ANTIBODY AGAINST PROGESTERONE IN LOCAL RABBIT FOLLOWING LOW DOSE OF PROGESTERONE INJECTION

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ABSTRACT

Antibody against progesterone was produced from serum of local rabbit following low dose of progesterone injection: While a control group (Control; n=5) was injected with Freund's adjuvant solution in aquadest, the treatment groups were either firstly injected with progesterone conjugated to Freund's Adjuvant (P-CFA, 150 p.l : 150 pl) or progesterone conjugated to Freund's Adjuvant and bovine serum albumin (P-CFA-BSA; 135 p;l : 150 tt1 : 15 gl). Twice boostering injections were administered using incomplete Freund's Adjuvant on day 14 and 52 after first immunization. Weekly bleeding for serum collection were done from 1 week following first booster immunization to week 10, Using ELISA technique it was shown that the antibody titer to progesterone after first and second booster immunization in the P-CFA groug was higher than Control- and P-CFA-BSA groups. The antibody titer in the P-CFA-BSA remained low similar in the Control group. ***(JIIPB Vol 21 No: 48-53)***

Keywords: antibody, progesterone, rabbit

INTRODUCTION

In the animal production, antibody anti progesterone is valuable as a tool for detecting the presence of progesterone in peripheral blood serum/plasma or milk sample of ruminant animal by technique as ELISA, RIA or dot blot throughout the time (Donaldson, et al., 1970; Ginther, et al. 1976; Eissa, et al. 1994; Strongeab, et al. 2005; Mann, 2009), in manner of early pregnancy diagnosis. In the modern laboratories of developed countries; researech and production antibody to progesterone either polyclonal - or ever monoclonal are routine procedure to perform (Baradaran et el. 2005: Dubreuil, et al. 2005). However, in most developing countries like Indonesia, those procedures are believed as high value technique due to the lack laboratory equipment or minimum found to conduct research.

This preliminary study was aimed to evaluate the antibody titer against progesterone in the local rabbit following immunization with progesterone conjugated to Freund's Adjuvant or with additional conjugation of bovine serum albumin. Final goal of this study is to perform a

simple kit diagnostic to determine parly pregnancy in beef or dairy cattle that could be practiced by small farmers in the field.

MATERIALS AND METHODS

Animals

The animal used in this study was male small local rabbits aged range from 3 – 4 months at the begining of study and weighed 1.5 - 3.0 kg. Genetically this animal is correctly unknown, persumably originated from brown Rex or The Netherlands brown dwarf rabbits. Animal was cared traditionally in single cage and fed with grass supplemented with low protein concentrates.

Animal treatments and blood samples collection

A total of 15 animals was divided into 3 groups at the same number. While animal in Group 1 were injected with 150 ul complete Freund's adjuvant (CFA) diluted in 150 ul aquadest (Control group), animal Group 2 and 3 were either immunized with 150 ul progesterone conjugated to 150 ul CFA (P-CFA Group) or with 135 ul progesterone + 150 ul CFA + 15 ul bovine serum albumin (P-CFA-BSA), respectively. For enhancing the immunological rensponse, booster with the same procedures above - instead CFA, incomplete Freund's adjuvant (IFA) was used - was performed twice on day 14 and day 52 after the first immunization.

Blood samples were collected weekly of about 1 - 3 ml of each commeced on day 14 after first booster injection and continued until bleeding-10, by fine needle from vena on the ear apex. Serum was isolated from other part of blood by elevately placing the tube containing sample for 3 - 4 h at temperature 5°C. Serum as supernatant was then isolated and purified to collect antibody and other protein molec~les with molecule size more than 20 nm using Selovan dialysis filter bag overnight in buffer solution: To the molecules remaining in the bag was then added absolute ethanol to precipate protein molecules. Antibody protein remained was removed and transfered to 1.5 ml eppedorft vials and freez stored until assay.

