

Acknowledgement Letter # 227/19

From: ivj83@yahoo.com

To: rma_fispro@yahoo.com

Date: Thursday, June 27, 2019 at 11:34 AM GMT+7

ACKNOWLEDGEMENT

Reg. No: 227/19

Dated : 21/06/2019

Dear Dr. Erma Safitri,

We acknowledge the receipt of the following articles entitled "Profile of Progesterone on Dairy Cow which Experiences The Failure of Artificial Insemination (AI)." (Erma Safitri, et al.).

For any further correspondence, please always quote the Registration Number of the Article.

Editorial Office,
Indian Veterinary Journal,
11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
email : ivj83@yahoo.com
Web : www.ivj.org.in



erma safitri <erma-s@fkh.unair.ac.id>

Article # 227/19 for revision. Referee comments attached.

1 message

Ind Vet Journal <ivj83@yahoo.com>
Reply-To: Ind Vet Journal <ivj83@yahoo.com>
To: Erma Safitri <erma-s@fkh.unair.ac.id>

Thu, Jul 18, 2019 at 10:05 PM

Sir / Madam,


Revise the paper according to the referee's comments and corrections marked on the manuscript. Resubmit the revised article as per IVJ format for further action.

Sincerely

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THE INDIAN VETERINARY JOURNAL

(The Official Organ of the Indian Veterinary Association)

Dr. S. SUKUMAR
MANAGING EDITOR

No.11, Chamiers Road, Nandanam
Chennai – 600 035, India.

ARTICLE NO: 227/19

Date: 17.7.19

Author is requested to note :

- Revise the paper according to the referee's comments and corrections marked on the manuscript.
- Return the original manuscript and the referee's comments sent herewith.
- Resubmit the revised article as per IVJ format – one hard copy and one soft (CD) for each article separately.

EDITOR'S COMMENTS

- 1) Title of the article may be revised as shown.
- 2) Fig-1 to be deleted
- 3) Result & Discussion to be abridged.
- 4) References should be in IVJ format. Entries reference and those more than 35 years old may be deleted.
- 5) Carrying out all corrections and following editor's comments, revised article and ~~soft copy~~ may be submitted as full research article of 5.6 pages, after going thru' the enclosed IVJ guidelines and with the help of a person proficient in English. The article shall be processed further only if all that fall are rectified.

To
..... Dr. Emma Sabitri

.....
.....
.....


Managing Editor

RETURN THIS PAPER WITH YOUR REPLY WITHIN 90 DAYS

Comments on Article A-227/19

"Profile of progesterone on dairy cow which experiences the failure of AI"

By Sri Pantjal Madyawati *et al.* from Surabaya, Indonesia

1. The authors estimated milk progesterone profile on days 0, 7, 14, 21 and 28 after AI in 15 dairy cows which had repeated to estrus once, and conclude fertilization failure in 3, early embryonic death in 9 and pregnancy in 3 cows. However, the data in Table and interpretation are ambiguous, and raise several queries as indicated on the text.
2. Title also needs to be refined as "Progesterone profile of dairy cows which experienced the failure of pregnancy to AI". English language throughout the manuscript in very poor and needs to be corrected in consultation with English proficient scientist. Some statements are not clear. These are pointed out in the manuscript.
3. Abstract need to be made clear and self-explanatory with proper language. In M&M and R&D sections also language corrections and scientific way of interpretation of data are required. In R&D, Figure 1 is not clearly visible. Moreover, the same data are reproduced in Table and Figure, hence the Figure 1 be deleted. Other corrections/editing required are indicated on the manuscript itself. The conclusion drawn based on progesterone alone is not valid.
4. The revised improved concise manuscript may be considered to publish in IVJ.

