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PRELIMINARY STUDY ON THE USE OF INHIBIN TO IMPROVE THE WATER BUFFALO SUPEROVULATION

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ABSTRACT

Seventeen female buffaloes with normal estrus cycle were randomly divided into 10 heads for the experimental group and 7 heads for the control. In the experimental group 1mg/head of the Recombinant porcine inhibin α subunit fusion protein was administrated as the first immunization. After 28 and 56 days, the immunization was strengthened by the dose of 0.5 mg/head. At the same time, an adjuvant consisting to the mixture of mineral oil and physiological saline was administrated to the control group. In both the experimental group and the control, at the days 28 (first strengthened immunization) and 56 (second strengthened immunization), the follicles size and number were monitored and counted respectively by B-mode linear array ultrasound scanner .At eight days after the super ovulation the follicles and corpus luteum count were performed by B-mode linear array ultrasound scanner and palpation. The results showed that, in the experimental group compared with the control one the average follicles number enhanced (from 8.8 to 15.0) after strengthening the immunization, but the difference was not significant ($P>0.05$). After the super ovulation, in the experimental group the mean number of the follicles and corpus luteum and ovulation rate were 12.2±0.79, 9.0±1.06 and 73.77 % respectively, compared to the control, the difference was significant ($P<0.05$). There was no difference between the total embryo recovery number and available embryo number. These results demonstrated that the re-immunization of inhibin can be used to enhance ovarian follicular development and ovulation rate and it is suitable to superovulation in buffaloes.

Key words: water buffalo; inhibin immunization; follicle development, super ovulation

INTRODUCTION

In 1983, Drost reported the first calving of water buffalo calf in USA resulted to the success of the use of the super ovulation in embryo transfer. Subsequently water buffalo calves resulted to the success of the use of the embryo transfer were reported in Bulgaria and India (Drost M, 1988; Misra, A.K., 1988). So far these researchers from each breeding water buffalo countries preformed large scale systematic studies on the water buffalo super ovulation and embryo transfer. In China, there were some studies on the buffalo super ovulation and embryo transfer (Chen et al. 2002, 2008; He et al. 2005; Jiang et al. 2006). Although there is a widespread study on the water buffalo MOET technique but the in vitro embryo production effectiveness was not improved. The widespread use of the MOET technique in water buffaloes is severely restricted due to many unbeneficial factors such as the lower reaction to the hormones, the lesser embryo production and the lower embryo transfer rate and so on. According to Mei cheng et al. (2008) report, the active immunization of the Holstein by the use of the recombinant porcine inhibin α subunit mimmunogen could improve the super ovulation effect and embryo quality. In this study, in order to improve the buffalo super ovulation effectiveness, the recombinant porcine inhibin α subunit fusion protein was used twice to strengthen the immunization, to combine the normal super ovulation treatment, to use a non-surgical method for embryo flushing and to count the obtained and available embryo number. This study was to investigate the application of the recombinant porcine inhibin α subunit on buffalo super ovulation and embryo production availability.

MATERIALS AND METHOD

Experiment reagents and materials: Oil emulsion was made by a mixture of the purified recombinant inhibin protein (favored by professor Shi Zhen-dan) and mineral oil. The concentration of the recombinant inhibin protein was 0.5mg/mg. For the control group, the immunization source was an oil emulsion consisting to a mixture of mineral oil and physiological saline.

Selection of donors: River dairy buffaloes with good reproductive system and normal reproductive function were chosen from Nanning Ovagene Biotechnology Co., Ltd experimental farm in Guangxi. At any day of the reproduction cycle the PGc (prostaglandin chloride, made in Shanghai Family Planning Research Institute) 0.6 mg per head was administrated intramuscularly to perform the synchronization treatment. The animals with estrus and in ovulation and having luteum corpus were chosen as donors. The donors were 17 heads with average age, body weight 4.54±1.86, and 456.78±64.58kg, respectively.

Experimentation method

Inhibin immunization treatment: In the experiment group, at the 1st day 1ml per head of the recombinant porcine inhibin α subunit fusion protein with a concentration of 1mg/ml was administrated intramuscularly. At the 28th day, 1ml per head with the concentration of 0.5mg/ml was administrated for the first immunization strengthening .At the 56th day the same dose with same concentration was done for the second immunization strengthening.

In the control group, 1ml per head of the mixture of mineral oil and physiological saline was administrated following the same step in the experiment group.

Super ovulation treatment: In the experiment group, at 12 and 16 days after the second immunization strengthening (day68 and 72) the progesterone releasing intravaginal devices (PRID, Duoxi, CO-MATE, made in Shanghai Family Planning Research Institute) was placed and the FSH (Denka Pharmaceutical Co., Ltd., Japan; Folltropin-V, Canada) began to be used by the diminishing method (for the super ovulation program and the FSH dose see Table1) to perform the super ovulation treatment, at 18 days after the second immunization strengthening 0.8mg/head of prostaglandin chlorine was administrated and the PRID was removed 12h after the injection of PGc. And according to the status of experiment buffalo, the estrus determination, the artificial insemination was done. At the first and second insemination, LHRH-A3（GnRH analogues, Ningbo Hormone Products Co., Ltd.). After 6 days, the embryos were collected by the non-surgical method.

