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Application of Embryo Transfer Technology to Improve Fertility Using GnRH Plus Progesterone and Estradiol Combination (FTAI) of Local South Pesisir Cow in West Sumatra

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Abstract*— The objective of this research was to evaluate the effect of FTAI on embryo production that used GnRH plus progesterone combination to synchronize ovulation of 3 different parity of donor from local pesisir cows in west Sumatra. This study used timed embryo transfer program using GnRH plus progesterone combination in 3 local pesisir cows and 2 cows in time embryo program using estradiol plus progesterone of donor. Ovarian ultrasonography was performed on d 0 (time of AI) and at flushing to determine ovulatory diameter and ovulation. On d 0 at the time CIDR implant + PGF2α injection and on d 7 started ovsynch protocol administrated by injection GnRH and FSH for FTAI without detection estrus. The number and the quality of embryo was evaluated to determine the fertility of pesisir selatan cows. The result showed that the expression of estrus cows tend to high ovulation rate. The diameter size of a follicle was range from 5 to 15 mm with 10.67±2.96 mm in GnRH plus progesterone and 5 to 14 mm with mean was 8.75±2.05 mm in estradiol plus progesterone (P<0.005). The number of follicle ovulation was 27 in treatment A and 18 in treatment B (P<0.05). The number of CL and embryos was higher in treatment A. CL was 19, with 18 embryos and in treatment B was 9 with 6 embryos. The quality of embryo range from 1-4 grade with the transferable embryos was higher in treatment A than treatment B (P<0.05). There was the linear regression between ovular follicle, CL and number of embryos recovery of local pesisir cows. It was concluded that equal numbers of embryos and transferable embryo by using GnRH plus progesterone combination of local pesisir cows.

***Keywords*— embryo transfer; FTAI; synchronization; ovulation.**

I. INTRODUCTION

Reproductive technologies in cow by the application of in vivo embryo production have been used to enhance genetics progress and fertility of local cow. The application of embryo transfer (ET) technique in local pesisir selatan cows could improve fertility such as pregnancy rate. The diameter of local pesisir selatan cows has a small category as a key to determine the fertility of cows [1]. Regarding the low fertility of repeat breeders and low ovulation rate, the embryo transfer becomes essential tools reproductive efficiency in local cow in West Sumatra. The main advantages of ET in local cows are to produce more calf per selection donors. This biotechnology help bypassing some of the greatest challenges of dairy cow reproduction: the difficulties of estrus detection, and low fertility [2]. According to [3], it is recommended to have enough process for production, selection of donor and recipients, and transfer procedures of the embryo as well as in recipient management before embryo transfer. It can be an effective to improve fertility in herds with reasonable fertility.

FTAI protocols were developed with the goal of synchronizing follicular grow, luteolysis and ovulation. The different protocol for synchronization of follicular wave emergence and ovulation with self- appointed management has been developed to avoid estrus detection and enhance pregnancy rate. According to [4] that strategy established to manipulate follicular wave dynamics (synchronization of the follicular wave emergence and super stimulation) can optimize the efficiency of embryo production techniques. The most common treatment to selectively induce follicular wave emergence involves the use of estradiol and progesterone (P4), especially in Bos indicus cow [5]. Recent protocols have been designed to control follicular wave emergence and ovulation, allowing the initiation of super stimulatory treatments and the AI of donor at a self-appointed time [6] Protocol for superovulation (SOV) without estrus detection are especially important when working with bos indicus donor, due to inherent difficulties with estrus detection [7],[8] The efficacy of estradiol and P4 administration, followed by the initiation of FSH treatment at the expected time of follicular wave emergence (4 days later), has been demonstrated in several studies with bos

indicus [9]. Futhermore, the protocols that synchronize the time of ovulation use estradiol (E2), progesterone (P4). and PGF2α to synchronize ovarian fuction and time of ovulation to allow FTAI [10]. According to [11] that the use of EB at the time of CIDR insertion in a Heatsynch protocol in dairy cow proved to be an alternative to GnRH which can an important tool to increase the TAI utilization by farmers. Furthermore, in heatsynch protocol cows showing estrus have pregnancy rates improved and lower pregnancy losses.