Change title

Profile of Progesterone on Dairy Cow which Experienced The Failure of Artificial Insemination (AI)

of pregnancy

Sri Pantja Madyawati, Isnaini Fadilah, Trilas Sardjito, Mas'ud Hariadi, Pudji Srianto, Suherni Susilowati, Erma Safitri*
Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, 60115

Abstract

The study aim was to know the profile of progesterone in milk samples of dairy cow that experienced failure of AI. Fifteen AI failure of dairy cows were taken milk samples on days 0, 7, 14, 21 and 28 (day 0 = estrus). Progesterone analysis was performed by ELISA method. On day 7th and 14th progesterone concentrations were increased in all dairy cows, on 21th day were decreased in 3 dairy cows and they returned to estrus, while in 12 cows were still increased. On 28th day were increased and indicated pregnant in 3 cows, while 9 other cows were declined and experienced early embryonic death.

Key words : Dairy cow, Progesterone, Estrus cycle, Artificial insemination (AI).

AI Failure can be an economically important problem on dairy farms (Canuel et al., 2010), such as increased costs for mating, long calving intervals, rejects of cow and birth of calves each year (Rustamadji et al., 2007). The reproductive disorder was caused by imbalance of progesterone and estrogen. The concentration of progesterone in the blood and milk can determine in animal the infertile, estrous and pregnant state, so that it can be used for estrous detection and other pathological conditions (Samik and Safitri, 2019).

Materials and Methods

Milk samples were taken directly on days 0, 7, 14, 21 and 28 (day 0 = estrus) in the morning from the udders of 15 AI failure of dairy cows aged 3.5-5.5 years who had breeding

make it clear & self explanatory

Seen

Very poor and ambiguous write up, needs sound english and proper interpretation of results.

3.5-5.5

at least one time, with healthy body condition and normal estrous cycle. The sample ^{3 were} was ~~to~~

~~kept~~ ^{at} into the ice box 4°C , and ~~then~~ transferred into the freezer. Before analyze the samples

were thawed at room temperature until it melts. Furthermore ~~taken~~ ^{of each} each of 6 mL ~~and~~ ^{sample}

~~was taken~~ inserted into a 10 mL reaction tube and then centrifuged with 3500 rpm for 15 minutes. ~~The~~

~~sample will~~ be separated ~~between~~ ^{at} fat and skim milk. Then ~~take~~ skim milk as much as 1 mL

~~then move~~ into microtube. Hormonal analyzes were performed using the sandwich ELISA

method. The data obtained ~~then presented~~ ^{were} and analyzed using ~~the~~ ANOVA test and

~~processed~~ using SPSS (Statistical ^{Package} Programs For Social ^{Sciences} Scientific) ~~software~~ ^{software}.

Results and Discussion

^{milk} Progesterone hormone profile ^{determined} in 15 dairy cattle with aged 3.5- 5.5 year that experienced failure of AI ~~from~~ ^{from} milk samples ~~were taken~~ on day 0 (estrous), 7, 14, 21 and 28,

^{is shown} ~~can be seen~~ in Table I.

Table I. ^{milk} Progesterone ^{bc} concentrations of ^{in d} Milk Dairy ^{cons} Samples ~~were Taken~~ on days 0, 7, 14, 21, 28 ^{of estrous} ^{cycle}

	Progesterone Concentration (ng/mL)				
	DA-0	DA-7	DA-14	DA-21	DA-28
Cow-1	2,923	4,718	7,265	3,023	5,326*
Cow-2	3,122	5,898	7,138	3,028	4,102*
Cow-3	2,855	4,307	8.12	15.81	16,521**
Cow-4	3,148	5,551	8,879	15,341	11,815***
Cow-5	2,453	4,301	7,622	2,677	4,237*
Cow-6	3,202	5,379	9,381	16,024	13,181***
Cow-7	3,019	5,417	8,992	15,821	12,193***
Cow-8	2,971	5,209	9,095	15,722	11,892***
Cow-9	3,305	5,281	9,198	15.38	11,913***
Cow-10	3,199	5,362	8,683	15,653	13***
Cow-11	3,258	4,583	8,216	15,483	17,028**
Cow-12	3,348	5,189	8.86	16,256	17,803*
Cow-13	3,325	5,382	8,897	15,89	12,574***
Cow-14	3,099	5,324	8,989	15,206	13,011***
Cow-15	2,989	5,541	9,079	15,153	12,796***

Note:

Cow * = Failure of fertilization

How did you confirm?

