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## REPRODUCTIVE DISORDER STUDIES USING RADIOIMMUNOASSAY (RIA) PROGESTERONE ON DAIRY CATTLE

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### ABSTRAK

**REPRODUCTIVE DISORDER STUDIES USING RADIOIMMUNOASSAY (RIA) PROGESTERONE ON DAIRY CATTLE.** Two intensive systems of husbandry practices, Garut West Java and Yogyakarta Central Java, were chosen for this study. Both areas have been voluntarily made into a pilot farm for the application of RIA progesterone to improve reproductive performance. Five dairy cattle from Garut West Java, which according to Health Extension and Artificial Insemination Technicians anamneses and according to farmers who own the animal, were showing reproductive failure and were selected from those cattle for the study. Other fifteen dairy cattle from Yogyakarta area, with anamneses of having low reproductive performance, were also selected for this study. Milk progesterone sample were collected twice a week for five consecutive weeks period of time to follow the biological reproductive status of every animal, while samples from dairy cattle at Yogyakarta were collected three times post Artificial Insemination (AI) services, as according to Artificial Insemination Database Application (AIDA) procedure, to monitor the failure of AI, success rate of AI, and ovarian activities of the cattle. Result of the study in Garut shows that RIA progesterone indicates that animals need special treatments and most AI failed due to lack of historical information of the dairy cows. RIA progesterone leads to a suggestion that it can be use as a tool to monitor the reproductive disorder, as the recommendation made for those cows to anticipate reproductive disorder overcome the problems. Similar result found in Yogyakarta, which almost 50% of the observed animals failed to AI due to miss-estrus detection. Furthermore, from the RIA for milk progesterone, information of the reproductive disorder figures can be drawn and early suggestion could be made to anticipate losses. Overall, beside the reproductive historical record, RIA progesterone is important tool to be applied in the animal husbandry system in Indonesia as to improve the herd productivity and has an economical value to reduce operational cost at waiting period for feeding animal up to INS Rp 224,000 - 336,000 per head animal.

Keywords: Reproductive disorder, RIA Progesterone

### 1. INTRODUCTION

Almost in the decade, management in the reproductive system together with animal health section, especially in dairy cattle, has become a focus to be improved, in parallel beside the nutrition. In practice management of all parts can integrate to improve not only production but also in attempt to increase the efficiency of reproductive rate as well.

Indonesia as one of developing country that practicing small scale farming system, which each farmers is only capable in maintaining 3 - 4 animals, has many factors to be reviewed in relation to the increase the production dairy cattle farming system. Recording system is identified as the key role to improve the reproductive rate in dairy cattle, and this requires collaboration between farmers, as an individual owner, and the field technician (i.e. the AI technician and/or Veterinarian Assistant). However, understanding the importance of recording, for background information for the field technician in early diagnosis of biological status of the animal, of the farmer often requires improvement. Therefore, in parallel with more training given to the farmer about reproductive traits of dairy cattle, a more comprehensive recording system used by field technician has to be applied. An expectation by a new provided recording system is more information can be known for early diagnosis of reproductive disorder, of the dairy cattle before confirmation from local veterinarian, may be given to the farmer for early anticipation.

Progesterone hormone is known to be reproductive hormone, which may be used to predict a biological status in term to diagnose reproductive disorder performance of dairy cattle [1]. Results of the progesterone hormone, after interpretation, will then be confirmed by veterinarian. Due to progesterone hormone will be the subject to predict the reproductive status, this study will use radioimmunoassay technique for progesterone hormone content in milk. The study is carried out in two different location, where the introduction of recording system is been introduce, and a RIA progesterone technique application requires a demonstration in the pilot farm. Therefore, this study would be emphasized to demonstrate RIA progesterone in terms of early reproductive disorder diagnosis among the animals that were already in the programme.

## **2. MATERIAL AND METHOD**

### **2.1. Animal and location**

To be eligible for the study, animal that have low reproductive performance (*i.e.* fail to AI, repeat breeder, silent heat, lack of individual historical record) were chosen from two locations; Garut district of West Java and Yogyakarta in Central Java. A total of 20 dairy cattle were used in this study, 5 animals from Garut and 15 animals from Yogyakarta, with body condition score performance is between 1.75 - 2.5 and 2 - 2.5, respectively. All animals in this study are sampled from those of a 312 animals

envisaged in the improvement of recording system programme (AIDA) from the two locations. Information of these animals, as their anamneses, was recorded by local field technician and the farmers for their basic biological status to identify the animals, which will be included in the study. The anamneses for all animals are summarized in the Table 1.

Table 1. Anamneses of each dairy cows with the reproductive disorder observed.

