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The efficacy of an inhibin DNA vaccine delivered by attenuated Salmonella choleraesuis on follicular development and ovulation responses in crossbred buffaloes

Qing Liu, Li Han, Zia Ur Rehman, Xingang Dan, Xiaoran Liu, Dinesh Bhattarai, Liguo Yang*

Key Lab of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agriculture University, Wuhan, Hubei, China

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ABSTRACT

The aim of this study was to evaluate the efficacy of an inhibin DNA vaccine delivered by attenuated Salmonella choleraesuis on follicular development and ovulation responses in crossbred buffaloes. A total of 158 crossbred buffaloes divided into four groups and were intramuscularly injected with 1×10^{10} (T1, n = 41), 1×10^{9} (T2, n = 37), 1×10^{8} (T3, n = 37) or 0 (C, n = 43) CFU/ml bacteria delivered inhibin vaccine in 10 ml PBS on day 0 and 14, respectively. All animals were administered with 1000 IU PMSG on day 28, 0.5 mg $PGF_{2\alpha}$ on day 30 and 200 μg GnRH on day 32. The results showed buffaloes immunized with the bacteria delivered inhibin vaccine had significantly higher titers of anti-inhibin IgG antibody than control group ($P < 0.01$). The number and diameter of large follicles (≥10 mm) as well as ovulatory follicles in group T1 was significantly greater than group C ($P < 0.05$). The growth speed of dominant follicles in group T1 was significantly faster than groups T3 and C ($P < 0.05$), resulting in a greater conception rate in buffaloes with positive antibodies. These results demonstrate that immunization with the bacterial delivered inhibin vaccine, coupled with the estrus synchronization protocol, could be used as an alternative approach to improve fertility in crossbred buffaloes.

1. Introduction

Buffaloes have lower reproductive activities than those in cattle, including poor estrus expression, longer postpartum anestrus and a low conception rate (Campanile et al., 2010; Perera, 2011). In order to improve reproductive efficiency, various synchronization protocols for buffalo have been made to regulate the estrous cycle and ovulation. It has been reported that following gonadotropin-releasing hormone (GnRH) associated with $PGF_{2\alpha}$ administration and timed artificial insemination, the percentage of ovulating buffaloes were 60–90% (Chaikhun et al., 2010; Oropeza et al., 2010),with conception rates recorded as 32.7%–60% during the breeding season(Paul and Prakash, 2005; Konrad et al., 2013). Buffaloes were treated with progesterone (P4)-releasing intravaginal device (PRID) along with pregnant mare serum gonadotropin (PMSG) and $PGF_{2\alpha}$, the ovulation rate during the low breeding season and breeding season was 58.3% and 91.7%, respectively

Abbreviations: HBsAg-S, hepatitis B surface antigen S; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent; PMSG, assay pregnant mare serum gonadotropin; PRID, progesterone releasing intravaginal device; GnRH, gonadotropin-releasing hormone.

* Corresponding author.
E-mail address: yangliguo2006@qq.com (L. Yang).

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(Barile et al., 2015), and pregnancy rate was between 28% and 52.7% during the non-breeding season (Neglia et al., 2003; Carvalho et al., 2013). However, the two main protocols are unable to control ovulatory follicle size and the concentrations of progesterone production by the corpus luteum (CL), which are essential conditions for the production of a viable embryo (Perry et al., 2007). Previously reported that ovulated small and premature follicles in cows might have decreased pregnancy rates and increased embryonic mortality associated with decreased circulating concentrations of progesterone (Santos et al., 2004; Perry et al., 2005). Therefore, it is necessary to develop an alternative approach to enhance follicular development and improve pregnancy rate in crossbred buffaloes.

As previous researches indicated that immunization with inhibin DNA vaccines could increase the number of large follicles and litter sizes in rats and sheep (Han et al., 2008; Wang et al., 2012). However, the amplification and purification of DNA vaccines are inefficient and labor-intensive(Greenland et al., 2007; Yang et al., 2010), and traditional DNA vaccines have antibiotic-resistance-based plasmid selection systems, which may induce antibiotic resistance in animals (Galen et al., 2010; El-Attar et al., 2012). To overcome these problems, we developed the recombinant inhibin vaccine crp$^-$/asd$^-$ C500/pVAX-asd-IS, which was equipped with the asd− balanced lethal chromosome–plasmid system and without antibiotic resistance gene (Han et al., 2014). It has been proved to be a safe tool for increasing ovarian follicular development and improving reproductive traits in mice and rats (Han et al., 2014; Hui et al., 2014). Therefore, the objective of this study was to evaluate the effects of the bacteria delivered inhibin vaccine on follicular development and ovulation responses in crossbred buffaloes coupled with a synchronization protocol, and to investigate its feasibility in improving reproductive performance.

