

Using Follicle Stimulating Hormone (FSH) for Superovulation in Buffalo

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ABSTRAK

SITUMORANG, P. 2006. Penggunaan *follicle stimulating hormon* (FSH) untuk superovulasi pada kerbau. *JITV* 11(4): 310-316.

Superovulasi pada ternak kerbau telah dilakukan dan sejumlah progres sudah dicapai tetapi jumlah embrio yang tertampung masih kecil. Dua penelitian dilakukan untuk mengevaluasi penggunaan *Follicle Stimulating Hormone* (FSH) pada ternak kerbau. Penelitian pertama limabelas ekor kerbau dibagi secara acak ke dalam tiga grup dengan masing-masing 5 ekor kerbau dan disuperovulasi menggunakan tiga macam FSH (FSH-P, FSH-china dan Folltropin). Hormon disuntikkan intra-muskular dua kali per hari selama 4 hari dengan dosis menurun. Penyuntikan pertama pada hari ke-10 dari siklus berahi, diikuti prostaglandin pada hari ke-12 dan diinseminasi buatan (IB) dua hari kemudian. Embrio ditampung dengan metoda tanpa operasi pada hari ke-6 setelah IB. Penelitian kedua, 10 ekor kerbau dibagi secara acak ke dalam dua kelompok masing-masing 5 ekor kerbau. Kelompok pertama disuperovulasi menggunakan FSH dan metodologi superovulasi seperti penelitian pertama (kelompok kontrol) dan kelompok kedua diberi suntikan FSH hari ke-1 dari siklus berahi sebelum superovulasi (kelompok perlakuan). Setelah tiga bulan superovulasi diulang dimana kelompok kontrol menjadi kelompok perlakuan dan kelompok perlakuan menjadi kontrol. Jenis FSH tidak mempengaruhi respon donor terhadap superovulasi. Rataan total corpus luteum (TCL), total embrio (TNE) dan total embrio berkualitas baik (TVE) adalah 6,8, 3,3 dan 2,2; untuk FSH-P; 6,2, 3,0 dan 2,1 untuk FSH-China dan 7,2, 3,6 dan 2,4 untuk Folltropin. Penyuntikan FSH pada hari ke-1 siklus berahi sebelum superovulasi nyata secara statistik ($P<0,05$) meningkatkan jumlah embrio yang tertampung. Rataan TCL, TNE dan TVE adalah 6,9, 2,8 dan 2,1 dan 8,2, 3,3 dan 2,5 berturut-turut untuk kelompok kontrol dan perlakuan. Konsentrasi progesterone tertinggi nyata lebih tinggi ($P<0,05$) pada perlakuan ($6,8 \text{ ng ml}^{-1}$) dibandingkan kontrol ($5,6 \text{ ng ml}^{-1}$). Pengulangan superovulasi setelah 3 bulan tidak nyata mempengaruhi jumlah embrio tertampung. Kesimpulan, kerbau dapat disuperovulasi dengan berbagai jenis FSH dan pemberian FSH pada hari ke-1 siklus berahi dapat meningkatkan jumlah embrio yang tertampung dan superovulasi dapat diulang setelah 3 bulan.

Kata Kunci: FSH, Superovulasi, Embrio

ABSTRACT

SITUMORANG, P. 2006. Using follicle stimulating hormone (FSH) for superovulation in buffalo. *JITV* 11(4): 310-316.

Studies of super ovulation have been carried out in buffaloes and the progress has been achieved but the number of embryo collected was still small. Two studies were conducted to evaluate the use of Follicle Stimulating Hormone (FSH) for super ovulation in buffalo. First study, fifteen buffaloes were randomly divided into 3 groups of five buffaloes of each group and super ovulated using three type of FSH (FSH-P; FSC-China and Folltropin). Hormones were given twice a day for 4 days in decreasing doses methods. First injection initiated on day 10 of estrus cycle, followed by prostaglandin on day 12 than artificially inseminated (AI) two days later. Recovery of embryo was conducted in un-surgically method by flushing uterus horn on day 6 after AI. Second study, 10 buffaloes were randomly divided into 2 groups of each five buffaloes First group was super ovulated with FSH following methodology in the first study (control group) and second group were given a prime FSH on day 1 of estrus cycle before super ovulation (treatment group). After 3 months the super ovulation was repeated in the reverse condition where the control become treatment and the treatment become a control group. The type of FSH did not significantly affect the super ovulation response. The mean total corpus luteum (TCL), total number of embryo (TNE) and total number of viable embryo (TVE) were 6.8, 3.3 and 2.2, for FSH-P; 6.2, 3.0 and 2.1 for FSH China and 7.2, 3.6 and 2.4, for Folltropin respectively. A single injection of FSH significantly increased ($P<0.05$) the number of embryo collected. The mean TCL, TNE and TVE were 6.9, 2.8 and 2.1 and 8.2, 3.3 and 2.5 for control and treatments respectively. The peak progesterone level was significantly higher ($P<0.05$) in treatment group (6.8 ng ml^{-1}) than those in control (5.6 ng ml^{-1}). Repeated the super ovulation after 3 months did not significantly affect the number of embryo collected. In conclusion, super ovulation in buffaloes can be performed using a type of FSH and prime injection of FSH on day 1 of estrus cycle increase the number of embryo recovered. The super ovulation can be conducted after 3 months.

