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EFFECTS OF DIFFERENT HORMONE COMBINATIONS ON SUPEROVULATION IN RIVER BUFFALOES

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ABSTRACT

This study was conducted to evaluate the effects of different hormone combinations with FSH, PGC, LHRH-A3 and LH on superovulation in river buffaloes. Thirty-five heads of river buffaloes were divided into six groups as follows: Group I, FSH (Japan, total doses 26 AU) + PGc (Shanghai, 0.6 mg); Group II, FSH (Japan, total doses 26 AU) + PGc (Shanghai, 0.6 mg) + LHRH-A3 (Made in Ningbo, 50 µg) ; Group III, FSH (Beijing, total doses 20 mg) + PGc (Shanghai, 0.6 mg) + LHRH-A3 (Ningbo, 50 µg) ; Group IV, FSH (Beijing, 20 mg) + PGc (Shanghai, 0.6 mg) + LH (Ningbo, 50 µg); Group V, FSH (Canada, 800 mg) + PGc (Shanghai, 0.6 mg); Group VI, FSH (Canada, 800 mg) + PGc (Shanghai, 0.6 mg) + LHRH-A3 (Ningbo, 50 µg). The results showed that superovulation rate was 97.14% (34/35). There were 8.71 mature follicles per head in superovulation (296/34). The average number of CL was 5.0 (170/34). The average ovulation rate was 57.3% (170/296). The average number of embryo collection was 2.72. Average transferable embryos were 1.33 (24/18). Recovery rate and transferable rate were 39.84% and 48.98 %, respectively. The mean number of CL in Group II (6.86±5.96) and the ovulation rate in Group VI (76.92 %) were the highest among the six groups. The results showed that the ovulation rate in Group VI with LHRH-A3 was higher than those in the other groups without LHRH-A3 (12.12%) and that with LH treatment (28.64%), respectively.

Key words: River buffalo, Superovulation, FSH, LHRH-A3

INTRODUCTION

There are more than 172 million heads of buffaloes in the world in 2006 (FAO). More than 95 percent of the world population is found in Asia where buffaloes play a leading role in rural livestock production. According to statistical data (FAO, 2006), the total number of buffaloes in China in 2003 was 22.28 million. Most of them are swamp buffalo. Buffalo is an important livestock resource, which provide milk, meat, and work power. The swamp buffalo has the characters of low milk production and slow growth. It is urgent to improve species by using embryo biology technique. One possibility for enhancing their reproduction efficiency is through genetic improvement of the existing population. Superovulation and embryo transfer are commonly used techniques for improving genetic potential. The use of the super ovulation to obtain the transferable embryo and perform the embryo transfer can completely highlight the potentiality of the excellent female cattle, reduce the generation's interval and is one of the ways of rapid reproduction of the female cattle bearing a special gene. But in buffalo, because of that the effect is not ideal, that technology has not been applied. This trial aimed to optimize the study scheme by the use of the different hormones combination treatment to improve the effectiveness of the superovulation of the dairy water buffalo.

MATERIALS AND METHODS

Animals and management: Water buffaloes reared at Guangxi Buffalo Research Institute with known good reproduction records and in normal reproductive function were examined and selected for use in this experiment. At any day of the reproductive cycle, uterine infusion of chlorine cloprostenol (PGc, Chlorine cloprostenol, Shanghai Institute of Family Planning) 0.6 mg/head was done to perform the synchronization treatment. Female buffaloes observed in estrus, with ovulation and corpus luteum on the ovaries were chosen as donors. The average age and body weight of the donors were 6.31±2.14 years old and 607.44±81.33 kg., respectively.

