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A Controlled Intra-Vaginal Device Releasing Hormone for Superovulation Program in Buffalo

(*Controlled Intra-Vaginal Device Releasing Hormon* dalam Program Superovulasi Kerbau)

Polmer Situmorang

National Research Institute for Animal Production, Ciawi Bogor PO Box 221

ABSTRAK : Dua penelitian telah dilakukan untuk mengevaluasi hubungan antara konsentrasi progesteron dan respon superovulasi serta penggunaan CIDR pada program superovulasi kerbau. Penelitian pertama, sebelas kerbau disuperovulasi dengan hormon gonadotropin (Folltropin) yang disuntikkan intra muscular selama empat hari dengan dosis menurun dan penyuntikan dua kali sehari (jarak penyuntikan 12 jam). Penyuntikan pertama dilakukan pada hari ke 10 dari siklus berahi, diikuti dengan pemberian prostaglandin pada hari ke 12, dua hari kemudian diinseminasi buatan (IB). Penampungan embrio dilakukan dengan cara menguras setiap tanduk uterus pada hari ke enam setelah IB. Penelitian kedua, 10 ekor kerbau dibagi secara acak ke dalam dua grup (kontrol dan perlakuan CIDR). Pemberian pertama hormon pada grup kontrol dilakukan pada hari ke 10 dari siklus berahi dan 6 hari setelah pemberian CIDR untuk perlakuan CIDR. Dosis dan metode pemberian hormon gonadotropin mengikuti penelitian pertama. Tujuh kerbau memberikan respon positif terhadap superovulasi sedangkan empat ekor tidak memberikan respon. Didapat hubungan nyata antara konsentrasi progesteron pada waktu pemberian pertama hormon dengan respon donor terhadap superovulasi. Rataan konsentrasi progesteron grup yang tidak memberi respon (1,15 ng/ml) nyata ($P<0,05$) lebih rendah dibanding grup yang memberikan respon (2,51 ng/ml). Rataan konsentrasi progesteron setelah superovulasi, total corpus luteum (TCL), total embrio (TE) dan total embrio dengan kualitas baik (TVE) adalah 1,64; 1,0; 0,0; 0,0 dan 6,86; 5,9; 4,0; 2,9 berturut-turut untuk grup yang tidak memberi respon dan yang memberi respon. CIDR nyata meningkatkan ($P<0,05$) rataan diameter ovari (DO) dan konsentrasi progesteron setelah superovulasi yaitu 4,5; 4,84 dan 7,0; 7,85 berturut-turut untuk kontrol dan CIDR. Rataan TCL, TE dan TVE cenderung lebih tinggi pada perlakuan CIDR dibanding kontrol adalah 6,0; 2,8 dan 2,0 dan 9,0; 4,0 dan 3,3 untuk kontrol dan CIDR.

Kata Kunci : Superovulasi, embrio, progesteron, CIDR

Introduction

Although effort given to buffalo for increasing their production were minor comparing to cattle, the world buffalo population in the last decade increased significantly and now is approximately 172.6 million spreads over in 129 countries (FAO, 2004). The most of these farm animals are found in Asia (97.1%) and play an important role in livestock economy by providing meat, milk and draught power. Now in a country that traditionally non buffalo regions such as Australia, has reared the buffalo for producing carabao beef with quality comparable to beef cattle and moreover the milk of buffalo contains higher fat and protein than those cattle but with low cholesterol. There are two major types of domestic buffalo which is the river buffalo with 50 pairs of chromosome which is mostly found in Indian continental for producing milk and the swamp types of the Southeast Asia region with 48 pairs of chromosome for meat and drought power. In Indonesia the population of this farm animal was reported slightly decreased in the last 10 years and now the population in the year 2004 is around 2.572 million (Statistik Pertanian, 2005). Almost all the Indonesian buffaloes are of the swamp type (*Bubalus bubalis*), a few hundreds of the river buffalo are found in North Sumatera (Situmorang *et al.*, 1990a, 1990b, 2005).

