APLICATION OF ANTI PREGNANCY-ASSOCIATED GLYCOPROTEIN (ANTI-PAG) AS A MOLECULAR MARKER OF EARLY PREGNANCY DETECTION IN DAIRY HEIFER

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Abstract

Research on Aplication of Anti Pregnancy-Associated Glycoprotein (Anti-PAG) as a molecular marker of early pregnancy detection in dairy heifer was conducted to support the dairy farmer in order to achieve efficiency from their heifer reproduction. The goal of research was to proove that anti-PAG could recognize antigen PAG in early pregnant dairy serum. Specificity test was done qualitatively by Dot Blot method. The collected data were visualized with purple color which derived from specific reaction between antigen (PAG) and antibody (anti-PAG). Expression of color was converted to numbers by NIH Image software, Version 1.62. Numeric average represent result of Dot Blot reaction. Collected data were statistically analysed by Nested Completely Random Design software. Result showed Anti-PAG recognition on antigen PAG in pregnant dairy heifer blood serum, from the 15th day of pregnancy. Consequently, Anti-PAG can be used as a molecular marker of an early pregnancy detection in dairy heifer.

Key words : Anti-PAG, Dot Blot, early pregnancy, detection, dairy heifer

INTRODUCTION

Early pregnancy detection in dairy farm is one of important keys to achieve a success breeding program. In a dairy farm, replacement of heifer should be 25 % per year [3] and it is costly. Therefore it is important to control fertility of the heifers as soon as possible. To achieve that, it is required a technique of an accurate and early detection of pregnancy for the heifers soon after they are mated, in order to get an efficient replacement program.

An ideal pregnancy diagnosis is sencitive, specific, cheap, simple in field application and precise to define pregnancy at the time of examine. Development of early and accurate pregnancy detection method is still in progress. Pregnancy detection based on immuology can be done by measuring specific protein of blastocyst using an anti of its protein itself.

Pregnancy-Associated Glycoprotein (PAG) as a specific protein of blastocyst is a glycoprotein substance produced by trophoblastic cells of blastocyst prior to implantation [11]. By radio immuno assay (RIA), the existence of PAG can be detected on day 22 after inseminated [12]; [7]. PAG is an immunogenic protein and has 67 kDa of molecular weight [11]; [12]; [6]; [8]. All immunogenic protein and have molecular weight over 10,000 Dalton, if they are injected into animal laboratories will be stimulate an antibody, anti of the protein themselves [4];[1]. Anti-PAG has produced from immunization on rabbit and this anti-PAG further can be used as a molecular detector of early pregnancy in dairy heifer.

MATERIAL AND METHOD

The research was a reaction test of anti-PAG yielded from imunisation of rabbit to the standard antigen (bovine PAG MDBiomed Cat. 101-7963-13-3) and to heifer serum of 15,20,25 and 30 days of pregnancy. Anti-PAG was produced in previous research. It has specifisity tested by Western Blot. Obtained Anti-PAG was 1.192 µg and has titer of 1.044 [9]. Further test used Dot Blot method to detect the existency of antigen (PAG) in the serum. Dot Blot test was started by compose Dot Blotter membrane.

Statistic Research method was experimentally using Nested Completely Random Design. Treatments were anti-PAG dilution i.e. : P1 : 1/100 : P2 : 1/200 and P3 :

1/400. Samples were pregnant heifers serum of 15, 20,25 1nd 30 days of gestation, each derived from four heifers. Each heifer was collected six serum samples as replication. Mathematically, equition model is $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \epsilon_{k(ij)}$.

RESULTS AND DISCUSSIONS

The research was aimed to proove that anti-PAG could recognise protein PAG within pregnant heifer serum from 15, 20, 25 and 30 days of gestation. Specifisity test was done qualitatively by Dot Blot method. Datas collected were visualized by blue- purple colour as shows in Figure 1.