Embryo transfer is a reproductive technology used to increase production of offspring from selected donor cows. Superovulation and embryo transfer are used to increase the number of offspring from genetically outstanding females as well as superior sire [3]. Multiple ovulation and embryo transfer (MOET) have been widely implemented in breeding to target the exploitation of cow genetics. The difficulties initially encountered for the reliable induction of superovulation in donor animal have been overcome through the use of FSH and LH hormonal treatments and the manipulation of follicular waves by steroid treatment. Thus , three important aspects should be considered when developing SOV protocol: 1); control of ovarian follicular dynamics and the follicular wave emergence to initiate gonadotropin treatments; 2):. Time of ovulation induction and AI in super stimulation donors; 3). Type of (FSH or eCG), dosage, and frequency of gonadotropin treatments for SOV. Generally, all the factors influencing embryo production is essential for breeding and achieving a satisfactory level of embryo recovery and transfer [9]. Even though the embryo transfer technology (ETT) has several benefits and requires consistent promotion, challenges such as lack of trained personnel and equipment for ovulation and packaging of embryos at the farm site need to be addressed [12] According to [13] that embryo production was affected by donor parity, and P/ET was affected by embryo type, embryo stage, embryo quality, recipient estrous cycle day at ET. Many research said that ET can be applied to beef herds to improve fertility and calving rate. However few research have focused on the effect of FTAI protocol as a potential factor for embryo production.

This research focused on the embryo production of local pesisir cow by introducing FTAI plus progesterone and estradiol combination without the need to detect estrus. The specific objectives of this present study are : 1). To increase the follicular dominant, ovulation rate and corpus luteum of local pesisir selatan cow; 2). To determine the number of embryo and transferable embryo of local pesisir selatan cows; 3). To compare FTAI plus progesterone combination and FTAI using estradiol plus progesterone combination on embryo production of pesisir selatan cows. This study is relevant and unique since there have not been any previous researches showing the use of FTAI and steroid combination on fertility of pesisir selatan cows in West Sumatra.

II. Material and Methods

This research was conducted at BPTU-HPT Breeding Farm in Padang Mengatas Payakumbuh, West Sumatera in July 2018. The five selected donors of local Pesisir Selatan were used in four different age such 2.5; 3; 3.5 and 4 years old, second parity and 2 months postpartum. The cows were housed in the same building and fed the same diet and all cows used were cycling before to start of protocol. The donors were divided into two groups: Treatments A). Time embryo transfer program using GnRH plus progesterone combination in local south pesisir cows. The protocol consists of the administration PGF2α (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 36 h after GnRH, with twice daily administration of FSH (Folltropin-V) 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 ;PGF2α) to synchronize ovulation and FTAI without estrus detection. Finally, embryo are collected on Day 20 (Fig.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 (FIG.2). The embryos were recovered by standard nonsurgical uterine flushing 7 days after AI.

The number of the 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 respond to the super ovulation treatment (SOV).

TABLE I
TIMED EMBRYO PRODUCTION PROGRAMS USING GNRH PLUS PROGESTERONE COMBINATION IN LOCAL PESISIR SELATAN COWS (A)

Treatment Day	AM	PM
0	P4 device + PGF2α	-
7	GnRH	
8		FSH (20%)
9	FSH (20%)	FSH (15%)
10	FSH (15%)	FSH (10%)
11	FSH(10%)	FSH (5%) +PGF2α
12	P4 device removal+FSH (5%)	
13	GnRH	FTAI
14	FTAI	
20	Flushing And Evaluation	Freezing

TABLE II
TIMED EMBRYO PRODUCTION PROGRAMS USING ESTRADIOL PLUS PROGESTERONE COMBINATION IN LOCAL PESISIR COWS (B0)

Treatment Day	AM	PM
0	P4 device + EB (2 mg)	-
4	FSH (20%)	FSH (20%)
5	FSH (15%)	FSH (15%)
6	FSH (10%) + PGF2α	FSH (10%)
7	FSH (5%)	FSH (5%)+P4 device removal
8	GnRH	FTAI
9	FTAI	
15	Flushing And Evaluation	Freezing

After collection, the embryos were classified according to the criteria defined by the international embryo transfer

society (Robertson and Nelson, 1998). The quality grade was assessed as good (grade1), 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.

