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3	Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women to Manipulate in vivo Ovulation and Pregnancy of Madura Cows	2019
4	Anti Early Embryonic Protein (EEP) for Pregnancy Test by Microtiter Strip in Dairy Cows	2019
5	The Effect of Feeding High Level of Protein on Reproductive Performance of Bali Starling.	2019
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7	Diagnosis of Single and Twin Pregnancy, and Early Embryo Mortality Through Progesterone Level Test on Local Does.	2019
8	Improvement of Pregnancy Rate in Bali Cows with the Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF _{2α} .	2019
9	Progesterone Profile of Dairy Cows which Experienced the Failure of Pregnancy to Artificial Insemination (AI).	2019
10	Effect of Heat Shock Protein (HSP) in Post Thaw Baluran Bull Semen	2018
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13	Implementation of fotogrametry techniques as body mass estimation of indo-pacific bottle nose dolphin (Tursiops aduncus) in bali dolphin lodge	2020
14	Uji Sensitivitas Kebuntingan Sapi Perah Menggunakan Pregnancy Specific Protein B (PSPB) Microtiter Strip dan Progesteron sebagai Gold Standard	2007
15	Estimation of Equine Chorionic Gonadotropin (eCG) concentrate in the Blood Sera of Pregnant Mare	2014
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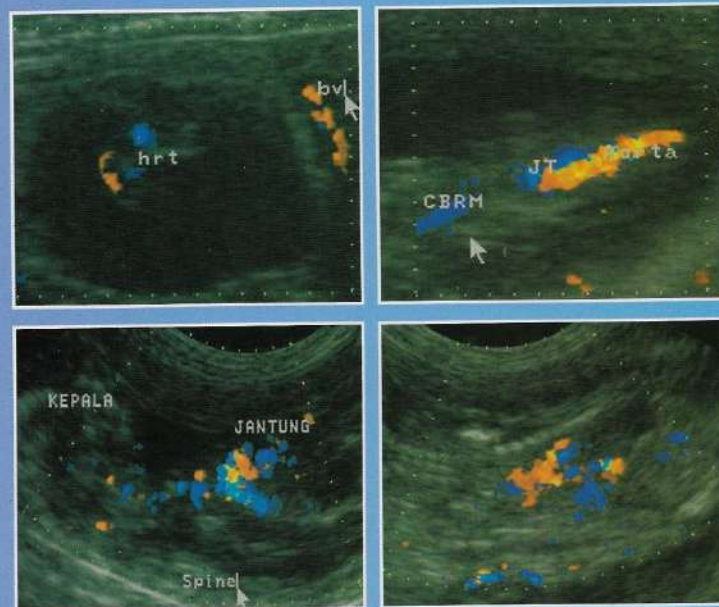


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Estimation of Equine Chorionic Gonadotropin (eCG) concentrate in the Blood Sera of Pregnant Mare

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Abstract

Equine Chorionic Gonadotropin (eCG) is a special hormone found in the blood of pregnant mare at 40 – 120 days pregnancy. The objective of this research was to identify and estimate of Equine Chorionic Gonadotropin (eCG) concentration from pregnant mare blood sera at 7–19 weeks of pregnancy. The research showed that a molecular weight of eCG between 55.6–66.4 kDa. While average concentration of eCG from pregnant mare at 7, 11, 15, 19 week were 7412 ± 1865.94 $\mu\text{g/ml}$, 9112 ± 1532.88 $\mu\text{g/ml}$, 16696 ± 1885.02 $\mu\text{g/ml}$ and 5636 ± 1245.67 $\mu\text{g/ml}$ respectively. Based on the results, it could be concluded that eCG could be detected in the blood sera of pregnant mare at 7-19 weeks of pregnancy.

Key words: pregnant mare, eCG blood sera

Introduction

The development of horse population in Indonesia has not reached the great condition moreover in the East Java decreasing of it occurred from 26.449 horses on 2002 became 18.333 horses on 2006 (Dijenak, 2006)

The obstacle often faced the horse farmer was about reproduction problem, such as the long distance of inter birth and the low pregnancy number therefore the high level of productivity difficult reached.

