Synchronization of GnRH and PGF2α on estrus response, pregnancy, progesterone hormones in crossing of swamp and water buffalo in West Sumatra, Indonesia

Abstract. This study aims to determine the effect of GnRH and PGF2α synchronization on estrus emergence, pregnancy percentage, progesterone hormone levels and blood profile from artificial insemination (AI) of swamp and water buffalo crossing in Sijunjung, West Sumatra. The samples were 21 female swamp buffaloes with criteria clinically healthy, age ≥ 2.5 years and not pregnant. All buffalos on the first day were synchronized using 250 µgGnRH (Fertagyl®, Intervet International). All of the buffaloes received 12.5 µg PGF2α on the seventh day after GnRH injection. On the second day after injection of PG2α, the observation of estrus was carried out, the buffalos with estrus symptoms appeared after performing AI for 18 hours which the estrus symptoms was seen using a 0.5 ml Murrah buffalo semen with a sperm concentration of 500 million. Blood serum of 3-5 ml for the examination of progesterone levels was taken on days 21, 24, and 27 after appeared in AI. Hormone analysis was performed using the Enzyme Linked Immunoabsorbant Assay (ELISA) method. The variables measured were the percentage of estrus, pregnancy, progesterone hormone levels, and blood profile. Pregnancy examination (PE) was carried out after 90 days in AI through rectal palpation. The data were analyzed descriptively. The results showed synchronization of GnRH and PG2α hormones in buffalo cattle which had 100% estrus, 66.67% pregnancy after AI, pregnant buffalo progesterone concentration 5.09-8.87 ng/ml and non-pregnant 1.11 – 3.45 ng/ml, total blood protein 7.9 g/l and blood glucose 86.86 mg/dl. The conclusion of this study is that the combination of GnRH and PG2α gives a clear appearance of estrus, progesterone hormone levels, and optimal buffalo blood profile.

Keywords: GnRH and PGF2α synchronization, estrus, progesterone, swamp, and water buffalo crossing, artificial insemination

INTRODUCTION

In West Sumatra, buffalo cattle act as a producer of meat, milk, labor and a complement in traditional ceremonies. As a milk producer, the role of buffaloes is quite important, contributing to 12.8% of world milk production (FAOSTAT 2015).~~that buffalo milk is processed into products for daily consumption~~. Buffalo milk production is still low with an average of 1 - 2 liters/day (Ibrahim 2008 dan Roza et al. 2017) because most of the buffalos used for milk production are not swamp/water type buffalo. Water buffalo is a milk-producing buffalo that is only found in North Sumatra Province and needs to be conserved as local livestock germplasm considering its population is less than 1000 individuals. Water buffalo has the potential as a milk producer developed in tropical regions such as Indonesia because of its high adaptability. Buffalo milk has the advantage of the fat content of 6-10% and protein 4-6% compared to the fat and protein content of cow's milk by 3-4% and water buffalo milk production ranging from 6-8 liters/head/day (Mihaiue *et al.* 2011, Roza *et al*., 2015).

Buffalo cattle have enormous potential to be developed in Indonesia to increase national milk availability. The population of buffalo in 2008 was 2.2 million, of which more than half (51%) were on the island of Sumatra. During the last five years (2011-2015), the population of buffalo in West Sumatra has fluctuated and tends to increase by around 18.8% (Direktorat Jenderal Peternakan, 2015). This proves that the natural and socio-cultural conditions of the people of Sumatra Island provide a decent place for the development of buffalo cattle. The buffaloes that many Indonesians maintain are swamp buffalos that are not dairy types even though in some areas farmers do milking.

To increase the production of meat and buffalo milk, it is necessary to make genetic improvement efforts through selection and cross-breeding. Increasing productivity of buffaloes through cross-breeding has not much being done in Indonesia. Buffaloes from the cross-breeding process produce high-quality meat and produce more milk than their mothers. The main obstacle that inhibits the productivity of buffaloes is the length of the calf because the heat of buffalo is not easily identified (silent heat), so it is difficult to detect the heat (Senger, 2005; De Rensis dan Lopez-Gatius, 2007). One way to overcome this problem is by applying reproductive biotechnology, namely the technique of estrus synchronization using the hormones of GnRH, FSH and Progesterone and Prostaglandin (PGF2α), whose purpose is to manipulate progesterone to the lowest level (Rensis dan Lopez-Gatius, 2007).