Low reproductive efficiency of the buffalo is thought to be due to delayed puberty, low conception rate, silent heat, long calving interval periods, higher gestation periods, etc. Therefore the biotechnology of reproduction of embryo transfer (ET) may become a key element of the effort to increase the production of the buffalo. As the integrated part of ET *in vivo* embryo production become very necessary to provide an embryo. Embryos are recovered by a super-ovulation 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 embryo produced then implanted/transferred into

surrogate mothers (recipients). A technology of embryo transfer (ET) in cattle has been established since 1970`s (Seidel and Seidel, 1982) but in buffalo is just recently reported by a number 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), in India (Kurup, 1988). For swamp buffalo was reported by Parnpai *et al.*, (1985) in Thailand and their crosses (Swamp x River) by Situmorang (2005) in Indonesia. Until today the technology of superovulation has achieved a certain progress however the number of embryos collected was still limited. In isolated trials up to mean 5.9 transferable have been recovered (Misra *et al.*, 1999). Baruselli and Carvalho (2005) in their trials since 1990 reported acceptable follicular response during superovulation (10-15 follicles ? `8 mm) moderate ovulate rate (60%) but in contrast a low embryo recovery rate is observed. Treatment with recombinant bovine somatropin (rBST) significantly increased the superovulation efficiency (Lucy, 2000; Baruselli *et al.*,2003) Recently, Hesheng *et al.*, (2006) also reported recovery of over 4 transferable embryos in buffalo in China. A major obstacle of the application of superovulation program is still the variability and the unpredictability of donor response to gonadotropin hormone. Most studies conducted that the first injection of hormone is initiated in mid-luteal phase (Drost *et al.*, 1983; Parnpai *et al.*, 1985; Misra, 1993; Kurup,1998; Situmorang, 2005) where the corpus luteum (CL) is present. It is well documented that there is correlation between CL quality and size and its progesterone production (Kastelic *et al.*, 1990; Assey *et al.*, 1993). The objective of this present study is to evaluate a relation of progesterone level and donor response to superovulation and the using of CIDR in the method of superovulation in buffalo.

Research Methods

The experiment were conducted at Indonesian National Research Institute for Animal Production (INRIAP) Ciawi using twenty one (21) of mature buffaloes with a live weight between 350 to 420 kg. The buffalo cows were housed individually in 2 x 3 m pens, drinking water and elephant grass were offered *ad lib*. Each cow was offered a concentrate (containing 14 % crude protein) 4 kg/day as a supplementation.

Superovulation

The first trial was conducted to evaluate the relationship between plasma blood progesterone level and the response of donor to superovulation. Eleven (11) buffaloes were given two intra muscular injections of 2 ml (500 µg chloprostenol) estrumate, Troy Lab. Pty. Ltd, NSW, Australia, 11 days interval to synchronize of estrus. When animals are in estrus, it is designated to be day 0 of cycle. The buffaloes were superovulated using Folltropin, V and the first injection was initiated on day of 10 of estrus cycle and administrated twice daily (12 hours interval) intramuscularly in decreasing doses for 4 days (2.5; 2.5; 2.0; 2.0; 1.0; 1.0 and 0.5; 0.5 ml Folltropin). In the morning of day 12 of estrus cycle (after the fifth injection), 2 ml estrumate was injected intra muscularly and again repeated after 12 hours with similar doses. After detection of estrus in the morning of day 14 of estrus cycle, all buffaloes were inseminated artificially using frozen semen and again repeated on the evening (12 hours from the first insemination) and the next day (Day 15 of estrus cycle). The schedule of treatments is shown in Fig 1. Flushing was performed early in the morning of day 20 of estrus cycle (6 days after insemination) and all buffaloes from which the embryo collected were defined as responsive group while the unresponsive group was those buffalo failed in producing an embryo.

In the second trials 10 buffaloes were divided randomly into 2 groups with 5 buffaloes in each group. The first group (control) was superovulated following the method of superovulation in the first trials. The second group (treatment group) CIDR, Douglas Phar. Ltd. Aukland, NZ containing 1.9 g progesterone was inserted in any days of cycle and it is designated to be day 0 of treatment. The superovulation was initiated on day 6 and the method of superovulation following the methods for the control group. In day 8 (after the fifth injection of folltropin), the CIDR was withdrawn from all buffaloes and 2 ml estrumate was injected intramuscularly. The estrumate were repeated injected after 12 hours with similar doses. Following estrus detection, all buffaloes were artificially inseminated on day 10 by using frozen semen and again repeated 12 and 24 hours from the first of insemination. The schedule of treatments is shown in Figure 1.