The effort done to increase productivity provided is by repair reproduction operational contains estrus detection, good mating, and good pregnancy diagnose.

Early pregnancy diagnose was need after mating occurred to early identified thus the loss productivity time can be decreased.

Early pregnancy diagnose at horse can be done in two ways, that is (a) by detection specific substance provided in the maternal blood such as *Chorionic Gonadotropin* (eCG), *early pregnancy factor* (EPF) and (b) by detection non specific substance in the blood, urine, or milk during pregnancy such as, progesterone, oestrone sulphat (Hafez, 2000)

Until now something done to early pregnancy diagnoses at the horse was by detection of providing non specific substance during pregnancy. Early pregnancy detection used non specific substance such as progesterone and oestrone sulphate by RIA

technique (Hunter, 1995; Nalbandov, 1990) have not accomplished fast in the field because some factors such as difficulties of application, high price of Kit, and difficult get materials for RIA (Dobson, 1983)

The aim of this research was to identify *Equine Chorionic Gonadotropin* (eCG) from mare blood serum on 7-19 weeks old pregnancy (Partodiharjo, 1992; Toelihere, 1985) and estimate content of *Equine Chorionic Gonadotropin* (eCG) from horse blood serum on 7-19 weeks old pregnancy.

Materials and Methods

In this research used 5 pregnant mares (Torough Breed) on pregnancy age 7-19 weeks.

Materials used was pregnant mare blood serum, methanol, PBS, whatman paper, stacking gel, e buffer, laimly buffer, silver coloring, BSA, biuret reactor, and aquades.

Some equipment used was disposable syringe, reaction tube, centrifuges, Millipore, vial, freezer, SDS PAGE, comb, kuvet spectrophotometer, spectrophotometer Bausch-Lombs.

Isolation and Spreading eCG Pregnant Mare Blood Serum

Five pregnant mares (on 7-19 weeks) were used for this research. Blood harvested from vena jugulars utilized disposable syringe 20 ml, then intercepted in the reaction tube and closed. Reaction tube made a slant 45° and let it for 2 hours at the room

temperature, then centrifuges on speed 3000 rpm for 10 minutes. Serum received filtered by Millipore 0.22 μm . serum harvested intercepted in the vial and saved in the freezer at 20°C or entered in the reaction tube and added methanol in comparison 1:5 and shacked for 3 minutes then let for 15-20 minutes until 2 fluid layer providing. Supernatant was taken by disposable syringe as much as 5ml and entered into vial to make dry frozen preparation. Before used to next examination, it needs added PBS 5ml.

eCG Identification by SDS-PAGE and Western Blot

Running gel entered into SDS-PAGE tool through wall tube about less from above limitation. Added butanol 1 ml and let it for 25 minutes, then butanol though after gel freeze and cleaned by PBS and dried utilized whatman paper. Then added stacking gel through wall tube until full and after it entered comb and waited until right set (25 minutes). Then comb taken and cleaned form gel utilized e buffer. Sample (pregnant horse serum) 15 μl mixed with 5 μl laimly buffer and heated at 100°C for 5 minutes. Then sample 10 μl entered into pole mold with tip 200 μl . Mold entered into Biord, started power supply on 125V, 4mA for 1 hour, if the gel reaction have reached base then switched off and plate opened and spread, than washed by buffer and the result colored by silver coloring.

