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Effect of using GnRH plus P4 and estradiol plus P4 on ovarian response of embryo production of local Pesisir Selatan cows in West Sumatera

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Abstract. The objective of this study to evaluate the effect of GnRH plus P4 and estradiol plus P4 on superovulation responses and to know the relation between ovarian activity of local Pesisir Selatan cows. The data were obtained from 4 local Pesisir cows superovulated on breeding centre in west Sumatera. The treatment in superovulation was using GnRH plus P4 and estradiol plus P4 and data were analyzed using SPSS16 to determine the effect of treatment on ovarian structure and the regression correlation. The result showed that using GnRH plus P4 was greater ovulate rate, transferable embryos and lower the percentage of degenerate and unfertilized ($P < 0.05$) than using estradiol plus P4. Linear regression between dominant follicle, the number of CL and the number of embryos in coefficient correlation $R_{fcl} = 0.950$; $R_{fe} = 0.829$ and $R_{cle} = 0.980$, respectively. In conclusion the using of GnRH plus P4 for FTAI is greater the response in embryos production of local Pesisir Selatan cows.

Keywords – GnRH, estradiol P4, embryo production, FTAI.

1. Introduction

Embryo transfer can be used to enhance genetic improvement and to increase marketing opportunities with purebred cattle. Embryo transfer is used for cattle, is caused low productivity rate and long generation interval. The primary use of embryo transfer in cattle has been to amplify reproductive rate of valuable cows. In local cattle transfer embryo technique is potential to apply in order to increase the offspring and population as genetic resources of beef cattle in west Sumatera. However, the local cattle has been correlated to the lower fertility of beef herds. The advantages of using ET are acceleration and dissemination of local cows to increase the population as a beef cattle. In previous study showed ovulating follicle size has a greater effect on fertility of local Pesisir cow ranged from 6 mm – 11 mm and largest follicle diameter has the highest ovulation rate and conception rate [1]. ET technique has been introduced as an efficient tool to improve fertility for repeat breeding dairy cows.

Multiple ovulation and embryo transfer (MOET) is a reproductive technology used to increase embryo production of cows, this technology has been used to increase the number of embryos for embryo transfer program. Currently, there are several protocols for fixed time artificial insemination (FTAI) and embryo transfer with different combinations. Ovulation synchronization protocols such as ovsynch allow timed ET without the need for estrus detection in donor and recipient. Protocol for SOV without estrus detection are especially important when working with *Bos indicus* donor and high-yielding dairy *Bos Taurus* cows, due to



the inherent difficulties with estrus with these animals [2] [3]. According [4] that delaying the timing of an ovulatory stimulus (GnRH or pLH) resulted in an increased superstimulatory response and embryo production following FTAI of lactating Holstein cows. Expression of estrus during protocols for TAI or TET is associated with an increase in fertility and reduction in pregnancy loss. During TAI programs, optimizing follicle diameter and increasing circulating P4 on day 7 after AI were also associated with increased fertility [5].

The timed-AI protocol commonly referred to as Ovsynch consists of three treatments; two injections of GnRH given 9nd apart, with an intervening injection of PGF2 α given 7 d after the 1st GnRH treatment. According to [6] that the application timed – AI and timed-ET can improve reproductive efficiency in dairy cows. Furthermore, various mechanism for improvement fertility due increase estradiol concentration associated with estrus expression exist. The improve embryo quality and advancement in stage of development among heifer that exhibited estrus could also be a result of improved environment for embryo development. Exhibiting estrus before TAI resulted in improved embryo quality and advanced embryo stage on d 6 [7]. A recent embryo transfer experiment that examined cows that did not exhibit estrus before TAI demonstrated that donor cows with increased concentration of estradiol at AI were more likely to yield a fertilized embryo and increased estradiol tended to improve embryo quality and viability [8].

Protocols have now been developed that allow AI to be performed at a known time in relation to expected ovulation without the need for detection of estrus even in anovular cows [9] [10] [11]. Fixed time artificial insemination are important tools to improve reproductive efficiency in cattle. Therefore, this present study was designed to compare the protocol of time embryo transfer using GnRH plus P4 and using estradiol plus P4 to increase the fertility of local Pesisir Selatan cows in West Sumatera. The objective of this study were to evaluate the ovulatory response of local Pesisir Selatan cows and to compare two protocol of time embryo transfer.

