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Acknowledgement Letter # 237/19

1 message

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Reply-To: ivj83@yahoo.com
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Fri, Jun 28, 2019 at 5:16 PM

ACKNOWLEDGEMENT

Reg. No: 237/19

Dated : 25/06/2019

Dear Dr. Erma Safitri,

We acknowledge the receipt of the following articles entitled "The Pregnant improvement of Bali cow through the use of a combination of equine chorionic gonadotropine (eCG) from local pregnant mare with PGF2 ." (Erma Safitri, et al.).

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

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Article # 237/19 for revision and referee comments attached

2 messages

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 10:02 PM

Sir,

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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erma safitri <erma-s@fkh.unair.ac.id>
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Thu, Jul 18, 2019 at 4:53 AM

ARTICLE # 237/19**TITLE :**

Improvement of Pregnancy Rate in Bali Cows Through The Use of A Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF2 α

Dear Editor,

Aurhor Answer for Editor's comments

1. We have revised the title of article as recommended from referee
2. We have revised of Result and Discussion in an abridged form.
3. We have revised of References and following IVJ format.
4. 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

Note : my revision in red colour

Author Answer for page in RR 16.7.19

1. We have revised the title of article as recommended from referee
2. We have revised and conying out all correction in English language
3. We have changed some statements that not clear → now become clear
4. We have revised the abstract → become self-explanatory
5. We have revised the objective of introduction → for fertility improvement
6. We have revised the materias and method → complete protocol of synchronization with time schedule and dose of drugs used as well as isolation,purification and standardization of eCG from local mare sera
7. We have revised in R&D:Table 2 last column have deleted, Text that same with Table have deleted and irrelevant and doule statements have deleted
8. We have cross-verified of references between text and list → we have added some regerences (red colour?)

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

Corresponding Author
Erma Safitri

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MANAGING EDITOR

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ARTICLE NO: 237/19

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.
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EDITOR'S COMMENTS

- 1) Title of the article to be revised as shown in the manuscript.
- 2) Results and Discussion may be presented in an abridged form.
- 3) References to follow IVJ format. Extra ref. and ref. in press to be deleted.
- 7) Only if all corrections pointed out are meticulously carried out and all comments rectified and revised article submitted as full research article of 5-6 pages, going thro' the enclosed IVJ guidelines, the article could be taken up for further action.

To
Dr. Erma Sapitri

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


Managing Editor

RETURN THIS PAPER WITH YOUR REPLY WITHIN 90 DAYS

Comments on Article A-237/19

"The pregnant improvement of Bali cow through the use of combination of equine chorionic gonadotropin (eCG) from local pregnant mare with PGF2 α "

By Herry A. Hermadi *et al.* from Surabaya, Indonesia

1. The manuscript describes the comparative estrus efficacy and pregnancy rate of patented eCG (Folligon) and eCG isolated & purified from local pregnant mare @ 400 IU following double PGF2 α injections 11 days apart in 45 Bali cows, and concludes that there was no significant difference between groups for estrus induction intervals (55-56 hrs) and pregnancy rate of both eCG groups was 86.66%, which was however significantly higher than only double PG group (53.33%). The findings are novel and worth appreciating.
2. Title however needs to be changed as *"Improvement of pregnancy rate in Bali cows through.... PGF2 α "*. The write up and English throughout the manuscript is also very poor and needs to be improved by consulting English proficient scientist/ teacher. Some statements are not clear/ambiguous. These are pointed out in the manuscript.
3. Abstract must be self-explanatory. The objective in introduction is unclear/wrong. In materials and method complete protocol of synchronization with time schedule and dose of drugs used as well as isolation, purification and standardization of eCG from local pregnant mare sera should be described.
4. In R&D, Table 2 last column be deleted. Similarly data on estrus achievement are given in Table 1, hence the same be deleted from the text. Moreover, Irrelevant discussion in R&D be deleted and so also the duplication of same statements/results at multi-locations.
5. References be cross-verified in text and list after concising the manuscript.
6. The revised concise improved manuscript may be considered to publish in IVJ.

