The Effect of Gonadotropin Releasing Hormone on Superovulation in Elite Swamp Buffalo Cows (*Bubalus bubalis*)

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ABSTRACT. The effect of gonadotropin releasing hormone (GnRH) supplement was investigated in twenty eight FSH-treated buffalo cows. Animals were assigned to three groups; Group I: GnRH was given at standing heat (n=9), Group II: GnRH was given 8–12 hr after standing heat (n=8) and Group III: Control group with FSH alone (n=11). The responses (no. of corpora lutea and no. of anovulatory follicles), the number of recovered embryos and transferable embryos among the three groups were compared following slaughter of the animals on days 6 to 7 after first mating. The results indicated that the application of GnRH in FSH-treated animals gave no advantage by increasing in the number of ovulations or recovered embryos in all the treatment groups ($P>0.05$); 4.33 ± 3.35 vs 3.88 ± 4.09 vs 4.5 ± 2.68 for corpora lutea, and 2.33 ± 2.24 vs 2.0 ± 3.20 vs 1.91 ± 2.74 for recovered embryos respectively. GnRH treatment tended to reduce the number of anovulatory follicles but the finding was not significant; 6.11 ± 3.3 vs 7.38 ± 4.84 vs 10.18 ± 2.74 follicles ($P>0.05$). The supplementation of GnRH at 8–12 hr after standing heat seemed to produce more transferable embryos than those of treated at standing heat or the controls 1.63 ± 2.77 vs 1.25 ± 1.67 vs 1.36 ± 1.69 embryos respectively.

KEY WORDS: FSH, GnRH, superovulation, swamp buffalo.

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The superovulation in swamp buffalo does not provide a large number of embryos per donor [1, 5, 7], the average number of recovered embryos per donor is often less than two, with number of transferable embryos per donor averaging less than one [1]. The major factor appears to be the relatively small numbers of primordial and graafian follicles in the buffalo ovary which are about 50% of those found in cattle [8, 22] and there is also a high rate of atresia [17]. In our previous findings, a low number of ovulations and a high number of anovulatory follicles with diameter of >10 mm were evident after superovulation [19]. It was found that 8 to 10 follicles were still at the anovulatory stage and on the surface of ovary, after superovulation either by PMSG or FSH. Application of human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH) can increase the numbers of ovulations in cattle [3, 23]. There is a limited information about these hormones in buffalo and according to available information [11, 13, 16]. They seem to give no advantage. From our recent findings, GnRH supplementation in the ovarian stimulation program either using FSH or PMSG, provided a higher rate of ovarian response than those which used FSH or PMSG alone [20, 21]. The present research was conducted to determine the effect of GnRH supplementation in a superovulation program, using FSH, in swamp buffalo cows from one elite herd.

MATERIALS AND METHODS

Animals: Twenty eight swamp buffaloes cows, which had calved 2 to 5 times aged 6 to 10 years old, were examined as potential donors which was then, based on their health and their normal reproductive condition as perceived by rectal

palpation. The animals were raised at the Surin National Buffalo Breeding Center, the Department of Livestock Development, in the North-East of Thailand and were allowed to graze in the pasture during daytime. Roughage, concentrate and mineral blocks were provided at night with fresh water *ad libitum*. The animals were divided into 3 treatment groups; Group I: FSH+GnRH at standing heat (n=9), Group II: FSH+GnRH 8–12 hr after standing heat (n=8) and Group III: FSH alone, control (n=11).

Oestrous cycle control treatment and superovulation: The oestrous cycle of each buffalo cow was synchronized by intravaginal administration of 1.9 g progesterone in an insert silicone elastomer^a together with 10 mg estradiol benzoate^b. The superovulation treatment was started on day 9, after CIDR-B insertion. The modified protocol of hormonal treatment [19] is shown in Table 1. Follicle Stimulating Hormone (FSH)^c was given twice a day (8.00 and 18.00 hr) in divided doses (280 mg; 50×50 , 40×40 , 30×30 , 20×20), for four consecutive days. Oestrus was detected after the CIDR-B treatment and one injection on PGF2 α ^d. One hundred μ g GnRH^e was given intramuscularly either on the day of standing heat (Group I) or 8 to 12 hr afterwards (Group II). When showing standing heat the animal was inseminated twice, first, by natural mating with a proven fertile bull and the second time by artificial insemination 12 hr later.

^a CIDIROL[®] capsule, InterAg, Hamilton, New Zealand.

^b EAZI-BREED CIDR-B[®], InterAg, Hamilton, New Zealand.

^c Follitropin[®]-V, Vetapharm Canada Inc., London, Ontario, Canada.

^d Prostavet[®], Gifavet, Virbac, France.

^e Gonadorelin diacetate tetrahydrate, Cystolerin[®], Sanofi, France.