- Replace H with D, and
- Coma (g) with full stop (.) in values.

How could you say (oo) marked cases a pregnant or EEA?

Cow ** = Pregnant
 Cow *** = Early Embryonic Death

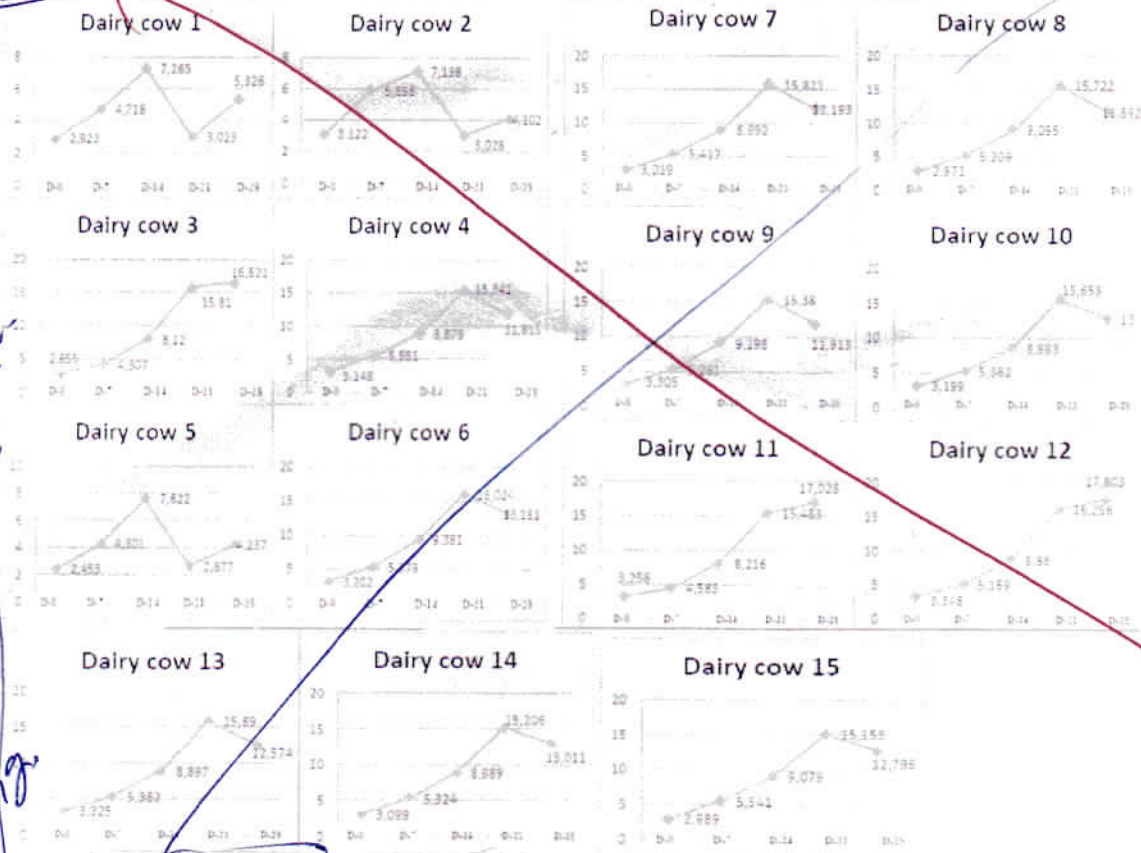


Figure 1. Profile of progesterone in milk samples on day 0, 7, 14, 21 and 28.

The hormone concentration on the day 0 (estrous) of 15 dairy cow $\left\{ \begin{array}{l} \text{was consistent} \\ \text{(2.2-3.5 ng/ml)} \end{array} \right.$ with the results of some researchers suggesting that the concentration of progesterone in milk, the animal is expressed when the concentration of progesterone between 2.2- 3.5 ng/ml milk and not estrous when the concentration of progesterone less than 2.2 ng/ml milk (Hoffman *et al.*, 1983).