if pregnancy rate in R.R 237/19
16.7.2019 11

The Pregnant Improvement of Bali cow through the use of a combination of equine chorionic gonadotropine (eCG) from local pregnant mare with PGF2 α

Herry A. Hermadi, R.T.S. Adikara, Sunaryo H. Warsito, Erma Safitri*
Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, 60115

Abstract

The study aim was improved of Bali cow pregnancy through use of a combination of eCG from local pregnant mare with PGF2 α . 45th Bali cows were ~~treated using~~ injected 25 mg PGF2 α twice and divided into 3 groups: without eCG (Control); patent eCG 400 IU from Folligon (Intervet-Holland) (T1) and eCG from local pregnant mare sera (T2). After the time of estrus achievement was done with AI and 60 days later the pregnancy rate was evaluated using two dimensional ultrasound. The results were indicated no significant differences ($p > 0.05$) between the T1 and T2 at the pregnancy rate, but between T1 and T2 were significant difference ($p < 0.05$) with control.

Handwritten notes: "rate" circled; "to" written above "aim"; "11 days apart" written above "twice"; "specific interval?" written to the right; "when injected?" written below; "better" written above "difference"; "both" written above "T1 and T2"; "than" written below "with control".

Keywords : Bali cow, eCG, PGF2 α , Time of estrus, Pregnancy rate.

Bali cattle are native Indonesian germplasm and still represents 27% of the total cattle population in Indonesia and it is considered the pillar breed for small farmers (Purwantara *et al.*, 2011). Therefore, the population needs to be improved through the use of a combination of eCG with PGF2 α . The eCG can support the growth of follicle in ovarium, such as FSH (Baruselli *et al.*, 2003) and combination of eCG and PGF2 α is important for pregnancy rate (Dias *et al.*, 2009).

Handwritten notes: "fertility rate of this" written above "population"; "was obtained" written above "from local pregnant mare"; "50-90 days of gestation" written above "3 months age"; "Blood samples were collected" written above "get was accumulated"; "pellet for" written above "with charcoal"; "this approach is not for improvement of population but for fertility improvement." written on the left; "give time schedule each injection when it was given?" written on the bottom left.

Materials and Methods

The eCG in this research from local pregnant mare of Indonesia at 3 months age that get was accumulated from jugular veins, and then purification with charcoal was done by chromatography Sephadex G100. Furthermore, eCG was evaluated of molecular weight with sds-page and examination of optical density absorbancy by ELISA method (Hermadi *et al.*, 2018). The Pregnant improvement of Bali cow through the use of a combination of eCG from local pregnant mare with PGF2 α . 45th Bali cows were treated using injected 25 mg PGF2 α twice and divided into 3 groups: without eCG (Control); patent eCG 400 IU from Folligon (Intervet-Holland) (T1) and eCG from local pregnant mare sera (T2). After the time of estrus achievement

Handwritten notes: "was obtained" written above "from local pregnant mare"; "50-90 days of gestation" written above "3 months age"; "Blood samples were collected" written above "get was accumulated"; "pellet for" written above "with charcoal"; "this approach is not for improvement of population but for fertility improvement." written on the left; "give time schedule each injection when it was given?" written on the bottom left; "given on 14 day of pregnancy" written on the bottom right; "clarify" written on the bottom right.

Corresponding Author, Email : erma-s@fkh.unair.ac.id

AI was done with ~~AI~~ and 60 days later the pregnancy rate was evaluated using two dimensional ultrasound (Samik and Safitri, 2019).

Results and Discussion

After isolation and purification with chromatography Sephadex G100, the identification was determined of protein profiles of the local pregnant mare sera. Based on the protein bands of molecular weight, that appear on sds-page ^{ed} were 63, 43 and 28 kDa (Figure 1).

