Superovulation in Different Buffalo Genotypes

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(Diterima dewan redaksi 04 Pebruari 2003)

ABSTRAK

SITUMORANG, P. 2003. Superovulasi pada kerbau dengan genotipe yang berbeda. *JITV* 8(1): 40-45.

Penelitian dilakukan untuk mempelajari respon kerbau dari genotipe yang berbeda terhadap perlakuan superovulasi. PMSG sejumlah 2500 IU disuntikkan intra muskular sekali suntik pada hari ke 10 siklus birahi sedangkan 12 ml Folltropin diberikan 2 x penyuntikan sehari dengan jarak antar penyuntikan 12 jam selama 4 hari dengan dosis menurun (2,5; 2,5; 2,0; 2,0; 1,0; 1,0 dan 0,5; 0,5 ml). Embrio ditampung dari setiap tanduk uterus, pada hari ke 6 dari siklus birahi dengan metodologi tidak membedah menggunakan 500 ml Dubelco Buffer Phosphat Saline (DBPS). Parameter yang diukur adalah diameter ovari (DO), total corpora lutea (TCL), total embrio (TE) dan persentase tingkat penampungan (% RR). Hormon sangat nyata secara statistik (P<0,01) mempengaruhi tingkat ovulasi kerbau. Rataan DO sangat nyata lebih tinggi (P<0,01) pada PMSG dibandingkan dengan Folltropin (9,5 *vs* 4,4 cm) akan tetapi TCL, TE dan % RR sangat nyata lebih rendah (P<0,01) pada PMSG (2,0; 0,0 dan 0,0 *vs* 5,5; 2,6 dan 43,7). Rataan DO nyata lebih tinggi (P<0,05) pada kerbau sungai dibandingkan dengan kerbau lumpur maupun persilangan (6,0 *vs* 4,3 dan 3,3 cm). Tidak didapatkan perbedaan yang nyata antara kerbau lumpur dan persilangan. TCL, TE dan % RR tidak nyata secara statistik pada setiap genotipe kerbau. Rataan TCL, TE dan % RR adalah 6,2; 2,5 dan 48,0; 5,8; 2,3 dan 40,0 dan 5,7; 3,3 dan 52,8 secara berturut-turut untuk kerbau sungai, lumpur dan persilangan. Baik ovari kiri maupun kanan memberikan respon yang sama terhadap perlakuan superovulasi. Kesimpulan, respon kerbau dari genotipe yang berbeda tidak nyata terhadap perlakuan superovulasi dengan Folltropin.

Kata kunci: Superovulasi, kerbau, genotipe, embrio

ABSTRACT

SITUMORANG, P. 2003. Superovulation in different buffalo genotypes. *JITV* 8(1): 40-45.

Studies has been conducted to evaluate the response of different buffaloes genotype to superovulation treatments. PSMG (2500 IU) was injected intra muscular in a single injection on day 10 of estrus cycle while a total of 12 ml Folltropin was administrated twice a day with 12 hours interval in a decreasing doses for 4 days (2.5; 2.5; 2.0; 2.0; 1.0; 1.0 and 0.5; 0.5 ml). Embryos were collected by non-surgically technique on day 6 of estrus cycle by flushing each horn of uterus with 500 ml Dubelco's Phosphat Buffer Saline (DBPS). Diameter of ovary (DO), total corpora lutea (TCL), total embryo (TE) and percentage of recovery rate (% RR) were recorded as parameters. Administration of hormone highly significantly (P<0.01) affected the ovulation response of buffalo. The mean DO was highly significantly higher (P<0.01) in PMSG than that in Folltropin (9.5 *vs* 4.4 cm), but TCL, TE and % RR were significantly lower (P<0.01) in PMSG (2.0; 0.0 and 0.0 *vs* 5.5; 2.6 and 43.7). The mean DO was significantly higher (P<0.05) in river than both in swamp and crosses (6.0 *vs* 4.3 and 3.3 cm). No significantly different was observed between the mean DO of swamp and crosses. TCL, TE and % RR were not statistically significant among different buffalo genotype. The mean of TCL, TE and % RR were 6.2, 2.5 and 48.0; 5.8; 2.3 and 40.0 and 5.7; 3.3 and 52.8 for river, swamp and crosses buffalo, respectively. Both left and right ovary gave a similar response to superovulation treatments. In conclusion, the response of a different buffalo genotypes following superovulation with Folltropin was not significant.

