



## ANTI-HCG PRODUCTION IN RABBIT WHICH IS CONFIRMED BY RADIAL IMMUNODIFFUSION TECHNIQUE

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### ABSTRACT

The presence of human chorionic gonadotropin (HCG) was defined as marker of pregnancy. Various methods have already been known in HCG detection such as latex agglutination and gold immune chromatography as well as radial immunodiffusion. HCG can be isolated from urine of pregnant woman therefore can function as detection material in confirmation of anti-HCG production.

The aim of this study was to confirm the generation of anti-HCG by immunized rabbit (*Oryctolagus cuniculus*) which was confirmed by radial immunodiffusion technique.

This experimental study use rabbit strain white new Zealand as animal model which was primarily subcutaneous immunized by HCG followed by secondary after four weeks and tertiary at sixth weeks. Blood collection was before immunization and respectively of each two weeks. Detection of antibody titer using radial immunodiffusion technique was performed to serum from week 2, 4, 6, 8 and 12 after immunization.

The results show that there is no anti-HCG can be detected from pre-immune serum and the presence of anti-HCG was confirmed by radial immunodiffusion technique two weeks after immunization with various titers. The anti-HCG titer was 1/8 from week 2 and 4 serum, while on week 6, 8, and 12 the titer of anti-HCG were respectively 1/16, 1/32 and 1/64.

As results, we conclude that generation of anti-HCG by immunized rabbit of white new Zealand strain was confirmed by radial immunodiffusion technique since two weeks after immunization.

**Keywords:** pregnancy, anti-HCG, radial immunodiffusion, antibody production.

### INTRODUCTION

Human chorionic gonadotropin (HCG) is generated by placenta at early stage of pregnancy. The presence of HCG in urine can be detected by various methods including immunologically based techniques which depend on antigen-antibody biochemical interaction and well known as immunoassay. In case of pregnancy test, antibody to HCG widely used as capture material on many detection systems as well as other molecules that function as indicator of immune complex formation including latex and gold colloidal.

Radial immunodiffusion is one of Immunoassay techniques to confirm immune complex formation based on migration of molecule in semisolid medium such as agarose gel where antigen and antibody molecules diffuse toward each other and form precipitation line at equilibrium point.

Anti- HCG production on rabbit had previously conducted by Lisawati (1992) by intravenous injection of HCG and was successful confirmed by agglutination technique as well as resulted in specific reaction for pregnancy detection. This study was aimed to generate anti- HCG in rabbit subcutaneously injected by HCG with confirmation by radial immunodiffusion technique.

### METHODS

The study was conducted at Immunology Laboratory of School of Health Sciences Jenderal Achmad Yani Cimahi.

**The pre-immune serum detection prior to immunization.** Blood collection was performed to the rabbit prior to the primary immunization and followed by radial immunodiffusion technique analysis to confirm the presence of anti-HCG in rabbit serum.

**The rabbit immunization.** Primary immunization was conducted by subcutaneous injection of 0.3 mL HCG at three dorsal regions of rabbit and then was continued by secondary immunization after four weeks as well as tertiary immunization after six weeks. Antibody generation was evaluated by radial immunodiffusion technique for every week with exception on serum from eighth and twelfth week.

**The radial immunodiffusion technique analysis.** HCG-containing urine which collected from pregnant woman was added to 1% agarose and poured into glass object to form gel layer with several wells (Darmawi, 2010). Collected serum was then filled into the well of agarose gel and followed by incubation for 24 hours at refrigerator in order to form precipitation line (Rantam, 1998).

**Titration of rabbit anti-HCG sera.** Serial dilution of collected serum from immunized rabbit was performed for week 2, 4, 8 and 12 after immunization. Each of the samples was then analysed by radial immunodiffusion technique as above.

## RESULTS AND DISCUSSION

### Rabbit pre-immune sera analysis:

Radial immunodiffusion technique analysis on rabbit pre-immune sera can be seen in figure 1 as follow:



Figure 1. Legend: No precipitation line was formed after 24 hours indicated no exposure of HCG to the rabbit before immunization.

### Antibody generation:

Anti-HCG generation by immunized rabbit had been confirmed at second week and indicated by precipitation line around the well of agarose gel as follow:



Figure 2. Legend: precipitation line which indicated by red arrow arise almost in every well of agarose gel except in pre-immune and first week serum.



Figure 3. Legend: The highest concentration of anti-HCG was achieved at twelfth week (red arrow).

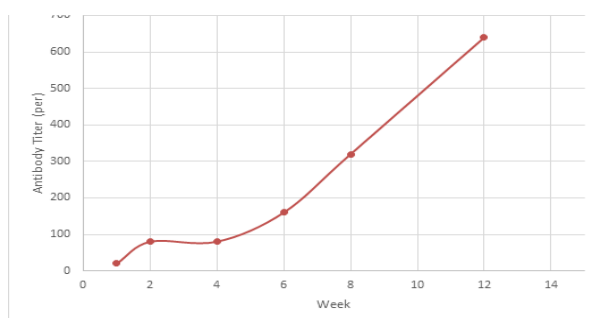


Figure 4. Legend: The graphic shows an increase of antibody generation of each week and indicates the function of adaptive immune response in anti-HCG generation.

Radial immunodiffusion technique is an antigen-antibody reaction that result in precipitation and involves several factors including concentration, pH and temperature. For instance, our results indicated that previously no ring of precipitation was formed when we use 0.5% of HCG (pure HCG) in contrast with 5% of HCG (HCG containing urine) thus confirm that interaction of antigen-antibody

can be seen as a complex of precipitation in equal proportions (Haryadi, 2003).

The presence of anti-HCG in this study was detected by the technique at second week after immunization with the highest serum dilution at 1/8. Corresponds to Clancy (1998) that antigen administration for the first time will induce antibody response in serum at measurable level after approximately six to twelve days. Lisawati (1992) also detected anti-HCG by agglutination method at second week with the highest serum dilution at 1/128, in case of no adjuvant combination to the antigen our level of concentration was lower than that Lisawati had. This difference corresponds to the function of adjuvant as an additional substance can evoke and modulate the immune response thus very useful to increase and stimulate antibody generation (Hau and Van Hoosier, 2003).

The dynamic and development of immune response against antigen in mammalia takes approximately 10-17 days for lymphocytes to arrange adequate and maximum responses where the B cells differentiate into plasma cells and memory cells. This primary immune responses will develop faster and stronger when the host get secondary antigen administration.

The same titer of antibody was achieved at second and fourth week namely 1/8 where the host still in primary immune response in contrast to sixth and eighth week namely 1/16 and 1/32 indicated that higher titer of antibody was detected after antigen booster administration. The highest titer of antibody in this study was achieved at 1/64 of twelfth week immunization and indicated that the host actively generate antibody response during the time of experiment.

This corresponds to the function of antigen booster administration in stimulation of memory cells as Clancy (1998) reported that at first to second week after primary immunization the IgG level

began to arise and will accelerate to increase the level after four weeks after antigen booster administration. We found that anti-HCG level was higher after second booster and indicated that the host develop stronger immune response. As results, finally the host then will have high antibody level for several months and maintain high affinity to the antigen (Agoes, Wurlina and Hariadi, 2009).

## CONCLUSION

Anti-HCG production in rabbit of New Zealand White (*Oryctolagus cuniculus*) was successfully confirmed and visually detected by radial immunodiffusion technique since second week of immunization.

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