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Improvement of Pregnancy Rate in Bali Cows with the Combination of Equine Chorionic Gonadotropine (eCG) from Local Pregnant Mare with PGF2α

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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 PGF2α. 45 Bali cows were injected with 25 mg PGF2α twice 11 days apart and devided 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 AI was done and 60 days later the pregnancy 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.

Key words : Bali cow, eCG, PGF2α, Time of estrus, Pregnancy rate.

Bali cattle are native Indonesian of breed importance regarding its direct ancestry from Banteng (Purwantara *et al.*, 2011). However, their fertility and pregnancy rate is very low (Lindell, 2013). There is a need for the improvement of fertility and pregnancy rate through the use of a combination of eCG and PGF2α. The eCG can support the growth of follicle in ovary, such as FSH (Baruselli *et al.*,2003) and combination of eCG and PGF2α is useful for successful pregnancy rate (Dias *et al.*, 2009).

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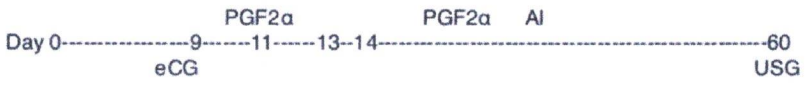

Fig 1. Time schedule of treatment and observation

Materials and Methods

The eCG was obtained from local pregnant mare at 50-90 days of gestation with charcoal (30 mg/100 mL) by chromatography Sephadex G100 column eluted with 0.05M NH4 HCO3. The eCG was evaluated for 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 horse radish peroxidase, Bioscience, San Diego, USA) (Hermadi *et al.*, 2018). 45 Bali cows were injected with 25 mg PGF2α twice 11 days apart and devided 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 Fig I. At the time of estrus AI was done and 60th day on the pregnancy rate was evaluated using two dimensional ultrasound (Samik and Safitri, 2019).

300ml Blood was taken from the jugular vein on the 50, 70 and 90th day of pregnancy. The plasma was separated and the phenol solution was been added and stored in the refrigerator until eCG extraction. The eCG purification techniques of sera include pH fractionation precipitation with metaphosphatic acid and absolute ethanol and dialysis by fixed chromatography.

Results and Discussion

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

After injected with 25 mg PGF2α twice on 11 days intervals and eCG treatment with second PGF2α, the results of the time of estrus and pregnancy verification after 60 days are presented in Table I and II.

The extract of eCG obtained from local pregnant mare sera was given to Bali cows at the same doses given to Bos indicus cows to improve the pregnancy rate Noqueira *et al.*, 2014 used hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates.

The utilization of eCG improved pregnancy rate of Bradford (26.7 - 34.6%), Nellore (38.9 - 45.7%) and crossbred Nellore cows (46.8 - 59.1%) with doses 400 IU eCG Bergamaschi *et al.* (2006 also concurred that the estrus of cows has improved by eCG.

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

Based on the Table I, there is no statistically significant difference in the rate of estrus of Bali cows either between the administration of patented eCG or pregnant mare sera and PGF2α (Control). Estrus synchronization in cows, facilitate simultaneous insemination in all the cows. Based on the findings the 400 IU eCG is recommended for the onset of estrus. The variation in the onset of estrus is most likely a reflection of differences in ovarian follicular growth phase so that during luteolysis

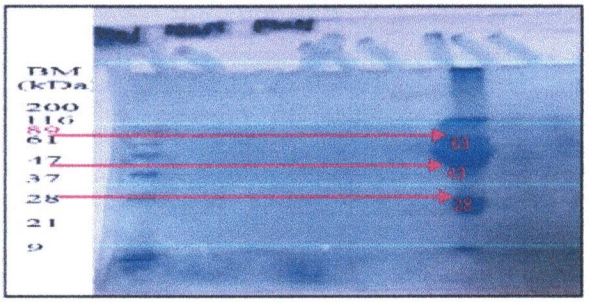

Fig 2. Molecular weight of eCG from local pregnant mare sera : 63, 43 and 28 kDa

Table I. Estrus interval (hours) after two Injections 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 PGF2α on the 60th days

Treatment	Pregnant	Not Pregnant
Twice injection of PGF2α without eCG (C)	8[a] (53.33%)	7[b](46. 66%)
Twice injections of PGF2α + patented eCG 400 IU from Folligon (T1)	13[b] (86.66 %)	2[a] (13. 33%)
Twice injections of PGF2α + 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 ($p < 0.05$)

after PGF2α injection resulting in ovulation at different times. Synchronization process using prostaglandin preparations (PGF2α) caused CL regression due to luteolysis.

Reproduction of cows is one of the major factors that affect the efficiency of the female productivity, which is determined by the level of fertility, pregnancy and calving (Martemucci and D'Alessandro, 2011). Factors that limit the expression of pregnancy rate is irregular estrus, which in turn results in low fertilization. The results of pregnancy examinations with ultrasound method on day 60 post-insemination presented in (Table II). The higher pregnancy rates in the T1 and T2 groups were due to the increased levels of progesterone. Low progesterone levels atributed to the early embryonic death. 5-10% of cattle in estrus could not ovulate, 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.

Ultrasound pregnancy examination 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 due to the low progesterone levels in the luteal phase which was 0.49 ± 0.41 mg/ mL reacting to 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 the use of a combination eCG from local pregnant mare and PGF2α was not significantly different ($p>0.05$) with a combination of patent eCG and PGF2 confirming equal efficacy of pregnant mare sera and the patented eCG.

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