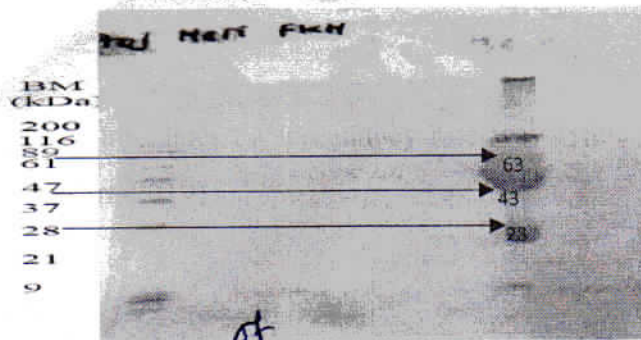


Figure 1. Molecular weight ^{of} eCG from local pregnant mare sera : 63, 43 and 28 kDa

After injected with 25 mg PGF2 α twice with 11 days intervals and eCG treatment, the results of the time of estrus can be seen in Table I.

Table I. Rate of estrus ^{interval} (hours) after twice injection of PGF2 α and eCG treatment

| Treatment | n | Mean of Estrus Time \pm SD (hours) |
|---|----|--------------------------------------|
| Twice injection of PGF2 α without eCG (C) | 15 | 56,54 ^a \pm 08,57 |
| Twice injection of PGF2 α + patent eCG 400 IU from Folligon ^{11 days apart} (Intervet Holland) (T1) | 15 | 55,15 ^a \pm 12,86 |
| Twice injection of PGF2 α + eCG 400 IU from local pregnant mare sera (T2) | 15 | 55,60 ^a \pm 12,08 |

Note: The same superscript in the same column indicates no significant difference ($p > 0.05$)

After the time of estrus achievement was done with AI and 60 days later the pregnancy rate was evaluated using two dimensional ultrasound (Table II)

Table 2. Pregnancy rates after the administration of combination of equine chorionic gonadotropine (eCG) from local pregnant mare with PGF2 α on the 60th days

| Treatment | Pregnant | Not Pregnant | Total |
|--|--------------------------|-------------------------|-----------|
| Twice injection of PGF2 α without eCG (C) | 8 ^a (53.33%) | 7 ^b (46.66%) | 15 (100%) |
| Twice injection of PGF2 α + patent eCG 400 IU from Folligon (Intervet-Holland) (T1) | 13 ^b (86.66%) | 2 ^a (13.33%) | 15 (100%) |
| Twice injection of PGF2 α + eCG 400 IU from local pregnant mare sera (T2) | 13 ^b (86.66%) | 2 ^a (13.33%) | 15 (100%) |

Note: The different superscript in the same column indicates significant difference ($p < 0.05$)

The ~~results~~ ^{extract obtained} of eCG from local pregnant mare sera was ~~extracted~~ ^{could} applied to Bali cow with the same doses with Bos indicus cow ^{s that} was ~~could~~ ^{improved} the pregnancy rate (Noqueira *et al.*, 2014). They used ~~of~~ hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates.

The utilization of eCG ~~was~~ ^{increased} pregnancy rates of Bradford (26.7 - 34.6%), Nellore (38.9 - 45.7%) and crossbred Nellore cows (46.8 - 59.1%), ^{with the same doses 400 IU eCG} (Bergamaschi *et al.*, 2006), also ~~was~~ ^{showed} that the estrus of cow ^{that} received 400 IU eCG was increased.

eCG is included in the aspartate proteinase group with amino acid levels of more than 50% identical to pepsin, cathepsin D and cathepsin E. The concentration of eCG from pregnant mare sera ~~was~~ ^{analyzed} by ELISA had ~~the~~ ^{Optical Density (OD)} in between range 0.957 - 1.069 on the sample with 400 IU/ml concentration.