Key Words: FSH, Superovulation, Embryo

INTRODUCTION

Buffaloes are farm animals with a great economic importance by its potential to provide meats, milk and draught power especially for farmer. World buffalo population was estimated to be 150 million (BOYAZOGLI, 1996), and now has been slightly increased to be around 170 millions (FAOSTAT, 2003), of which 97.1% are in Asia. India is reported to have a highest population (75 million) followed by Pakistan with just under 18 million. There are two major types of domestic buffaloes which are the river buffalo with 50 pairs of chromosome and the swamp types of the Southeast Asia region with 48 pairs of chromosome. In contrast, in Indonesia the population of this farm animal was reported slightly decreased in the last 10 years and the population in the year 2004 was around 2.572 million (STATISTIK PERTANIAN, 2005). Almost all of the Indonesian buffaloes are of the swamp type (*Bubalus bubalis*), a few hundreds of the river buffaloes are found in North Sumatera (SITUMORANG *et al.*, 1990a, 1990b). Decreasing of the buffalo population is thought to be related to the increasing demand for meats of buffalo by the increasing of people income. Low buffalo production is due to a low reproductive efficiency such as delayed puberty, longer gestation periods, low conception rates, silent heat, etc. Therefore the biotechnology of reproduction of embryo transfer (ET) is might becomes a key element of the effort to increase the production of the buffalo. Recover of embryo in superovulation program becomes an important role to determine the success of ET. The superovulation is a technique in which the genetically superior female is treated with hormones to induce her to produce many eggs simultaneously. These eggs can be fertilized with sperm *in vivo* or *in vitro* and the embryos produced then implanted/transferred into surrogate mothers (recipients). A technology of ET in cattle has been established since 1970's (SEIDEL and SEIDEL, 1982) but in buffalo is just recently reported by a numbers of researchers. The first success results of ET in river buffalo was reported by DROST *et al.* (1983) and followed in Bulgaria (KARAIVANOV, 1986, ALEXIEV *et al.*, 1988) and in India (KURUP, 1988). For swamp buffalo was reported by PARNPAI *et al.* (1985) and CHANTATRAPRATEEP *et al.*, 1988 in Thailand. Until today the technology of superovulation have achieved the certain progress however the number of embryo collected was still limited. A major obstacle of superovulation program is still the variability and the unpredictability of donor response to gonadotrophin hormone. FSH was widely used for superovulation in cattle. Initial study of using FSH resulted in a poor response of donor (DROST *et al.*, 1993; KURUP, 1988; MISRA, 1993). FSH-P (FSH prepare from porcin) was the most of FSH used for superovulation, however a

number of preparation and type of FSH was also has been available in the market. Preparation and standard of FSH was reported affect the number of embryo collected. The objective of this present study is to evaluate a type of FSH and single injection of FSH on day 1 of cycle on superovulation treatment in buffalo.