Progesterone is one of the important reproductive-related hormones secreted by Luteal corpus luteum (CL) cells (Hafez, 2000 and Hafez, 2000). Corpus luteum is an endocrine organ that is responsible for producing the hormone progesterone. Blood serum progesterone concentration can determine the state of the animal in an infertile, normal, estrus, or pregnant state so that it can be used for estrus detection, pregnancy examination and knowing other pathological conditions. Early pregnancy diagnosis based on progesterone hormone concentrations has been carried out in cattle (Amiruddin et al. 2001).

The AI program for synchronizing estrus in buffalo cattle is essential. The advantages of estrus synchronization include increasing reproductive efficiency (Herdis, 2011). Several studies have been conducted on buffalo abroad using GnRH and PGF2α as a method of synchronization in Mediterranean buffalo (Berber et al. 2002), Egyptian buffalo (Bartolomeu et al. 2002) and Italian buffalo (Neglia et al. 2003).

~~Increased productivity of buffaloes through crossing in Indonesia has not been done much~~, but in other countries such as the Philippines, China, Australia, Vietnam, and Bangladesh, a lot has been done to get dual-purpose buffaloes. Crossing of swamp buffalo and water buffalo is conducted to form new breeds with a genetic composition of water buffalo ~~blood~~ above 32.5%. The productivity of crossing between 32.5% water buffalo 67.5% and swamp buffalo results on 40% body weight which is higher than swamp buffalo (Lemcke, 2004). The buffalo produced by this crossing method is a strong working animal, produces high-quality meat and produces more milk than its mothers. The purpose of this study was to detect estrus, pregnancy, and progesterone hormone levels after synchronization of GnRH and PGF2α in crossing swamp and water buffalos in Sijunjung, West Sumatra.

MATERIALS AND METHODS

The material used was female swamp buffalo milked in Pematang Panjang village, Sijunjung District, West Sumatra with the total number of 21, aged ≥ 2.5 years old with GnRH hormones (Fertagyl®, Intervet International, Europe) and PG2α (Noroprost® Noorbrok, Northern Ireland).

This study uses an experimental method in buffalo cattle which produce dadih/dadiah in Pematang Panjang village, West Sumatra. The location and breeder selection uses purposive sampling method. The buffalo used by the selection was based on good health; reproduction was not interrupted and was not pregnant, carried out by health workers and sub-district staff of Artificial Insemination (AI).

On the first day, the female buffalos were injected with GnRH (Fertagyl®, Intervet International, Europe) intramuscularly (I m) with the total number of 250 µg/head. On the seventh day 12.5 mg of PG2α was injected (Noroprost® Noorbrok, Northern Ireland) intramuscularly (I m). On the second day after injection of PG2α, the observation of estrus was carried out. According to Siregar (2008), ~~Lust~~ symptoms in buffaloes are generally not as clear as in cows, which are characterized by changes in the external genitals, vulva reddened, swollen and mucus coming out and changes in behavior. AI could be done after 18 hours of estrus symptoms seen using a 0.5 ml Murrah buffalo semen with a sperm concentration of 500 million. The frozen semen used was from the North Sumatra Artificial Insemination Center. On the 21^{st}, 24^{th} and 27^{th} day, 3 - 5 ml of blood was taken from each buffalo. Blood sampling was performed by manual technique using a venoject needle and vacuum tube, assisted by technicians from the local Animal Science Office. Pregnancy examination was conducted through rectal palpation 90 days after AI. The tools used were AI equipment, syringes, and venoject for collecting buffalo blood, coolboxes, kit and chemicals for analysis of blood and progesterone hormones. Blood samples were taken to the Biomedical Laboratory of the University of Andalas Medical School in Padang to analyze blood progesterone concentrations with the Enzyme-Linked Immunosorbent Assay (ELISA) method using a progesterone kit (Diagnostic Products Corporation, Los Angeles, CA), and test sensitivity of 0.24 n.mol/liter (Technical Reports Series, 1984). Moreover, blood profile analyzed using the Reflotron Plus method with modification of Reflovet Plus (Roche).

Variables measured: percentage of estrus, percentage of pregnancy, pregnancy number by looking at the number of pregnant females divided by the number of inseminated females multiplied by 100%, progesterone hormone levels, protein and blood glucose levels. The data obtained were analyzed descriptively by displaying percentages, calculating averages and standard deviations (Sudjana, 2005).