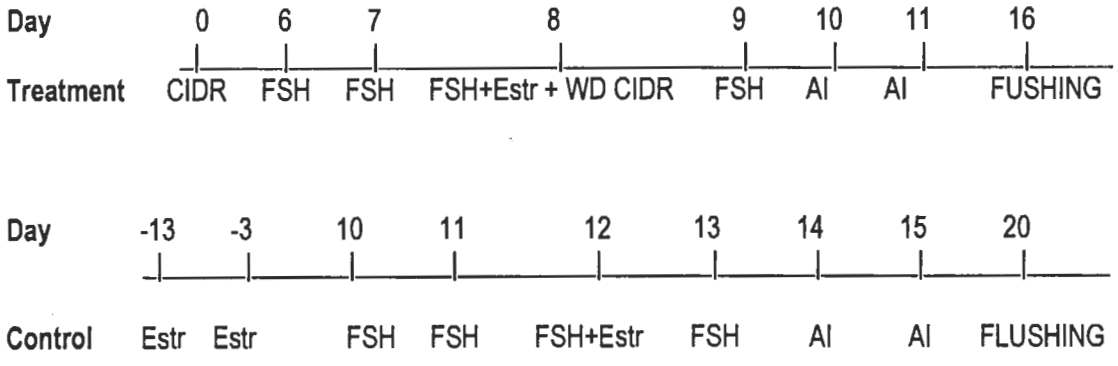

Figure 1. Methods of superovulation using FSH and CIDR in buffalo

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 2 ml 2% Xylocaine Hydrochloride to prevent straining and defecation was given. Through rectal palpation, a preliminary observation of the ovary diameter and number of CL was determined by skilled technician. A Foley catheter was used to collect the embryo by non-surgically technique. Each horn of uterus was flushed with a 500 ml of Dulbecco's Phosphate Buffered Saline (DBPS, Sigma Aldrich Chemical Co., Inc. Milwankee, USA) containing 0.04 % Bovine Serum Albumin (BSA, Sigma Chemical Co, Inc. Milwaukee St. Louis, USA). Immediately after flushing, the embryo was searched in collected media. The embryos collected were then transferred to fresh DPBS containing 0.4 % BSA for evaluation. Two (2) ml estrumate was given 2 days after flushing in avoiding the pregnancy due to the failure of embryo collection

Blood Collection.

Blood was collected from jugulars vein 3 times a week to measure progesterone level. Concentration of plasma progesterone was analyzed by radio immunoassay technique (RIA).

Parameter Recorded

Parameter recorded was diameter of ovary (DO), total number of CL (TCL), total number of embryo (TNE), total number of valuable embryo (TVE) which is the embryo graded as a good and transferable embryo, the percentage of recovery rate (%RR) calculated by dividing no of embryo to number of CL and progesterone level (Pr). Buffalo was categorized as a responsive donor if at least one embryo was able to be collected. In the first trial the mean value of each parameter recorded of each group (responsive and unresponsive group) was analyzed by t-test. Experimental designed was complete randomly designed with two treatments (control and CIDR) and 5 replications for the second trial. All data was statistically analyzed according to Steel and Torrie (1993).

Results and Discussion

Seven out of eleven buffaloes in this study gave a positive response to superovulation treatment and the mean DO, TCL, TNE, TVE and peak progesterone level is shown in Table 1. The un-responded group (4 buffaloes) has the mean concentration of progesterone before superovulation significantly ($P<0.05$) lower than those responded group. The mean DO,TCL, TNE, TVE and peak progesterone was also significantly lower in un-responded ($P<0.05$) than those responded group This present result proven the significant relationship between the level of progesterone at the initiation of superovulation and superovulatory response (Fig.2).

Table 1. Mean of DO,TCL,TNE, TVE and peak progesterone level before and after superovulation treatment of un-responded and responded group

Parameter	Un-responded (n=4)	Responded (n=7)
DO, cm	$3.00^{a} \pm 0.70$	$5.50^{b} \pm 1.15$
TCL	$1.00^{a} \pm 0.81$	$5.90^{b} \pm 2.27$
TNE	$0.00^{a} \pm 0.00$	$4.00^{b} \pm 1.29$
TVE	$0.00^{a} \pm 0.00$	$2.90^{b} \pm 0.90$
Peak Prog1, ng/ml	$1.15^{a} \pm 0.67$	$2.51^{b} \pm 1.10$
Peak Prog2, ng/ml	$1.64^{a} \pm 1.01$	$6.86^{b} \pm 2.02$

[a,b] Means in the same line with different superscript are significantly different (P<0.05). DO: diameter of ovary, TCL: total number of corpus luteum, TNE: total number of embryo, and TVE: total number of valuable embryo.