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 ºC 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 - 30ºC at the rate of – 8.3 ºC min ¹, and then the straws were plunged into liquid nitrogen and stored in liquid nitrogen.

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

III. Result and Discussion

A. *Ovarian responses of cows treated using GnRH plus progesterone (A) and using estradiol plus progesterone (B) combination on number of ovulating follicle , number corpus luteum, number of embryo and diameter size of dominance follicle in local pesisir cows in West Sumatra*

1) Diameter size of ovulating follicle and corpus luteum

The diameter size of ovulating follicles at AI in GnRH plus progesterone combination (treatment A) ranges from 5 to 15 mm and the mean of the ovulating follicle size is 10.67±2.96 mm. The diameter size of ovulating follicle at AI in estradiol plus progesterone (treatment B) ranges from 5 to 14 mm and the mean is 8.75±2.05 mm.The diameter size of corpus luteum in treatment A range from 3 to 5 mm and the mean is 3.53±0.63 mm and in treatment B ranges from 3 to 5 mm and the mean is 3.56 ±0.72 mm (Fig. 1). The number of corpus luteum in treatment A ranges from 1 to 5 and the total is 14 CL and in treatment B the number of corpus luteum ranges from 1to 3 and the total of corpus luteum is 9 CL(Fig.2). This present study indicated that superovulation protocol with GnRH plus Figures 1, 2 and 3 show that the highest soluble fiber, insoluble fiber, and total fiber were 60 minutes of reaction time and 400C reaction temperature. The temperature and duration of extraction affect soluble fiber, insoluble fiber, and total fiber. This is presumably because a lot of soluble fiber, insoluble fiber, and total fiber extracted in corn hair powder. and P4 – releasing devices allows to development of more follicle, corpus luteum and of local pesisir cow. Both treatments require lesser managements and shorter duration of embryo production in local cows. The diameter size of ovulating follicle and diameters size of corpus luteum was no significant different (P>0.05). This both combinations resulted similar in diameter size of follicle and corpus luteum of local pesisir cows, caused the protocols used can improved ovarian function, ovulatory stimulus and without estrus detection, but in treatment A combination between GnRH with PGF2α improving the synchrony of ovulation. However, due to differences in diameter size of ovulating follicle and CL that appropriate time to induce ovulation may differ in local cows. According to [14] that CIDR-PG and co-sycnh –CIDR protocols provide the opportunity to expedite genetic improvement through FTAI and facilitate enhanced reproductive management within the herd. This is clearly that the most successful hormone therapy to synchronize follicular wave emergence in cow. In addition, strategies established to manipulate follicular wave dynamics can optimize the efficiency of embryo production techniques.

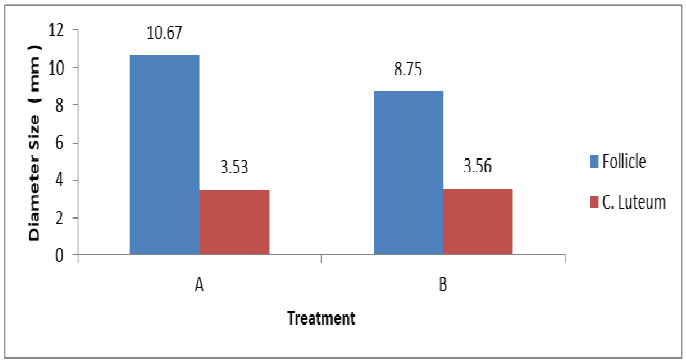

Fig. 1 The diameter size of ovulating follicle and corpus luteum of GnRH plus P4 and Estradiol plus P4 in local pesisir cows .