Key words: Superovulation, buffalo, genotype, embryo

INTRODUCTION

Domestic buffaloes are farm animals of great economic importance. They provide meat, milk and draught power in many countries. World population of this species is about 150 millions which is distributed 4 millions in Europe and Mediterranien basin, 102 millions in Southern and Western Asia, 43 millions in the eastern Asia and Australasia and 1.7 millions in Americas (BOYAZOGLU, 1996). India is reported to have a highest buffalo population (75 millions) followed by Pakistan with just under 18 millions. There are two major types of domestic buffalo: the river buffalo of the Indian subcontinent with 50 pairs of chromosome and the swamp buffalo of the South Asian region with 48 pairs of chromosome. The Swamp water buffalo, *Bubalus bubalis* is indigenous to Southeast Asia and they play an important role to the farmer by providing a draught power. Crossbreeding by mating the river buffalo and swamp buffalo has been practiced in many countries. This mating produces an F1 hybrid with a chromosome complement of 2n = 49, the combination of the haploid sets of each parent (BONGSO and HILMI 1982). Almost all of the Indonesian buffalo are of the swamp type, a few hundred of the river buffalo are found in North Sumatera (SITUMORANG *et al.,* 1990a). The performance, semen quality and draught capacity of the Indonesian swamp buffalo and its crosses were reported (SITUMORANG and SITEPU, 1988; SITUMORANG *et al.,* 1990b; SITUMORANG and SITEPU, 1991). Technology of embryo transfer (ET) in cattle has been established since 1970's and the major limiting factor for developing this technology is the variation response of the donor to superovulation, therefore understanding of technology of superovulation is still the priority to evaluate (SEIDEL and SEIDEL, 1982). ET in buffalo is just recently reported by a number of workers. First success result in ET technology in river buffalo was reported in America by DROST *et al.* (1983) and followed in Bulgaria (KARAIVANOV, 1986), India (KURUP, 1988). For swamp buffalo was reported by PARNPAI *et al.* (1985) in Thailand. Many factors were reported to affect the ET in buffalo resulted the numbers of embryo collected are very limited (DROST *et al.,* 1983; KAIVANOV *et al.,* 1987). An optimum rate of release of oestrogen and duration of the peak, coupled with the low circulatory progesteron, have been observed to be major determinants for obtaining good ovulatory response (MADAN, 1990). Although a numbers of study were reported, until this present study there is no any information to compare the response of different buffalo genotypes, particularly the crossbred to superovulation treatment which is conducted in once time and place.

MATERIALS AND METHODS

Location, the experimental animals and management

The study has been conducted at Research Institute for Animal Production (RIAP) Ciawi, using 15 mature buffaloes (6 river, 6 swamp and 3 crosses) with live weight between 350 to 420 kg. The buffalo cows were housed individually in 2 x 3 m pens, drinking water and elephant grass was offered *ad lib*. Each buffaloes was offered 4 kg/day of commercial concentrate (14% crude protein) as a supplementation.

Superovulation

Following two consecutive estrus cycles, all buffaloes were given 2 intra muscular injections of 2 ml prostaglandin (Estrumate) in 11 days interval to synchronize of estrus. When animals are in estrus, it is designated to be day 0. In the first study, the effect of hormone for superovulation was evaluated. On day of 10 of estrus cycle, a single injection of 2500 IU PMSG were given to 5 buffaloes (PMSG Treatment), while a total of 12 ml of Folltropin were administrated to 10 buffaloes twice daily (12 hours interval) intramuscularly in decreasing doses (2.5; 2.5; 2.0; 2.0; 1.0; 1.0 and 0.5; 0.5 ml) for 4 days. In the morning of the third day (after the fifth injection of Folltropin), 2 ml estrumate was injection intra muscular and was repeated again after 12 hours. All estrus buffaloes were artificially inseminated with frozen semen on day 14 and 15 of estrus cycle and the schedule of treatments is shown in Table 1. In the second study a different buffalo genotype were superovulated using the better hormone resulted from first study. The protocols of superovulation following the doses and procedure stated in the first study.

Technique of embryo collection

The embryos were collected by non-surgically technique on day 6 of estrus cycle. All buffaloes were fasted for 24 hours prior to collection to reduce bowel. Buffalo was confined in a chute and 2ml of 2% Xylocaine Hydrochloride was injected to prevent straining and defecation. Through rectal palpation, a preliminary estimate was made for observing the diameter of ovary and number of corpora lutea (CL). The size of ovary was determined by skilled technician that has been previously trained. A Foley catheter was used to collect the embryos by non-surgically technique. Each horn of uterus was flushed with 500 ml Dulbecco's Phosphate Buffered Saline (DBPS) containing 0.04% Bovine Serum Albumin (BSA). Immediately after flushing, the collected flush media was observed for embryos and embryos collected was transferred to fresh DPBS containing 0.4% BSA for evaluation.