RESULTS AND DISCUSSION

The results of observing the percentage of estrus in synchronized buffalo cattle with GnRH and PG2α show excellent results for the appearance of 100% estrus (Table 1.), marked by discoloration of the vulva to red and swollen, mucous discharge from the vulva and changes in animal behavior to become agitated. This shows that GnRH given can respond to buffaloes synchronized with PG2α to cause estrus; GnRH will help uterine involution. ~~This is in accordance with the opinion of Irikura *et al* (2003) that the hormone PGF2α can lyse the luteal corpus in buffalo which results in all estrus buffalo cattle (100%).~~ This condition occurs because GnRH will stimulate FSH to stimulate follicle growth and stimulate LH to ovulate and form Corpus Luteum (CL) and respond well to PG2α; this is in accordance with the statement of Metwelly *et al*. (2001) and Irikura *et al*. (2003) that the combination of giving GnRH and PG2α gave estrus 100% in virgin and adult buffaloes. The results of this study are similar to those of Yenriza *et al.* (2012) that the giving of 300 μgGnRH synchronized with 12.5 mg PGF2α can show signs of estrus in postpartum buffalo cattle with a percentage of pregnancy 100%. This is confirmed by Neglia *et al*. (2003), Paul and Prakash (2005) that the combination of the use of GnRH and PGF2α will accelerate the emergence of heat in buffalo.

This situation shows that the reproductive conditions of acceptor animals are fertile and have a regular reproductive cycle so that they respond well to the PGF2α hormones. Brito *et al*. (2002) reported that reactivation of prostaglandin hormone (PGF2α) to livestock that has regular cycles in the luteal phase would be effective in stimulating estrus, due to the nature of prostaglandins which lyses CL. Generally, the luteal phase (diestrus phase) is around 17 days from the buffalo estrus cycle (on average of 21-22 days), so it is estimated that in one buffalo population, female buffaloes in the luteal phase can reach 60-80% (De Rensis dan Lopez-Gatius, 2007).

The appearance of estrus is caused by Gn-RH, which is responsible for stimulating FSH release. This FSH hormone plays an important role to stimulate follicle growth in the ovary. The growth of follicles will stimulate the formation of estrogen. According to Fricke and Shaver (2007), the emergence of estrus is caused by the effect of increasing the hormone estrogen in the body produced by the ovum. ~~This is supported by~~ Hafez, (2000) Gn-RH which functions to stimulate the release of FSH and LH in anterior pituitary will stimulate the development of Follicle and ovulation and the formation of the corpus luteum. Rajamahendran *et al.* (2002) stated that the number of recruited follicles to develop further to de Graaf is highly dependent on FSH concentration in the blood.

Percentage of pregnancy and hormone levels of progesterone

The hormone progesterone is one of the reproductive hormones that are very important in the sexual development and reproductive performance of female mammal. The concentration of the hormone progesterone in blood of Pregnant and not pregnant swamp buffalo in AI after estrus synchronization can be seen in Table 1.

Table 1. Percentage of Estrus, Levels of Progesterone and Hormones of Pregnant and non-pregnant Buffalo swamps inseminated artificially after Estrus Synchronization

~~Number of~~ Buffalo No.	**~~Percentage of Estrus (%)~~**	**PE**	**Progesterone Hormone Profile (ng / mL) After AI (day)**		
			21	**24**	**27**
1	~~100~~	Pregnant	5.87	6,22	8.69
2	~~100~~	Not-Pregnant	2.22	2.41	1.92
3	~~100~~	Pregnant	5.32	5.61	7.13
4	~~100~~	Pregnant	5.78	5.99	7.02
5	~~100~~	Not-Pregnant	1.29	1.11	0.89
6	~~100~~	Not-Pregnant	2.68	2.27	1.07
7	~~100~~	Pregnant	5.61	6.70	8.07
8	~~100~~	Pregnant	5.58	6.84	7.75
9	~~100~~	Pregnant	5.21	6.34	8.62
10	~~100~~	Pregnant	5.09	5.98	7.41
11	~~100~~	Not-Pregnant	2.13	2.76	1.99
12	~~100~~	Pregnant	5.35	6.57	7.98
13	~~100~~	Not-Pregnant	1.57	2.34	3.45
14	~~100~~	Pregnant	5.87	7.03	8.87
15	~~100~~	Pregnant	5.65	6.79	8.03
16	~~100~~	Not Pregnant	1.62	2.51	1.89
17	~~100~~	Not Pregnant	2.34	3.22	2.45
18	~~100~~	Pregnant	5.69	7.12	8.48
19	~~100~~	Pregnant	5.90	6.91	8.37
20	~~100~~	Pregnant	5.41	6.89	8.35
21	~~100~~	Pregnant	5.19	7.02	8.57