The diameter size of ovulating follicle in this present study is smaller than in crossbred cows was 13.6 ±0.5 mm in ECP protocol and 13.5± 0.7 mm [15], was 13 mm [2]. However this study is similar to [16] that diameter size of ovulating follicle range from ≤6mm to ≥ 9 mm with mean is 13.1±0.26 mm in the GnRH-FSH analogue, 8.2 ±1.16 mm in the hMG, and 4.2±1.07 mm in the eCG-FSH. The diameter of follicle dominant on d2 was similar among primiparous (12.9±0.56 mm than the dominant follicle of multiparous cows that became pregnant (15.6±0.37 mm) [17]. In this result showed no significant effect of treatment GnRH plus P4 (A) and estradiol plus P4 (B) on diameter size of ovulating follicle of local cow (P.0.05). This indicated that the GnRH plus P4 and Estradiol plus P4 were allows development of follicles then acquire the capacity of ovulation and increasing the number of embryo production. According to [5] that program for ET estradiol plus P4 or GnRH plus P4 for B. indicus are proposed. The use of FTAI in super ovulated donor eliminates the need to detect estrus with satisfactory result. Reducing the number of animal

handlings and improving the efficiency of protocols using estradiol or GnRH and P4 – releasing devises to control follicular wave emergence and ovulation for SOV and ET in B. indicus and B. Taurus cow [6],[18] In addition, that the largest follicle did not differ significantly in diameter between treatment on day 0 in PGF is 10.9±1.0 mm and ovsynch + CIDR is 10.9± 0.5 mm. the CL diameter did not differ significantly on day 7 were 16.6±1.00 mm and 16.2±0.9 mm in ovsynch +CIDR [19].

2) The Number of Dominant Follicle, The Number of Corpus Luteum and the number of embryo

The number of dominant follicle range from 8 to 19 embryo in area of ovarium and the mean is 13.3±2.121 embryo with total number of embryo is 27 in treatment A. In the treatment B the number embryo range from 3 to 7 and the mean of follicle dominant is 9.00±1.41 (Fig.2). The number of CL in treatment A range from 1 to 13 and the mean of CL is 9.50± 2.82. In the treatment B the number CL range from 2-7 and the mean of CL number is 4.50± 2.11 (Fig.3). The number of embryo in treatment A range from 3 to 9, and the mean the number of embryo is 9.00± 2.33 (Fig. 4). In the treatment B the number of embryo range from 1 to 5 and the mean the number of embryo is 3.00±0.70 (Fig. 4). The number of dominant follicle, the number of corpus luteum and the number of embryo in treatment A (GnRH plus progesterone) is significant higher than treatment B ($P<0.05$) in all area of ovarium. This study indicated that the using of GnRH plus P4 (treatment A) in embryo transfer program is essential to FTAI. This presence study is supported by [20] that GnRH treatment improved ovarian function rather than embryo quality and the administration of GnRH at the first insemination time resulted in a greater number of CL and fewer unovulated follicles at the time of embryo collection ($P<0.01$). there are two types of FTAI protocols currently used in beef cow ; GnRH –based and estradiol –based protocols, both of which are combined with progesterone – releasing devise. Protocols that control follicular development and ovulation using GnRH or estradiol and progesterone – releasing devices provide for the opportunity to apply FTAI in beef herds without the need for detecting estrus [21].