A sample of blood ^{was} ~~taken~~ ^{as much as 300 mL} ~~was~~ ^{taken} from the jugular vein of the mare on the 50th, 70th and 90th day of pregnancy. The sample plasma was aspirated and the phenol solution ^{has} ~~been~~ ^{added} to the plasma and stored in the refrigerator until eCG extraction. The classical eCG purification techniques of pregnant mare sera include pH fractionation with precipitation step of metaphosphatic acid and absolute ethanol and dialysis followed by fixed chromatography.

Based on the Table I, it can be seen ⁱⁿ ~~that~~ the rate of estrus of Bali cows ~~by~~ ^{using} anova ~~test~~ ^{there is no} statistically significant difference ($p > 0.05$) ~~between~~ ^{and} the administration of eCG with patent product and local pregnant mare, ~~also~~ ^{with} PGF2 α only (Control). Estrus synchronization aims to increase the number of estrus cows simultaneously, allowing

simultaneous insemination. After signs of estrus appear was needed insemination to improve pregnancy. The rate of estrus onset in this study in cows was received of PGF2 α twice injected of 11 days intervals. PGF2 α without eCG injection (Control) was $56.54 \pm 0.8.57$ (C); PGF2 α + patent 400 IU eCG from Folligon (Intervet-Holland) (T1) was 55.15 ± 12.86 ; PGF2 α + 400 IU eCG from local pregnant mare sera (T2) was 55.60 ± 12.08 . The rate of estrus onset was not significantly different ($p > 0.05$). This suggests 400 IU eCG is recommended in onset of estrus.

The variation in the onset of estrus is most likely a reflection of differences in ovarian follicle growth phase so that during luteolysis after PGF2 α injection there was no uniformity in the maturity of ovulatory follicles, resulting in ovulation at different times. Synchronization process using prostaglandin preparations (PGF2 α) was caused CL regression due to luteolysis.

Prostaglandin F2 α is released naturally by the uterus of non-pregnant animals on day 16 to 18 of the cycles which serves to destroy the CL. Estrus onset after PGF2 α administration results from CL lysis due to PGF2 α vasoconstriction so that blood flow to the CL drastically decreased, leading to decreased levels of progesterone produced by the CL. The decrease in progesterone levels will stimulate the anterior pituitary to release FSH and LH, which are responsible for the process of folliculogenesis and ovulation resulting in the growth and maturation of estrogen-producing follicles that may manifest estrus symptoms. The action of estrogen hormone to improve the sensitivity of female sex organs is marked by changes in the vulva and the presence of transparent discharge (Murphy, 2012). Effect of eCG on luteal function and reproductive performance of high-producing lactating Holstein dairy cows, human chorionic gonadotropin has an activity similar to that of LH and binds to LH receptors on the membrane of small luteal cells to activate a second messenger, which enhances progesterone synthesis. Corpus luteum formation and development is highly responsive to LH, and small luteal cells synthesize more progesterone when stimulated by LH (Santos *et al.*, 2001).

An overview of the positive pregnancy in the diagnosis of pregnancy with an ultrasound, showing anechoic spherical formations on the dorsal side of the bladder Pregnancy Rate Examination on day 60 post-insemination in heifers by using USG (Romano and Magee, 2001). Pregnancy was marked with black amniotic bag and white fetus within the amniotic bag, so that bone formation can be seen, although still not perfect. Reproduction of female cows is one of the major factors that affect the efficiency of the cow productivity, which is largely determined by the level of fertility, pregnancy and birth (Martemucci *et al.*, 2011). Factors that

calving and space

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limit the expression of pregnancy rate is low estrus, which in turn results in low fertilization.

