

PROFILE OF BLASTOCYST PROTEIN : PREGNANCY-ASSOCIATED GLYCOPROTEIN (PAG) AS AN INDICATOR OF PREGNANCY IN LIVESTOCK

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The research on Pregnancy-Associated Glycoprotein (PAG), a blastocyst protein isolated from pregnant cow serum was conducted to find out its characterisation. The aim of the research was to study the PAG profile as an indicator of pregnancy in dairy cattle. PAG protein was isolated from serum from cows during 274-279 days of gestation, prior to deliver through an isolation and characterisation technique such as SDS-PAGE, Western Blot, Electroelusion and ELISA. The results of research show that PAG isolated from pregnant dairy cows in East Java had molecular weight of 67.34 kDa.

Key words : Pregnant Cow Serum, Blastocyst Protein, PAG and Characterization

INTRODUCTION

Early pregnancy detection based on immunological technique can be done by measuring blastocyst protein which is exist in maternal blood circulation. Several protein-like substance have been identified in maternal blood circulation during pregnancy. This substance is produced by conceptus and could be used as an indicator of pregnancy (Jainudeen dan Hafez, 2000).

Protein produced by blastocyst which also specific exist in maternal blood circulation during early pregnancy is pregnancy-associated glycoprotein (PAG). PAG first described as placenta antigen of cow, in maternal blood circulation after implantation (Green, *et al*, 1998). Zoli *et al*, (1991) have been purified PAG from specific antigen named pregnancy-associated glycoprotein (PAG). PAG is synthesized from mono and binucleat cells of ruminants trophoblast. Most of PAG is secreted into maternal blood circulation at the time of blastocyst attached to the uterin wall and started to create placentom (Wooding, 1992). PAG can be assayed by RIA or ELISA.

Garbayo, *et al*. (1998) reported that PAG is an immunogenic antigen detected in pregnant goat serum. This PAG is isolated

from placenta. There are three types of PAG which are differed by their amino acid sequences and molecular weight i.e. 62; 59 and 55 kDa. According to Green, *et al*. (2000), the existancy of bovine PAG can be used as pregnancy diagnose and this method mostly used to detect early pregnancy in dairy cow, under one month pregnancy. This research is aimed to isolate PAG from pregnant cow and learned its characterization.

MATERIAL AND METHOD

This research used serum from cows during 274-279 days of gestation, prior to deliver. As a control using serum from non pregnant cow. Method of research consisted of isolation, purification, and characterisation of PAG. Monoclonal Antibodi was Anti bovine PAG US Biological Cat. P2008-02 as a standard.

Blood were collected from jugular vein of six pregnant cows. After centrifused 1500 rpm, supernatant was collected as serum. Serum were run by SDS PAGE to get their protein bands. Molecular weight of PAG was define by calculate its Retardation Factor (Rf) (Sumitro, *et al*.,1998). Protein standard curve was made in order to get sample relatively molecular

mass. Relative molecular mass of each protein was defined by converted data of Rf value in accordance with simple linear equation $Y = b_0 + b_1X$ (Gaspersz, 1995).

Gel from SDS PAGE will be confirmed as PAG through Western Blot test. Several band which were exist then to be transferred to nitrocellulose membrane, added by Anti bovine PAG US Biological Cat. P2008-02. This technique recognised PAG molecule specifically.

RESULTS AND DISCUSSIONS

Protein profile of PAG isolate derived from pregnant cow serum by SDS PAGE is showed in **Figure 1**. There were several band of protein from six samples in **Table 1**.

Table 1 showed control and pregnant cow serum have the same band protein profile. In pregnant cow serum there was a protein with molecular weight of 67.34 kDa. It was supposed as specific protein PAG.

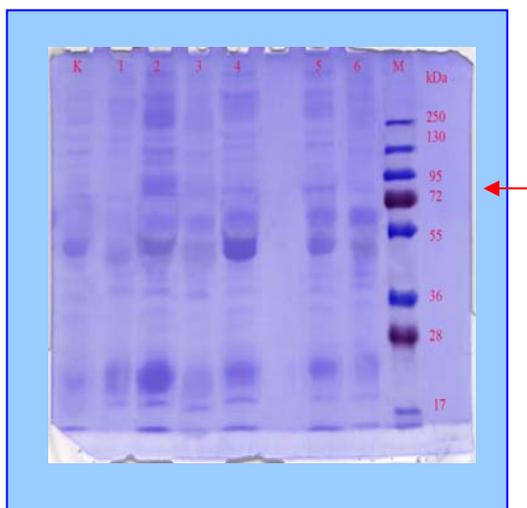


Figure 1. Protein band profile of isolate PAG from pregnant cow serum (←)

M : Protein Marker

K : Control

1 - 6 : Sample of pregnant cow serum

Table 1. Molecular Weight of Protein Sample

Serum Sample	Protein Molecular Weight (kDa)											
	229.08	177.01	136.45	122.74	105.19	89.94	76.91	67.34	48.19	43.45	28.64	18.92
Control	√	√	√	√	√	√	√	-	√	√	√	√
Pregnant	√	√	√	√	√	√	√	√	√	√	√	√

This research obtained PAG with molecular weight of 67.34 kDa. This PAG was in accordance with Green, *et al*, (2000); Zoli, *et al*, (1991); Xie *et al*, (1994), who found PAG with molecular weight of 67 kDa. According to Kiewisz, *et al*, (2006) PAG has variety of molecular weight between 54 to 70 kDa. PAG is an acid glycoprotein (pH 4.4 – 5.4). This protein could be detected in maternal blood circulation at the time of embryo implantation (Gordon, 2004).