Statistical analysis

Parameter recorded was diameter of ovary (DO), total number of corpora lutea (TCL), total number of embryos (TE) and the percentage of recovery rate (% RR) which is calculated by dividing number embryo to number of corpora lutea. Experiment designed of study was completely randomized designed (CRD) with 2 types of hormone for first study and 3 different buffalo genotype for second study. All data recorded was analyzed according to STEEL and TORRIE (1993).

Tabel 1. Regimen for superovulation treatments in buffalo

Day	Time	Treatment 1	Treatment 2
10	am	2.5 ml Folltropin	2500 IU PMSG
	pm	2.5 ml Folltropin	
11	am	2.0 ml Folltropin	
	pm	2.0 ml Folltropin	
12	am	1.0 ml Folltropin	
	pm	2.0 ml Estrumate	2.0 ml Estrumate
	pm	1.0 ml Folltropin	
		2.0 ml Estrumate	2.0 ml Estrumate
13	am	0.5 ml Folltropin	IM
	pm	0.5 ml Folltropin	IM
14	am	AI	AI
	pm	AI	AI
15	am	AI	AI
20	am	Flushing	Flushing

RESULTS AND DISCUSSION

The present study showed that the embryos were success collected from buffaloes following methodology of superovulation practiced in cattle, however ovulation rate and total number of embryos collected were lower compared to cattle. This result was very logic since there is an evidence that reproductive characteristics of buffalo was comparable to those of cattle. The lower result obtained in buffalo comparing to those cattle due to a lower number of primordial follicles. These different and the addition to the high number of follicles become atretic and degenerate, resulted the total number of follicles with diameter more than 1 mm is only 30 % in buffalo than those in cattle (DANNEL, 1987). Since the number of healthy follicles over 1.7 mm has been found to range from only one to five in buffaloes, in comparison with a range of 17-32 in cows contribute to the lower superovulatory responses in buffaloes (MONNAUX *et al*., 1983; LEVAN *et al*., 1989). The effects hormone gonadotrophin for superovulation in buffaloes is shown in Table 2. Hormone highly significant (P<0.01) affect the means diameter of ovary, total corpora lutea, total embryo and percentage of recovery rates of embryo. Although the diameter of ovary was significantly higher (P<0.01) in PMSG than those Folltrophin but in contrast, the total corpora lutea, embryo and percentage of recovery rate were significantly lower (P<0.01). This present result is comparable with a recent report on the status of embryo transfer programmers in buffalo (MISRA, 1993) revealed that 91.2% of buffaloes responded to the superovulation treatment, the number of responding buffaloes being highest with Folltropin (95.3%) and the lowest with PMSG (80.8%). This result is in an agreement with those reported by earlier workers in cattle (LASTER, 1973; ELDSEN *et al.,* 1978; SITUMORANG *et al.,* 1994), and buffalo (CHANTARAPRATEEP *et al., 1988*). Enormous evidence showed that PMSG results in a much larger ovary, generally double the volume of one treated with FSH. MCINTOSH *et al.* (1975) reported the sialic acid content in PMSG prolonged the half-life of hormone, which results in continued recruitment of follicles after ovulation. Long requirement of those follicles secrete oestradiol far in excess of the normal preovulatory concentration cause a poor superovulatory responses (KARAIVANOV, 1986). This, in combination with an increasing of an unovulated follicles as a consequence of the low magnitude of the preovulatory LH surge and a low progesterone concentration during the early luteal phase of the superovulatory cycles, leads to a lower embryos recovered (SCHALLENBERGER *et al*., 1990).

Table 2. The effects of hormone on diameter of ovary (DO), total corpus luteum (TCL), embryo (TE) and percentage of recovery rate (% RR)

Parameter recorded	Hormone	
	PMSG (n=5)	Folltropin (n=10)
DO (cm)	9.5[a] (9-12)	4.4[b] (2-8)
TCL	2.0[a] (0-4)	5.5[b] (0-8)
TE	0.0[a] (0-0)	2.6[b] (0-6)
% RR	0.0[a] (0-0)	43.7[b] (0-75)

Different values in rows showed a highly significant difference (P<0.01)