Western Blot was done used fragment of eCG string of pregnant horse which have accomplished running on SDS-PAGE and transferred at the Nitrocellulose membrane. Membrane blocked utilized 3% BSA in the 20 mM Tris-HCL pH 7.5 and 150 mM NaCl for an hour, then incubated in the Tris/NaCl contained 1% BSA with monoclonal antibody anti-eCG as primary antibody. Then washed in the Tris-Cl contained 0.05% Tween 20. Then membrane incubated with secondary antibody (anti-rabbit IgG label AP, on dilution 1: 1000) and added western blue substrate. Arising string was eCG protein string, thus it could known molecule weight (WM) of eCG isolation.

eCG Isolation by Electro Elution

SDS-PAGE gel without coloring sliced as long as string wanted. Every gel slice entered into nylon pouch. Then entered into block glass contained PBS and then sterer for 24 hours, every 6 hours PBS changed. To know that protein have realized elution, gel slice colored by silver coloring, if there was not the string it was mean protein have eluted. After that Biuret test done to know titer of the protein.

eCG Isolation Examination by Biuret

Total content of protein can be determined by biuret reagent with adding protein standard solution

(BSA). 3 spectrophotometer kuvet prepared, the first kuvet signed "S" as sample kuvet will be measured. In the S kuvet entered 0.05ml eCG isolation and 2.5ml biuret reactor. The second kuvet signed "ST" as standard kuvet, entered 0.05ml standard protein solution and 2.5 biuret reactors. The third kuvet signed "BL" (blank), entered 2.5ml biuret reactor and 0.05ml aquadest. All kuvet were let for 30 minutes and then read at spectrophotometer Bausch-Lombs spectronic 20 in the wave length 540 nm.

Formulation as follow: total content of protein ($\mu\text{g}/\text{ml}$): $Y = 5.10 \cdot X$; Exp.: Y, absorbance value; X, Protein content ($\mu\text{g}/\text{ml}$)

Results and Discussion

The Profile of eCG Identification by SDS-PAGE

SDS-PAGE result from pregnant mare blood serum could be seen at Fig. 1.

The string of pregnant mare blood serum arose at the examination by SDS-PAGE after compared with marker protein that were: 42.7 kDa, 55.6 kDa, 66.4 kDa. Those protein strings suitable with eCG molecule weight, about 45-65 kDa. Protein Strings with molecule weight 42.7-66.4 kDa accomplished specify test by Western Blotting Method to determine the validity of protein which was been running by SDS-PAGE method as eCG protein, then purification also done at protein SDS-PAGE result by electro elution method to spread with other protein and after it protein content examination done by biuret method.

The Result of Specify Test of eCG Protein by Western Blotting Method

The result of specify test of eCG protein by Western Blotting method could be seen at Fig. 2.

According to the specify test by Western Blotting method showed that the protein examined was right eCG protein, it signed by the providing string from Western Blotting Method presented at 55.6 - 66.4 kDa.

The Result of eCG Protein Examination by Biuret Method

The result of eCG protein examination by Biuret method could be seen at Table 1.

Base on Table 1. upper received absorbance average of eCG protein from pregnant mare serum in age 7 weeks, 15 weeks, and 19 weeks were: 0.371, 0.456, 0.835, 0.282 and the protein content average of pregnant mare serum were: 7412 $\mu\text{g}/\text{ml}$, 9112 $\mu\text{g}/\text{ml}$, 16696 $\mu\text{g}/\text{ml}$, 5636 $\mu\text{g}/\text{ml}$.

The average and the standard deviation of eCG protein content of pregnant mare blood serum could be seen at Table 2.

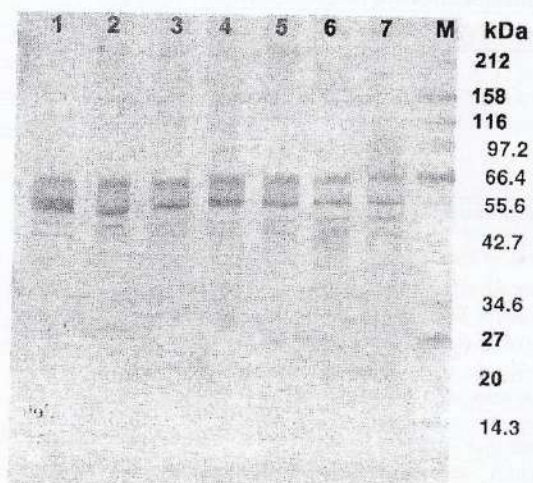


Figure 1. Protein strings from pregnant mare blood serum.