No.	Animal ID.	Location	Anamneses
1.	A	Garut	Lack individual record; parity 6; 7 – 8 years of age; calving date not available.
2.	B	Garut	Lack individual record; parity 7; age unknown; calving date not available.
3.	C	Garut	Lack individual record; parity 2; age unknown; BCS: 3.25; fail of AI.
4.	D	Garut	Lack individual record; parity 3; fail of AI; BCS: 3.00.
5.	F	Garut	Lack individual record; parity 3; silent heat or no oestrus sign; BCS: 1.75.
6.	Sartono (Krisna)	Yogyakarta	Lack individual record; beef cattle; AI before milk first sampling.
7.	Saeri (Krisna)	Yogyakarta	Lack of Individual record; recorded to have retensio secundinae; AI but then natural mating at 24/9/2003 at sampling first date.
8.	Suyanto (Ngipiksari)	Yogyakarta	Lack of individual record; age: approximately 5 years; BW: $\pm$ 300 kg; 3 times failure of AI; AI at first sampling date.
9.	Seno (Ngipiksari)	Yogyakarta	Lack of individual record; parity 3; approximately 5 years; BW: $\pm$ 350 kg & BCS: 2; 3 times failure of AI; 12 l/d milk production; AI at first sampling date.
10.	C9 (UNT)	Yogyakarta	Lack of individual record; parity 4; AI at first sampling date.
11.	C8 (Untung)	Yogyakarta	Lack of individual record; parity 2; Embryo transfer acceptor.
12.	C14 (Untung)	Yogyakarta	Lack of individual record; parity 2; embryo transfer acceptor.
13.	Kota	Yogyakarta	Lack of individual record; 3 times fail of AI.
14.	Dwi (BDK)	Yogyakarta	Lack of individual record; recorded to have abortion; several times fail of AI; date of AI unrecorded.
15.	Yos (BDK)	Yogyakarta	Lack of individual record; recorded to have abortion; BCS: 2.00; several times fail of AI.

No.	Animal ID.	Location	Anamneses
16.	Witanto	Yogyakarta	Lack of individual record; age $\pm$ 4 years; last calving date: June 2002; Low milk yield; no sign of estrus; 4 times fail of AI.
17.	Aris (BPMBPT)	Yogyakarta	Lack of individual record; parity 1; no heat or oestrus sign.
18.	Unip (Kaliadem)	Yogyakarta	Lack of individual record; parity 1; 4 months post calving; No heat or oestrus sign; BCS: 2.5.
19.	Poskeswan Sanden	Yogyakarta	Lack of individual record; 10 months post calving; no heat or estrus sign; BCS: 2.75.
20.	Poskeswan Turi	Yogyakarta	Lack of individual record; 4 times fail of AI.

## 2.2. Housing and feeding system

All animal were fully restrained and were fed with a combination cut and carry road side grass with additional of local concentrate, with water available for 24 hours. Animals were fed averagely 10% of body weight, with the average proportion of feed fed to the animals is 60 – 70% basal feed (*i.e.* road side grass, banana stems, sorghum straw, rice straw), 20 – 25% green forages (*i.e.* legume), and 10 – 15% local concentrate. The proximate composition of these feed is shown in the Table 2.

Table 2. Partial proximate analysis (dry matter: D.M.; organic matter: O.M.; crude protein: C.P.; crude fiber: C.F.; and gross energy: G.E.) of several agricultural waste products used as complimentary basal diets, local concentrate and UMMB.

Kind of grass, forages and shrubs:	D.M. (%)	O.M. (%D.M.)	C.P. (%D.M.)	C.F. (%)	G.E. (kJ/g D.M.)
<i>Pinnesetum purpureum</i> (Elephant grass)	18.5	89.7	13.4	37.8	17.6
<i>P. purpurhoides</i> (King grass)	25.3	89.1	12.0	27.0	17.2
<i>Leucaena leucocephala</i>	28.9	91.1	26.3	20.4	18.8
Rice straw	33.1	83.6	5.5	29.6	13.4
Corn leaves	17.7	83.4	15.3	25.6	14.5
Tapioca chopped	34.3	95.5	2.9	4.0	-
Banana stem	10.0	88.2	4.2	24.6	13.4
Mixed road-side grass	17.1	85.5	14.5	30.4	13.7
Local concentrate ("Puri")	90.5	88.4	14.4	14.0	14.5
UMMB	81.1	82.7	27.0	7.3	18.4

### 2.3. Sampling

Sampling procedures are not uniform at both locations. Due to reproductive disorder identified animals used in this study, sampling from animal at Garut was made to be more frequent, which is twice a week for a period of five weeks, as it aims to monitor ovarian activities due failure of AI and the incidence of silent heat. At Yogyakarta area, the sampling procedure is following the method of sampling in the programme of Artificial Insemination Database Application (AIDA), as its purpose in monitoring the failure, or the success rate, of AI using RIA technique. Sampling time at Yogyakarta area is 0 Day; 10 - 11 Day; and 20 - 21 Day post AI services.

All samples were recorded for animal ID, farmer, location, and date of sampling time. Milk samples were preserved using sodium azide tablet, where each sampling tube of 10 ml is added with one tablet  $\text{NaN}_3$ , and then freeze and preserve until assayed.

### 2.4. Data analysis

Milk samples are assayed for progesterone using RIA technique as described in RIA protocol of the Joint FAO/IAEA Division, from where the RIA kit progesterone supplied. The progesterone milk samples are assayed using a self-coating Radioimmunoassay employing  $^{125}\text{I}$ -progesterone as tracer and standard (0, 1.25, 2.5, 5, 10, 20, and 40 nmol/l) prepared in skim milk. The intra-assay and inter-assay coefficients of variation were 3.4% and 6.9%, respectively. Data were recorded and then plotted using a Grafit programme for further interpretation and then cross check with veterinarian diagnosis, which conducted by rectal palpation, for confirmation of the result before it is inform to the farmers.

### 2.5. Data interpretation

Data interpretation is based on the progesterone hormone profile as it is guided in the data interpretation of AIDA [2]. The interpretation will then be cross checked with the result from veterinarian examination *as per* rectal palpation of the individual animal. The interpretation on the level of milk progesterone hormone will follow the justification of the recommendation from IAEA, and also result from observation of ZDUNCZYK, S. et al. [3], on progesterone level interpretation as it is summarize in Table 3.