Since hormone Folltropin is superior than PMSG, these hormone was used for the second study and the response of different buffaloes genotype to superovulation treatment is shown in Table 3. Although the mean diameter of ovary and total number of corpora lutea were lower in crosses however the mean number of embryos collected was higher than those of their parents swamp and river buffaloes and the consequence the percentage of recovery was also higher. It was interesting to note that the diameter of ovary was not a good indication to predict the number of embryo collected. Moreover, there was an evidence that the bigger diameter ovary the lower percentage recovery rate. The highest recovery rate (75%) was found from a relatively smaller ovary (3.0 cm). In general, the superovulation using gonadotrophin increase the diameter of ovary and somehow will affect the success to collect an embryo. Although the mechanism is not very clear, it is probably the increase in diameter of ovary without followed by a changes in oviduct will affect the capability of *fimbriae* to collect the ovum particullary in buffalo. These condition, become a good explanation why the percentage of recovery rate was higher in crosses buffalo and left ovary (Table 4). The failure to get embryo from PMSG treatments may be also related to the size of ovary where the diameter of ovary is extremely big and therefore the *fimbriae* is fail to collect the ovulated ovum. Only a few worker succeed to collect embryo non-surgically in swamp (TECHAKUMPHU *et al.,* 1991) and river buffalo (DROST *et al.,* 1983; ALEXIEV *et al.,* 1988; KURUP, 1988). The results found in this present study was slightly higher than those reported earlier. A comparable results reported by MADAN *et al.* (1996) who stated that the early recovery rate of 0.15 embryos has now increased to 2.0 with altered superovulation protocols. Although the reproductive performance of crosses buffalo has been studied for female (MAHYUDDIN *et al.,* 1991; PARKER *et al.,* 1991) and male (HILMI, 1991; SITUMORANG and SITEPU, 1991), the present study is the first report of multiple ovulation embryo transfer (MOET) in those buffalo genotype. The higher embryo from crosses was comparable to that reported by MAHYUDDIN *et al.* (1991) who found that percentage of pregnancy was higher in crosses than those of both river and swamp buffalo. It could be concluded that the reproductive rate of crosses is normal and it is slightly higher compare to their parents, and therefore the program crossbreeding in effort to get a F_1-hybrid for increasing the productivity of buffalo become inevitable. The response of both left and right ovary to superovulation was a similar (Table 4). There was a tendency that the diameter of ovary, number of corpora lutea and embryos were higher in right ovary than those left ovary but this different were not significant. In contrast, the percentage of recovery rate was higher in left ovary. This result was comparable to results reported in lactating dairy cattle (SITUMORANG *et al.,* 1994) who found that total corpora lutea and embryo were higher in right than those left ovary of dairy cattle. The better response of right ovary is related to arterial blood supply to the needs of the organ.

Table 3. Response of different buffalo genotype to superovulation treatments with Folltropin

Parameter recorded	Genotype		
	Swamp (n = 6)	River (n = 6)	Crosses (n = 3)
DO (cm)	4.3[a] (3-6)	6.0[b] (4-10)	3.3[a] (3-4.)
TCL	5.8[a] (2-9)	6.1[a] (1-10)	5.6[a] (3-8)
TE	2.3[a] (1-4)	2.5[a] (1-4)	3.3[a] (1-6)
% RR	40.0[a] (25-50)	47.9[a] (25-75)	52.7[a] (50-75)

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

Table 4. Response of left and right ovaries to superovulation treatment

Parameter recorded	Ovary	
	Left (n = 15)	Right (n = 15)
DO (cm)	4.7 (2-9)	4.9 (3-10)
TCL	2.9 (1-5)	3.1 (0-6)
TE	1.4 (0-3)	1.2 (0-4)
% RR	41.5 (25-75)	32.0 (25-50)

CONCLUSION

Response of buffalo to superovulation treatments using PMSG was inferior than those Folltropin. The mean diameter of ovary is significantly higher for PMSG but in contrast the total corpora lutea, embryo and percentage of recovery rate of embryo was lower than those Folltropin. The embryos were successfully collected from different buffalo genotype following superovulation with Folltropin. The mean total embryos and percentage of recovery rate were higher in crosses than those of its parent swamp and river buffalo. Left and right ovary gave a similar response to superovulation treatments. Diameter of ovary was not a good indication to predict the number of embryo collected. There was an evidence that the failure and the low embryo collected from buffalo in superovulation treatments was more affected by the failure of fimbriae to collect the ovulated ovum rather than the negative response of the ovary.

ACKNOWLEDGEMENTS

The author would like to thank the Australian Centre for Animal Agricultural Research (ACIAR) for the funding support. I remain indebted to Dr. Endang Triwulanningsih and Drh. Adriana Lubis, MSc for searching and evaluating embryo. I also recorded my thanks to all technician in reproductive physiology for preparing media and handling the experiment animals.

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