3(day74) and 14(day83) days after the injection of the FSH, the follicles were observed by B-mode ultrasound (HS-101 V, Japan) to compare with the control group and to determine whether follicles number or size were correlated.

Follicles number and corpus luteum inspection: In the immunized and control group, at the first, second and third immunization, before superovulation and after embryos flushing the numbers of embryos, follicles and luteum corpus were checked by ultrasound and. According to their diameter, the follicles are classified to big (≥10mm), medium (≤5<10mm) and small (<5mm）follicles.

Statistical analysis: T-test and Chi-square test were performed to analyze the follicles, corpus luteum and the embryo recovery number and ovulation rate respectively .All statistical analyses were performed with SAS8.0 software.

RESULTS AND ANALYSIS

Follicles development change in immunized and control groups: The follicles development in the immunized group before the immunization, after the first and second immunization strengthening and in the control group and statistical results were shown in the Table2.

From the Table2 we could observe that in the immunized group, the follicles number continuously increased after the first and second immunization strengthening(from 8.8 to 15.0 follicles).There were more 2.6 follicles in the immunized group (15.0) than the control group (12.4) after the second strengthened immunization. And the big and medium follicles number continuously increased but compared to the control group, the difference was not significant （P>0.05）. As a result the number of the medium follicles increased and the foundation for the further superovulation was laid.

Comparison of the superovulation effectiveness of the immunized and control group: The data about the mature follicle, corpus luteum, unovulated follicle, recovered and available embryo number from the immunized and control group at the 16th day after the second immunization strengthening and from the experiment group in which the FSH was used by diminishing method to perform the superovulation and non-surgical embryo collection were registered and shown in Table 3 and 4.

In Table 3 it showed that the immunized group mature follicle, corpus luteum number and the ovulation rate was 12.2±0.79, 9.0±1.06, 73.77% respectively. All were significantly higher than the control (P<0.05). The result indicated that in the immunized group, the immunization by the inhibin could significantly enhance the mature follicle and corpus luteum number.

As result, in this experiment only 5 buffaloes with best superovulation effect were chosen to perform the non-surgical embryo collection. These data were shown in Table 4. Compared to the control, the recovered and available embryo number and the recovery rate were not significant in the immunized group. But the embryo availability in the experiment group was worse than in the control group.

Table1: super ovulation program and hormone total quantity

		Control group	Immunized group	*P*
Before immunization（0d）	Follicle total number	6.3±1.02（n=7	8.8±1.20（n=10	0.1538
	Small	4.6±0.90	5.6±0.75	0.3920
	Medium	1.3±0.29	3.2±0.74	0.0566
	Large	0.4±0.20	0	0.0212
First immunization strengthening (28d）	Follicle total number	10.9±1.50	13.1±1.86	0.3965
	Small	5.9±0.74	7.7±1.29	0.3145
	Medium	4.6±1.13	5.3±0.96	0.6303
	Large	0.4±0.20	0.2±0.13	0.3394
Second immunization strengthening (56d)	Follicle total number	12.4±1.53	15.0±1.53	0.2688
	Small	6.0±0.87	7.6±0.76	0.1912
	Medium	6.1±0.74	7.1±1.12	0.5283
	Large	0.3±0.18	0.3±0.15	0.9531

Table2: follicles development in the immunized and control groups

M S	M S	M S	M S	M S	M S	M S	MS	M S
Morning	PRID placement	FSH (Japan) 2.6ml+FSH (Canada) 2.0ml	FSH (Japan) 1.8ml+FSH (Canada) 1.5ml	FSH (Japan) 1.2ml +FSH (Canada) 1.0ml+PGc0.8mg	FSH (Japan) 0.7ml+FSH(Canada) 0.5ml	AI+LHA3 (200mg)	AI	Embryo flushing
Afternoon		FSH（Japan) 2.6ml+FSH (Canada) 2.0ml	FSH（Japan) 1.8ml+FSH (Canada) 1.5ml	FSH (Japan) 1.2ml+FSH (Canada)1.0ml, CIDR removal	FSH (Japan) 0.7ml+FSH (Canada) 0.5ml	AI+LHA3 (100mg)		

Table3 Comparison of the super ovulation effectiveness of the immunized and control group

Group	Number	Mature follicle number	Corpus luteum number	Unovulated follicle number	Ovulation rate
Immunized group	n=6	12.2±0.79[a]	9.0±1.06[a]	4.5±0.76	73.77[a]
Control group	n=6	7.7±0.61[b]	4.3±0.67[b]	4.7±1.28	55.84[b]