^f AB technology Inc., Pullman, WA, U.S.A.

Table 1. Program of FSH-GnRH treatment in swamp buffalo cows

Day of treatment	Program
Day-12	A health check and reproductive tract examination by rectal palpation CIDR-B insertion + Estradiol benzoate (8)
Day-9	FSH 50 × 50 (8 and 18)
Day-8	FSH 40 × 40 (8 and 18)
Day-7	FSH 30 × 30 (8 and 18)
	Removal of CIDR-B + PGF 2α (8)
Day -6	FSH 20 × 20 (8 and 18)
	Heat detection and insemination + GnRH(8)
Day 0	Embryo collection on day 6-7

() time of injection.

Determination of the response to superovulation: The number of ovarian corpora lutea and anovulatory follicles were counted directly immediately after the animals were slaughtered. The animals possessing two corpora lutea were judged to have responded to the treatment.

Embryo collection: Six to seven days after first mating, embryos were flushed from the oviducts and uterus using Vigro uterine-base solution^f, supplemented with 50X Dulbecco' modified supplement and their stage of development was determined under a microscope. The morula or early blastocyst stages were assigned to be normal, according to the previous observation [6] and the normal embryos were classified as being transferable. The number of ovulations, anovulatory follicles, recovered embryos and transferable embryos were presented in means ($\bar{X} \pm SD$) and compared between groups using Student's *t* test.

RESULTS

In our program, twenty nine of thirty six buffaloes (80.6%) showed oestrus 45.0 ± 6.85 hr after CIDR-B removal and 28 of them were used for embryo collection.

Four cows were found with CIDR-B loss and they were excluded from the experiment. The number of animals responding to superovulation treatment were the same for all the three groups; FSH+GnRH (0 h), FSH+GnRH (8 hr) and FSH, being 88.9% (8/9), 50% (4/8) and 72.7% (8/11) respectively (Table 2) ($P>0.05$). The number of ovulations among the groups did not differ significantly ($P>0.05$), 4.33 ± 3.35 in Group I, 3.88 ± 4.09 in Group II and 4.5 ± 2.68 in Group III, respectively. Supplementation with GnRH, appeared, though it was not significant ($P>0.05$), to reduce the number of anovulatory follicles to 6.11 ± 3.3 in group I and 7.38 ± 4.84 in group II compared to 10.18 ± 5.96 in the control group. There was no difference in the number of embryos recovered from each per donor among the three groups; 2.33 ± 2.24 , 2.0 ± 3.02 and 1.91 ± 2.74 respectively and in transferable embryos per donor; 1.25 ± 1.67 , 1.63 ± 2.77 and 1.36 ± 1.69 . The recovery rate and normality rate of the embryos from each group was of 53.8, 51.5, 42.4%; and 53.6, 81.5, 71.2%. No difference was found between the left and right ovaries.

Among the recovered embryos, 53.4% were in the morula stage while early blastocyst, blastocyst and hatched blastocyst stages were 8.6% (5/58), 8.6% (5/58) and 3.4% (2/58), respectively (Table 3). Retarded, unfertilized and degenerate embryos were 3.4% (2/58), 17.2% (10/58) and 5.1% (3/58). The embryos at morula and blastocyst stages are shown in Fig. 1.

DISCUSSION

The study showed that GnRH supplement in the superovulation regimen at or 8 to 12 hr after standing heat had no advantageous effect on ovulation rates and embryo yield in swamp buffalo. Our result is in agreement with observations in murrah type buffalo [11, 13], in which FSH+GnRH gave the same response as FSH alone. However the number of anovulatory follicles observed directly after slaughter in this study was evident higher than that from the study in

Table 2. Ovarian response and embryo production in buffalo cows after superovulation with FSH+GnRH or FSH

Group	N	Nr	Corpora lutea	Follicles >10mm.	Embryos recovered	Embryos transferable
FSH+ GnRH (0 hr)	9	8 (88.9%)	4.33 ± 3.35 Range: 0-10	6.11 ± 3.3 Range: 2-12	2.33 ± 2.24 (53.8%)* Range: 0-5	1.25 ± 1.67 (53.6%)* Range: 0-3
FSH+ GnRH (8 hr)	8	4 (50%)	3.88 ± 4.09 Range: 0-9	7.38 ± 4.84 Range: 0-16	2.00 ± 3.02 (51.5%)* Range: 0-9	1.63 ± 2.77 (81.5%)* Range: 0-5
FSH	11	8 (72.7%)	$4.5 \pm 2.68^{**}$ Range: 1-10	10.18 ± 5.96 Range: 2-20	1.91 ± 2.74 (42.4%)* Range: 0-2	1.36 ± 1.69 (71.2%)* Range: 0-8

N= number of buffaloes, Nr = No. of buffalo cows with 2 or more corpora lutea.