The hormone concentration on the day 7 of 15 dairy cow explain that during the cycle of estrous, progesterone concentrations can already be detected by the third day (Valdez *et al.*, 2005) and increase until day eight after estrous and continue to increase until

Data already given in Table
 Avoid duplication

delete fig

not clear

earlier observation

Progesterone

of cycle /
21st day of pregnancy (McDonald, 2000). This statement is consistent with Frestantie (2017) that the formation of the corpus luteum has occurred after ovulation, so the hormone progesterone begins to be produced.

The ^{high} hormone concentration on the day 14 of 15 cow explain the luteal phase, the hormone progesterone inhibits ~~an~~ gonadotropin hormone secretion that is Folicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) ^{from} in the anterior pituitary gland.

Prevent These barriers ~~cause~~ the follicles of the ovaries to develop and the hormone estrogen is not produced, so the animals do not show symptoms of estrous (Hafez and Hafez, 2013).

The ^{higher} hormone concentration on the day 21 explains ^{that} the concentration of progesterone in pregnant dairy cow rises in line with positive pregnancy age so that ~~progesterone concentration measures to determine the success of IB earlier.~~

Budiasa (2014) suggested that ^{milk} progesterone concentrations above 15 ng / ml can be stated positive. This study showed that 12 dairy cow ^{that had} results above 15 ng /mL while three cow that returned to the cycle ^{probably had} of estrous or the failure of fertilization ~~can be seen in~~

Table 1 Increased concentrations of progesterone occur gradually from day 4 to peak on day 14 after estrous, while decreased concentrations of progesterone begin to occur after day 14 and approach concentration during lunacy from the day 20. Increased and decreased concentrations of progesterone are in line with the development of the corpus luteum during the estrous cycle (Frestantie, loc. cit). The decrease in progesterone concentration is due to the ^{endogenous} luteolytic properties of ⁽²⁰¹²⁾ Prostaglandin F₂ α (PGF₂ α).

The hormones concentration on the day 28 of 15 dairy cow showed 3 cows with a concentration of progesterone that increased above 15 ng/mL as reported by Pemayun and

Budiasa (Loccit). The high concentration of progesterone during pregnancy due to the corpus luteum still serves to stimulate the endometrial cells to produce uterine milk which is the initial nutrient for the embryo ^{before} after implantation (Hafez and Hafez, loccit). The corpus luteum will be maintained during pregnancy so that the concentration of the hormone progesterone in milk remains high (Drajat, 2002). The formation of the placental membrane begins to develop at 15-17 days after fertilization which is the period of maternal recognition of pregnancy and aims to prevent the release of ~~prostaglandin~~ prostaglandin F_{2α} so that no regression of the corpus luteum and progesterone can be maintained properly in maintaining pregnancy (Hafez and Hafez, loccit). Besides, 9 cows with concentration below 15 ng/mL which can be seen in (Table I) show that cows experience early embryonic death. In animals that fail ^{to become} pregnant the concentration of progesterone will decrease due to regression of the corpus luteum on the 18-24 days after estrous (Drajat, loccit).

One of the factor affecting early embryonic death is non-aseptic gun or plastic sheet th and additional feed ~~is waste of bread~~ containing fungal. The implementation of AI in the field is also not supported with supporting infrastructure and poor sanitation conditions so that the implementation of AI becomes the medium of entry of bacterial and fungal infections. The presence of bacteria in the uterus may result in mild endometrial infections or subclinical endometritis (Madyawati *et al.*, 2019). Gautam *et al.* (2010) states ^{ed} that the failure of AI of cattle in Indonesia is generally subclinical ~~due to~~ bacterial and fungal infections in female reproductive organs.