Table4: embryo recovery of the immunized and control group

Group	Number head	Corpus luteum number	Recovered embryo number	Available embryo number	Recovery rate (%)	Availability (%)
Immunized group	n=3	7.7±1.73	5.3±2.19	3.0±2.08	68.83	56.60
Control group	n=2	6.0	5.0±1.00	4.5±0.50	83.33	90

DISCUSSIONS

This study was performed to investigate the feasibility of the Recombinant porcine inhibin α subunit fusion protein in the buffalo super ovulation. The results showed that the use of the recombinant porcine inhibin α subunit fusion protein to immunize water buffaloes, combined with the traditional superovulation technology, could significantly enhance the buffalo follicle number and ovulation rate. Mei et al. (2008) reported immunized eight Holstein heifers of 16-17 months age with the inhibin as an experiment group and ten Holstein with the physiological saline as a control group, after twice immunization strengthening, the superovulation was performed .The results showed that the average embryo number obtained was 15.8±2.8 and 8.3±1.5 per head in the immunized and control group respectively, the difference was significant (P<0.05) in the immunized group. The average transferable embryo was 9.6±3.1 and 5.8±1.6 per head in the immunized and control group respectively, the immunized group was higher than the control but the difference was not significant (P>0.05). This result was similar with other report (Takedomi et al. 1997), in which the inhibin was used to immunize Japanese black cattle which could enhance its superovulation.

The most basic biological action of the inhibin is to inhibit the synthesis and secretion of the FSH and regulates its plasmatic level by the complex feedback mechanism. Kanecko et al. (1995a; 1995b) used 32kD bovine serum inhibin for a passive immunization of the dairy cattle and after, 48, 72, and 96 hours, then its small, medium and large follicles number enhanced. That conclusion was also further confirmed when he used a combination of estradiol and anti-inhibin serum. At the same time, the heifers were singularly administrated by anti-bovine inhibin at 9d of estrus synchronization and discovered that the medium. As a result the large and small follicle number in the immunized group was higher than that in the control group (Takedomi et al., 1997). The beef cattle were immunized three times consecutively with a vaccine made from Oil-coated α inhibin and the FSH, E2 and P4 concentrations increased significantly. The large follicles (≥10mm), medium follicles (≥7<10mm) and small(≥ 4<7mm)follicle number and the ovulation rate were higher in the immunized than those in the control group (Medan et al. ,2004). In China, crude inhibin extracted product from boar sperm was used to actively immunized the dairy and beef cattle and the experiment results showed that the product could improve the ovulation rate, which could reach 36% on average (Ye et al., 1996; Niu et al., 1996). Sang et al. (2000) used inhibin and gonadotropin to perform the cattle super ovulation treatment and the embryo number and transferable one per head were 5.6 and 3.2 respectively. In this study after the first and second immunization strengthening, the total follicle number of the buffalo immunized with the recombinant porcine inhibin α subunit fusion protein continuously increased (from 8.8 to 15.0), higher than that in the control group (12.4) by 2.6 follicles. And the medium and big follicle number continuously increased but compared to the control, the difference was not significant ($P > 0.05$). All in all, whether by the active or passive immunization, the inhibin can promote livestock follicular development, increase ovulation rate, and the similarity and purity of the sequence of amino acid synthesized inhibin were greater and higher, thus after the immunization the anti serum titer and neutralization capacity were higher. And FSH level and the unovulation rate remarkably increased.

As we all know, the water buffalo superovulation effectiveness and the embryo recovery rate are bad and lower respectively for the reason that the primordial follicle number on either the swamp or river buffalo ovary is lower than that of the dairy cattle 20% and 30%, respectively. Compared to the dairy cattle, the water buffalo follicle atresia rate is higher. In the recent years the embryo recovery rate greatly improved in water buffaloes, the available embryo number reached 2.5-3.0, but the most were in the vicinity of 1.0－2.0. In this study, recovered and available embryo average number was over 3 in either the immunized or control group, which exceeded the average level. It confirmed that the immunization of the buffalo with the inhibin can improve its super ovulation effectiveness. But many factors such as the donors, the reagents and the embryo flushing skill influenced the super ovulation effectiveness. Misra et al. (1990) reported that even if the embryo recovery was done from a slaughtered buffalo after the superovulation, it could still have 50% of receptor on which the embryos were not recovered. The results in this study also indicated that the mature follicle and corpus luteum number and the ovulation rate in the immunized group were 12.2±0.79, 9.0±1.06, 73.77% respectively. And all were significantly higher than those in the control group. The immunization of the water buffalo with the inhibin could enhance its mature follicle and corpus luteum number. But the embryo recovery was not necessarily high. In addition, in the immunized group, even though the ovulation rate was improved but the unfertilized ovum number was still higher, that might be because of that during the superovulation of the water buffalo, the ovulation speed varied or because of the insemination time was not well mastered. Then even though during the superovulation, the follicle growth and development was stimulated, but the reasons of unovulation of some follicles were still unknown. Therefore, further studies would focus on strengthening the reproductive mechanism to improve superovulation in buffaloes.

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