2. Material and methods

2.1. Location, animal and management

This study was conducted in breeding centre (BPTU-HPT) Padang Mengatas during June – August 2018 using multiparous cows local Pesisir Selatan (n=5). Then were housed during the treatment and then maintained in intensive pasture that allowed for continuous grazing, and salt and water was available all the time. The cows were selected on the basis of ovarium function (transrectal ultrasonography) and postpartum period, normal estrus cycles, and normal health status.

2.2. Experimental design and FTAI

The donors were divided into two groups: Treatments A). Time embryo transfer program using GnRH plus progesterone combination in local cows Pesisir Selatan cows. The protocol consists of the administration PGF2 α (lutalyse) concurrent with P4 device insertion (Day0), followed by the administration of GnRH (fertagyl) on Day 7 AM. Treatment with gonadotropins is then initiated on Day 8 PM 936 h after GnRH, with twice daily administration of FSH until Day 12 AM. Donors are given GnRH on Day 13 AM, with FTAI 12h and 24 later. All donors was used Ovsynch protocol (GnRH; PGF2 α) to synchronize ovulation and FTAI without estrus detection. Finally, embryo are collected on Day 20 (Figure 1) (Treatment B). Timed embryo transfer program using estradiol plus progesterone, the treatment of P4 implanted concurrent estradiol on Day 0. Treatment of FSH on Day 4 twice daily until Day 7 PM, and the GnRH treatment should be given 24 h after the P4 device removal (Day 8 PM), followed by FTAI both 12 h (Day 9 AM) later. Embryo are then collected (flushing) on Day 15 PM (Figure 2). The embryos were recovered by standard nonsurgical uterine flushing 7 days after AI. The number of ovulatory follicles were assessed by Transrectal Ultrasonography immediately before GnRH and corpus luteum (CL) immediately before embryo collection. The cows with 3 or more CL were considered to have responded to the superovulation treatment (SOV).

After collection, the embryos were classified according to the criteria defined by the international embryo transfer society [16]. The quality grade was assessed as good (grade 1), fair (grade 2), poor (grade 3), or degenerated (grade 4). Only good and fair quality embryos at the morula and blastocyst stage were used for transfer and freezing. The number of ovulatory follicles were assessed by transrectal ultrasonography examination performed before GnRH injection and 7 days later immediately before embryo collection to assess the number of CL. Percentage of transferable embryos was calculated by dividing total number of grade 1, 2, and 3 embryos by the total numbers of embryos. The percentage of degenerated embryos was calculated by dividing the total number of generated embryos by the total number embryo. Percentage freezable embryos was calculated by dividing total number of grade 1 and 2 embryos by the total number of embryos. Ovulation rate was calculated by dividing the number of CL by the number of ovulatory follicle.

Table 1. Timed embryo production programs using GnRH plus progesterone combination in local Pesisir Selatan cows (A).

Treatment day	AM	PM
0	P4 device + PGF2 α	-
7	GnRH	
8		FSH (20%)
9	FSH (20%)	FSH (15%)
10	FSH (15%)	FSH (10%)
11	FSH(10%)	FSH (5%) +PGF2 α
12	P4 device removal+FSH (5%)	
13	GnRH	FTAI
14	FTAI	
20	Flushing and evaluation	Freezing

Table 2. Timed embryo production programs using estradiol plus progesterone combination in local Pesisir cows (B).

Treatment day	AM	PM
0	P4 device + EB (2 mg)	-
4	FSH (20%)	FSH (20%)
5	FSH (15%)	FSH (15%)
6	FSH (10%) + PGF2 α	FSH (10%)
7	FSH (5%)	FSH (5%)+P4 device removal
8	GnRH	FTAI
9	FTAI	
15	Flushing+evaluation	Freezing

The freezing medium consisted of 5 % ethylene glycol and 6% propylene glycol in Dulbecco's phosphate-buffered saline (PBS) and 20 % fetal bovine serum. Embryo at the morula and blastocyst stage were transferred directly into the freezing medium, and each embryo was then loaded into a 0.25 ml plastic straw and allowed to equilibrate for 15 min. the straws were then placed in an alcohol bath of programmable freezer and cooled at -6.5 °C for 5 min. subsequently, seeding was induced by touching the straws with forceps pre-cooled in liquid nitrogen. Five min later, the straws were cooled to - 30°C at the rate of - 8.3 °C min⁻¹, and then the straws were plunged into liquid nitrogen and stored in liquid nitrogen.

To compare the two timed embryo transfer program was analyzed by using SPSS 16 program. The results in table are expressed as a mean \pm standard deviation, graphs and regression correlation between the parameter was used.