According to Table 1, the results of pregnancy examination by looking at the concentration of the progesterone hormone carried out on 21^{st}, 24^{th} and 27^{th} day after AI showed that from 21 swamp buffaloes in AI with Murrah buffalo frozen semen, 14 of them (66.67%) got pregnant, and 7 were not pregnant (33.33%). Buffalos that are not pregnant may be due to the condition that is different from the pregnant one, which is the first time pregnant, while the pregnant cattle have given birth two and three times. This is supported by Belstra (2003) that parity is positively correlated with the life span or age of livestock. The pregnancy rate is similar to the results of research by Lietman *et al*. (2009) which reached 61%.

Pregnancy testing and the ability of progesterone to maintain pregnancy are more effective if it is conducted on the 21^{st} day or more after AI is performed because progesterone levels at that time have stabilized. In pregnant animals, the level of progesterone hormone will tend to be high while the non-pregnant cattle have lower levels. Thus, no embryonic death occurs after the 21st day after on AI can be used as pregnancy indicator.

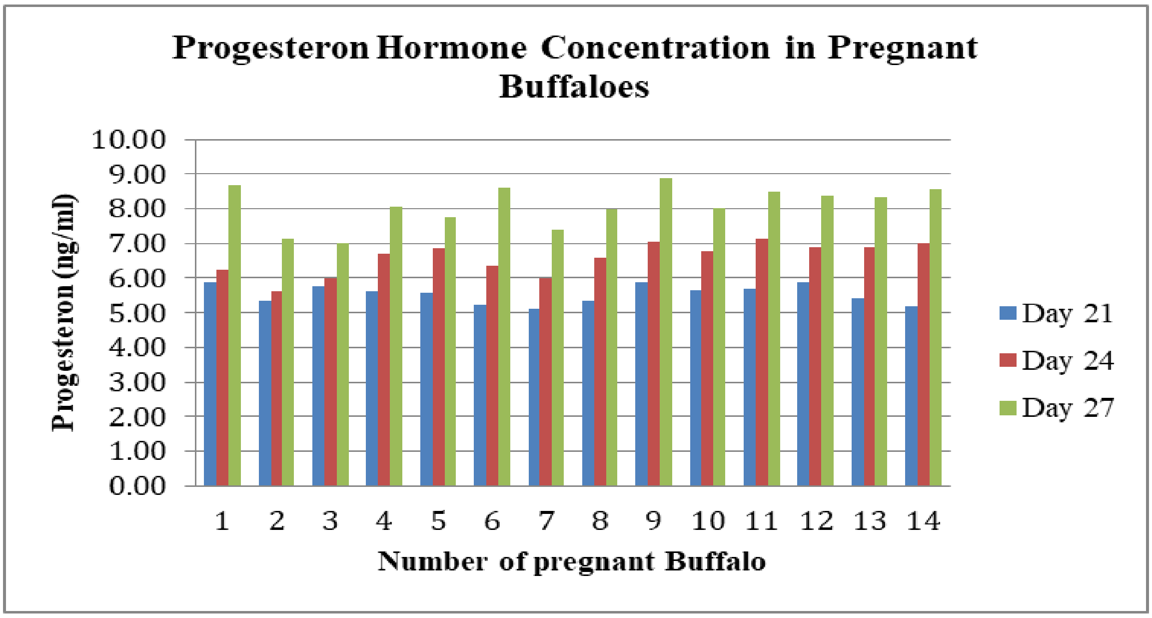

Figure 1. The concentration of Progesterone hormone of Pregnant Buffalo (21, 24 and 27 days after AI)

