Accuracy of Frozen Dry Equine Chorionic Gonadotropin (E Cg) Production from Local Horse in Increasing Bali Cattle Pregnanacies Rate

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ABSTRACT

The goal of this research was to produce an e CG from local Indonesian horse pregnant mares sera. This research identified e CG by explaining the glycoprotein characteristic. Equine Chorionic Gonadotropin or e CG were collected from 10 horses jugular vein. The sera material was filtrated by using sephadex G-100. The result of SDS-PAGE showed protein bands which ranged between 65, 55 and 28 k Da and kept on revealing immune-reactivity by monoclonal antibody of e CG of the 55 k Da bands. The level of e CG 400 mIU/ml used indirect elisa. The result of e CG product it can be frozen as dry e CG. The continuation of this study was to evaluate potency of e CG from local Indonesian horse and e CG from patent product (Folligon Intervet Holland) on the onset of estrus time, and pregnancy rate of Bali cattle in Buleleng. This study used 24 female Bali cattle divided by 4 treatment groups. All of the cattle were being synchronized of their estrus cycle. After that the control group (K) was injected with 600 IU Folligon Intervet Holland, P1, P2 and P3 were injected by e CG local horse product with dosage of 600 IU, 300 IU and 150 IU respectively. After the injection of e CG the onset of estrus time was evaluated. Then, using two dimensional USG, the follicle numbers were examined. The cattle were then inseminated by using fresh semen seven days after artificial insemination. Forty days later the pregnancy rate was evaluated by using two dimensional USG. The result of this study showed that there was no significant difference on the onset of estrus time, and pregnancy rate between control, P1, P2 and P3 group.

KEYWORDS: pregnant mares sera, frozen dry e CG, estrus, pregnancy rate

INTRODUCTION

The research on large animals, the Bali cattle in this case, has become the main engine for the discovery of reproductive biology. Bali cattle is germ plasma from Indonesia, especially Bali Island, in which its existence is maintained until now. Characteristics and advantages of quality beef Bali cannot be doubted, but what should we be aware that increased of Bali cattle beef is native Indonesian natural resources.

Improved quality of Bali livestock is one of the main aspects in the development of beef cattle farms in Indonesia. One of the cutting-edge technology that has been used to improve the reproductive efficiency of livestock is induced estrus, the technology is used in handling cases of infertility or reproductive disorders, artificial insemination, super ovulation and embryo transfer. Bali has a beef type adaptability, meat production and reproduction which are quite good in Indonesia. Bali beef production has reached more than 50% higher than other beef carcass production. With the management of Bali is cattle for 305 days and the 60 day dry period is expected to achieve calving interval of 12 months the Bali cattle can give birth once a year.

German anatomist Wilhelm Schauder one hundred years ago, published a seminal paper of work he had carried out while undertaking his veterinary training at the University of Giessen. In it, he described the existence of unusual structures in the endometrium of pregnant mares or female horse pregnant. This is the first known description of the endometrial cups, which constitute a circle of raised, ulcer-like protuberances at the base of the gravid horn in the equine uterus in early pregnancy. Therefore, alternatives to e CG dose hormone need to be sought e CG (equine chorionic gonadotropin) in domestic animals being discussed is the e CG derived from horses, namely e CG, or named Pregnant Mare Serum Gonadotrophin by the inventor harlod cold [1,2,3]. Until now research that seeks to examine the horse serum levels of e CG Indonesia (Sandel, CBG2 and CBG4) is rarely performed [4].

This research isolated e CG protein from pregnant horse serum with the addition of charcoal in the extraction and ultra centrifuge 4 degrees C and was added with absolute ethanol in 1: 1 composition compare and purified by chromatography columns technique CM Sephadex G-100. E CG identification of proteins used SDS-PAGE showed protein bands which ranged between 65, 55 and 28 k Da and kept on revealing immune-reactivity by monoclonal antibody of e CG of the 55 k Da bands.