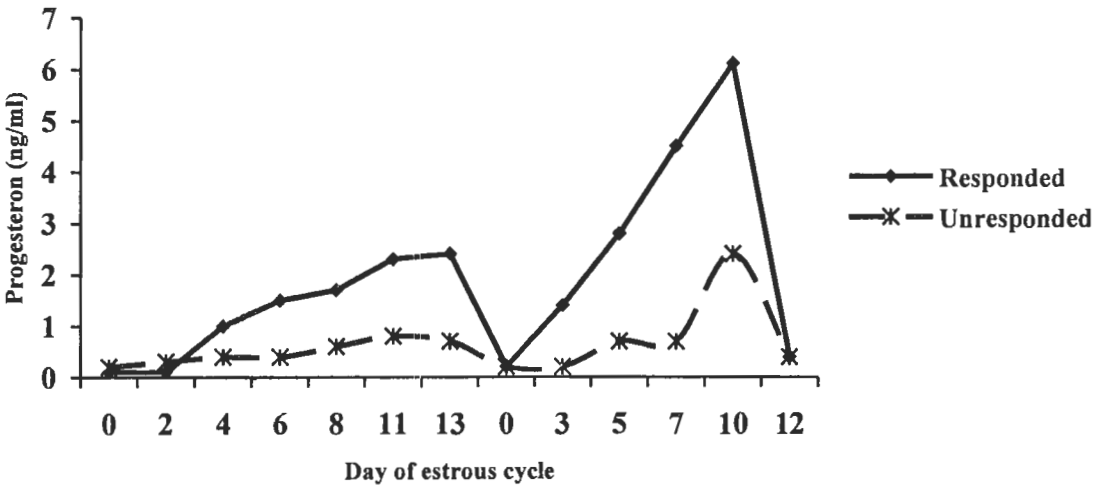

Fig 2: Progesterone level of responded and unresponded donor

There was evident that the presence of good CL both quality and quantity will significantly increase superovulatory response. In general, the superovulation is conducted during mid-luteal phase and the first administration of hormone normally initiated on day 10 of the cycle. Superovulatory response of buffalo which is superovulated either on day 9-12 or days 13-15 of the cycle did not differ (Misra *et al.*, 1990). Bulgarian worker reported that better superovulation rate was obtained when PMSG was administrated on day 10 than those on day 6 or 14 of cycle. The necessary of the initiation of superovulation in mid-luteal phase is related to the nature of the condition of ovary where the CL is present. In nature, many intrinsic factors influenced the results of superovulation. But on the time of the initiation of superovulation treatment, at least there are three major factors which are CL, dominant follicle and follicular population play a significant role for the success of recovered embryo. The presence of CL is necessary in producing progesterone to avoid the releasing of the premature LH as the consequence of the elevating of estrogen level due to superovulation treatment. Early LH surge will result the premature ovulation and decreasing superovulatory rate. Moreover there was a significant role of LH in superovulatory response (Greve *et al.*, 1984). The number of study showed that the presence of CL on the time of initiation of superovulation which is observed by ultrasonography was critical to ensure the success of collecting

embryo in cattle (Purwantara, 1995) and in buffalo (Singh *et al.*, 2003).

A similar results was reported by Misra *et al.*, (2002) who found that progesterone level at the time of initiation of the superovulation treatment in buffalo can be a good criteria for further selection for the day of superovulation. It is well documented that there is correlation between CL quality and size and its progesterone production (Kastelic *et al.*, 1990; Assey *et al*, 1993).