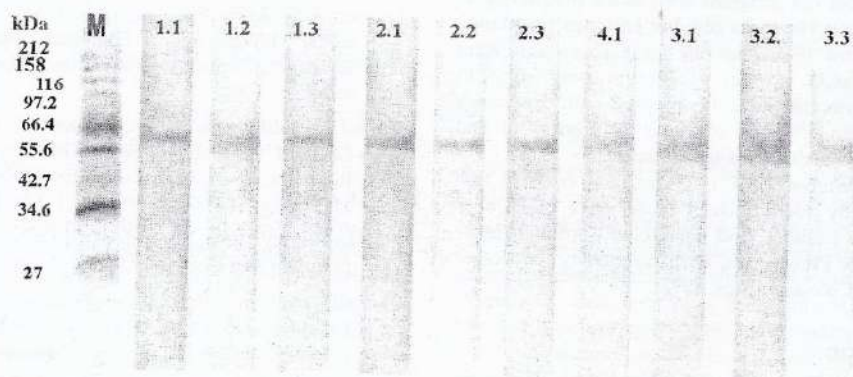


Figure 2. The figure showed eCG protein string by Western blotting method. Exp.: 1.1 – 1.3, Pregnant mare 7 weeks old; 2.1 – 2.3, Pregnant mare 11 weeks old; 3.1 – 3.3, Pregnant mare 15 weeks old.

Table 1. The Result of eCG Protein Examination by Biuret Method

Mare	Pregnant Age							
	7 Weeks		11 Weeks		15 Weeks		19 Weeks	
	Abs	PC	Abs	PC	Abs	PC	Abs	Pc
1	0.332	6640	0.407	8140	0.956	19120	0.360	7200
2	0.257	5140	0.526	10520	0.836	16720	0.331	6620
3	0.504	10080	0.346	6920	0.834	16680	0.208	4160
4	0.415	8300	0.512	10240	0.692	13840	0.248	4960
5	0.345	6900	0.487	9740	0.856	17120	0.262	5240
Means	0.371	7412	0.456	9112	0.835	16696	0.282	5636

Exp: Abs, Absorbance; PC, Protein Content

Table 2. The Average of eCG Protein Content of Pregnant Mare Blood Serum

No.	Pregnancy Age	N	Average (µg/ml)
1	7 weeks	5	7412.00 ^{ab} ± 1865.94
2	11 weeks	5	9112.00 ^b ± 1532.88
3	15 weeks	5	16696.00 ^c ± 1885.02
4	19 weeks	5	5636.00 ^a ± 1245.67

^{a-c} different superscript at same column showed significant difference (p<0.05).

According to the analysis of ANOVA statistic presented difference of eCG protein content of blood serum due to pregnancy age of mare. eCG protein increased at pregnancy 7 weeks old until 15 weeks old, but after pregnancy 19 weeks old the protein content decrease. It was synchronous with the statement of Knobil *et. al.*, (1998) that eCG begin arise in the maternal blood circulation at pregnancy 40 days old, it increase until pregnancy 55 – 65 days old and decrease slowly until loss at pregnancy 150 days old (Peters, and Ball, 1987).

Statistically eCG protein content at pregnancy 7 weeks old did not different with at the pregnancy 11 weeks old and 19 weeks old, but between pregnancy 11 weeks and 19 weeks old there were significant difference. eCG protein content at pregnancy 15 weeks old was the highest compared with pregnancy 7 weeks old, 11 weeks old, and 19 weeks old and specifically was different significantly.

The difference of eCG protein content was influenced by some factors those were body size, parity, fetus number, and genetic. eCG content more found at the Pony mare, twin pregnancy, and often gave birth (Cole and Cups, 1977).

Conclusion

According to the result received could be concluded the *Equine Chorionic Gonadotropin* (eCG) could be detected at the pregnant mare 7 -19 weeks old.

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