control (○, n=13) groups of water buffalo cows. Vertical bars represent the standard error of the mean. ** indicate that the means of the immunized group were significantly different from those of the controls (P<0.01). The arrow indicates a single dose of anti-inhibin immunization. The same as below.