3. Result and discussion

3.1. Effect of treatment on ovulation rate and quality of embryos of Pesisir Selatan cows

The ovulation rate was higher in treatment A is 70.37 % that in treatment B is 50 % ($P < 0.05$), with the number follicular ovulation was 19 from total follicular ovulation was 27 in treatment A and 9 follicle was ovulate in total follicular follicle 18. This present study showed that treatment A using GnRH plus P4 is more effective to superstimulation ovary than the estradiol plus P4. Ovulation rate was increase by using GnRH plus progesterone, this association with the function of GnRH induce the follicle emergence wave and ovulation. FTAI is used of GnRH 2x and PGF2 (ovsynch protocols) to stimulate of follicle emergence wave ovulation. Administration of GnRH protocol depended to ovarium status (medium anestrus is better response than deep anstrus). Ovsynch plus CIDR protocol can induce synchronized ovulation and luteal formation with a normal life span in early postpartum beef cows even though they are in non- cycling status [12] [13]. Meanwhile, estradiol and progesterone- based synchronization protocols have been successfully used to control follicular and luteal dynamics and to synchronize ovulation enabling AI without estrous detection [14] [15]. Many factors may influence how donors respond to superovulation and generate a high number of fertilized good to excellent quality embryos. According to [16] founded that the ovulation in different protocols superovulation range from 38.7 ± 10.6 % to 88.0 ± 4.9 %. According to [17] and [18] that treatment with GnRH on day 6 and 7 of the estrus cycle is the optimal time for ovulation and fertility, however, treatment GnRH at random reproductive stage generally results in ovulation in 50 % or less. [9] reported that regression of dominant follicle does not occur in all dairy cows following the standard treatment with 2 mg EB ant P4 implant and 3 mg EB does not improve follicle wave synchronization. Furthermore, treatment GnRH that can cause ovulation of CL and thus increase circulating P4 during growth of the ovulating follicular wave. Treatment with E2 esters can induce CL regression, potentially decreasing circulating P4 during ovulatory follicle growth. This present study is supported by [19] that the increased P4 was due to greater ovulation to the GnRH than the Eb at the star of the protocol and reduced premature CL regression for GnRH compare with EB.

The percentage of transferable embryo is higer in treatment A (61.11%) than treatment B (0%) transferable embryo ($P < 0.05$) and the number of transferable embryo in GnRH plus P4 is 11 embryos and did not have the transferable in using estradiol plus P4. [20] that the number of transferable embryo dairy cows in different postpartum period range from 1.7 ± 0.74 – 4.4 ± 0.93 . the quality and stage of the embryo development influenced the CR. [21] [22] founded the number of transferable embryo was 2.01 ± 0.39 . In treatment A greater the percentage of transferable embryo caused GnRH protocol can induce an LH surge, ovulate a dominant follicle and thus initiated a synchronized new follicular wave, although the magnitude of these responses depend on stage of the estrus cycle when GnRH treatment is given. That percentage of embryos classified as grade 1 and 2 were 21.4 % vs 32.8 % ($P < 0.05$) and lower than the percentage s of embryos with a quality code excelent and /or good were 81.5 % to 83.8 %. In addition, that on day 6 of estrus cycle a reduction of the dose of GnRH to synchronize follicular wave emergence as a pretreatment for superstimulation promote transferable embryos [23].

Table 3. Effect of treatment on ovulation rate and embryo quality of local Pesisir cows.

Superovulation Response	Time Embryo Transfer Program	
	GnRH +P4 (treatment A)	Estradiol+P4(treatment B)
Ovulation rate (%)	70.37	50
Transferable embryo (%)	61.11	0
Degenerated embryo (%)	22.22	66.66
Unfertilized (%)	16.66	33.33

The degenerate embryo percentage in using GnRH plus P4 (22.22 %) was lower ($P < 0.05$) than using estradiol plus P4 (66.66). This present study indicated that GnRH plus P4 is also effect in quality of embryo

associated with FSH. The number of degenerate of embryo in using GnRH plus P4 was 4 and in using estradiol plus P4 was 2 ($P < 0.05$). The number of generated embryo in the present study was higher than reported by [22] that 0.93 ± 0.25 and [24] was founded the transferable embryo in Holstein 12.46 % and higher in inbreeding group. According to [25] that a lower number of transferable embryos in cows with Fx = 9.0%-30.0% compare to those with Fx = 0%-8.9% ($P < 0.05$).