The number of dominant follicle and corpus luteum is lower to [22] that the follicle number range from 7-51 and CL number range from 7-32 using Folltropin and using B50-LArFSH the range of dominant follicle from 4—37 and 2-39 of CL According to [23] found the number of large follicle (>9 mm) , the number of CL and the number of embryo were 5.1±1.4; 15.4±2.5 and 12.9±1.4, respectively. The number of dominant follicle ,and the number of CL in precent study is higher than earlier studied [24] that 13.4±5.5 and 8.0±1.3, respectively. The number of dominant follicle and corpus luteum to measure the superovulation response of local pesisir cows. In this study found that the number of dominant follicle, corpus luteum and the number of embryo is higher in treatment A (GnRH plus progesterone) than treatment B, (estradiol plus progesterone). This means that the combination of GnRH and PGF2α in FTAI protocol (named ovsynch) can induce synchronized ovulation and luteal function with a normal life span. This result is higher than reported by [25] that the 3FSH and the 8FSH had the similar number follicle > 8 mm at ovulation induction with pLH were 15.8±0.9 vs 16.1±1.1, and the number of embryo were 7.0±0.6 vs 7.2 ± 0.5, respectively, the number of CL at flushing were 11.8±0.8 vs 12.8±07,respectively. However, the number of follicle were 8.9±0.5. CL at flushing 7.1±0.6 in 2FSH. In addition, using Brahman donors showed the number of follicle at LH injection was 17.7±7.8, the CL number at flushing was 14.1±7.6. and the number of transferable embryo was 8.4±0.3[26].

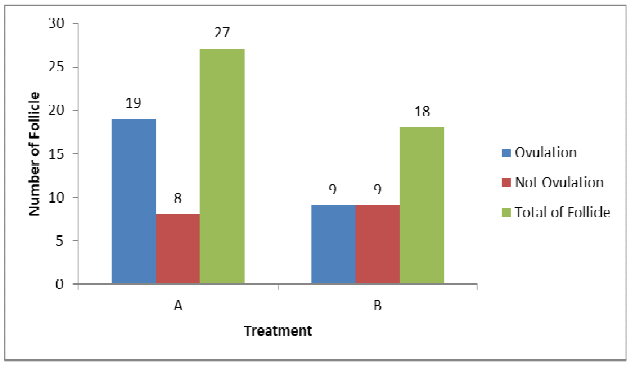

Fig. 2 The number of dominant follicle in treatment A and treatment B of local pesisir cows.

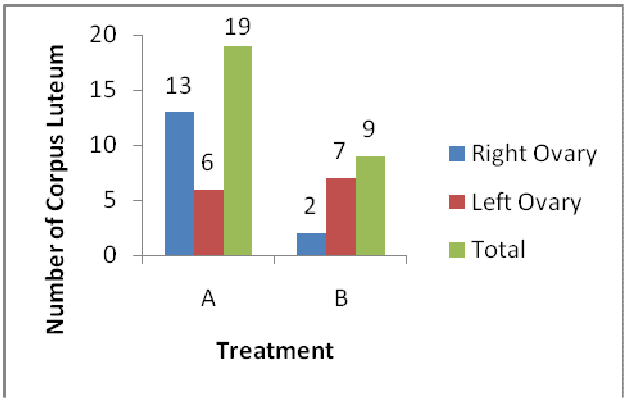

Fig. 3. The number of CL in treatment A and treatment B of local Pesisir cows

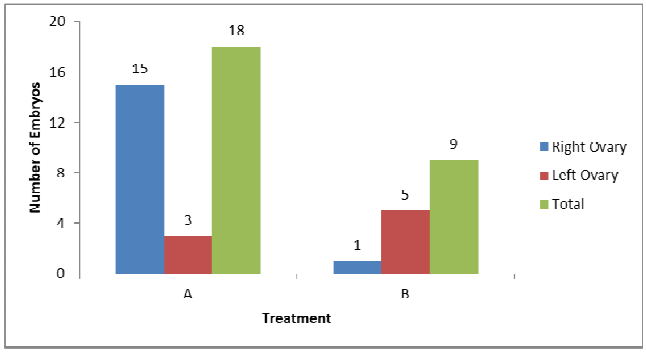

Fig. 4 The number of embryos in treatment A and treatment B of local pesisir cows