## 2.6. Economical evaluation

The study is also conducting economic evaluation on the application of RIA milk or serum/plasma progesterone for measuring reproductive failure in the field. Parameter observed on this study included estimation of cost per progesterone sample using RIA kits of NNEA production. Calculation for comparison is made on cows with reproductive failure and which the milk is sampled, with if no samples being taken and no anticipation treatments given.

Table 3. Interpretation system use to determined ovarian activity in relation to progesterone hormone level.

Level of progesterone (nmol/L)	Interpretation
< 1	No corpus luteum available; no activity found in the ovarium.
1 - 3	Doubtful range; progesterone level hormone found in this area could means anything. At this stage, no recommendation can be given. A further examination (i.e. re-assay sample or clinical examination by the veterinarian) is necessary and should be correlated with physiological status.
> 3	Indicating an activity in the ovarium, and could be an indication of pregnancy.

## 3. RESULTS

Result of milk progesterone profile on the observation of dairy cattle A and B maintained by local farmer at Garut West Java is drawn in the Figure 1. These two animals were suspected to have reproductive clinical disorder as they never showed the onset of estrus or heat and never conceived due to AI services. The graphs in the Figure 1 indicate no ovarian activities can be seen for these two cows, as no milk progesterone hormone could be detected (*trace* nmol/L) during the period of observation. It is then leads to a suggestion that animals were anoestrus and there is nothing could be informed to the farmer and/or local AI technicians to provide the next AI services. Lack of individual historical data of reproductive performance and the early anamneses of these cows, as animal age already reach to be more than 7 years, may confirm suggestion for culling.

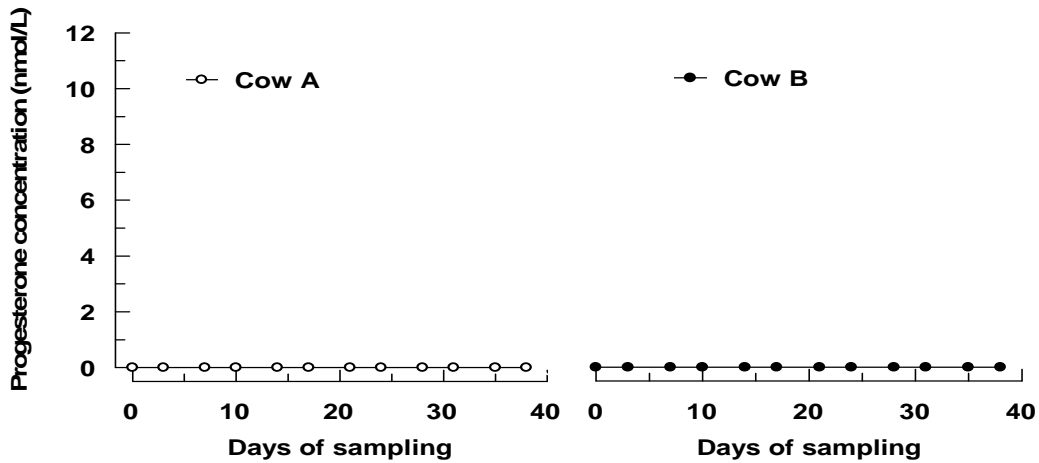


Figure 1. Milk progesterone profile from two dairy cows that were suspected to have reproductive clinical disorder. Day 0 indicating the first of sampling day.

The plotted concentration of milk progesterone from cow D and cow F, indicate that there is an ovarian activity post calving. However, no heat sign were detected at these two cow prior AI services, as it's shown in the Figure 2. No information on the exact last calving date from these two cows. The progesterone hormone plotted in the Figure 2, suggesting that the day of first estrus is on Day 25 - 26 and 30 - 31 of sampling days, respectively for cow D and cow F. Days of estrus in the figure are adjusted to the date of sampling days and due to the average length of normal estrus cycle is 21 days; therefore it is possible to suggest that the next AI services could be conducted 21 days after the shown estrus days as illustrated in the figures. Confirmation of this AI services should also be conducted, per rectal, as to confirm the conception. Result of rectal examination on Day 45 post AI indicated that both cows were conceiving.

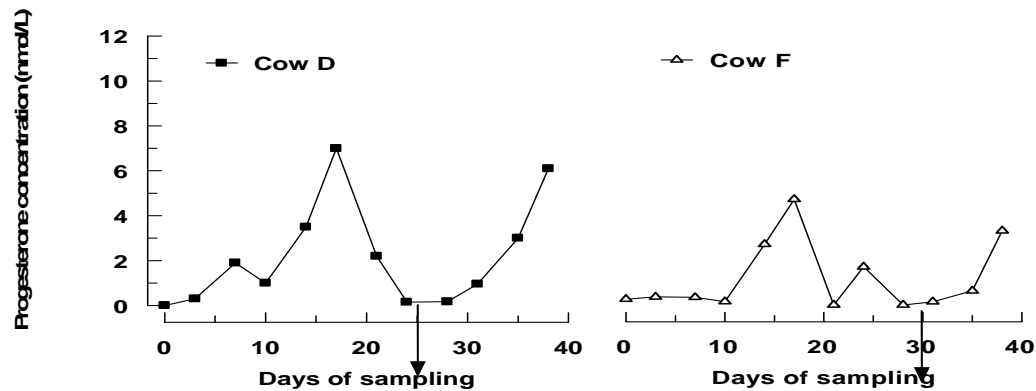


Figure 2. Milk progesterone profile of 2 dairy cows suspected to have late ovarian activity post partum prior AI.

