# RINGKASAN

## IDENTIFIKASI, ISOLASI DAN KARAKTERISASI RESEPTOR FERTILISASI (ZONA PELUSIDA-3) KAMBING SEBAGAI KANDIDAT BAHAN IMUNOKONTRASEPSI

### Penelitian Eksploratif Laboratorik dan Fertilisasi *in vitro* pada Hewan Model

**Imam Mustofa**

Penelitian imunokontrasepsi telah dilakukan pada sejumlah spesies, namun belum pernah dilakukan pada kambing. Sebagaimana diketahui sampai saat ini kontrasepsi hormonal mempunyai efek samping. Penelitian pendahuluan menunjukkan bahwa *crude* zona pelusida kambing efektif menghambat kebuntingan pada mencit. Penelitian ini bertujuan untuk menemukan protein reseptor fertilisasi kambing (gZP3) dan uji potensi imunokontraseptifnya secara *in-vitro* pada pada mencit (*Mus musculus*) sebagai hewan model.

Pada penelitian pertama identifikasi dilakukan untuk mengetahui konstituen protein zona pelusida kambing, dilanjutkan dengan isolasi protein yang diduga sebagai reseptor fertilisasi, yaitu gZP3. Karakterisasi dimaksudkan untuk mengetahui massa molekul relatif (Mr), uji densitometri, uji imunogenisitas dan spesifisitas terhadap antibodi asal mencit (*Mus musculus*) dan kelinci (*Oryctolagus cuniculus*) serta uji imunofluoresen. Pada penelitian kedua dilakukan uji fertilisasi *in vitro*, untuk membuktikan bahwa isolat yang diperoleh benar-benar *sperm receptor* kambing. Isolat gZP3 disuplementasikan pada media kapasitasi spermatozoa kambing, sedangkan antibodi gZP3 disuplementasikan pada media maturasi oosit kambing, selanjutnya masing-masing secara terpisah dilakukan fertilisasi *in vitro*. Pada penelitian ketiga, antibodi gZP3 disuplementasikan dalam media untuk inkubasi oosit, selanjutnya dilakukan uji fertilisasi *in-vitro* dan uji *Binding (Binding assay)* pada oosit mencit.

Hasil penelitian pertama menunjukkan bahwa zona pelusida kambing terdiri dari tiga konstituen, yaitu gZP1, gZP2, dan gZP3 dengan massa molekul relatif (Mr) berturut-turut 120, 94, dan 82 kDa. Analisis Densitometri gel hasil SDS-Page menunjukkan bahwa masing-masing *band* gZP1, gZP2, dan gZP3

pada gel sesuai dengan puncak-puncak densitograf. Berdasarkan luas daerah di bawah kurva densitograf, kompoisisi gZP1, gZP2 dan gZP3 berturut-turut adalah 6,93 %, 29,60 % dan 63,47 %. Uji SDS-PAGE ulang dan analisis Densitometri menunjukkan bahwa protein yang diisolasi adalah benar dari *band* ketiga (gZP3) hasil SDS-PAGE protein zona pelusida kambing. Protein gZP3 bersifat imunogenik pada mencit (*Mus musculus*) betina dan kelinci (*Oryctolagus cuniculus*) jantan, menghasilkan titer antibodi ($p<0,05$) setelah imunisasi. Analisis *Dot blot* menunjukkan bahwa protein gZP3 dapat dikenali oleh antibodi gZP3 asal mencit betina dengan intensitas lebih tinggi dibandingkan antibodi gZP3 asal kelinci jantan. Analisis imunofluoresen menunjukkan bahwa antibodi gZP3 dapat mengenali homogenat zona pelusida kambing, dan protein gZP3 dapat mengenali membran plasma spermatozoa kambing.

Penelitian kedua membuktikan bahwa isolat protein gZP3 merupakan reseptor fertilisasi. Uji biologis dengan teknik fertilisasi *in-vitro* : antibodi gZP3 dalam media maturasi oosit kambing maupun protein gZP3 dalam media kapasitasi spermatozoa kambing menurunkan ($p<0,05$) angka *cleavage*.

Penelitian ketiga menunjukkan potensi imunokontraseptif protein gZP3 pada hewan coba model. Antibodi gZP3 menghambat ($p<0,05$) fertilisasi oosit mencit secara *in-vitro* dan menurunkan ($p<0,05$) *Binding Index* antara spermatozoa dengan oosit mencit (*Mus musculus*).