MATERIALS AND METHODS

Location, the experimental animals and management

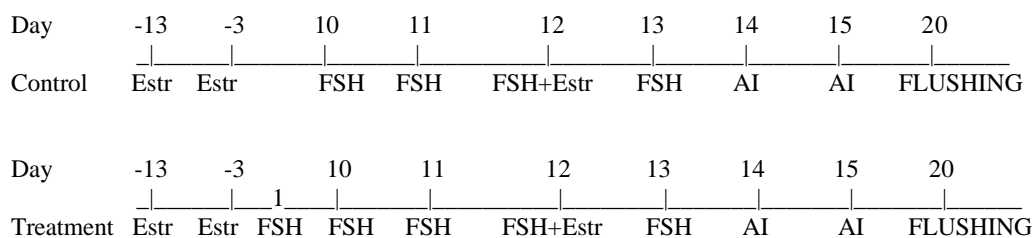
Two studies were conducted at the Indonesian Research Institute for Animal Production (IRIAP) Ciawi. Fifteen and 10 of mature buffaloes with a live weight between 350 to 420 kg were used for first and second study respectively. The buffalo cows were housed individually in 3 x 2 m pen, drinking water and elephant grass was offered *ad lib*. Each cow was offered a commercial concentrate (containing 14% crude protein) 4 kg/day as a supplementation.

Superovulation

Following two consecutive of estrus cycles, all buffaloes were given 2 intra muscular injections of 2 ml estrumate in 11 days interval to synchronize of estrus. When animals are in estrus, it is designated to be day 0 of cycle. In the first study, buffalo was randomly divided into three groups with each 5 buffaloes per group. Each group was superovulated with three types of FSH (FSH-P; FSH China and Folltropin) as a treatment. First injection of FSH was initiated on day 10 of estrus cycle and administrated twice daily (12 hours interval) intramuscularly in decreasing doses for 4 days. In the morning of day 12 of estrus cycle (after the fifth injection), 2 ml estrumate was injected intra muscular and again repeated after 12 hours. After detection of estrus in day 14 of estrus cycle, all buffaloes were inseminated artificially using frozen semen and again repeated on day 15 of estrus cycle. The schedule of treatments is shown in Table 1. In the second study, the effect of single injection of gonadotrophin on day 1 of estrus cycle following superovulation was evaluated. The best hormone in the first study was used. Buffaloes were randomly divided into two groups of 5 buffaloes. Group 1 was a control (without single injection) while the second group was given a single injection of gonadotrophin hormone on day 1 of cycle as the treatment and the procedure of hormone treatment is shown in Figure 1. After 3 months superovulation was repeated where the first group become a treatment and group 2 become control group to bring a total of 10 buffaloes for each group of treatment.

Table 1. Regimen for superovulation using 3 types of FSH as a treatment in buffalo

Day	Time	TREATMENT		
		FSH-P	FSH-CHINA	Folltropin
-13	Am	Estrmate 2.0 ml	Estrmate 2.0 ml	Estrmate 2.0 ml
-3	Am	Estrmate 2.0 ml	Estrmate 2.0 ml	Estrmate 2.0 ml
10	Am	FSH 6mg	FSH 2.5 ml	FSH 2.5
	Pm	FSH 6 mg	FSH 2.5 ml	FSH 2.5 ml
11	Am	FSH 5 mg	FSH 2.0 ml	FSH 2.0 ml
	Pm	FSH 5 mg	FSH 2.0 ml	FSH 2.0 ml
12	Am	FSH 4 mg,Estr 2.0ml	FSH 1.0 ml;Estr 2.0 ml	FSH i.o ml;Estr 2.0ml
	Pm	FSH 4 mg,Estr 2.0ml	FSH 1.0 ml;Estr 2.0 ml	FSH i.o ml;Estr 2.0ml
14	Am	AI	AI	AI
	Pm	AI	AI	AI
15	Am	AI	AI	AI
20	Am	Flushing	Flushing	Flushing

**Figure 1.** Methodology of single injection of FSH on day 1 of estrus cycle following superovulation

Technique of embryo collection

The embryos, were collected by non-surgical technique on day 20 of estrus cycle. All buffaloes were fasted for 24 hours prior to collection to reduce bowel. Buffalo was confined in a chute and 2ml 2% xylocaine hydrochloride was given to prevent straining and defecation. Through rectal palpation, a preliminary estimation was made to measure the ovary diameter and number of CL. The size of ovary was determined by skilled technician. A foley catheter was used to collect the embryo by non-surgical technique. Each horn of uterus was flushed with a 500 ml of Dulbecco's Phosphate buffered saline (DBPS) containing 0.04% Bovine Serum Albumin (BSA). Immediately after flushing, the media collected was observed for embryo then transferred to fresh DPBS containing 0.4% BSA for evaluation.