Summary

Based on the results ^{it is} ~~and discussion were~~ concluded that the concentration of progesterone hormone on day 0 (estrous), 7 and 14 have increased in 15 dairy cows. On the day 21, three dairy cow failed fertilization and on the day 28, the progesterone concentration continued to rise above 15 ng/mL in three dairy cows and were declared pregnant while progesterone concentrations decreased in 9 dairy cows that experienced early embryonic death.

how?

not valid

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2. **Manuscripts are accepted for publication on the clear understanding that :**
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 - c) All authors are jointly and severally responsible to the various authorities for the contents of the manuscripts. The Editorial Committee shall not be held responsible in any manner whatsoever to the contents of the manuscript and the views and interpretations expressed by the authors in the manuscripts. All disputes regarding any legal proceeding pertaining to the journal shall be within the jurisdiction of the High Court of Judicature at Chennai.
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 - c) **Clinical articles :** Abstract (not more than 100 words), Keywords (not more than 4 words), Introduction (with no subtitle), Case History and Observations, Treatment and Discussion, Summary, Acknowledgement (optional) and References (not exceeding 15). The Clinical articles should not exceed 3 printed pages.
 - d) **Short Communications:** There is no specific format for Short Communication. Articles which do not comply to either Full Papers or Clinical Articles either on quality basis or on page-wise basis will be allotted under Short Communication.
 - e) The page limits are inclusive of figures, photographs and tables.
 - f) All tables should be numbered with upper case roman numerals (I, II...) and all figures should be numbered with arabic numerals (1, 2, 3...).
9. **Citing references:** In the text, references should be cited by names of the authors followed by the year of publication in parenthesis. In case of more than two authors, the name of the first author must be followed by *et al.* For the references repeated in the text, the year must be replaced by *loc cit.* in successive references. At the end of the text, the references must be furnished in alphabetical order. Each author's name must be followed by initials. Before the name of the last author, the word 'and' must be included. The year of publication must be in parenthesis. The title of the referred article must be given in full. The Journal's name must be in approved abbreviated form and must be in italics. The volume number of the journal must be given in bold letters. It should be followed by a colon and the starting and ending page numbers. While citing books, the Title of the book, Year, Publisher, Place of Publication, Number of the Edition and the relevant page number(s) must be provided. Website, 'in press' and 'personal communication' should be avoided as references.
10. All correspondence regarding the status of the articles must be made with The Editor, Indian Veterinary Journal at the Official Address of the Journal.



Revision Article 227-19

1 message

erma safitri <erma-s@fkh.unair.ac.id>
To: Ind Vet Journal <ivj83@yahoo.com>

Sat, Jul 20, 2019 at 8:50 PM

27 July 2019

ARTICLE # 227/19

TITLE :

**Progesterone Profile of Dairy Cows which Experienced The Failure of pregnancy
to Artificial Insemination (AI)**

Dear Editor,

Author Answer for Editor's comments

1. We have revised the title of article as recommended from Editor and Referee
2. We have deleted Figure 1
3. We have revised of Result and Discussion in an abridged form.
4. We have revised of References and following IVJ format.
5. We have deleted of reference more than 35 years old and change with reference at 2007
6. We have responded of queries revised by referee and conying out all correction pointed out and we have revised the article as full research article in 6 pages include of table following the IVJ guidelines and with the help of person professional in English.

Note : my revision in red colour

Author Answer for page in RR 16.7.19

1. We have revised the data in Table and interpretation to be clear, and we have answer several queries as indicated on the text
2. We have revised the title of article as recommended from referee
3. We have revised and conying out all correction in English language
4. We have changed some statements that not clear → now become clear
5. We have revised the abstract → become self-explanatory
6. We have deleted Figure 1 → the same data with Table
7. We have revised in M&M and R&D: language corrections and scientific way of interpretation of data have revised.
8. We have revised other corrections/ editing required that indicated on the manuscript
9. We have revised the conclusion.

Note : my revision in red colour

Thank you for your considered and chance for me to revise our article

Corresponding Author
Erma Safitri

2 attachments

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27 July 2019

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Corresponding Author
Erma Safitri

Progesterone Profile of Dairy Cows which Experienced The Failure of pregnancy to Artificial Insemination (AI)

Sri P. Madyawati, Isnaini Fadilah, Trilas Sardjito, Mas'ud Hariadi, Pudji Srianto, Suherni Susilowati, Erma Safitri*
Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, 60115

Abstract

The **aim of study** was to know the profile of progesterone in milk samples of dairy cows that experienced failure of AI. **Milk samples of fifteen** AI failure dairy cows were taken on days 0, 7, 14, 21 and 28 (day 0 = estrus). Progesterone analysis was performed by ELISA method. On day 7th and 14th progesterone concentrations were increased in **all** cows, on 21th day **the levels** were decreased in 3 cows and they returned **to** estrous, while in 12 cows **the progesterone levels** were still **increase**. On 28th day **the levels** were **high** and indicated pregnant in 3 cows, while 9 other cows **progesterone levels** declined and experienced early embryonic death.