Plotting of milk progesterone assay for cow C drawn in the Figure 3, suggesting that this animal has more than 21 days of estrus cycle, which from the Figure 3, the length of estrus cycle for this cow is  $\pm 28$  days. This graph is suggesting of prolonged estrus cycle incidence, and therefore resulting in miss-heat detection and failure of AI, if the animal is bred during luteal phase. Day of the onset estrus is then adjusted into the date when the sample is taken. Recommendation is then made that AI can be performed at 21 days after the Day when the onset of estrus, where it is monitored from the progesterone profile (Day 31 - 32 of Figure 3), after it is adjusted to the respective date.

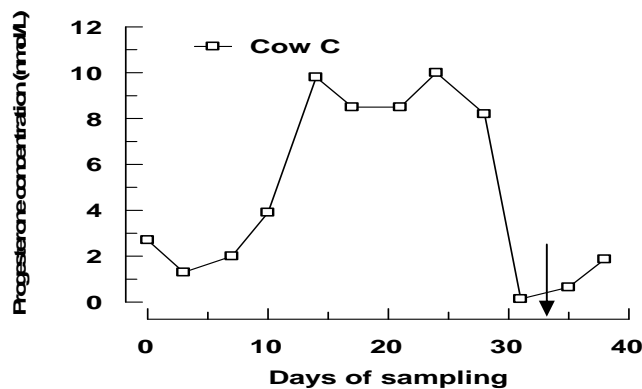


Figure 3. Milk progesterone profile of a cow suspected to have prolonged estrus cycle (28 days).



Similar information of anoestrus cows obtained from plotting progesterone level is also found for animals at Yogyakarta area, as it is drawn in the Figure 4 and Figure 5.

Results from utilization of RIA progesterone at Yogyakarta area are presented in the Figure 4 to 12. Almost all result of the assay, which are plotted in the Figure 4 and 5 shows that milk progesterone concentrations are below 1 nmol/l, and this lead to an interpretation that there is no ovarian activity recorded in those cows. These indications are all confirming the earlier anamneses, that those cows showed the incidence of reproductive disorder, *i.e.* 2 times failure of AI, 4 times failure of AI and no sign of estrus, acyclic, and post partum acyclic, respectively for cow coded Yos BDK, W, Saeri (Krisna GK), and Poskeswan Sanden.

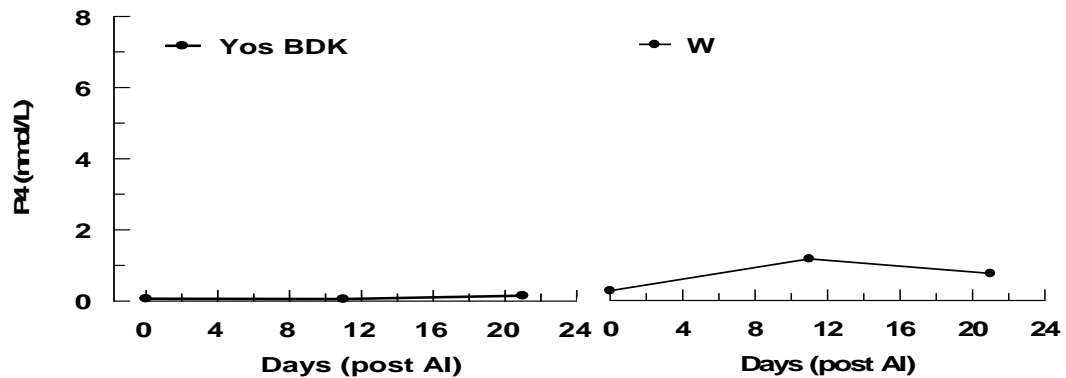


Figure 4. Milk progesterone profile of anoestrus cow.

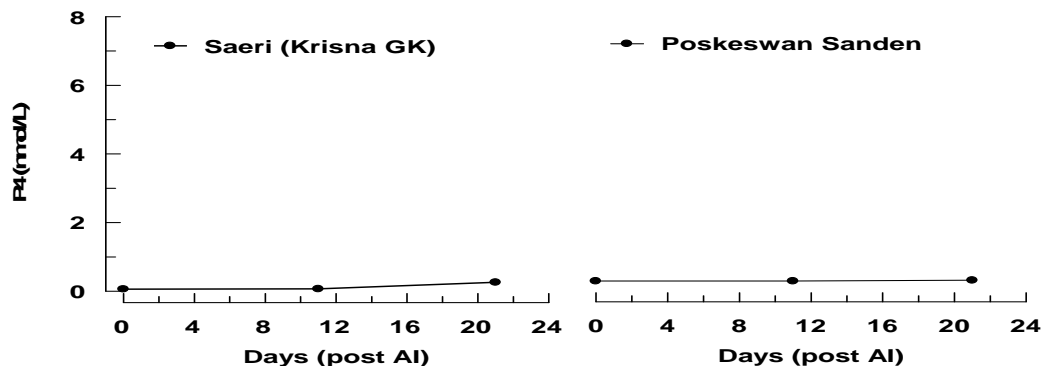


Figure 5. Milk progesterone profile of anoestrus cow.

Two cows coded C8 UNT and C14 were used as embryo transfer acceptor and were sampled for milk progesterone to be assayed. The result from this assay, as plotted in the Figure 6, leads to an indication that both cows have cyclic ovary than pregnant post embryo transfer. The figure suggesting no implantation occur post ET, which then implicate to no pregnancy, or a possibility of EED post ET. Valid data of time and date for this embryo transfer is not available when milk samples are taken, suggesting of low reproductive management recording system in this herd.