Antibody assay

Antibody titer anti progesterone was determined by indirect ELISA technique (Suyadi, 1999): Progesterone as antigen was diluted to make concentratiw on of 10 ul/ml in carbonate-bicarbonate solution (coating buffer). For coating plate well, progesterone solution was then transfered to well 50 ul/well, and incubated overnight at 4°C. The well was then washed with PBS Tween-20 (washing buffer) 4 times, and decanted at each washing: Blocking well was performed by incubating the well containing 50 ul blocking buffer each well for 2 h at room temperature; and then washed with washing buffer 4 times as above. Serum collected from animal was then pippeted into wells of microplate of 50 ul each well, and incubated overnight at 4°C to let conjugation reaction of antigen-antibody complex for sample serum containing antibody to progesterone and then the microplate was washed 4 times using washing buffer as procedure before. For secondary antibody, sheep-anti rabbit antibody (AP-IgG) labelled with alkaline phosphatase (AP) was used. This antibody was dissolved in PBS Tween 20 in dilution rate of 1 / 2500. The mixture was then transferred to

microplate wells containing antigen-antibody (primer) complex or 50 uUwell, incubated at room temperature for one hour. After incubation, the wells were washed with PBS Tween 4 times as before; and substrate of pNPP ((p-Nitrophenyl Phosphate, Disodium Salt, in 10% diethanolamine) 50 uUwell was added to develop the color. Incubation of this reaction should be run in dark condition. The reaction was stopped by adding 3.0 M NaOH of 50 uUwell. The antibody titer (optical density= OD) was determined by ELISA reader at wave lenght of 405 nm.

Data analysis

The basal level of antibody was defined as the lowest optical density (OD) in each of group. Quantitative data were analyzed by ANOVA using SPSS Version 11.1 (Microsoft®).

RESULTS AND DISCUSSION

Bleeding to collect serum sample was done to the all animals in the volume of 1 - 3 ml throughout 10 times collection at a week interval (Table 1).

Table l: Serum sample (ml) collected from rabbit in different groups throughout bleeding no 1 to 10

Bleeding No.	Control	P-CFA	P-CFA-BSA
1	1,40 ± 0,35	1,56± 0,91	1,20 ± 0,42
2	1,40 ± 0,35	1,08± 0,50	1,35 ± 0,57
3	1,50 ± 0,42	1,32± 0,27	0,90 ± 0,42
4	0,80 ± 0,35	1,20± 0,42	1,30 ± 0,14
5	0,80 ± 0,35	0,84± 0,33	1,35 ± 0,21
6	1,00 ± 0,35	0,64± 0,11	1,20 ± 0,14
7	1,00 ± 0,35	0,75± 0,30	1,20 ± 0,14
8	1,00 ± 0,35	0,84± 0,33	1,15 ± 0,07
9	0,90 ± 0,42	0,84± 0,33	0,65 ± 0,07
10	1,00 ± 0,35	1,20± 0,16	0,90 ± 0,42

Control: animal injected with Freund's adjuvant diluted in aquadest
P-CFA: animal group injected with progesterone conjugated to Freund's adjuvant P-CFA-BSA: animal group injected with progesterone conjugated to Freund's adjuvant and bovine serum albumin.

There was no significant difference in the volume between groups and between the courses. This was also shown at the volume of the purified antibody protein (Table 2).

Table 2. Antibody recovered by filtering with Selovan bag (ul) in defferent groups throughout the bleeding procedure.

Bleeding No.	Control	P-CFA	P-CFA-BSA
1	20 ± 2	22 ± 3	17 ± 4
2	22 ± 2	28 ± 3	18 ± 3
3	23 ± 3	16 ± 2	21 ± 2
4	12 ± 2	28 ± 4	23 ± 3
5	24 ± 3	24 ± 2	24 ± 3
6	23 ± 4	20 ± 2	24 ± 3
7	21 ± 5	21 ± 2	25 ± 2
8	16 ± 2	27 ± 4	26 ± 2
9	27 ± 3	28 ± 5	20 ± 2
10	21 ± 4	15 ± 4	15 ± 2