Blood collection

In one estrus cycle, blood was collected from jugularis vein 3 times a week to measure progesterone level. The collection of blood was performed up to day of 16 of estrus cycle following superovulation treatment. In avoiding the pregnancy due to the failure of embryo collection, 2.0 ml of estrumate was given on day 16 of estrus cycle. Concentration of plasma progesterone was analyzed by radio immunoassay (RIA) technique.

Parameter recorded and statistical analysis

Parameter recorded was diameter of ovary (DO), number of CL (TCL), number of embryo (TNE), number of valuable embryo (TVE) which is the embryo graded as a good and transferable embryo, the

percentage of recovery rate (calculated by dividing no of embryo to number of CL) (%RR) and progesterone level (Pr). Experimental design was complete randomly design with three treatments (FSH-P; FSH-China and Folltropin) for the first study and 2 treatments (Control and single injection of FSH) for second study. All data was statistically analyzed according to STEEL and TORRIE (1993).

RESULTS AND DISCUSSION

The ovulation rate and the mean TCL, TNE and TVE of buffalo collected in this study were much lower than those of cattle. This is very logic since in a nature the number of primordial follicle in those both species was different. Total number of follicles with diameter more than 1 mm was only 30% in buffalo than those in cattle (DANNEL, 1987). Most of the embryo collected has been well developed to morula and blastocyst stage with only one found in hatch blastocyst stage. This result is in an agreement to the previous study who stated that buffalo embryo development in female tract was faster than those in cattle (CHANTATRAPRATEEP *et al.*, 1989; DROST and ELDSSEN, 1985; MISRA *et al.*, 1990; OSMAN and SHEHATA, 2002). Therefore flushing embryo in buffalo was earlier (day 6 of estrus cycle) than those normally practiced in cattle (day 7). Delaying of flushing to day 7 will results a difficulty of collecting of embryo as in this stage most of embryo has developed to be a hatched blastocyst stages which is very past to be disintegrated. The effect of type of preparation of FSH on superovulatory response is shown in Table 2.

There was an evident that DO, TCL, TNE and TVE was higher in Folltropin than those in FSH-P or FSH-China but this difference was not significant. Superovulatory response was also increased from 75% in the both FSH preparation to 87.5% in Folltropin. This present result is in an agreement with the previous

report that Folltropin has certainly increased the superovulatory response and embryo yield in cattle (CHUPIN *et al.*, 1984; AMSTRONG and OPAVSKY, 1986) and in buffalo (MISRA, 1993). FSH from the pituitary of pigs (FSH-P) is the most widely used gonadotropin for superpopulation, however preparation from other animals have also been used. Preparation of FSH become more critical since the purity and LH content will affect the effectiveness of this hormone for superovulation. Commercial preparation of FSH is known to have or contaminated with LH content. There is an evident that LH content of FSH is correlated with superovulatory response where the increasing of LH content will decrease the superovulatory response (MURPHY *et al.*, 1984). Removal of LH from FSH increased fertilization rated in superovulated cows from 52 to 74% (DONALDSON and WARD, 1986). Now preparation of FSH with low content in LH such as Folltropin and Super-Ova has been available for superovulation treatment.

Effect of ingle injection of FSH on ovulation rates is shown in Table 3. There was an evidence that single injection of FSH on day 1 of estrus cycle following superovulation treatment significantly increased ($P<0.05$) TCL, TNE, TVE and concentration of peak progesterone. This results were comparable to the result previously reported that injection of single injection of FSH following superovulation increased TCL, TNE and TVE collected from dairy cattle (SITUMORANG *et al.*, 1994). The result is differ from the previous reports by JAILKHANI *et al.*, 1990 and JOSHI *et al.*, 1992 who found that injection 4-5 mg FSH on day 3 and 4 of estrus cycle before their superovulation on day 10 and 11 failed to increase ovulation rate in buffalo. The differences results might be affected by a different in methodology of superovulation where in the previous study injection of FSH was conducted on day 3 and 4 while in our study the FSH was given on day 1 of estrus cycle. Estrus cycle is divided into 2 phases which are 1) follicular phase and 2) luteal phase and normally the

Table 2. Effect of FSH preparation on the mean of diameter ovary (DO), total corpus luteum(TCL), total number of embryo (TNE), total valuable embryo (TVE) and percentage of response donor