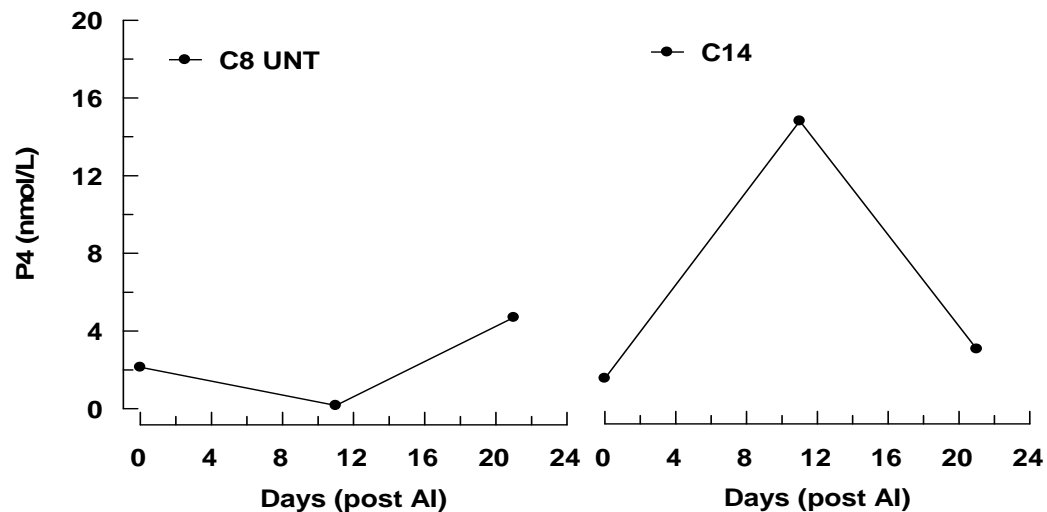


Figure 6. Milk progesterone profile of cows with active ovaries and as acceptors for embryo transfer.

Milk progesterone assay on cows coded Sartono Krisna and Adisupono Boyong, indicated that AI were performed during luteal phase. However, due to longer period of estrus cycle recorded at both cows (more than 21 days), due to high concentration on Day 0 sampling, a sign of EED might also be interpreted from cow drawn its status in the Figure 7. The occurrence of EED is confirmed at both cow, and due to previous anamneses, the AI services were performed several days before the first sampling time of Day 0.

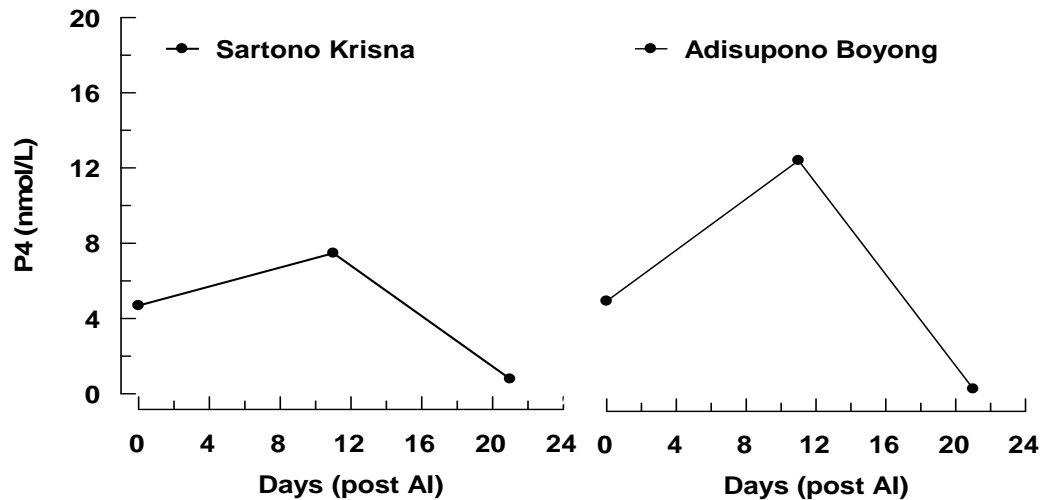


Figure 7. Milk progesterone profile of cows with EED incidence. AI was performed before D - 0 sampling time.

Different result is observed in cows coded Suyatno (Ngipiksari) and Seno S. (Ngipiksari) as shown in the Figure 8, where the presence of progesterone hormone, which is  $> 3$  nmol/l, was recorded along the three sampling times. These two cows had been recorded to have 3 and 4 times failure of AI, respectively for cows coded Suyatno (Ngipiksari) and Seno S. (Ngipiksari). According to the progesterone hormone plotted in the graph, interpretation suggests that the previous AI services were conducted at luteal phase. The high concentration of progesterone hormone is indicating the presence of corpus luteum. Furthermore, both cows, which have been failed to conceived for more than three times of AI, lead to a high possibility that these cows suffer from corpus luteum persistent (CLP).