This present study found that ovulation synchronization protocol of GnRH plus progesterone for FTAI were developed to maximize the number of dominant follicle, the number of CL and the number of embryo than the estradiol plus progesterone combination on number of dominant follicle, number of CL and number of embryo. The delaying

the timing of an ovulatory stimulus (GnRH or pLH) resulted in an increased super stimulating response and embryo production following FTAI of lactating Holstein cows [24]. The percentage of follicular ovulation in treatment A is 70.37 % and in treatment B is 50 % (P<0.05). This present study is similar to Mikola and Taponen were 64.0 % and 62.4 % in falltropin and pluset , respectively. Cows that were treated with GnRH rather than EB at the initiation of a synchronization protocol tend to have better P/AI, probably due to increased circulating P4 concentrations during growth of the ovulatory follicle. The increased P4 was due to greater ovulation to the GnRH than EB at the start of the protocol and reduced premature CL regression for GnRH compared with EB.[8],[27].

B. Embryo Quality, transferable embryo percentage, and degenerated embryo of Local pesisir cows

The grade of embryo after flushing of local pesisir cows range from very good (6 embryos) , good (6 embryos) , unfertilized embryo (3 embryos) and degenerate embryo (4) in treatment A (Fig. 5), however in treatment B from 6 embryos recovery 2 grade of embryo were unfertilized (2 embryos) and degenerate embryo (4 embryos). The quality of embryo of pesisir cow is significant higher (P<0,05) in treatment A than treatment B. In this study found that all embryo in treatment B were the nontransferable embryo, caused the unsuccessful of embryo flushing. This result supported by [28] that there was no correlation between circulating concentration of estradiol at TAI and embryo or embryo stage. Embryo evaluation is one of the most critical steps of the embryo production. Grade 1 embryos survive well to freezing and/ thawing, whereas grade 2 and 3 must be transferred fresh into recipients [29]. The farm management and donor age are the main factors that should be considered when implementing a program of embryo transfer in Nelore cows submitted to superovulation treatment. Senile cows (≥14 years) produced on average 5.0±0.2 fewer total embryo and 3.0±0.1 fewer transferable embryo than young cows (P<0.01). donor age negatively effected the number and quality of embryo [9]. The quality of embryos and the developmental stage were similar between the group (falltropin vs pluset) and there was no difference in the proportion of low – responding donors in group F and group P, and also equal numbers of transferable embryos and pregnancies can be achieved with Folltropin and pluset [30].

The number of transferable embryo in treatment A was highly significantly (P<0.01) on grade 1, grade 2,and transferable embryo . The percentage of transferable embryo in treatment A is 66.11 % higher than in the treatment B is 0 %. The percentage of degenerate embryo is 22,22 % is and lower than treatment B 66.66 %. This result found that the treatment A is the best grade of embryo and than treatment B. The number of transferable embryo in this study was lower than reported by [24] is 66.1±8.9 % to 88.5±89 %. The present study found the is similar result of transferable embryo range from 37.8 % in cow and 69.5 % in heifer [31]. This result indicated that the quality of embryos affected by using combination of protocol to induce development of emergence wave of diameter size of dominant follicle in embryo production in donor cows.

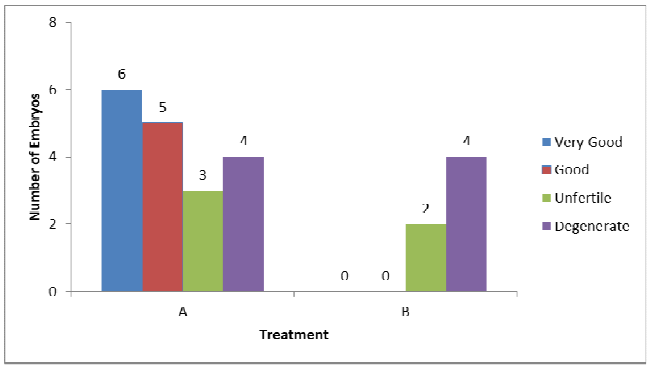

Fig. 5 The quality of embryos of local pesisir cows in GnRH plus progestetone (Treatment A) and estradiol plus progesterone (treatment B)