Figure 1. Blue-purple color of Dot

Figure 1 shows the representatives of color derived from Dot Blot Test. The dots come from 288 samples. The first four represented rection of anti-PAG and antigen PAG within 15 day of gestation, followed by reaction of 20, 25 and 30 days gestation serum. The dot color of 1:100 dilution Anti-PAG were thicker than 1:200. Dilution of 1:200 were thicker than 1:400. This meant that smaller dilution of Anti-PAG detected antigen PAG more obvious or more clear. Color expression was converted into numbers through software of NIH Image Version 1.62. Average of numbers obtained from Dot Blot reaction test is showed in Table 1.

Tabel 1. Average Numbers of Specific Reaction between Antigen PAG and Antibodi Anti-PAG from pregnant heifer serum

Dilution Anti-PAG	Serum Sample	Gestation (day)			
		15	20	25	30
P1	1	31,63	45,25	86,48	154,23
	2	33,00	46,89	87,92	152,93
	3	32,62	43,39	87,41	152,82
	4	35,63	43,21	89,29	149,97
P2	1	22,34	38,96	55,91	119,89
	2	27,56	33,79	57,51	116,59
	3	25,10	39,13	59,15	123,32
	4	26,27	40,04	62,06	121,36
P3	1	19,90	29,03	44,46	115,71
	2	21,36	32,18	56,56	114,60
	3	20,19	32,18	51,12	118,55
	4	24,06	30,99	56,04	118,23

Remark : Pregnant Heifers Serum of 15,20,25 and 30 days of gestation
P1 : Dilution Anti-PAG 1 : 100
P2 : Dilution Anti-PAG 1 : 200
P3 : Dilution Anti-PAG 1 : 400

Stastistically analysis of Nested Random Design shows there is signifant diffrent between dilutions. To know further about the differences, it was followed by Duncan test. The result is showed in Table 2.

Table 2. Duncan Test of Effect on Gestation Day to the Dilution of Anti-PAG Toward Reaction of Antigen (PAG) and Antibody (Anti-PAG)

Dilution Anti-PAG	Gestation (day)			
	15	20	25	30
P1	$33,28^{a}$	$44,68^{b}$	$87,78^{c}$	$152,49^{d}$
P2	$25,32^{a}$	$37,98^{b}$	$58,66^{c}$	$120,29^{d}$
P3	$21,38^{a}$	$31,09^{b}$	$52,15^{c}$	$116,77^{d}$

Remark: Superscript letters to the coulomb direction show highly significant different (P>0.01)

Based on Duncan test result, serum pregnant heifer of 15, 20, 25 and 30 day of gestation in Anti-PAG dilution dose of 1/100, 1/200 dan 1/400 show high significant different. The specificity test between anti-PAG and PAG standard through Dot Blot showes homolog nucleutide and high amino acid due to covalen tight between them. Antibody has ability to recognize special antigen [10]. This means Anti-PAG recognized antigen PAG.

After fertilization, prior day seven, zona pellucida surround balstocyst has hatched, therefore trophoblast cells directly contact to uterin epithel. Trophoblast cells develop rapidly construct folding trophoblast wall (Arthur, et al, 1989). This fact is also supported by the existency of Glycocilated Cell Adhession Molecule-1 (Gly-CAM-1) secreted by endothelium, which has function of process mediation adhesion of leucocyte endothel cell in order interaction between conceptus-maternal during pre-implantation. This occurred on day 11 – 15 after conception [5]. In addition, protein PAG as production of blastocyst, has high affinity, works with paracrine system, diffuse directly ino endometrium and enter maternal blood circulation. Consequently, PAG wich is produced by trophoblast cells can be recognized by Anti-PAG in maternal serum from day 15 of gestation.

Attachment of mammals embryo to uterin wall involves proliferation, differentiation and migration of both embryo cells and uterin. Prior to attach to the endometrium, ruminant embryo flying freely within uterin for several days. In cow attachment occured in dy 11 to 24 after fertilization [2]. Consequantly, antigen PAG can be recognized by Anti-PAG in pregnant serum from 15 to 30 days of gestation

CONCLUSIONS

1. Anti-PAG can be used as an early pregnancy detection in dairy heifer.
2. Anti-PAG recognized antigen PAG in pregnant heifer serum from15 days of gestation.

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