Experimental design: According to the method of the association of the hormones, the experiment is subdivided into 6 groups. There were 35 hds of animals used in this experiment and were randomly divided into six groups:
Group I: FSH - Japan (26 AU, Japan Denka Pharmaceutical Co., Ltd) + PGc – Shanghai (0.6 mg);
Group II: FSH-Japan (26 AU) + PGc - Shanghai (0.6 mg) + Ningbo LHRH-A3 (50 µg,Gonadotropin-releasing hormone, LH, Ningbo Hormones Products Co., Ltd.);

Group III: FSH - Beijing (20 mg, Chinese Science Institute Animal Research Institute) + PGc - Shanghai (0.6 mg) + Ningbo LHRH-A3 (50 µg);
Group IV: FSH - Beijing (20 mg) + PGc - Shanghai (0.6 mg) + LH (50 µg, Ningbo);
Group V: FSH - Canada (800 mg, Folltropin-V, Canada) + PGc – Shanghai (0.6 mg);
Group VI: FSH – Canada (800 mg) + PGc – Shanghai (0.6 mg) + LHRH-A3 (50 µg).

Superovulation treatment: On day 9 to 11 after estrus synchronization, FSH was administrated intramuscularly to the donors for four consecutive days at a decreasing dose for Groups I, II, III, IV or equal in dose for Groups V, and VI. On the third day, PGc was given twice intramuscularly. After standing estrus was observed to animal, first insemination was done. In the corresponding group, the intramuscular injection of LHRH-A3 or LH was done at the same time with the first insemination. Second insemination was done at an interval of 8~12 h from the first insemination. Recovery of embryo was performed on days 5.5 ~ 6.5 after the first insemination.

Superovulation started 10th days after the occurrence of estrus .With 26 AU of the FSH (Japan) as example, the superovulation methods and its dosage of hormones was presented in Table 1.

Statistical analyses: After embryo recovery, the number of corpus luteum and the unovulated follicles (≥10 mm) were counted by palpation of the ovaries per rectum and B-mode ultrasound equipped with a transrectal 5.0 MHz linear-array transducer (HS-101V, Japan, Honda Co.). The non-parametric ANOVA for a single factor (Kruskal–Wallis one-way ANOVA) of SAS/STAT was used in the analysis.

RESULTS

Superovulation response: After superovulation treatment, it was observed that 34 out of the 35 donors carried two or more mature follicles with an overall superovulation effectiveness rate of 97.14 % (34/35). It was also found out that all buffaloes in Groups I, III, IV, V and VI while only 7 hds of buffaloes in Group II had mature follicles and corpus luteum after superovualtion treatment. As indicated in Table 2, overall means obtained for mature follicles was 8.71 (296/34) with 5 (170/34) for corpus luteum and 57.43 % (170/296) for ovulation rate.

Effects of different hormonal treatments on the follicles and ovulation of the donors: The numbers of corpus luteum in Groups I (6.17), and II (6.86) were higher than those obtained from other Groups, but found to have no significant differences (P＞0.05) among the treatment groups. The average number of unovulation per head was highest for Group I (5.33±2.94) and the lowest was on Group VI (1.50±1.29). Group IV had the lowest ovulation rate which accounted to 35.48 % while other Groups had the rates 50% and above. Based on the results, it appears that Group II is the best treatment and superior over the other Groups.

Corpus luteum and ovulation rate of donors treated with FSH from different sources: The average corpus luteum of FSH-Japan (6.54±4.52) treated buffaloes was higher than those in FSH(Canada , 5.37±1.85) and FSH-Beijing(3.23±1.83), and was found to be significantly different (P＞0.05) from each other (Table 3). However, almost similar results were obtained on the number of unovulated follicles in each group. On the ovulation rate, FSH- Japan and FSH - Canada treated groups were significantly better (P＞0.05) than FSH – Beijing group.

Effects of LHRH-A3 or LH on the ovulation rate of the donors: From the Table 4 the results showed that the ovulation rate (67.91%) in LHRH- A3 Group was higher than one (55.79%) in no LHRH-A3 and LH Group. It was 12.12%. Also it was higher than one (39.29%) in LH Group. It was 28.62%. Those results could also be obtained from the Table 1, which the use of the LHRH-A3 in the superovulation experiment was beneficial in enhancing the ovulation rate. However, in this experiment, the use of the LH contrarily reduced the ovulation rate, which could be in relation with the fewer number of the female buffaloes in the experiment.