In Figure 1 it shows that the concentration of progesterone continues to increase from day 21, 24 and 27 days after IB. On the 21st day after at AI the lowest hormone progesterone concentration was 5.09 ng/ml and the 27th day after AI was increased to 7.41 ng/ml. This condition showed that buffaloes were likely to have the pregnancy and could be maintained until the 60th day because of the CL activity that produced the progesterone hormone. The results of this study are similar with the results of McDonald (2000) that the progesterone levels in pregnant cows levels above 6.6 ng/mL while not at the time of pregnancy were 0,1-2,2, ng/mL (Muhamad et al. 2000). In a study of Korean cows (Hanwoo) Ryu *et al.* (2003) found that progesterone levels in pregnant cows were more than 3 ng/ml while those that were not pregnant were less than two ng/ml. The concentration of progesterone in blood plasma decreases 60.42 - 50.88 nmol/L and in the last month of pregnancy it was 1.59 - 9.54 nmol/L at the time of delivery (Partodiharjdjo, 1992 and Ginther et al. 2010). Concentration non-pregnant cow progesterone typically decreases on day 17 to 20 of the estrus cycle, while pregnant cows, progesterone concentrations continue to be maintained until close to the end of pregnancy. According to Frandson (1996), progesterone can cause thickening of the endometrium and the development of the uterine gland preceding the implantation of the fertilized ovary.

Total of protein and blood glucose

The results of this study indicate that the total blood protein of pregnant buffalo is quite high, namely 7.19 g/dl. This indicates that sufficient total serum blood protein concentration in pregnant buffalo is a sign that the buffalo has sufficient protein in the ration so that the amino acids are working for the biosynthesis of gonadotropins and the gonadal hormone (Khan *et al.* 2010).

The biochemical profile of blood serum, especially the level of total protein and blood glucose levels indicates the fulfillment of nutrients in the rations given, both in terms of quality and quantity. Such conditions are very influential in the reproductive system. According to Pradhan and Nakagoshi (2008) cows fed with low-quality nutrition have a significant influence on the state of reproduction. Nutritional deficiencies in the ration can affect the ovulation and fertilization process, affecting the development of the embryo and fetus in the uterus, so that causing embryonic death and absorption of the embryo by the uterine wall, abortion or the birth of a weak child and neonatal death (Jainudeen dan Hafez, 2000; Bearden et al. 2004).

The results show that the concentration of glucose in the blood serum of pregnant buffalo was quite high at 86.68 mg/dl. The high serum glucose levels in pregnant buffalo indicate high energy (carbohydrates) in the ration. This study supports the opinion of Chandrahar *et al.* (2003), that pregnant dairy cows have high blood glucose levels. The blood glucose level of swamp buffalo in this study was higher than that of buffalo blood glucose levels reported by Fahlevi *et al.* (2017), which ranged from 34,00-114,00 mg/dL. The high blood glucose level indicates the fulfillment of nutrients in the rations given and affects reproduction. If blood glucose levels in the serum are low, besides being able to inhibit the synthesis or release of gonadotropin-releasing hormone (GnRH) it also inhibits the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), causing obstruction of follicle, ovum, estrogen and progesterone development. Nutritional deficiencies also have an impact on the death of the ovum, embryo, and fetus due to insufficient ovarian steroid hormones.

Glucose is one of the most critical metabolic substrates needed for functions that are compatible with reproductive processes in buffalo. The low serum glucose levels not only can cause high concentrations of non-esterified fatty acids

(NEFA) which have toxic effects on follicles, oocytes, embryos, and fetuses (Murray et al. 2003), and decreased hypothalamic GnRH secretion (Murray et al. 2003), but also decrease GnRH which inhibits FSH and LH synthesis and cause recurrence of mating (Mulligan et al. 2006).

To conclude, the results shows that the injection of GnRH hormone combined with PG2α in buffalo livestock gave 100% estrus appearance; pregnant buffalo progesterone concentration 5.32 -8.69 ng/ml and non-pregnant 1.11 - 2.68 ng/ml with pregnancy percentage of 62.5%, total blood protein 7.9 g/l and blood glucose 86.86 mg/dl. Combination of GnRH and PG2α gives rise to estrus and progesterone concentration and optimal buffalo blood profile. ~~From the research, it is highly expected that this study will continue to look at the results of the crossing between F1 swamp buffalo with the Murrah buffalo on the production and quality of F1 milk and other product performance.~~

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