Specificity test was done to ensure that the mentioned protein was PAG. The test was Western Blot. Result of Western Blot test is showed in **Figure 2**.

Figure 2. showed that band of 67.34 kDa is PAG molecule which has already confirmed by standard antibody Mab-bPAG (Anti bovine PAG US Biological Cat P2008-02). Visualization process using Western Blot resulted only one band of protein, while the others were not exist. This proved that the bands exist are PAG molecule.

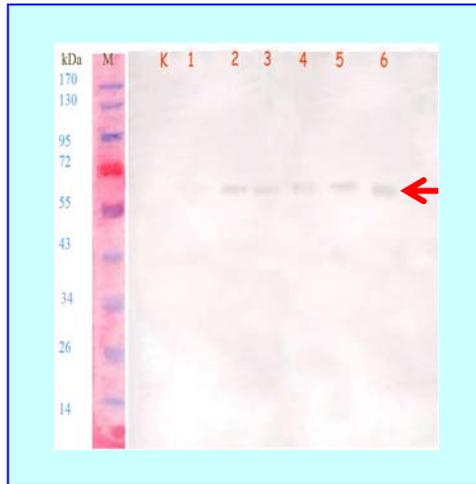


Figure 2. Western Blot Test. PAG isolated from Cow Serum Was recognised by Mab-bPAG (Anti bovine PAG US Biological Cat P2008-02)

Keterangan : M : Protein Marker
 K : Control
 → : Molecule PAG
 1 - 6 : Sample of pregnant cow serum

CONCLUSIONS

Specific protein in cow serum of 274-279 days of gestation, prior to deliver, has characterization as pergnancy-associated glycoprotein (PAG). PAG isolated from pregnant cow serum has molecular weight of 67.34 kDa.

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REFERENCES

- [1] Garbayo, J.M.; B.Remy; J.L. Alabart; J. Folch; R. Wattiez; P. Falmagne and J.F. Beckers. 1998. *Isolation and Partial Characterization of a Pregnancy-Associated Glycoprotein Family from The Goat Placenta*. Biol. Reprod. 1998 Jan; 58(1):109 -15.
- [2] Gordon, I. 2004. *Reproductive Technologies in Farm Animals*. CABI Publishing. Cambridge. USA
- [3] Green, J.A.; S. Xie; X. Quan; B. Bao ; X. Gan; N. Mathialagan; J.F. Beckers and R.M. Roberts. 2000. *Pregnancy-Associated Bovine and Ovine Glycoprotein Exhibit Spatially and Temporally Distinct Expression Patterns During Pregnancy*. Biology of Reproduction 62, 1624 – 1631.
- [4] Jainudeen, M.R. and E.S.E. Hafez. 2000. *Pregnancy Diagnosis*, dalam Hafez, E.S.E and B. Hafez. 2000. *Reproduction in Farm Animals*. 7^{ed}. Lippincott Williams & Wilkins. Philadelphia.
- [5] Kiewisz, J.; N.M. Sousa; J.F. Beckers; H.Vervaecke; G. Panasiewicz; B.Szafranska. 2006. *Isolation of pregnancy-associated glycoproteins from placenta of American Bison (Bison bison) at first half of pregnancy*. *Reprod Fertil Dev* (CSIRO Publishing, Australia) 2006; submitted.
- [6] Sumitro, B.S.; S.Rahayu; Fatchiyah dan S. Widyarti. 1998. *Materi Kursus Teknik-teknik Dasar Analisa Protein dan DNA*. Jurusan Biologi, FMIPA. UNIBRAW. Malang.
- [7] Xie, S. ; B.G.Lom; R. J. Nagel; J.F.Beckers and R.M.Roberts.1994. *A novel glycoprotein of the aspartic proteinase gene family expressed in bovine placental tropectoderm*.Biology of Reproduction, 1994, 51:1145-1153.
- [8] Zoli^a, A.P; J.F. Beckers and F. Ectors. 1990. *Evidence of a protein immunologically related to pregnant specific protein in ruminant gonads*. 3th International Ruminant Reproduction Symposium (Nice) 1990 ; 34.
- [9] Zoli^b, A.P.; F. Ectors and J.F. Beckers.1990. *Ruminant gonads as accessory source of Pregnant specific protein*. 2nd Annual Meeting of the Endocrine Society (Atlanta) 1990; 118.
- [10] Zoli, A.P; J.F. Beckers; P. Wouters-Ballman; J. Closset; P. Falmagne and F. Ectors.1991. *Purification and Characterization of A Bovine Pregnancy – Associated Glycoprotein*. Biology of Reproduction, 1991, 45(1): 1-10.