The degenerated embryo in treatment A is lower (22.22 %) than treatment B (66.665) , the proportion of degenerated embryos was similar between cows and heifer were 9.2 % and 11 % , respectively [31] the proportion of degenerate embryos was similar between cows and heifer were 9.2 % and 11.9 %, respectively [31] and to [30] is 65 in folltropin and 60 in pluset and the degenerated embryo range was higher is 19 % in folltropin and 25 % in pluset. In this result the quality of embryo in both treatment is lower , caused of the quality of CL i.e the size of CL is small category. The importance finding of the present study was the association between the environmental and production efficiency. In addition, that extending the period of follicular development during superovulation (from 6 to 9 days between wave emergence and ovulation) increases follicular maturation, and the number and synchrony of ovulations without compromising ova/embryo competence, and in turn, the potential for more transferable embryos [32]. Furthermore, that administration of (25mg) FSH for superovulation in donor of local Omani breed decreased number of unovulated follicles, decreased number of unfertilized ova, and increased number of transferable embryos and had beneficial effect on embryo quality compared with dose of (25 mg) and 30 mg) FSH. it could be suggested, that dose of (25mg) FSH for superovulation of the donor cows was the best and recommended for obtain higher number of good quality embryos for embryo transfer and realization of breeding programs [33]. In this present study the dose of (20mg) FSH per donor cows, depended to body weight of donors. The local pesisir cows was small category of body weight. This result was supported by (33) that un-ovulated follicles and number of unfertilized ova compared with the cows in G1(20mg), G2(25mg) and G3 (30mg) were (3.17, 4.16 and 6.83) and (3.00, 3.33 and 5.67/ flushed cow) , respectively.

The present study found that the strategies embryo production in local pesisir cows can promote superovulation donor without estrus detection (named ovsynch), synchronization of follicular wave emergence, timed of ovulation induction and AI and type, dosage and of hormone using for superovulation. The success of in vivo embryo production is closely associated to embryo quality. Therefore, factors related to breed, heat stress, and nutrition should be considered before applying SOP in the field [4].

C. Linear Regression between dominant follicle, CL and number of embryo of local pesisir cows.

The linear regression for the number of follicle dominant and the number of CL (Fig. 6), with high coefficient determination (R^2 = 0.950) and linear regression between dominant follicle with the number embryos and high coefficient determination (R^2 = 0.879). 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 produced 18 embryo. 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 depend to the number of follicular ovulation and the number of CL at the flushing. 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 influence of the side of previous pregnancy persisted until the second postpartum ovulation, and this affected postpartum dominant follicle selection and ovulation, but not the development of growing follicles [34]. In addition, postpartum ovarian activity imbalance was not association with the reproductive and productive performance of cows [35]. The relationship between the dominant follicle and CL due to the hormonal concentration such as estrogene and progesterone. Follicular diameter on the day of GnRH application similar to prematurely regressed CLs of the same group, but had the smaller area, this result in smaller number of luteal cells, a fact that was possible responsible for lower concentration of P4 produced by these CLs (36). This study showed the high coefficient of correlation between the number of follicular ovulation and the number of CL/ the number of embryos. Otherwise, a low correlation was observed between follicular diameter and CL category (R^2= 0.15), CL category and P4 concentration (R^2=0.19) [36]. The number of embryos collected per CL, however was significantly lower in the early versus late group (0.39±0.32 % vs 0.44±0.34%,respectively). The late collection allowed the retrieval of full concept uses (embryonic and extra embryonic tissues), even at very late stages such as days 18 to 21. [37].