Value presented in mean $\pm$ SD.

*overall recovery rate or overall normality rate.

**calculated from 10 to 11 buffaloes.

Table 3. Stages and quality of recovered embryos after superovulation

Treatment	Total	4-8 C	M	EB	B	HB	UNF	DEG
FSH+GnRH (0 hr)	21	1	10	2	3	—	4	1
FSH+GnRH (8 hr)	16	1	9	3	1	—	1	1
FSH	21	—	12	—	1	2	5	1
Total	58	2 (3.4%)	31 (53.4%)	5 (8.6%)	5 (8.6%)	2 (3.4%)	10 (17.2%)	3 (5.1%)

C=cell, M=morula, EB=early blastocyst, B=blastocyst, HB=hatched blastocyt, Unf=unfertilized ova, Deg=Degenerated ova.

Fig. 1. Swamp buffalo embryos at the morula stage collected after superovulatory treatment by FSH.

murrah [13], in which the count was done per rectum; 6 to 10 vs 2 anovulatory follicles. The GnRH treatment did not alter the variability of the superovulation response as it was the same as seen in our previous observations [19]. Although the number of corpora lutea in the 3 treatment groups ranged from 0 to 10, there was no difference in the means. Also there was no difference in the ovarian responses (CL and anovulatory follicles) between right and left ovaries. The number of anovulatory follicles after superovulation in these experiments were not preovulatory LH pattern [14] and subsequent ovulation. The high percentage of anovulatory follicles after gonadotropin treatment was probably due to the high progesterone levels, which led to a block of the ovulatory LH peak [2]. In dairy cattle, the time of GnRH supplement administration plays an influential role on the superovulatory response. The ovulations increased from 7.3 ± 0.7 to 11.7 ± 1.0 corpora lutea per animal if GnRH was given at standing heat, it further increased to 15.0 ± 0.7 when GnRH administration was

delayed by 8 hr later and the number of transferable embryos was also increased [3]. These results in dairy cattle are contradicted to our findings, as no difference in recovered and transferable embryos was found. Furthermore, a variation of response among the animals was still evident. Although there was no advantage using GnRH supplement in the superovulation program, it seemed that giving GnRH supplement 8 to 12 hr after standing heat had a tendency to produce a higher number of transferable embryos than when it was given at the time of standing oestrus. In our previous study in prepubertal buffalo calves [20, 21], GnRH supplement in FSH of the PMSG programs improved the ovarian responses, in terms of the number of recruited follicles and a higher number of an homogenous size. In adult buffalo, this benefit of GnRH was not observed, although more investigations should be considered to examine the appropriate dose, time of injection and other variables.

In swamp buffalo, a low number of responding animals was not significant because the number of buffaloes show-

ing oestrus after the end of superovulatory treatment was high (80% with the mean time interval of progesterone removal at 45 hr). This is in agreement with the findings in murre buffalo [11, 13, 15], but the poor superovulation rate and a high number of anovulatory follicles is a problem that remains to be solved. Our previous study in swamp buffalo [19] suggested that the factors involved in the success of superovulation depended on the hormones used, the hormone dose and the technique of embryo collection. PMSG provided only 0.22 embryos per donor, while the FSH group gave a higher response and a higher number of embryos were found. It is remarkable that the rate of embryo collection after slaughter is so low in the three experimental groups. It is possible that embryos may have degenerated in utero before collection or the embryo transport may have been altered by the treatment due to the high level of oestradiol produced from a number of existing-anovulatory follicles. Using a GnRH agonist may be an alternative way to increase the ovulations and improves embryo quality in cattle [9], while in buffalo this subject has not yet been studied. Recently, Songsasen *et al.* [18] described the pretreatment of recombinant bovine somatotropin (BST) which tended to increase the superovulatory response and significantly increase the production of transferable embryos, in response to superovulation.

Embryo quality after superovulation allowed 50 to 80% of recovered embryos to be transferable, depending on the program used. Around 70% of embryos recovered between days 6 and 7 were the morula (50%) to blastocyst stages (20%), which corresponded to previous reports [6, 12]. In swamp buffalo, 16 cell stage, compact, morula, blastocyst, expanded blastocyst and hatched blastocysts were recovered on days 5.5, 6.0, 6.5, 7.0 and 7.5 after the onset of standing oestrus [6].

In Thailand, the National Buffalo Breeding Center and buffalo performance testing was established in 1981, in order to select parent stock for replacement of the nucleus herd. The selected bull semen is collected and frozen and then distributed to the farmers [4, 10]. The application of embryo transfer techniques could be carried out in order to maximize the number of these improved animals. Development of the superovulation programs is necessary for the development of embryo transfer in this species.

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