The unfertilized in treatment A (16.66%) is low ($P < 0.05$) than treatment B (33.33%). The fertilized embryo is lower in treatment A than treatment B ($P < 0.05$). The number of unfertilized embryo in GnRH plus P4 was 3 and in using estradiol plus P4 was 2. This result was higher than reported by [22] that the the average unfertilized ova was 1.31 ± 0.37 and [24] was 15.77 %. According to [26] that E2 or P4 treatments are used at the initiation of protocols suppression circulating LH and FSH concentrations and regression the follicles in the current follicular wave, resulting in a synchronized follicular wave emergence 3 to 5 d later, depending on the dose type of estradiol. [13] founded that higher synchronization and transfer rates, and shorter postpartum interval to ET, can be achieved with timed ET following the Ovsynch plus CIDR protocol than after estrus with the single PGF2 α treatment.

3.2. Regression correlation of ovarian activity in two treatment of timed embryo transfer program

The average number of follicle dominant is 27, the number of corpus luteum is 19 and the number of embryo is 118 in GnRH plus progesterone combination. In estradiol plus progesterone combination the number of follicle dominant is 18, the number of CL is 9 and the number of embryo is 6. The average number of embryos in treatment A is 6.0 embryo and treatment B is 3.0 embryos /donor. This present study the number of embryos is lower than reported by [27] was 14.3 ± 2.1 90.5 % (in hyaluronan 0.5%) and 14.4 ± 2.0 (in hyaluronan 10%) and 10.2 ± 1.8 (in FSH). This present study the transferable embryos is lower than earlier study was range from 47.8 ± 8.4 to 62.5 ± 7.5 %. The largest number of cows showing estrus in GnRH group may be could be explained due to the induction of an earlier follicular wave. Thus, the greater size of follicle has more time to grow and therefore it was able to produce more levels of estradiol at the time of CIDR removal [28].

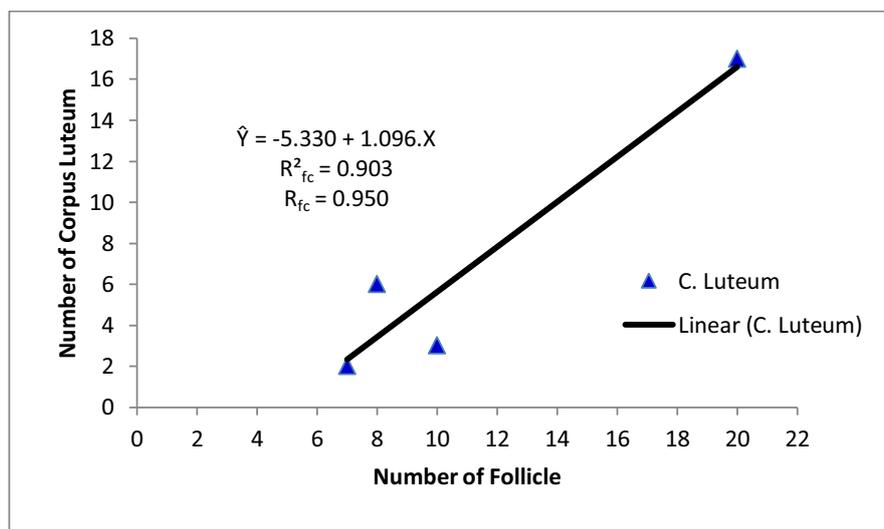


Figure 1. Linear regression lines for the number of follicle dominant with the number of CL of local Pesisir Selatan cows.

The linear regression for the number of follicle dominant in relation to the number of CL (Figure 1), with high coefficient correlation is $R = 0.950$ and linear regression (Figure 2.) with the embryos is $R = 0.879$. Furthermore, the linear regression for the number of CL in relation to the number of embryo (Figure 3), with the high coefficient correlation is $R = 0.980$. At the time of flushing not all the donor have the equal number of follicle with the CL and embryo is tend to decrease the number, even the linear regression. In treatment GnRH plus P4 from 27 follicle dominant produced 19 CL and and produced 18 embryos. In treatment B at the time of flushing the number of embryos was 6 from 9 and 10 follicle dominant. This present study showed that the ovarian activity response, tend to decrease at the step of cyclic status of reproduction of donor. This means the number of embryo production depended to the number of follicular ovulation and the number of CL at the flushing.