Table 1. Superovulation method, its processes and dosage of hormones

Time (h)	Estrus cycle (d)						
	10	11	12	13	14	15	20
8:00	FSH 5 AU	FSH 4AU	FSH 3 AU	FSH 2 AU PGc (0.3 mg)	insemination LHRH-A3 (50µg)	insemination	embryo recovery
18:00	FSH 5 AU	FSH 4 AU	FSH 2 AU	FSH 1 AU PGc (0.3 mg)	insemination		

Table 2. Comparison of the superovulation responses among the different groups

Group	No. of animals treated	No. responded to treatment	Response rate (%)	No. of mature Follicles	No. of corpus luteum	No. of unovulated follicles	Ovulation rate (%)
I	6	6	100	11.5±2.81	6.17±2.48	5.33±2.94	53.62ab
II	8	7	87.5	9.57±6.02	6.86±5.96	2.71±1.98	71.64a
III	9	9	100	6.89±2.26	3.44±2.13	3.44±2.19	50.00ab
IV	4	4	100	7.75±2.36	2.75±0.96	5.00±2.58	35.48c
V	4	4	100	10.25±0.96	5.75±1.71	4.50±1.73	56.10ab
VI	4	4	100	6.50±2.65	5.00±2.16	1.50±1.29	76.92ab
Average	/	/	97.14	8.71±3.65	5.00±3.35	3.71±2.41	57.43

Numbers with different letters (a, b) differed significantly ($P < 0.05$).

Table 3. Superovulation effects from different kinds of FSH

Group/Source	No. of animals used	No. of mature follicle	No. of corpus luteum	Unovulated follicle	Ovulation rate (%)
FSH - Japan	13	10.46±4.55	6.54±4.52a	3.92±2.72	62.51a
FSH - Beijing	13	7.15±2.14	3.23±1.83b	3.92±1.87	45.15b
FSH - Canada	8	8.38±2.55	5.37±1.85ab	3.0±2.14	62.26a

Numbers with different letters (a, b) differed significantly ($P < 0.05$).

Table 4. Effects of LHRH-A3 and LH in superovulation

Group	No. of animals used	No. of corpus luteum and unovulated follicle	No. of corpus luteum	Ovulation rate (%)
LHRH- A3	20	6.72±4.08	4.55±4.03	67.91a
LH	4	7.21±2.36	2.75±0.96	39.29b
Without LHRH-A3 and LH	10	9.46±2.15	5.21±2.11	55.79ab

Numbers with different letters (a, b) differed significantly ($P < 0.05$).

Correlation between the number of luteum corpus numbers and embryo recovery: The presence of corpus luteum in the ovary reflects strong or weak action of FSH on the ovary of the buffalo. On the other hand, one factor for worse or better result of the embryo recovery depends on the embryo flushing technical skill. The results presented in Table 5 showed that the embryo recovery and the corpus luteum were highly correlation to each other. It was experienced that even if there are many corpus luteum in the ovary, if the embryo flushing manipulation is not completely done can have a bad effect on the embryo recovery. The curved structure of the horn of uterus of the dairy buffalo made difficult also to collect embryos. As such, the average recovery rate obtained in this experiment was only 39.84 % from the Table 5.

Table 5. Embryo recovery in each group

Group	No. of animals	No. of corpus luteum	No. of embryo recovery	Available embryo	Embryo recovery rate (%)	Transferable embryo rate (%)
I	3	8.67±0.58	1.67±0.58	1.0	16.67	66.67
II	5	10.0±6.16	6.6±6.35	2.6±3.29	66.00	39.39
II	4	4.50±2.45	0.75±0.5	0.25±0.58	33.33	35.33
IV	2	3.5±0.71	1.0	1.0	33.33	100
V	2	4.5±0.71	2.0	1.0	50.0	50.0
VI	2	6.5±2.12	2.5±2.12	1.5±2.12	38.46	60.0
Average		6.92	2.72	1.33	39.84	48.98

DISCUSSION

Superovulation effect with FSH: In the comparison of results from Tables 2 and 3 revealed that in the series of experiments conducted to know the effect of different hormonal treatments to buffaloes, even to whether with mature follicles or corpus luteum, the FSH – Japan treatment was found to be most effective. The average head number was respectively 10.46 and 6.54, followed by the Canadian group with the mean head number of 8.38 and 5.37 from Table3. The Beijing FSH had a slightly bad effect, the average head number was only 7.15 and 3.23. The experiment results were similar with the reference (He *et al*, 2004).