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PAGE (Sodium Sulphate Deodecyl poly acrilamid gel electrophoresis) and then frozen dry preparations was made. The purpose of this study was to test the accuracy of the biological potential production applications of pregnant horse serum extract separated with sephadex G-100 against gonadotropin (e CG) in the form of frozen cleaning to improve the pregnancy rate of Madura cattle. This study also aimed to determine a model of technology infertility treatment and induction of estrus with other e CG in experimental animals and commercial livestock, in particular Bali cattle, demonstrated with high success.

METHODS

This research was conducted in two stages as follows: Isolation of horse serum proteins pregnant e CG was done with charcoal and extraction with the addition of absolute ethanol 1:1 in 4 degrees C ultra centrifuge and purified by chromatography techniques columns CM Sephadex G-100. eCG Identification of proteins used SDS-PAGE (Sodium Sulphate Deodecyl polyacrilamid gel electrophoresis) and made into dry frozen dosage forms [5,6].

In the first stage, a local horse blood serum was taken from the jugular vein as much as 10 ml per cattle with Venoject 10 ml. Centrifugation was performed at 3000 was sonificated and vortexed. Cold absolute ethanol 1 : 1 were incubated in refrigerator. With Cl 20 mM tris buffer will be obtained subsequent to the examination of protein isolates SDS-PAGE [5]. Identification of proteins e CG was performed by SDS-PAGE and protein bands followed examination used by examination of immune-reactivity with the monoclonal antibody of the e CG. The indirect elisa, e CG concentration on local horse was made in dry frozen dosage forms. In the second stage, 24 female Bali cattle with BSC (Body Condition Score) 2 (two) for synchronization of estrus were treated with dry frozen e CG from the study results.

Data from e CG extraction, identification, isolation, and characterization were processed descriptively. Obtained data on Bali cattle pregnancy rate after e CG injection were analyzed using Anova. If different treatment was present, the test was continued with Tukey test, while the data on ovarian cell maturity were analyzed using Chi-square test [7].

RESULTS AND DISCUSSION

This study aimed to determine the stage of protein profiles that exist on a local pregnant horse serum. Identification of proteins based on the protein bands that appeared on the electropherogram. Determination of protein molecular weight standards has made with the help of proteins (BIO-RAD). There were 3 bands identified in the serum of pregnant horses appearing in the local examination with 12% SDS-PAGE. After comparison with protein molecular weight marker, proteins were located between 65 k Da, 55 k Da and 28 k Da. According to Acris Antibody (2010), the e CG laboratory calibration results in the following specification it, that it had molecular weight of 53 k Da analysis by RP-HPLC, UV spectroscopy at 280 nm. Lyophilized e CG is only stable at room temperature for 3 weeks, so it should be stored in the refrigerator at 2-8°C. Pregnant horse serum content of 46.7% has an e CG and is rich in sialic acid molecules (13.5%) [8].

Early stage research was conducted to determine the characteristics or identity of e CG the existence of proteins based on molecular weight. The method used was the technique of SDS-PAGE 12% to determine the difference in the location of bands in the gel compared with marker proteins. The precise molecular weight can be calculated with marker proteins ranging from 65 k Da, 55 k Da and 28 k Da protein fraction profile shown in 3 columns namely columns M which is a marker. The molecular weight was obtained by calculating the linear regression between Rf values of marker proteins and sample results of running 12% SDS-PAGE. Calculations using linear regression would lead to the possibility of determining the relative differences in protein bands distance that has little difference with other studies, but the real question is the same protein bands [5].

e CG Measurement

This phase of the study was aimed to determine the content of glycoproteins, carbohydrates and protein isolates e CG. Having ascertained that the protein will be cut (elution) is the e CG through western blot test, the measurement of the levels of glycoproteins, carbohydrates and proteins was done by using the Glycoprotein Carbohydrate Estimation Kit 23260. The results obtained showed that the average value of the e CG protein absorbance absorbance of 0.2565 at the sample mean and sample 1.0,252 2.0,251 with concentration of 5.12501 mIU / ml. Using filtration sephadex G - 200 obtained concentration of 2,500 IU / ml using 4-12% SDS-PAGE (1 mg protein per lane). Major bands were observed at approximately 65 k Da and 28 k Da e CG [9].
Figure 1. Protein profiles by SDS-PAGE e CG (M = marker (k Da), columns 1-4 = blood sample)

Glycoprotein staining e CG research results with BM about 55 k Da. There are bands that are believed to be e CG molecule, then followed by Western blot method using a monoclonal antibody against PMSG (CSA Catalog 614 Bioreagen stress gene). The results confirmed that there is an e CG is the tape shows because the molecules were recognized by monoclonal antibodies as being against the e CG.