Table 3 showed that animals received immunological stimulation with progesterone conjugated to Freund's adjuvant (P-CFA) have higher response than in the groups of control and injected with progesterone conjugated to Freund's adjuvant and bovine serum albutmin (P-CFA-BSA): The slight rensponse of antibody anti progesterone production was observed after first booster injection of progesteron only for one week (21 d after first booster) and then declined dramatically to the basal level. The higher responses were observed after second booster injection starting day-7 until day-82 before declining to the basal level on day-89. Coupling reagent could effect on reactivity and sensitivity of progesterone to induce immune respons. Seeger et al (1979) reported that coupling of I1-a-OH *progesterone to --D*gadactosidase showed - lower sensitivity than those the use of lla-OH-progesterone-hemisuccinate-BSA as immunizing antigen. In contrast was found in this research that the presence of BSA did effect on the induction of antibody production, and even reduced the immune respons to progesterone injection: This may be caused the technique of conjugation between progesteron and BSA.

Other report showed that coupling of progesterone to BSA induced significant higher after booster injection. Repeating booster injection induced increase significantly anti-progesterone antibody in mice (Wang et al: 1991). In our study, additional BSA in conjugation progesterone-Freund's adjuvant was not effective to stimulate high response of antibody, probably due to low ineffective dose of progesterone in this group, compared to those reported by Wang et al. (1991) with ratio of 10:1 (progesterone : BSA).

Table 3: Antibody anti progesterone expressed in optical density (OD) at X=405 nm in defferent groups throughout the bleeding procedure.

Bleeding No.	Control	P=CFA	P=CFA=BSA
1	0,16 ± 0.06	0,119 ± 0.027	0,132 ± 0.002
2	0,13 ± 0,01	0,143 ± 0.009	0,130 ± 0.014
3	0,14 ± 0.01	0,192 ± 0.026	0,135 ± 0.007
4	0,12 ± 0.01	0,136 ± 0.006	0,125 ± 0.007
5	0,12 ± 0,01	0,124 ± 0.005	0,123 ± 0.019
6	0,14 ± 0.01	0,204 ± 0.020	0,158 ± 0.004
7	0,15 ± 0.01	0,221 ± 0.004	0,147 ± 0.006
8	0,15 ± -0.01	0,227 ± 0.013	0,144 ± 0.012
9	0,12 ± 0.01	0,203 ± 0.013	0,118 ± 0.008
10	0,11 ± 0.01	0,114 ± 0.003	0,107 ± 0.004

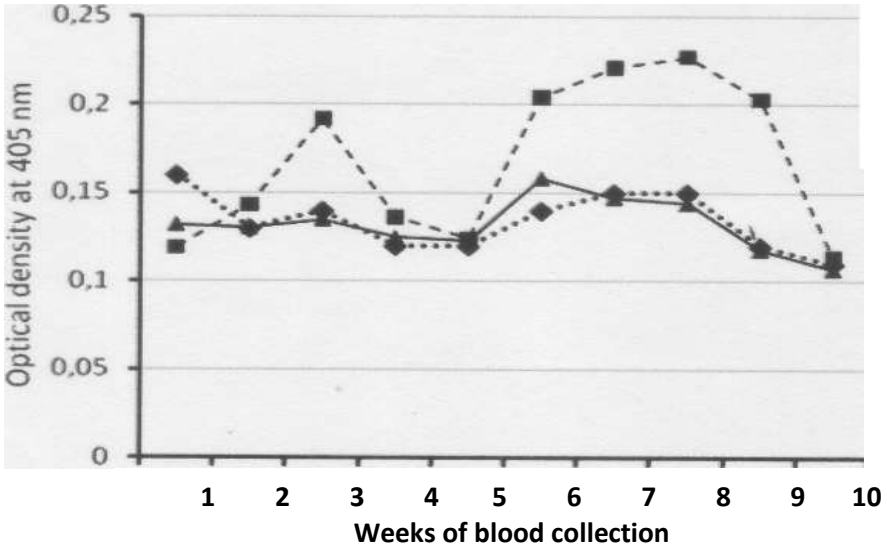

Figure l. Profile of antibody anti progesterone in Control, P-CFA and P-CFA-BSA groups. First booster injection was performed on day-0, and second booster was on day 35.

CONCLUSION

We concluded that conjugating of progesterone to Freud's adjuvant was more effective to stimulate the immune response to produce anti progesterone antibody than conjugated to Freund's adjuvant and BSA.

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