The results of
Pregnancy examinations with ultrasound method performed on day 60 post-insemination ~~on~~
are shown in Table-2
~~pregnancy rate was showed.~~ Pregnancy rate in artificial insemination technology is determined
by many factors, such as estrus detection and the right time for insemination. Higher pregnancy
rate in group T1 and T2 was likely caused by high levels of progesterone in luteal phase which
were approaching basal progesterone levels, and suspected the possibility of early embryonic
survivals, which is a normal process of natural selection and often occurs in cows with high
number of offspring or in cattle with high number of offspring in one pregnancy. Early
embryonic death is also caused by progesterone deficiency. 5-10% of cattle have estrus but not
accompanied with ovulation, resulting in lower pregnancy rate at first estrus. Lower pregnancy
rate is likely due to abnormal fertilization (Romano and Magee, ²⁰⁰¹ ~~loc cit~~). Not every ovulation is
always followed by fertilization and not all fertilization produces normal individuals.

An ultrasound pregnancy examination performed on the 60th days post-insemination at
~~the pregnancy rates~~ showed that group control group (C) had a pregnancy rate ~~of~~ (53.33%);
group (T1) = 100% (86.66%); group (T2) = 100% (86.66%). ~~The~~ *Significantly* lower pregnancy rate in the control
group may be caused by low progesterone levels in the luteal phase near the basal progesterone
level, which was 0.49 ± 0.41 *ng/ml* and predicts the possibility of early embryonic death, which is a
natural process. Selection ~~and~~ often occurs in pigs with high numbers of offspring or in cattle
and goats with high numbers of offspring in one pregnancy (Romano and Magee, ~~loc cit~~).

Summary :

obtained
The sds-page examination of eCG *yielded* from local pregnant mare sera ~~was obtained by~~
molecular weight = 63 kDa, 43 kDa and 28 kDa. The improvement of Bali cow pregnancy
through use of a combination of eCG from local pregnant mare and PGF2 α was not significantly
different ($p > 0.05$) with a combination of patent eCG and PGF2 α , *proving equal efficacy*

of local pregnant mare sera & patent eCG over control group.

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Article # 237/19 for revision and referee comments attached

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To: Ind Vet Journal <ivj83@yahoo.com>

Thu, Jul 18, 2019 at 4:53 AM

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18 July 2019

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2. We have revised of Result and Discussion in an abridged form.
3. We have revised of References and following IVJ format.
4. We have responded of queries revised by referee and conyng 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

Note : my revision in red colour

Author Answer for page in RR 16.7.19

1. We have revised the title of article as recommended from referee
2. We have revised and conyng out all correction in English language
3. We have changed some statements that not clear → now become clear
4. We have revised the abstract → become self-explanatory
5. We have revised the objective of introduction → for fertility improvement
6. We have revised the materias and method → complete protocol of synchronization with time schedule and dose of drugs used as well as isolation, purification and standardization of eCG from local mare sera
7. We have revised in R&D: Table 2 last column have deleted, Text that same with Table have deleted and irrelevant and doule statements have deleted
8. We have cross-verified of references between text and list → we have added some regerences (red colour?)

Note : my revision in red colour

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

Corresponding Author
Erma Safitri

Improvement of Pregnancy Rate in Bali cows through the use of a combination of equine chorionic gonadotropine (eCG) from local pregnant mare with PGF2 α

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Abstract

The aim of study was to improve of pregnancy rate in Bali cows through use of a combination of eCG from local pregnant mare with PGF2 α . 45 Bali cows were injected with 25 mg PGF2 α twice 11 days apart and divided into 3 groups: without eCG (Control); patented eCG 400 IU from Folligon (Intervet-Holland) (T1) and eCG from local pregnant mare sera (T2). After the estrus achievement was AI done and 60 days later the pregnancy rate was evaluated using two dimensional ultrasound. The results indicated non significant differences ($p>0.05$) between the T1 and T2 at the pregnancy rate, but both were significantly better ($p<0.05$) than control.

Keywords : Bali cow, eCG, PGF2 α , Time of estrus, Pregnancy rate.