This present study the number of embryos is lower than reported by[38] 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).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, 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 d 7 after AI were also associated with increased fertility, independent of expression of estrus [39]. This present study supported by [40] 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 [41] that a positive correlation was found between the number of follicle responsive to pFSH (2-8mm) at the beginning of treatments and the super ovulatory response, and no differences were found in these follicular population between two treatment groups. . In addition, that no effect of the number ovulation on the percentage of transferable embryos. 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. In addition by [42] that large ovulatory follicles have associated with the formation of the larger CL with greater capacity of synthesizing P4. The number of follicles of diameter 5 mm or less was negatively correlated with the number of follicles of diameter 6 mm or greater through the treatment period (r=-0.70,P<0.001 in the control group and r= -0.76, P<0.001). [43]. The number of large follicles at ovulations and CL occurred with lengthened protocol (7-day) than with the convention 4-day FSH treatment [32]. When animal are bred following a TAI protocol, where ovulation is induced by GnRH estrus response and increased pre-ovulatory concentration of estradiol before ovulation are critical determinants of subsequent embryo quality and potential pregnancy loss. According to [44] that inbred animals with a lower coefficient Fx had a very small and insignificant difference in quality and proportion of transferable embryos compared with group Fx=0. At greater inbreeding coefficients (3.1%- 25%),%), the reduction in embryo quality increased. Breeding management in local cow is needed to increase the reproduction efficiency by introducing AI and embryo transfer programme. This study found that the number of dominant follicle , the number of CL a significant impact on the yield of embryo production in local south pesisir cows. This means when the number of CL increase , the number of embryo significant will be increase. This present study supported by [46] that as a follicle size and concentrations of progesterone and estradiol in the follicular fluid showed significant correlations with the later development of a corpus luteum as well as morphology and progesterone concentration.

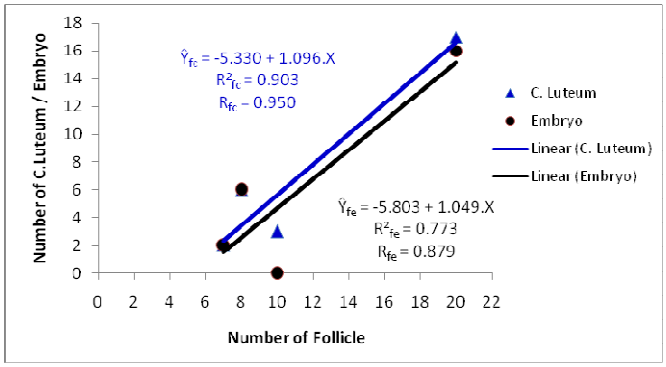

Fig. 6. Regression linear of dominant follicle, CL and embryo of local pesisir cows.

The donor of south pesisir cows had the high inbreeding without breeding management control, caused of limited bull to bred the cow at the time breeding seasons. This caused the low quality of embryos and untransferable embryo production. This result supported by [45] that a decrease in the count of transferable embryos in the inbreeding group

Fx= 3 %- 5.9%. The effect of inbreeding depression on ovarian activity in super ovulated cows. The regression equation was significant for transferable embryos was`R^2= 0.91 [46]. In this result indicated that uncontrolled of breeding programme in rural farm increased inbreeding depression caused the number of embryos production and the number of transferable embryos had a negative impact on reproduction. Based on the discussion above, we conclude that in embryo production program in local pesisir cows submitted to a superovulation protocol, special attention should be given to management and age of donors cows.In addition, when only a few CL are present in the ovaries, transrectal palpation is sufficient to accurately quantify the number;[30]. This result is indicated that the ovarian response is high relationship in fertility such as the embryo production and quality of local south pesisir cows.

IV. CONCLUSION

The strategies to increase the fertility of local Pesisir cows by using the GnRH plus progesterone combination (treatment A) in the embryo production program. This result indicated that the use of GnRH increase the embryo production and quality of embryo in local pesisir cow. Linear regression between the number of the dominant follicle, number of CL and the number of embryo with high determination coefficient (R^2) range from 0.8 to 0.9.

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