Key words : Dairy cow, Progesterone, Estrous cycle, Artificial insemination (AI).

AI Failure can be an economically important problem on dairy farms (Canu *et al.*, 2010), such as increased costs for mating, long calving intervals, rejects of cow and **fewer** birth of calves each year (Rustamadji *et al.*, 2007). The reproductive disorder **is** caused **by imbalance** of progesterone and estrogen. The concentration of progesterone in the blood and milk can determined in animal the infertile, estrous and pregnant state, so that it can be used for estrous detection and other pathological conditions (Samik and Safitri, 2019).

Materials and Methods

Milk samples were taken directly on days 0, 7, 14, 21 and 28 (day 0 = estrous) in the morning from the udders of 15 AI failure dairy cows **aged 3.5-5.5** years who had

breeding at least one time, with healthy body condition and normal estrous cycle. The samples were kept into the ice box at 4⁰C, and transferred into the freezer. Before analyze the samples were thawed at room temperature. Furthermore 6 mL of each sample was taken into a 10 mL reaction tube and centrifuged at 3500 rpm for 15 minutes to separate fat and skim milk. Then skim milk as much as 1 mL was transferred into microtube. Hormonal analyzes were performed using the sandwich ELISA method. The data obtained were analyzed for ANOVA using Statistical Package Programs Social Sciences Software (SPSS).

Results and Discussion

Milk progesterone hormone profile determined on day 0 (estrous), 7, 14, 21 and 28 in 15 dairy cattle aged 3.5- 5.5 year that experienced failure of AI is shown in Table I.

Table I. Milk Progesterone Concentrations in Dairy Cows on days 0, 7, 14, 21, 28 of estrous Cycle

Progesterone Concentration (ng/mL)					
	D 0	D 7	D 14	D 21	D 28
Cow-1	2.923	4.718	7.265	3.023	5.326*
Cow-2	3.122	5.898	7.138	3.028	4.102*
Cow-3	2.855	4.307	8.12	15.81	16.521**
Cow-4	3.148	5.551	8.879	15.341	11.815***
Cow-5	2.453	4.301	7.622	2.677	4.237*
Cow-6	3.202	5.379	9.381	16.024	13.181***
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Cow-8	2.971	5.209	9.095	15.722	11.892***
Cow-9	3.305	5.281	9.198	15.38	11.913***
Cow-10	3.199	5.362	8.683	15.653	13.00***
Cow-11	3.258	4.583	8.216	15.483	17.028**
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Cow-14	3.099	5.324	8.989	15.206	13.011***
Cow-15	2.989	5.541	9.079	15.153	12.796***

Note:

Cow * = Failure of fertilization (on 21th and 28th day the levels progesterone were decreased)

Cow ** = Pregnant (on 21th and 28th day the levels progesterone were still increased)

Cow *** = Early Embryonic Death (on 21th the levels progesterone were increased, but on 28th day were declined)

The **progesterone** hormone concentration on the day 0 (estrous) of 15 dairy cows **were** consistent (2.2 - 3.5 ng/mL) with the results of some researchers suggesting that the animals **were in estrous** (Petersson, 2007).

The hormone concentration on the day 7 of 15 dairy cow explain **earlier observtion** that during the cycle, progesterone concentrations can be detected by the third day (Valdez *et al.*, 2005) and increase until day eight after estrous and continue to increase until 21st day **of cycle** pregnantly (McDonald, 2000). This statement is consistent with Frestantie (2017) that the formation of the corpus luteum has occurred after ovulation, so the hormone progesterone begins to be produced.