The action of the LHRH-A3 and LH on superovulation: In the treatment of the superovulation, a lot of follicles rapidly develop and mature due to the stimulation of the ovary by the use of the FSH. If the LH secretion is not enough, it can not induce sufficiently to mature follicles and then turn into corpus luteum. LH level in the serum can be enhanced by the injection of the LH, or by the injection of the GnRH or LHRH-A3 to regulate the body FSH and LH synthesis and releasing. In this experiment LHRH-A3 and LH were used respectively to determine the effect of two kinds of hormones to stimulate superovulation. From Table 4, we can find that the application of LHRH-A3 has improved the ovulation rate, however, the use of LH compared to other groups without the ovulation stimulus, had low ovulation rates (39.29% and 55.79%), which was in close agreement with the reports of some researchers (Carvalho, *et al*., 2002). The reason to consider of the results indicated above might be due to the addition of an inappropriate dose of LH thus inhibited ovulation. In addition, the number of treated animals is just few to conclude something hence further studies with the increase number of animals is necessary. There was another report (M.A. Beg *et al*., 1996) to confirm that the priming of donor buffaloes prior to superovulation or administration of GnRH on the day of estrus had no effect on the onset or duration of estrus, ovulation rate or steroid profile. And progesterone levels on the day of initial FSH injections and on the day of palpation (per rectum) were positively correlated with ovulation rate.

The factors influencing the superovulation, embryo recovery and availability: Out of 34 female donors, 18 donors with many corpora lutea and with good ovulation effect were chosen. Embryo flushing was done by one person with different results obtained in each donor. The average embryo recovery per head was 2.72. The mean available embryo number per head was 1.33. The embryo recovery rate was 39.84 % and the availability was 48.98 %. The highest number recovered in one female was 17 and the lowest was zero. Results showed that under the same condition, the number of the embryo obtained could be influenced by the embryo flushing technical skills. The skilled manipulation not only can help to obtain more embryos, but also shorten the manipulation duration and lighten the stress and injury of the animal. When the three-ways embryo recovery tube was used, it was noted that the embryo recovery tube must be placed deeply to obtain good results. Because of the strong flexibility of the uterine horn, intubations deeply can involve the curling and tilting of the large ligament. To obtain a high embryo recovery rate, only the suitable intubation maintaining the embryo recovery point in the lower level of the uterine horn must be performed. Some researchers (M. Anwar *et al*., 1998) reported that it was hard to recover embryos completely if one buffalo was slaughtered to collect embryos from uterine horn. As for the transferable embryos, Guo *et al*. (1998) considered that in cattle superovulation must be performed with two to three times of insemination and in each insemination the effective spermatozoon number must not be less than to 50×10^6. It was also considered that the oocytes of development, ovulation and movement were disturbed by the use of the FSH, which affected fertilization and reduced the fertilization rate. Moreira *et al*., (2002) have shown that bST (bovine Somatotropin) increased the available embryo number by increasing the fertilization rate and the early embryonic development ability which was opposed in the study of Gray *et al*. (1993) who used bST in superovulation found to have no improvement in superovulation response in cattle. Chen *et al*. (1992) considered that the purity of the FSH was related with embryonic quality. Better embryonic development and excellent embryos were obtained with the FSH product. Its mechanism relates that maybe the FSH reagent was mixed with excessive LH. Oocytes were activated before it developed maturation thus fertilization rate was reduced and the rate of embryonic degeneration was increased. In this experiment, the lower embryonic availability obtained was due to the lower vitality and fertilization ability of the frozen spermatozoon and might be also to some other factors such as hormone quality. It was suggested that further research studies must be undertaken to elucidate hormonal effects and improve superovulation in buffaloes.

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