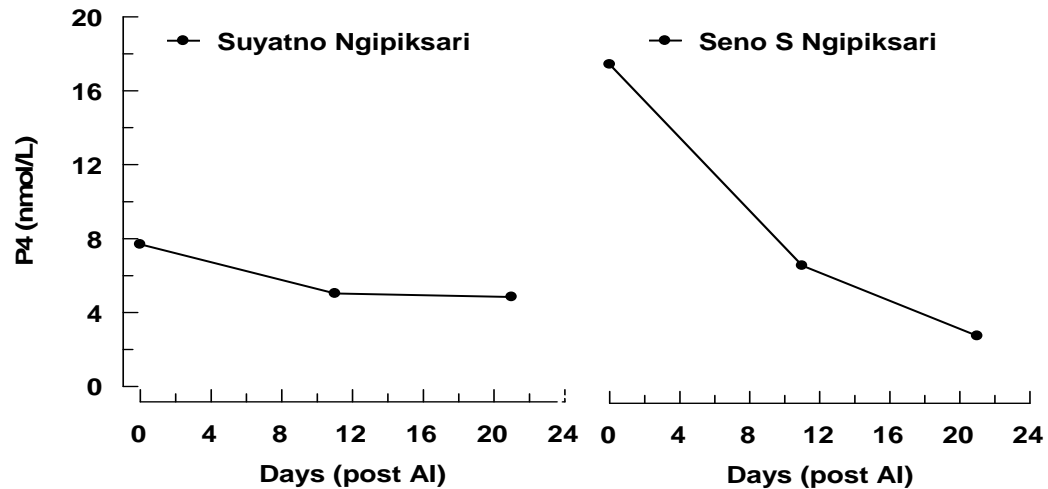


Figure 8. Milk progesterone profile of cows suspected with corpus luteum persistent (CLP) occurrence.

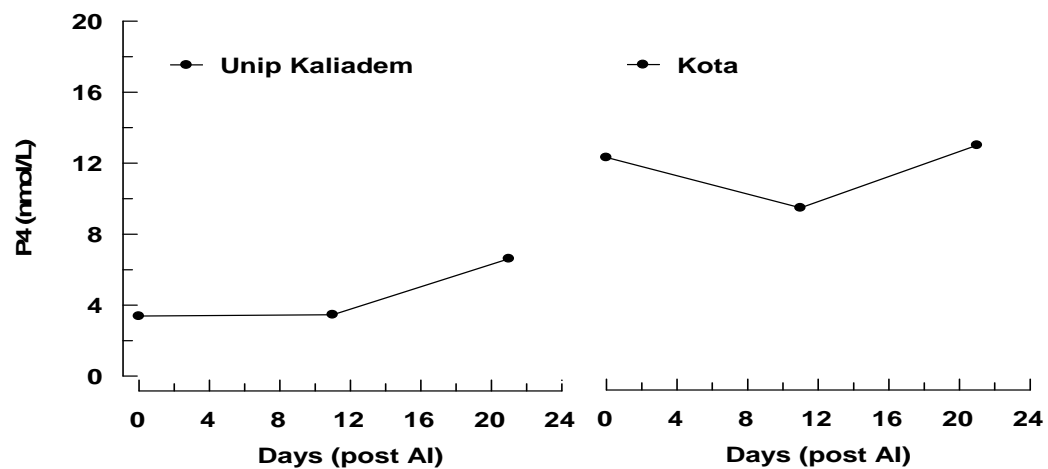


Figure 9. Milk progesterone profile of cows with active ovary and high possibility of AI performed on pregnant cow.

Pattern of the plotted data milk progesterone assay for cows coded Unip kaliadem and Kota in the Figure 9 and cows coded Poskeswan Turi and Aris BPMBTP in the Figure 10, shows that AI have been performed during the presence of corpus lutea, due to high level of progesterone concentration recorded on Day 0 of sampling

time or the day of AI services. The anamneses of cow coded Unip Kaliadem showed this animal had been calved four months before Day 0 sampling, and when AI conducted. The level of progesterone on the Day 0 sampling, which is  $> 3\text{nmol/l}$ , indicating there is an active ovary when the animal receiving semen. More over, the level of progesterone continuously increase as the sampling period time (i.e. sampling Day 11 and 21). This leads to a suggestion that due to no recording system applied for this cow, the reproductive performance would declined, particularly in cow coded Unip Kaliadem. Animal coded Kota has also received AI service when the animal has already pregnant as it is indicated in the progesterone profile in the Figure 9. Poor recording system in these two animals may be due to poor monitoring system post AI.

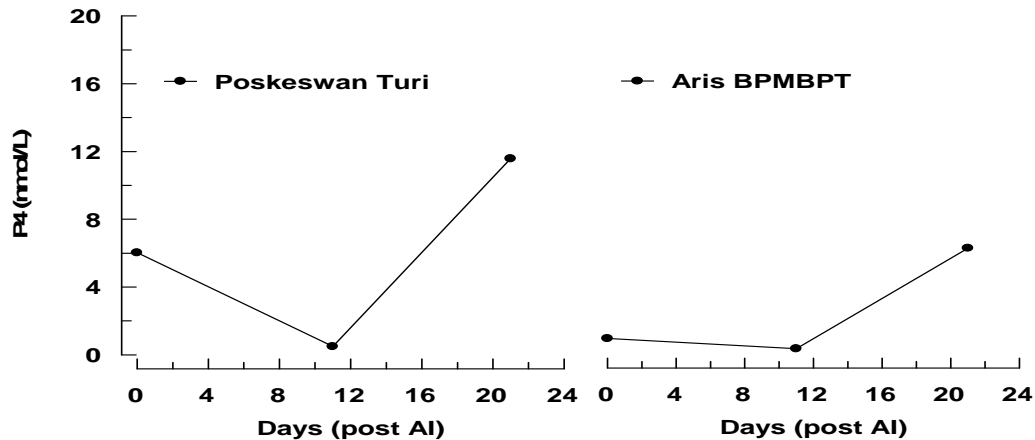


Figure 10. Milk progesterone profile of cows AI at luteal phase (Poskeswan Turi) and on pregnant animal (Kota).

