

## **PREGNANCY DETECTION OF GOAT USING PREGNANT SPECIFIC PROTEIN OF EARLY PREGNANCY FACTOR (EPF)**

*Abdul Samik*

Department of Veterinary Reproduction Faculty of Veterinary Medicine  
Airlangga University, Surabaya

### **ABSTRACT**

Early Pregnancy Factor (EPF) is a special antigen which can be found in the blood of pregnant goat starting at 7 day pregnancy. The objective of this research is to identify and determine concentration of Early Pregnancy Factor (EPF) of pregnant goat sera at 1-4 months of pregnancy. The result showed that the molecular weight of EPF between 43-67 kDa. While average protein of EPF pregnant goat at 1, 2, 3 and 4 months were  $612 \pm 721,75 \mu\text{g} / \text{ml}$ ,  $10208 \pm 879,61 \mu\text{g} / \text{ml}$ ,  $14976 \pm 1785,90 \mu\text{g} / \text{ml}$  and  $10848 \pm 996,758 \mu\text{g} / \text{ml}$ . This concentration of EPF can used for pregnancy detection in the goat.

*Keyword : Oestrus Synchronization, Artificial Insemination, Early Pregnancy Factor, Detect Pregnancy Detection.*

### **INTRODUCTION**

Growth of Population of livestock like ox, goat and sheep in Indonesia are not yet reached the circumstance seething with excitement even tend to downhill. This matter due to the number of protein request from flesh of ox livestock, goat and sheep increased but is not made balance to with the make-up of the livestock population.

East Java represent the province in Indonesia owning crosscut livestock with the highest population as well as representing biggest supply flesh requirement in national. Program the seed felt as very urgent requirement therefore pursuant to perception of livestock traffic especially livestock expenditure out big enough province so that felt concerned about to a period of/to to coming livestock population [in] East Java more and more to decrease. See the the condition or fact felt important to develop and improve the efficiency reproduce so that livestock population can be awaked. Constraint which often faced by the breeder concerning area reproduce, like length of calving interval and lower the pregnant level so that strive to reach the inaccessible high level reproductivity. Effort done by high goals to reproductivity can be reached by doing repair of reproduction management covering to detect the lechery and oestrus synchronization, correct fertilization and correct pregnant detection.

Early pregnant detection needed after the happening of fertilization to identify earlier so that time loss produce as effect of deductible infertility.

## **MATERIALS AND METHOD**

### **Oestrus Synchronization and Artificial Insemination of Goat**

As much 5 goat used in this research. Oestrus synchronization done by using 5 mg of PGF<sub>2</sub>α intra muscularly. Artificial insemination by using frozen cement after 12-18 hours sign of oestrus.

### **Collection of The EPF Sera from Pregnant Goat**

Blood taken away from pregnant goat by vena jugularis with the different pregnant age ( 1-4 month), then accomodated in tube react and closed. Tube keeled over 45° and hushed during 24 hours at room temperature. Later;then centrifugated with the speed 3000 rpm during 10 minute. Supernatan taken by disposable syringe and packed into tube.

### **Identification of EPF Protein by SDS-PAGE**

Put into gel running into appliance of SDS-PAGE through wall about less than boundary. Adding butanol about 1 ml and let during 25 minute. Butanol thrown after congelation gel and cleaned with PBS and dried with Whatman paper. Hereinafter added by gel stacking pass wall until full and is afterwards comb and awaited until really set ( 25 minute). Hereinafter comb taken and clean gel pickings with buffer e. Sample ( pregnant goat sera) counted 15 µl and mixed with 5 µl buffer laimli and heated 100 °C during 5 minutes. 10 µl sampel to printing;mould hole with tip 200 µl. Printing;Mould to appliance of biorad, supply power starter with strength 125 V, 40 mA during 1 hour. If gel reaction have until under, then power puss off and plate opened and dissociated, hereinafter cleaned with the buffer and the result coloration with silver or direct transferred to membrane of nitoselulose.

### **Spesifisity Test of EPF Protein by Western Blot**

Western Blot done by using ribbon fragment of EPF goat which have run in SDS-PAGE and transferred at membrane of nitroselulose. Membrane block by 3 % BSA in 20 mM Tris-HCl pH 7,5 and 150 mM NaCl during one hour, hereinafter incubated in Tris / NaCl concidering 1 % BSA with anti-EPF as primary antibody. ;Then cleaned in Tris-Cl concidering 0,05 % Tween 20. Hereinafter membrane incubated with second antibody ( IgG lable anti-rabbit of AP, thinning 1 : 1000) and added by blue western substrat.

### **Isolation of EPF Protein by Electroelution**

Gel of SDS-PAGE which do not colour to be cut as long as ribbon desired. Each gel cutting packed into nylon sack. Then packed into glass block concidering PBS and continued with sterer during 24 hour, each;every 6 hour done by replacement of PBS. To know that protein have experienced of elution hence gel cutting coloured with colour of silver, if do not there are ribbon mean protein have caressed. Afterwards to test Biuret to know concentrate of protein.