Bali cattle are native Indonesian germplasm and a breed importance regarding its direct ancestry from Banteng (Purwantara *et al.*, 2011). However, the fertility and pregnant rate is very low (Lindell, 2013). Therefore, there is a need for the improvement of fertility and pregnant rate through the use of a combination of eCG and PGF2 α . The eCG can support the growth of follicle in ovarium, such as FSH (Baruselli *et al.*, 2003) and combination of eCG and PGF2 α is importance for pregnant rate (Dias *et al.*, 2009).

Materials and Methods

The eCG in this research was obtained from local pregnant mare of Indonesia at 50-90 days of gestation blood samples from jugular veins were collected and then purification of eCG with charcoal (30 mg/100 mL) was done by chromatography Sephadex G100 column eluted with 0.05M NH₄ HCO₃. Furthermore, eCG was evaluated of protein profiles (molecular weight) and optical density absorbancy with SDS-PAGE (Sodium Sulphate Deodecyl polyacrilamid gel electrophoresis) 12% and sandwich ELISA (with monoclonal antibody eCG and conjugated with horseradish peroxidase, Bioscience, San Diego, USA) (Hermadi *et al.*, 2018). 45 Bali cows were injected with 25 mg PGF2 α twice 11 days apart and divided into 3 groups: without eCG (Control); patented eCG 400 IU from Folligon (Intervet-Holland) (T1) and eCG from local pregnant mare sera (T2), time schedule of injection can be seen in Figure I. After the time of

estrus achievement **AI was done** and 60 days later the pregnancy rate was evaluated using two dimensional ultrasound (Samik and Safitri, 2019)



Figure 1. Time schedule of treatment and observation

Results and Discussion

After isolation and purification with chromatography Sephadex G100, the identification was determined of protein profiles of the local pregnant mare sera. **The protein** bands of molecular weight, that **appeared** on SDS-PAGE 12% **were** 63, 43 and 28 kDa (Figure 2).



Figure 2. Molecular weight of eCG from local pregnant mare sera : 63, 43 and 28 kDa

After injected with 25 mg PGF2α twice with 11 days intervals and eCG treatment with **second PGF2α**, the results of the time of estrus can be seen in Table I. **Furthermore, 60 days later the pregnancy rate can be seen in Table II.**

Table I. Estrus Time (hours) After Twice Injection of PGF2α and eCG Treatment

| Treatment Groups | N | Mean of Estrus Time ± SD (hours) |
|--|----|----------------------------------|
| Twice injection of PGF2α 11 days apart without eCG (C) | 15 | 56.54 ^a ± 08.57 |
| Twice injection of PGF2α 11 days apart + patented eCG 400 IU from Folligon (T1) | 15 | 55.15 ^a ± 1.28 |
| Twice injection of PGF2α 11 days apart + eCG 400 IU from local pregnant mare sera (T2) | 15 | 55.60 ^a ± 1.20 |

Note: The same superscript in the column indicates no significant difference ($p > 0.05$)

Table II. Pregnancy Rates After The Administration of Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF2 α on the 60th days

| Treatment | Pregnant | Not Pregnant |
|--|--------------------------|-------------------------|
| Twice injection of PGF2 α without eCG (C) | 8 ^a (53.33%) | 7 ^b (46.66%) |
| Twice injection of PGF2 α + patented eCG 400 IU from Folligon (T1) | 13 ^b (86.66%) | 2 ^a (13.33%) |
| Twice injection of PGF2 α + eCG 400 IU from local pregnant mare sera (T2) | 13 ^b (86.66%) | 2 ^a (13.33%) |

Note: The different superscript in the same column indicate significant difference ($p < 0.05$)

The **extract** of eCG **obtained** from local pregnant mare sera applied to Bali cows with the same doses with *Bos indicus* **cows that could improve** the pregnancy rate (Noqueira *et al.*, 2014). They used hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates.