As it has mentioned earlier, similar false AI is also found in cows coded Poskeswan Turi and Aris BPMBPT. These cows received AI during the luteal phase as it is indicated in the Figure 10, suggesting of re-AI could be done on the next estrus, particularly for Poskeswan Turi on the adjusted date. However, progesterone profile of cow coded Aris BPMBPT leads to an indication that the animal was at the stage of ovarian recovery post calving.

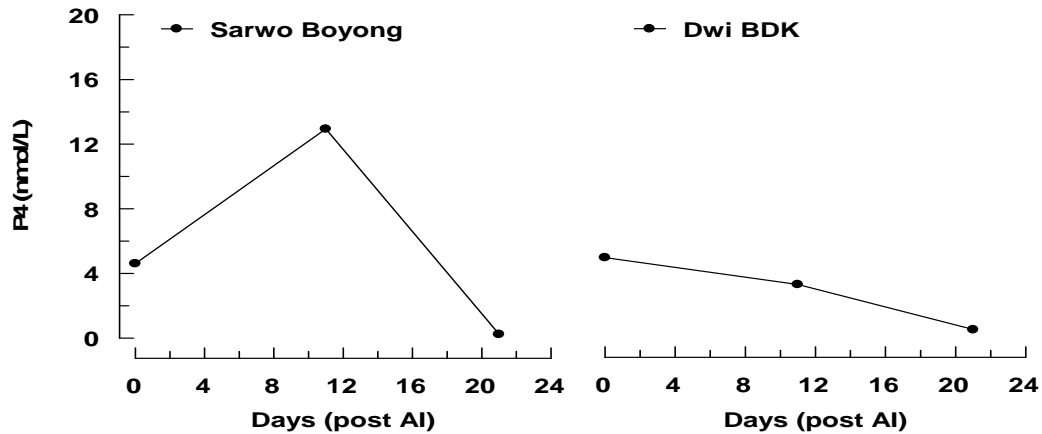


Figure 11. Milk progesterone profile of cows fail to conceived or the occurrence of EED.

Both recorded cows coded Sarwo Boyong and Dwi BDK, as shown in the Figure 11, was AI at luteal phase. Furthermore, progesterone level at Day 0 sampling, from both cows, were at the high level ( $>3$  nmol/l), which lead to a suggestion that both cows were already pregnant and had EED incidence. A possibility of CLP recovery might also cause of the sudden dropped of the progesterone concentration recorded, particularly on cow coded Dwi BDK.

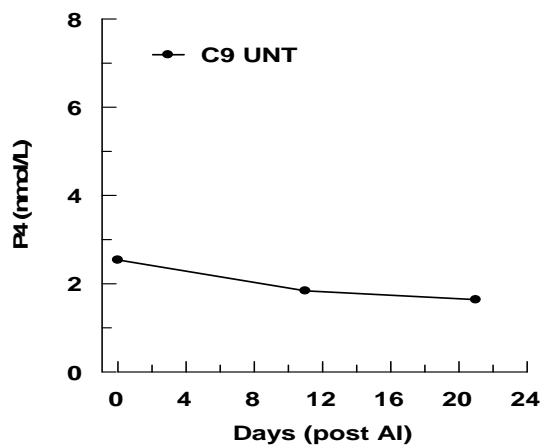


Figure 12. Milk progesterone profile of cow within a doubtful range of progesterone concentration, where additional examination is needed.

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The milk progesterone recorded in the Figure 12 indicated an uncertainty of the ovarium status of cow coded C9 UNT, due to the doubtful range of progesterone level ( $>1$  n mol/l and  $<3$  nmol/l) been recorded. Further observation for this cow's biological status is needed.

With regard to the social economic impact in the utilizing RIA technique, a preliminary evaluation has been made, for the progesterone Kit produced locally by the National Nuclear Energy Agency. Calculation has been made, which the cost of each sampling is INS Rp 37,500 or make up to INS Rp 112,500 with 3 sampling time post AI (assuming that US \$1.00 = INS Rp 9,300.-). The additional of this INS Rp 112,500,- is comparable to be spend by the farmers, whom their cattle have reproductive disorder and so required at least 3 AI services to be conducted. A new current system in Indonesia has been introduced in pricing semen straw for AI, which for every service value at INS Rp 20,000.- – 40,000.-, depending on the type, whether imported or locally produced of semen. The additional cost have to pay by the farmers, own cattle with reproductive disorder, with 3 AI services is INS Rp 60,000.- – 120,000.-. Additional cost, for maintaining cattle with reproductive disorder, to be considered is from the longer period of animals to be fed until the animal conceiving due to AI. This will be considered as additional variable cost of 56 – 84 days of for feeding, which make up to INS Rp 224,000.- – 336,000.-, with an assumption of daily feeding cost is INS Rp 4,000.-/head animal. Therefore, the economical value of utilizing RIA progesterone technique for early AI failure detection, is from the saving amount cost between the multiple AI with additional feeding cost and the cost 3 sampling for progesterone assay.

#### **4. DISCUSSION**

All animals observed in this study were animals that have been involved in the programme for the improvement of recording system using the introduced programme; Artificial Insemination Database Application (AIDA). The programme aims to records almost all major factors that influenced to success rate of AI, such as animal maintenance system, heat detection, semen quality, AI technician, and utilizing progesterone RIA in detecting the failure of AI. The utilization of RIA is not compulsory due to confirmation the success of RIA might be done by rectal examination on Day 50 post AI onward. However, as progesterone is a female and

reproductive hormone, the present of this hormone may lead to view the endocrinological and reproductive status of an animal (4).