The effect of CIDR for superovulation protocol in buffalo is shown in Table 2. The mean DO, TCL and peak progesterone level were significantly higher ($P<0.05$) in CIDR treatment than those in control. There was evidence that the mean of TNE and TVE was also higher in CIDR treatment but this difference was not statistically different. The non significant of treatment in this present study due to partly by a big variation between buffalo for both within and between group and the small number of buffalo for replication (only 5 buffaloes for each group). Future study using a big number of buffalo will be granted to evaluate of CIDR treatment. An increasing of TNE and TVE in CIDR treatment make the application of using CIDR for superovulation program in buffalo become more applicable and worthy. The presence of CIDR which contain 1.9 g natural progesterone during FSH treatment will guarantee the blood progesterone at a level around 4 to 5 ng/ml. These level of progesterone will be increased by the addition of progesterone produced by the presence of CL will suppress the frequency and amplitude of the LH surge without preventing the follicular waves development. The higher level of progesterone in CIDR group ensure the response of donor to superovulation treatments where the embryos was success recovered from all of buffaloes (100%) while one out of 5 buffalo was failed to response to superovulation treatment in control groups (80 %). The result was comparable to the report that significantly reduced diameter of CL is an indication of premature regression and concomitant decrease progesterone level is usually associated with a poor response (Callesen *et al.*, 1986). The results in this study was comparable to result early reported by Barusseli *et al.*, (2002) cited by Vale (2004) who found that treatment with CIDR combined with prostaglandin, eCG (equine chorionic gonadotropin) and hCG (human chorionic gonadotropin) gave percentage of conception rates higher than those GnRH (gonadotropin releasing hormone) and prostaglandin.

The ovulation rate and the mean TCL,TNE and TVE collected in this study was higher than those reported earlier (Drost *et al.*, 1983; Karaivanov, 1986; Alexiev *et al.*, 1988; Kurup, 1988; Parnpai *et al.*, 1985) and comparable to results obtained in the previous studies (Misra, 1993; Situmorang, 2003; Situmorang 2005; Baruselli *et al.*, 2003; Hesheng *et al.*, 2006) but much lower than those in 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 is only 30% in buffalo than those in cattle (Dannel, 1987). Misra *et al.*, (1999) in isolated study found the mean 5.9 valuable embryo in river buffalo. The lower results obtained in this study due to a different type of buffalo used where in the previous study using river buffalo but in this study using swamp type. Although there was no statistically different of response of different buffalo genotype to superovulation treatment but there was a tendency that TNE in river type higher than those swamp type (Situmorang 2003).

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 (Chantaraprateep *et al.*, 1989; Drost and Eldsen, 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 is day 7.

Delaying of flushing to day 7 will result a difficulty of collecting of embryo as in this stage most of embryo has developed to hatch blastocyst stages which is very past to be disintegrated. Therefore in this study it might be the embryo has been developed to hatch blastocyst stage resulting a difficulty to recovery. This hypothesis was supported by a low percentage of recovery rates which was only 46 and 44 for control and treatment group respectively. There was evidence that the increasing TCL will result a lower % RR its mean an increasing number of embryo uncollected. The explanation can be drawn from this evidence is the increasing DO ovary is might not be followed by the increasing of female tract resulted the failure of fimbriae to catch the ovulated oocytes. Baruselli *et al.* (2000} found a strong evidence that low embryos recovery in buffalo due to a high proportion of oocyte fail to enter the oviduct after superovulation treatment. However these fact needs to be further investigated.

Table 2. Effect of CIDR in superovulation of buffalo using Folltropin

Parameter	Treatment	
	Control (n=5)	CIDR (n=5)
DO, cm	4.50[a] ± 0.95	7.00[b] ± 2.38
TCL	6.00[a] ± 2.28	9.00[b] ± 2.71
TNE	2.80 ± 2.53	4.00 ± 2.71
TVE	2.00 ± 1.67	3.33 ± 1.89
Peak prog1, ng/ml	4.84[a] ± 2.11	7.85[b] ± 1.16
Recovery rate, %	46.00 ±32.00	44.00 ±17.00

[a,b] Means in the same line with different superscript are significantly different (P<0.05). DO: diameter of ovary, TCL: total number of corpus luteum, TNE: total number of embryo, and TVE: total number of valuable embryo.

Conclusion

Seven out of eleven buffalo gave a positive responses to superovulation treatment using gonadotropin hormone (Folltropin). There was evidence that concentration of progesterone at the time of initiation of superovulation play an important role and the high concentration of progesterone become a significant indication of the success of obtaining a valuable embryo and therefore selection of donor for superovulation program in buffalo could be firstly based on the presence of good corpus luteum (CL). CIDR used in superovulation program in buffalo significantly increased DO,TCL and peak concentration of progesterone after superovulation. The mean of TNE and TVE was also increased but it was not statistically different. The superovulatory response of donor was increased from 80% in control to 100% in CIDR treatment. Therefore it can be conclude that application of CIDR in superovulation program in buffalo become applicable and worthy.

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