This precense study supported by [16] that the number of ovulatory follicles increased, the number of CL, also increased ($r = 0.84$) and the number of CL increased, also increased the number of embryo ($r = 0.38$). Thus more total embryos collection because more total structure ovarian were recovered. According to [29] compared twice daily im FSH injections to once daily sc FSH injection to superovulate beef heifers, had the greater superovulatory response, more recovered structures, and more embryos grade 1 and (twice daily is greater than once daily). In addition, that no effect of the number ovulateion on the percentage of transferable embryos.

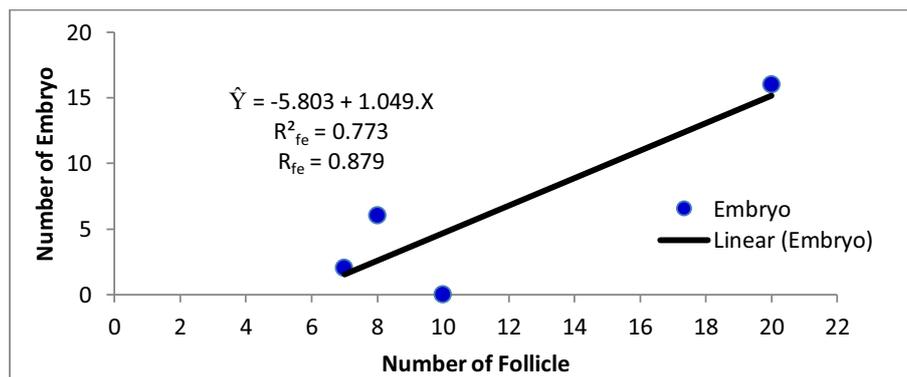


Figure 2. Linear regression for the number of follicle dominant on the number of embryo of local Pesisir Selatan cow.

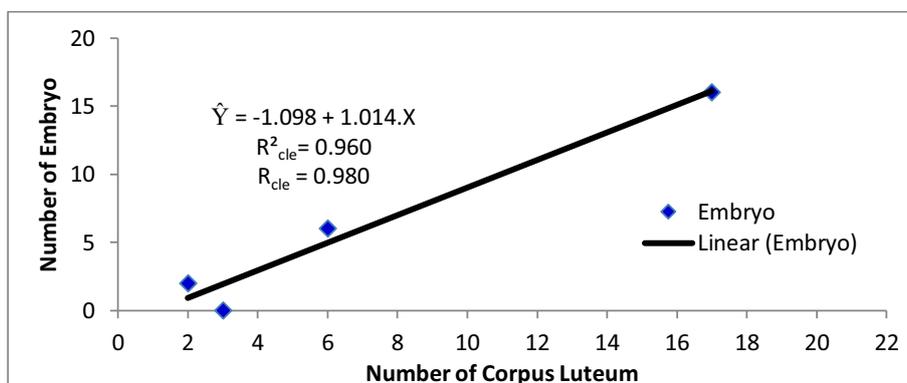


Figure 3. Linear regression for follicle dominant, the number of CL and the number of embryos local Pesisir Selatan cows.

In this result showed that the greater number of follicle, tend to greater number of CL and embryos production and treatment GnRH plus P4 more embryo production than treatment estradiol plus P4. According to [30] that large ovulatory follicles have associated with the formation of the larger CL with greater capacity of synthesizing P4. The manifestation of estrus behavior prior to timed-AI was associated with large ovarian follicle, greater plasma P4 concentrations during the luteal phase and greater conception rate [31]. When animal are bred following a TAI protocol, where ovulation is induced by GnRH, estrus response e and increased preovulatory concentration of estradiol before ovulation are critical determinants of subsequent embryo quality and potential pregnancy loss. According to [24] that inbred animals with a lower coefficient F_x had a very small and insignificant difference in quality and proportion of transferable embryos compared with group $F_x = 0$. At greater inbreeding coefficients (3.1%- 25%), the reduction in embryo quality increased.

The donor of Pesisir Selatan cows had the inbreeding without breeding management control, caused of limited bull to bred the cow at the time breeding seasons. According to [25] that a decrease in the count of transferable embryos in the inbreeding group $F_x = 3\% - 5.9\%$. The effect of inbreeding depression on ovarian activity in superovulated cows. The regression equation was significant for transferable embryos was $R^2 = 0.91$ [24], founded that increased inbreeding of embryos had a negative impact on reproduction.

4. Conclusion

The ovulation rate, and the quality of embryos were greater in using GnRH plus P4 than Estradiol plus P4. The response of ovarium activity have the linear regression between number of follicular ovulation, the number of CL and the number of embryo of local Pesisir Selatan cows in west Sumatera. linear regression of ovarian activity was high in donor local Pesisir Selatan cows.

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