As it has been presented in the previous country report (5, the location of dairy farm in Garut where the programme was introduced, has proportion number of AI conducted on pregnant and/or luteal phase up to 35% and service per conception up to 3.0 (conception rate  $\pm$  33.3%). A number that would have meant to the improvement of reproductive efficiency if this can be reduced. The five consecutive animals in this study were chosen by the farmers as to detect the possible cause of repeat breeder or reproductive disorder. Two from the animals observed (cow coded A and B, Figure 1) were showing of anoestrus and possibly cystic ovarian diseases (Varner and Majeskie, 1988). Furthermore, according to the anamneses, the two animals has reach 6<sup>th</sup> and 7<sup>th</sup> parity, and physiologically, a possibility for the animals have reach the stage of a decline in reproductive efficiency (6 and 7). Culling was the recommendation made for these two cows and inform to the farmer through the extension worker of the Livestock Services.

Anoestrus cows were also found in cow D and F (Figure 2), as according to the anamneses from the field technician and farmer, these animals have not been reach resumption of ovarian activity post calving, and both cows have been three times failed to conceive due to AI. However, the resumption of ovarian activity has been detected by the time of sampling period. Recommendation for the time of AI to be carried out has been made on 21 days after the onset of estrus detected (arrows in the Figure 2). Both animals were conceived post AI and was confirmed by rectal examination by local qualified veterinarian. This incidence often occurs for animal post calving in dairy farms of Garut area and mostly in Indonesia. According to the earlier anamneses, cow coded F had BCS 1.75 - 2.00, and this might only be overcome by nutritional management post calving (8). Furthermore, it was stated that appropriate nutritional management of the prepartum transition dairy cow with the objective of reducing the incidence of calving related disorders (i.e. milk fever, dystocia, retained fetal membranes, ketosis and metritis) which alone or collectively reduce reproductive success is an integral component of the herd's reproductive herd health program (8 and 9). However, in most dairy herds, attention to transition cow nutrition and management occur after problems occur. Therefore, to prevent calving related disorders form occurring at incidences which result in major economic losses, periodic evaluation of prepartum and post partum transition cow management is recommended.



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The plotted milk progesterone profile in the last animal observed in Garut, shows that this cow coded C has more than 21 days of estrus cycle post partum (Figure 3). This lead to a physiological condition that the animal will not be conceived post AI, due to miss detect the heat. The prolonged ovarian cycle or prolonged diestrus often found in the animal, which has persistence of the CL and may be attributed to a failure of PGF2 $\alpha$  release (10). The lack of PGF2 $\alpha$  is often due to the incidence of metritis or the uterine pathology. If there is adequate endocrine stimulation, the CL might regress and ovulation may occur followed by the onset of estrus, as it is also shown in the Figure 3. Recommendation for AI services has been made at 21 days after the detected estrus interpreted from the figures. Rectal examination has been taken on day 50 post AI, and confirmed that the animal had conceived.

Result from plotting progesterone data for the first four animals observed from Yogyakarta area (Figure 4 and 5) suggest that those animals were in the stage of anoestrus. No further recommendation made to these animals until the apparent onset of heat, which indicate the resumption of ovarian activity. Anoestrus often happen for those animals post calving. As the CL regresses post partum, the progesterone hormone remains at basal level in the plasma and milk until the resumption of the ovarian cyclicity (11). However, due to the anamneses for each individual cows, a possible diagnose for those animals might be due to nutritional miss-management. Rectal palpation has already been conducted, and suggesting of no ovarian activities was found. Other case observed from the cows that were use as ET acceptor (C8 UNT and C14), which result from the Figure 6 leads to an indication that both cows have cyclic ovary rather than pregnant animal post embryo transfer or implant. The figure suggesting no implantation occur post embryo transfer, which then implicate to no pregnancy, or a possibility of EED. Possible explanation might be failure in the process of estrus synchronization. Valid data of time and date for this embryo transfer is not available when milk samples are taken, suggesting that poor maintenance and reproductive management occur in the herd and preparation for the process of ET suggesting more improvement.

The rest of the animals observed in this study had similar reproductive disorder, such as: fail to conceive due to AI, repeat breeder, CLP, EED, and prolonged the resumption of ovarian activity post partum (Figure 7, 8, 9, 10, and 11). All those reproductive failure are related to the reproductive management system as a whole, which related to the component of transition of nutritional management (8), health

management, (9 and 12), as an integrated factors that require improvement. It can be easily prove by the anamneses for each animal, which provided not adequate data for early clinical examination and prediction for anticipation to the animal. Difficulties have been found in introducing such a new method of recording and management system, due to culture, different recording system in practices between the field technician and the farmers, and lack of understanding of dairy herd management by the farmers. However, by the demonstration of this recording system and the utilization of progesterone by RIA, inappropriate recording and management system that had been practice may be understood and, therefore improvement in the management system of dairy cattle farm can be done. Moreover, improvement of the quality for field technician and/or AI technician may also be the aspect to be focus in terms of improvement results of conception rate due to AI.

## 5. CONCLUSION

Almost all reproductive disorder observed in Garut and Yogyakarta, which caused the failure of AI, is due to poor management reproduction system, especially the recording system.

The present study illustrate that the most effective system to improve the efficiency of livestock reproduction is by applying a better recording system with no matter the size of the herd. The application of recording system requires coordination between the farmers and field technician, so then historical information of individual animal can be obtained. The improvement of recording system will affect an efficient of AI programme, where acceleration on the genetic may progress and maximize profitability on dairy farming practices.

Progesterone radioimmunoassay technique can be use as a tool for early diagnosis of reproductive disorder, which may result suggestion for early anticipation and overcome problems. The technique can be implemented especially for those animals, which lack of historical background and therefore support the AI programme.

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