The utilization of eCG increased pregnancy rates of Bradford (26.7 - 34.6%), Nellore (38.9 - 45.7%) and crossbred Nellore cows (46.8 - 59.1%). **With the same doses 400 IU eCG** (Bergamaschi *et al.* (2006) also showed that the estrus of cows was increased.

eCG is included in the aspartate proteinase group with amino acid levels of more than 50% identical to pepsin, cathepsin D and cathepsin E. The concentration of eCG from pregnant mare sera analyzed by ELISA **had** the Optical Density (OD) in between range 0.957 - 1.069 on the sample with 400 IU/ml concentration.

Based on the Table I, it can be seen there is no statistically significant difference ($p > 0.05$) in the rate of estrus of Bali cows between the administration of eCG with patented product and local pregnant mare, and also with PGF2 α only (Control). Estrus synchronization aims to increase the number of estrus cows simultaneously, allowing simultaneous insemination. This result suggests 400 IU eCG recommended in onset of estrus. The variation in the onset of estrus is most likely a reflection of differences in ovarian follicle growth phase so that during luteolysis after PGF2 α injection there was no uniformity in the maturity of ovulatory follicles, resulting in ovulation at different times. Synchronization process using prostaglandin preparations (PGF2 α) caused CL regression due to luteolysis.

. Reproduction of cows is one of the major factors that affect the efficiency of the female productivity, which largely determined by the level of fertility, pregnancy and calving (Martemucci and D'Alessandro, 2011). Factors that limit the expression of pregnancy rate is low estrus, which in turn results in low fertilization. The results of pregnancy examinations with ultrasound method performed on day 60 post-insemination are shown in Table II. **The higher pregnancy rates in the T1 and T2 groups were thought to be due to increased blood levels of progesterone. The previous research was showed that eCG in 40-120 days pregnant mare has a function like the folliculo stimulating hormone (FSH), which acts to increase follicular growth, but the follicle did not become de Graaf's follicle and did not ovulate, but was luteinized, so that become corpus luteum (CL) accessory. This CL accessory is produce progesterone which maintains the mare's pregnancy at the age of 40-120 days when placenta's mare has not produced progesterone (Ismudiono *et al.*, 2010; Hafez and Hafez, 2013). In this study, the injection of eCG was caused growth of follicularly and luteinization become CL accessory form. CL accessory that were formed to cause an increase in the level of progesterone in the blood, so that pregnancy can be maintained, whereas in the control it does not occur. Low progesterone levels was suspected the possibility of early embryonic death. Early embryonic death is also caused by progesterone deficiency. 5-10% of cattle have estrus but not accompanied with ovulation, resulting in lower pregnancy rate at first estrus. Lower pregnancy rate is likely due to abnormal fertilization (Romano and Magee, 2001). Not every ovulation is always followed by fertilization and not all fertilization produces normal individuals.**

An ultrasound pregnancy examination performed on the 60th day post-insemination showed that control group (C) had a pregnancy rate of 53.33%; group (T1) = 86.66%; group (T2) = 86.66%. Significantly lower pregnancy rate in the control group may be caused by low progesterone levels in the luteal phase near the basal progesterone level, which was 0.49 ± 0.41 mg/ mL and **low progesterone** can caused the early embryonic death (Samik and Safitri, *loc cit*).

Summary :

The sds-page examination of eCG obtained from local pregnant mare sera molecular weight = 63 kDa, 43 kDa and 28 kDa. The improvement of Bali cow pregnancy through use of a combination of eCG from local pregnant mare and PGF2 α was not significantly different

($p > 0.05$) with a combination of patent eCG and PGF₂, proving equal efficacy of level pregnant mare sera and patented eCG over control group.

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Mon, Jul 22, 2019 at 2:09 PM

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| 237/19 | Improvement of Pregnancy Rate in Bali Cows through the Use of a Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF ₂ α . | Herry A. Hermadi, R.T.S. Adikara, Sunaryo H. Warsito, Erma Safitri |

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**Managing Editor,
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