Concentration of e CG by ELISA

Production of anti- e CG done by induction research results. Immunogenit as evidence proved by counting OD (Optical Density) ELISA as in table 1.

Table 1. e CG concentration from analysis results with a mean absorbance of 0.957 at the sample 1.069 with concentration of 400 IU/ml

<table>
<thead>
<tr>
<th>Coating e CG</th>
<th>Titer Rabbit Antibodi e CG</th>
<th>Optical density OD</th>
<th>X Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 IU/ml</td>
<td>0.957 – 1.069</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>control neg</td>
<td>0.264</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control pos</td>
<td>0.719</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 IU/ml</td>
<td>0.546 – 0.639</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>control neg</td>
<td>0.255</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control pos</td>
<td>0.496</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of the study of various treatments and control group with intramuscular administration e CG injection of the Bali cow pregnancy [2,10]. Estrus synchronization aims to increase the number of cows in estrus at the same time which allows the simultaneous insemination. Observation of signs of estrus insemination is needed in determining the appropriate time so as to improve pregnancy.

Examination of Gestation Rate on Day 35 Post Insemination

The result in figure 2 shows an amniotic bag black, white fetus in the amniotic bag, enabling bone formation to be seen although still not perfect. Reproduction of cows is one of the biggest factors that affect the efficiency of the cow productivity is largely determined by the level of fertility, pregnancy and birth. Factors that limit a low pregnancy rate is low expression of estrus, which in turn resulted in low fertilization.

Examination of pregnancy with ultrasound method performed on day 35 post-cattles insemination. In group this study, the rate of pregnancy in the group (Control) Folligon Intervet Holland 600 IU, 5 pregnant (83.33%); in the group (P1) 600 IU local horse e CG, 5 pregnant (83.33%); in the group (P2) 300 IU local horse e CG, 5 pregnant (83.33%); the group (P3) 150 IU local horse, 5 pregnant 83.33%). Statistically by using the Chi-Square test there was no significant difference between treatment groups (p>0.05).

Figure 2. Ultrasound Images Gestation Age 35 Days
Table 2. Events of Pregnant and not-Pregnant due to e CG Administration from Local Horse

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pregnant</th>
<th>Not Pregnant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Folligon Intervet Holland 600 IU</td>
<td>5 (83.33%)</td>
<td>1</td>
<td>6 (100 %)</td>
</tr>
<tr>
<td>(P1) e CG local horse 600 IU</td>
<td>5 (83.33 %)</td>
<td>1</td>
<td>6 (100 %)</td>
</tr>
<tr>
<td>(P2) e CG local horse 300 IU</td>
<td>5 (83.33 %)</td>
<td>1</td>
<td>6 (100 %)</td>
</tr>
<tr>
<td>(P3) e CG local horse 150 IU</td>
<td>5 (83.33 %)</td>
<td>1</td>
<td>6 (100 %)</td>
</tr>
</tbody>
</table>

The table an overview of the positive results in the diagnosis of pregnancy with an ultrasound examination that showed an anechoic spherical formations on the dorsal side of the bladder.

CONCLUSION

SDS-PAGE results of the examination showed e CG molecular weights of 65 k Da, 55 k Da and 28 k Da. Western blot assay using a monoclonal antibody against e CG. The results are e CG glycoprotein molecule as recognized by the monoclonal antibody against e CG. With a molecular weight of 55 k Da, e CG concentration from analysis results with a mean absorbance of 0.957 at the sample 1.069 with concentration of 400 IU/ml.

The results of e CG from local horse are presented in frozen dry form. Biopotential between e CG® and e CG from local horse against e CG®. Folligon Intervert Holland with e CG from local horse against pregnancy were not significantly different.

REFERENCES