2. Materials and methods

2.1. Bacteria delivered inhibin vaccine

The recombinant inhibin vaccine crp$^-$/asd$^-$ C500/pVAX-asd-IS was constructed by using the attenuated crp$^-$/asd$^-$S. choleraesuis C500 strain transfected with a recombinant plasmid pVAX-asd-IS (named crp$^-$/asd$^-$C500/pVAX-asd-IS) (Han et al., 2014). The recombinant pVAX-asd-IS plasmid contained IS fusion gene and the pVAX-asd eukaryotic expression plasmid (Invitrogen, USA). The IS gene encoded inhibin α (1–32) gene inserted into the hepatitis B surface antigen (HBsAg) S gene. The attenuated crp−/asd−S. choleraesuis C500 strain was the kind gift of Dr. Guo Aizhen at Huazhong Agricultural University (Liang et al., 2014) and was used as a carrier for plasmid pVAX-asd-IS by using the asd$^-$ balanced lethal host-vector system.

2.2. Preparation of bacterial vaccine

The crp$^-$/asd$^-$C500/pVAX-asd-IS strains were cultivated in Lysogeny Broth (LB) medium at 37 °C overnight with shaking at 200 rpm. Bacteria were harvested by centrifugation at 6000 rpm for 5 min at 4 °C, and re-suspended in sterile phosphate buffered saline (PBS). Before immunization, bacteria were plated on LB agar to determine the number of colony-forming units (CFU) in triplicate, and the values were expressed as CFU/ml.

2.3. Animals and immunization protocol

The experimental crossbred buffaloes (River × Swamp) were raised in the dairy farm of JinNiu Farm, Jingmen, central China (30°32′N, 111°51′E). The experiment was carried out from November to February. The average ambient temperature varied between 4 and 19 °C, with relative humidity ranging between 44 and 72%. Animals were fed a total mixed ration that consisted of forage (corn silage, Peanut vine, Rice straw), and concentrate (corn, soybean meal, wheat bran). A total of 158 crossbred buffaloes of body condition scoring (BCS) 2.5–3.5 having normal estrous cycles. The proportions of buffalo with chromosome number in diploid of 2n = 50, 49 and 48 were 38.55%, 51.81% and 9.64% respectively. All animals were randomly divided into four groups: Group T1 (n = 41), T2 (n = 37) and T3 (n = 37) were intramuscularly immunized once a day with10 ml of 1×10^{10}, 1×10^{9}, 1×10^{8} CFU/ml of the bacteria delivered inhibin vaccine for 3 days, respectively. C group (n = 43) was intramuscularly injected once a day with 10 ml PBS for 3 days. All animals were immunized twice with an interval of 14d (Fig. 1).

2.4. Blood collection

The blood samples of buffaloes were collected from the jugular vein three times at two weeks intervals: before primary immunization, and at weeks 2 and 4 after the primary immunization. The serum was prepared by centrifugation at 1500 rpm for 10 min and stored at −20 °C for measurements of anti-inhibin antibody.

2.5. Estrus synchronization and ultrasonography

All buffaloes for each experimental group underwent the following protocol to synchronize estrus and ovulation: On day 28 all buffaloes received 1000 IU of a PMSG analogue (Sansheng Pharmaceutical Co. Ltd., Ningbo, China) regardless the stage of the estrous cycle, followed by 0.5 mg of $PGF_{2\alpha}$ (Sansheng Pharmaceutical Co. Ltd., Ningbo, China) was intramuscularly injected(i.m.) 48 h later, and 200 μg GnRH analogue(Sansheng Pharmaceutical Co. Ltd., Ningbo, China) i.m. on day 32 to guarantee ovulation.

From day 29(the second day of the synchronization PMSG treatment) until ovulation occurred, or up to 72 h after GnRH treatment, all buffaloes were ultrasound scanned (Shenzhen Well.D Medical Electronics Co. Ltd., Guangdong, China) for recording the development of follicles at 8-h interval to evaluate the folliculogenesis and ovulation (Fig. 1). Follicles on both ovaries were classified into three categories according to their diameters: small (≤6 mm), medium (7–9 mm) and large (≥10 mm). All buffaloes were observed twice a day (8:00 and 16:00) by ultrasonography to assess estrous status, such as the presence of large follicles (≥10 mm) on the ovary and visi-