Parameter	Type of FSH		
	FSH-P	FSH-China	Folltropin
DO,cm	4.4 (2-6)	3.5 (2-5)	5.0 (3-8)
TCL	6.8 (1-9)	6.2 (1-8)	7.2 (1-10)
TNE	3.3 (0-8)	3.0 (0-8)	3.6 (0-9)
TVE	2.2 (0-5)	2.1(0-4)	2.4 (0-5)
Response, %	75.0	75.8	87.5

Table 3. The effect of single injection of FSH on day 1 of estrus cycle, following superovulation treatment on ovulation rate and embryo recovery

Parameter recorded	FSH		
	Control (n = 10)	Treatment (n = 10)	Average
DO (cm)	4.5 (2.5-7.0) a	4.2 (2.5-7.0) a	4.4
TCL	6.9 (2-10) a	8.2 (3-10) b	7.1
TNE	2.8 (0-5) a	3.3(0-6) b	3.1
TVE	2.1 (0-4)	2.5 (0-4)	2.3
RR(%)	48 (20-57) a	58 (20-60) a	44
Peak P (ng ml ⁻¹)	5.6 (3.0-9.0) a	6.8 (4.6-9.2) b	5.7
Response (%)	80.0	80.0	80.0

Different alphabet in rows showed a significant difference (P<0.05)

superovulation is initiated on day 10 of estrus cycle when at the same time a dominant follicle is present. The presence of dominant follicle will suppressed the growth of the other follicle and resulting in a low response of donor to superovulation treatment. Injection of FSH on day 1 of estrus cycle has at least 2 objections which are avoiding the presence of a dominant follicle and the recruitment of follicles lead to decreasing number of follicle to become atresia. In avoiding the negative effect of dominant follicle the superovulation was initiated on day 2 or 3 of estrus cycle and has been reported to prevent the initiation of atresia (GOULDING *et al.*, 1990, MOOR *et al.*, 1984). Increasing of recruitment of follicles from the earlier stages of cycle (day 1 of estrus cycle) might be increase the number of follicles with a medium size in the time of superovulation initiated and therefore will increase the ovulation rate. This hypothesis was supported with the evidence that an increasing of peak progesterone level in treatment group (1.5 to 6.8 ng ml⁻¹) is significant compared in control group (2.7 to 5.6 ng ml⁻¹). The serious limitation to technology of superovulation are the highly variable and unpredictable superovulatory response and the level of progesterone, follicular population particularly with medium size and the presence of dominant follicle will affect the subsequent response of donor to superovulation treatment.

The effect of repeated superovulation after 3 months did not affect superovulatory response of buffalo and the mean number of embryo collected is shown in Table 4. It was an evident that the donor that give a good response in the first superovulation will also give a similar response in the subsequent superovulation. In contrast the donor do not give response in the first superovulation in general will also give a poor superovulatory response in subsequent superovulation. This present results are slightly differ from the previous results reported by RAO *et al.* (1994) who found that

there was a drop in embryo production after the first and seconds superovulation. The differences results obtained in this study was due to the different of time between first and second superovulation. In this study, repeated superovulation was done after 3 months while the previous study was 77 days. Therefore in our study the donor has been allowed to recover from any stress that might be occurred during superovulation treatment. It can be concluded that repeated superovulation after 3 months will not affect the response of donor and its means the donor can be superovulated 4 times in one year. RAO *et al.* (1994) also stated that the repetitive superovulatory treatments up to 6 times did not significantly affect the fertility of donors.

Table 4. Superovulatory response and embryo collected following repeated superovulation treatment in bubalis

Parameter recorded	Superovulation no	
	I	II
DO,cm	4.5 (2.6-7.0)	4.3 (2.5-7.0)
TCL	7.2 (2-10)	6.8 (2-9)
TNE	3.2 (0-6)	3.0 (0-6)
TVE	2.4 (0-4)	2.2 (0-4)
Peak P, ng/ml	6.4 (3.5-8.9)	6.2 (3.5-9.2)

CONCLUSION

Embryoes were successfully recovered from buffalo after superovulation treatment using a different types FSH. The total number of embryo was increased by injection of FSH on day 1 of estrus cycle following superovulation treatment. Considering the number of embryo recovered from each buffalo was still small (2-4

embryoes) and the cost for hormone, the selection of donor for superovulation program in buffaloes becomes very necessary. Repeated of superovulation for at least 3 month did not significantly affect the embryo recovery, and therefore superovulation 4 times a year can be practiced to increase the *in vivo* embryo production of each valuable donor.

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