The **high** hormone concentration on the day 14 of 15 cow explain the luteal phase, the hormone progesterone **inhibit** gonadotropin hormone secretion that is Folicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from the anterior pituitary gland. These barriers **prevent** the follicles of the ovaries to develop and the hormone estrogen is not produced, so the animals do not show symptoms of estrous (Hafez and Hafez, 2013).

The **highes** hormone concentration on the day 21 explainsthat the concentration of progesterone in pregnant dairy cow rises in line with positive pregnancy age. Pemayun and Budiasa (2014) suggested that **milk** progesterone concentrations above 15 ng / ml can be stated positive. This study showed that 12 dairy cows ha results above 15 ng /mL while three cow that returned to the cycle **probably had** failure of fertilization (Table I). Increased concentrations of progesterone occur gradually from day 4 to peak on day 14 after estrous, while decreased concentrations of progesterone begin to occur after day 14 and approach concentration during **pregnancy** from the day 20. Increased and decreased concentrations of

progesterone are in line with the development of the corpus luteum during the estrous cycle (Hermadi *et al.*, 2019). The decrease in progesterone concentration is due to the luteolytic properties of Prostaglandin F_{2α} (PGF_{2α}).

The hormones concentration on the day 28 of 15 dairy cow showed 3 cows with a concentration of progesterone that increased above 15 ng/mL as reported by Pemayun and Budiasa (Loc cit). The high concentration of progesterone during pregnancy due to the corpus luteum still serves to stimulate the endometrial cells to produce uterine milk which is the initial nutrient for the embryo **before** implantation (Hafez and Hafez, loc cit). The corpus luteum will be maintained during pregnancy so that the concentration of the hormone progesterone in milk remains high (Drajat, 2002). The formation of the placental membrane begins to develop at 15-17 days after fertilization which is the period of maternal recognition of pregnancy and aims to prevent the release of **prostaglandin** F_{2α} so that no regression of the corpus luteum and progesterone can be maintained properly in maintaining pregnancy (Hafez and Hafez, loc cit). Besides, 9 cows with concentration below 15 ng/mL (Table I) show that cows experienced **d** early embryonic death. In animals that fail **to become** pregnant the concentration of progesterone will decrease due to regression of the corpus luteum on the 18-24 days after estrous (Drajat, loc cit).

One of the factor affecting early embryonic death is non-aseptic gun or plastic sheeth and additional feed containing fungi. The implementation of AI in the field is also not supported with supporting infrastructure and poor sanitation conditions so that the implementation of AI becomes the medium of entry of bacterial and fungal infections. The presence of bacteria in the uterus may result in mild endometrial infections or subclinical

endometritis (Madyawati *et al.*, 2019). Gautam *et al.* (2010) stated that the failure of AI of cattle in Indonesia is generally subclinical bacterial and fungal infections in female reproductive organs.

Summary

Based on the results it is concluded that failure of fertilization in 3 cows because levels of progesterone were decreased on 21th and 28th day and they returned to estrous. The indicated pregnant in 3 cows because levels of progesterone were still increased on 21th and 28th day. The indicated Early Embryonic Death in 9 cows because on 21th the levels progesterone were increased, but on 28th day were declined.

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Demand Letter # 227/19

From: Ind Vet Journal (ivj83@yahoo.com)

To: rma_fispro@yahoo.com

Date: Friday, August 16, 2019 at 12:17 PM GMT+7

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Managing Editor
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Nandanam. Chennai .600035

Phone : 91 44 2435 1006
E Mail : ivj83@yahoo.com
Online : www.ivj.org.in

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Corresponding Address:

Dr. Erma Safitri,
Faculty of Veterinary Medicine,
Airlangga University, Surabaya,
Indonesia – 60115.
E-mail : rma_fispro@yahoo.com

Publication Address:

Dr. Erma Safitri,
Faculty of Veterinary Medicine,
Airlangga University, Surabaya,
Indonesia.
E-mail : erma-s@fkh.unair.ac.id

Sd/-
(S. SUKUMAR)
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11 Chamiers Road, Nandanam
Chennai 600035. India
Phone # 91 44 2435 1006
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Dated : AUGUST 19, 2019

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Sd/-

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