Berdasarkan hasil-hasil penelitian di atas, disimpulkan bahwa isolat gZP3 penelitan ini adalah protein reseptor fertilisasi pada zona pelusida kambing. Protein gZP3 tersebut efektif sebagai bahan imunokontrasepsi pada mencit (*Mus musculus*) sebagai hewan model. Penelitian lanjutan perlu dilakukan untuk menemukan peptida yan memiliki homologi sekuen asam amino gZP3 dengan ZP3 manusia (*Homo sapiens*), dan menguji potensi imunokontraseptifnya pada primata dengan pengamatan efek utama pada infertilitas serta, efek samping pada siklus menstruasi dan perubahan histologi ovariumnya. Perlu dilakukan penelitian lebih lanjut untuk pengujian peptida tersebut dengan teknik *Human sperm-oocyte binding assay* atau *Human Hemi zona assay*.

## SUMMARY

## IDENTIFICATION, ISOLATION AND CHARACTERIZATION OF FERTILIZATION RECEPTOR (ZONA PELUSIDA-3) OF GOAT AS CANDIDATE OF IMMUNOCONTRACEPTION SUBSTANCE

### Explorative Laboratoric Experiments and In-vitro Fertilization On Animal Model

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The researches of immunocontraception have done in several species, but haven't yet been done in goat. As well as known that hormonal contraception up to now is still having side effect. In preliminary study, crude of goat zona pellucida protein was effective prohibitted of graviditation of mice. The aim of this study was to find the fertilization receptor protein of goat (gZP3) and to test of its immunocontraceptive potential in-vitro on mice (Mus musculus) as animal model.

The first research was conducted to identify protein constituent of goat zona pellucida, before continuing with an isolation of protein wich was predicted as fertilization receptor protein i.e. ZP3. The characterization was aimed to identify relative molecular mass (Mr), gauging with densitometry, test of immunogenicity and specificity on mice (Mus musculus) and rabbit (Oryctolagus cuniculus) and test of immunofluorescence. The second research was in-vitro fertilization of goat, to prove that obtained isolate was representing receptor of sperm. Isolate of gZP3 was incubated on goat sperm, while antibody of gZP3 was incubated on goat oocyte, then each of them was in-vitro fertilized separately. The third research, antibody of gZP3 supplemented into M-16 media for oocyte incubation, continued with in-vitro fertilization assay and Binding Assay on mice (Mus musculus) as model.

Results of identification, isolation and characterization of fertilization receptor at goat zona pellucida (gZP), showed that zona pellucida of goat consisted of three constituents that were gZP1, gZP2, and gZP3 with relative molecular mass (Mr) were 120, 94, and 82 kDa respectively. Gel analysis result

of SDS-PAGE with densitometry indicated that each band of gZP1, gZP2, and gZP3 were in accordance with the tops of densitograph. Based on the wide of area under curve of densitograph, composition gZP1, gZP2 and gZP3 were 6.93 %, 29.60 % and 63.47 % respectively. The results of gel analysis by using SDS-PAGE and densitometry indicated that isolate protein was true from the third band (gZP3) of SDS-PAGE result of goat zona pellucida protein. Protein of gZP3 was immunogenic on female mice and male rabbit, it could produce antibody ($p<0,05$) after immunization. Result of dot blotting analysis showed that gZP3 protein could recognized gZP3 antibody of the female mice with higher intensity compared to gZP3 antibody of male rabbit. Immunofluorescence staining technique indicated that protein of gZP3 could recognize plasma membrane of goat sperm.

The second research indicated that gZP3 protein isolate was fertilization receptor in goat. Biological test by using in-vitro fertilization technique, antibody of gZP3 which was supplemented in the maturation media of goat oocyte in-vitro could decrease ($p<0,05$) of cleavage rate. Protein of gZP3 which was supplemented in the capacitation media of goat sperms decreased ($p<0,05$) of cleavage rate.

The third research indicated that by using in-vitro fertilization technique, antibody of gZP3 resulted in blocking of fertilization ($p<0,05$), and decreasing ($p<0,05$) The Binding Index between mice sperm and mice oocyte.

Based on the results of the research above, it was concluded that gZP3 isolate was fertilization receptor protein at the goat zona pellucida. This protein was effective as immunocontraceptive on mice (Mus musculus) as a model. Hence, it is suggested to conduct further research to find a peptide with amino acid sequence homology of gZP3 and human ZP3, and trial of its immunocontraceptive potency on primates to observe its main effect on infertility response and its side effects on menstruate cycle and ovarian histological changes. Further research is required to test its peptide by using Human sperm-oocyte binding assay or of Human Hemi zona assay.