### **Inspection Concentration of EPF by Biuret Method**

Concentration of protein determined to use biuret reagent with addition of protein standard condensation ( BSA). Drawn up by three spectrophotometer kuvet, said the word first kuvet S as sample kuvet to be measured. In S kuvet by 0,05 ml EPF1 and

2,5 ml of biuret reagent. Second kuvet of ST as standard kuvet, by 0,05 ml protein standard condensation and 2,5 ml of biuret reagent. Third kuvet of BL ( blanko) by 2,5 ml reagent of biuret and 0,05 ml aquadest. Third of the kuvet hushed during 30 minute and then read at spectrophotometer of Bausch-Lombs spektronic 20 with wavelength 540 nm.

Calculation : Total concentrate of protein (  $\mu\text{g} / \text{ml}$  ) =>  $Y = 5.10^{-5}X$

Description : Y = Value Absorbance

X = Concentrate of Protein (  $\mu\text{g} / \text{ml}$  )

## RESULTS AND DISCUSSION

Protein ribbons from pregnant goat blood sera inspection with SDS-PAGE after compared to protein of marker there is four protein ribbon with MW between 42,7-66,4 kDa. the Protein ribbons according to molecule weighing of EPF found by Atkinson et al. ( 1993) between 43-75 kDa. While Xie et al. ( 1996) finding molecule weight of EPF goat between 47-90 kDa. Karen et al. ( 2003) and Garbayo et al. ( 1998) reporting the invention of that MW EPF goat there is three that is 55 kDa, 59 kDa and 67 kDa. El Amiri et al. ( 2004) immunoreactive analyse with SDS-PAGE and find MW of EPF goat between 55-66 kDa. This Protein molecule and other protein separated and is hereinafter by inspection of the protein concentrate by biuret methode.

Result of Western Blot of pregnant goat blood sera can be seen at figure below this :

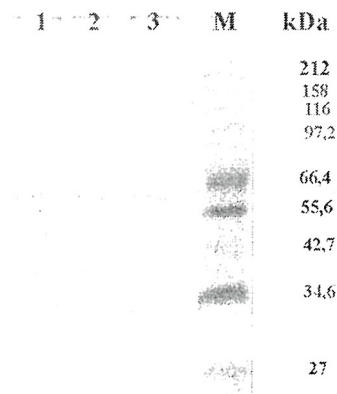


Fig 1. Protein ribbons of pregnant goat blood sera

Table 1. Average of concentrate of EPF protein of pregnant goat blood sera

No.	Age of Pregnancy (month)	N	Average ( $\mu\text{g}/\text{ml}$ )
1	1	5	<sup>a</sup> 612 $\pm$ 721,75
2	2	5	<sup>b</sup> 10208 $\pm$ 879,61
3	3	5	<sup>c</sup> 14976 $\pm$ 1785,90
4	4	5	<sup>b</sup> 10848 $\pm$ 996,758

<sup>a,b,c</sup>) Different superscripts indicate differences ( $p < 0.05$ )

### **Inspection Concentration of EPF with Method of Biuret**

Result of inspection of EPF protein by biuret method can be seen at tables below this Pursuant to statistical analyse of ANOVA there are difference of protein concentrate content of EPF blood sera according to age of pregnant goat. Protein concentrate of EPF progressively increased at pregnant 1-3 months and start downhill at 4 months pregnant. This matter is matching with the one which told by Cavanagh (1996) that EPF is first time found as substance pregnancy-associated and can detect at 6-24 hour after fertilization at all of specieses like mice, human being, pig and sheep. Duplants ( 2000) saying that EPF found after process of implantation and remain in pregnant goat blood to the last and disappear before parturition.

Statistically protein concentrate of EPF at 2-4 months pregnancy not differ reality but if between 1 months pregnancy with 2 and 4 months pregnancy there is a marked difference. And between 1 months pregnancy with 3 months pregnancy there are a marked difference. Gonzales et al. (2000) expressing that concentration of EPF will continue to go up reach maximal at 8 week pregnancy and start downhill 12-14 week pregnancy, then the concentration of constant until birth.

Difference of concentration of EPF protein influenced by some factor that is offspring amounts and weight born (Vandaele et al., 2003). Concentration of EPF more found at pregnant goats with offspring amount more than only one. Breed and total of weight born larger ones have positive correlation to concentration of EPF than totally heavy born lowerly. While mains and borne offspring gender do not have an effect on to concentration of EPF (Vandaele et al., 2005).

### **CONCLUSION**

Pursuant to obtained result can be concluded that Early Pregnancy Factor ( EPF) can detect pregnant goat at 1-4 month of pregnancy..

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