# ABSTRAK

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Penelitian pertama terdiri dari SDS-PAGE protein zona pelusida kambing, isolasi, penghitungan massa molekul relatif, densitrometri, dot blot, dan uji imunofluoresen protein gZP3. Penelitian kedua dilakukan uji fertilisasi *in-vitro*, untuk membuktikan bahwa isolat yang diperoleh adalah *sperm receptor* kambing. Isolat gZP3 disuplementasikan pada media kapasitasi spermatozoa kambing, sedangkan antibodi gZP3 disuplementasikan pada media maturasi oosit kambing, selanjutnya masing-masing secara terpisah dilakukan fertilisasi *in-vitro*. Penelitian ketiga, antibodi gZP3 disuplementasikan dalam media untuk inkubasi oosit, selanjutnya dilakukan uji fertilisasi *in-vitro* dan uji *Binding* pada oosit mencit (*Mus musculus*) sebagai model.

Penelitian pertama menunjukkan bahwa protein zona pelusida kambing terdiri dari tiga konstituen, yaitu gZP1, gZP2, dan gZP3 dengan massa molekul relatif (Mr) berturut-turut 120, 94, dan 82 kDa, dan dengan komposisi 6,93 29,60 dan 63,47 %. Hasil analisis *Dot blot* menunjukkan bahwa protein gZP3 dapat dikenali oleh antibodi gZP3 asal mencit betina dengan intensitas lebih tinggi dibandingkan antibodi gZP3 asal kelinci jantan. Analisis imunofluoresen menunjukkan bahwa protein gZP3 dapat mengenali membran plasma spermatozoa kambing. Penelitian kedua membuktikan bahwa isolat protein gZP3 merupakan reseptor fertilisasi. Uji biologis dengan teknik fertilisasi *in-vitro* : antibodi gZP3 dalam media maturasi oosit kambing maupun protein gZP3 dalam media kapasitasi spermatozoa kambing menurunkan ($p<0,05$) angka *cleavage*. Penelitian ketiga menunjukkan bahwa antibodi gZP3 menghambat ($p<0,05$) fertilisasi oosit mencit secara *in-vitro* dan menurunkan ($p<0,05$) *Binding Index* antara spermatozoa dengan oosit mencit (*Mus musculus*).

Berdasarkan hasil penelitian di atas, disimpulkan bahwa isolat gZP3 adalah protein reseptor fertilisasi pada zona pelusida kambing yang efektif sebagai bahan imunokontrasepsi pada mencit (*Mus musculus*) sebagai model.

Kata Kunci : Zona pellucida-3 kambing, imunokontrasepsi, fertilisasi *in vitro*.

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The first research was consist of SDS-PAGE of goat zona pellucida protein, isolation, calculation of relative molecular mass (Mr), densitometry, dot blotting and test of immunofluorescence of gZP3 protein. The second research was in-vitro fertilization, to prove that obtained isolate was representing fertilization receptor of goat. Isolate of gZP3 was incubated on goat sperm, while antibody of gZP3 was incubated on goat oocyte, then each of them was in-vitro fertilized separately. The third research, antibody of gZP3 supplemented into media for oocyte incubation, continued with in-vitro fertilization and binding assay on mice.

The result of the first research indicated that zona pellucida of goat consisted of three constituents that were gZP1, gZP2, and gZP3 with relative molecular mass 120, 94, and 82 kDa, and it composition were 6.93, 29.60and 63.47 % respectively. Dot blotting analysis showed that gZP3 protein could recognized gZP3 antibody of the female mice with higher intensity compared to gZP3 antibody of male rabbit. Immunofluorescence staining technique indicated that protein of gZP3 could recognize plasma membrane of goat sperm. Secondly research indicated that gZP3 protein isolate was the receptor of fertilization. Biologic test by using in-vitro fertilization technique : antibody of gZP3 which was supplemented in maturation media of goat oocyte of in-vitro decreased ($p<0,05$) of the cleavage rate. Protein of gZP3 which was supplemented in capacitation media of goat sperm could also decreased ($p<0.05$) the cleavage rate. The third research indicated that by using in-vitro fertilization technique, antibody of gZP3 blocked ($p<0.05$) the fertilization, and decreased ($p<0.05$) The Binding Index between mice sperm and mice oocytes.

The results of this study concluded that gZP3 protein isolate was the fertilization receptor protein at the goat zona pellucida. This protein was effective as immunocontraceptive on mice (Mus musculus) as an animal model.


Key Words : Goat zona pellucida 3, immunocontraception, *in-vitro* fertilization