Produksi antibodi poliklonal anti protein rekombinan voltage dependent anion channel-3 (VDAC3) terpurifikasi dan efeknya terhadap motilitas dan viabilitas sperma manusia = Production of polyclonal antibody anti purified voltage dependent anion channel-3 (VDAC3) recombinant and its effects on human sperm motility and viability / Amalia Shari

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Abstrak

ABSTRAK
Latar Belakang. Protein yang berperan penting dalam fungsi sperma berpotensi sebagai target molekul dalam upaya pengembangan bahan kontrasepsi pria. Salah satu protein yang terdapat pada sperma adalah protein kanal Voltage Dependent Anion Channel3 (VDAC3). VDAC3 berfungsi mengatur aliran ion dan metabolit termasuk ATP. Dari penelitian dengan menggunakan teknik knock-out mouse pada gen VDAC3 dilaporkan bahwa mencit jantan mutan VDAC3 homozigot mengalami penurunan yang signifikan dalam motilitas spermanya. Tujuan penelitian ini adalah memproduksi antibodi poliklonal VDAC3 melalui imunisasi protein rekombinan VDAC3 murni dan uji aktivitasnya terhadap motilitas dan viabilitas sperma manusia.

Metode. Verifikasi keberhasilan pemotongan His fussion tag beserta 31 asam amino plasmid dari protein rekombinan dilakukan dengan teknik Western blott. ELISA digunakan untuk mengetahui titer IgG anti VDAC3sedangkan uji efektifitas antibodi VDAC3 terhadap fungsi sperma dilakukan dengan menghitung prosentase sperma yang tidak bergerak, waktu yang ditempuh sperma dalam jarak 0,1 mm. Analisa viabilitas sperma dilakukan dengan metode pewarnaan eosin.

Hasil. Pada penelitian ini Western blotting dengan menggunakan antibodi Rabbit Anti VDAC Human menghasilkan pita tunggal dengan ukuran ~ 16 kDa, sedangkan penggunaan antibodi terhadap His (C-term) tidak menunjukan adanya pita. Hasil spektofotometri ELISA titer antibodi poliklonal VDAC3 yang berasal dari kelinci menunjukkan adanya peningkatan titer antibodi poliklonal VDAC3 setelah imunisasi dibandingkan dengan titer antibodi sebelum imunisasi (preimun serum). Hasil uji aktivitas antibodi poliklonal VDAC3 menunjukkan terjadi peningkatan jumlah sperma bergerak yang bermakna pada waktu 30 menit ($p<0,05$) dan 60 menit ($p<0,05$), juga terjadi peningkatan waktu tempuh sperma yang bermakna pada waktu 0-30 menit ($p<0,05$) setelah perlakuan. Penambahan antibodi poliklonal VDAC3 juga berpengaruh secara nyata terhadap persentase viabilitas spermatozoa yang hidup ($p<0,05$).

Kesimpulan. VDAC3 poliklonal antibodi berhasil diproduksi melalui imunisasi dari VDAC3 rekombinan murni. Antibodi poliklonal anti-protein rekombinan Voltage Dependent Anion Channel-3 (VDAC3) dapat menurunkan motilitas dan viabilitas sperma manusia invitro secara bermakna.
<hr><i>ABSTRACT
Background. Sperm-specific proteins that are important for sperm function can potentially be used as a target for developing a male contraceptive. One of the proteins found in the human sperm is Voltage

Dependent Anion Channel3 (VDAC3). VDAC3 regulates the flow of ions and metabolites including ATP in the mitochondrial membrane and cell membrane of the eukaryotes. A previous study showed VDAC3 knockout mice had significant reduction in sperm motility. The purpose of this study was to produce polyclonal antibodies through immunization of pure VDAC3 recombinant protein and analyze its effect towards sperm motility and viability.

Methods. Removal of the His fussion tags plus 31 amino acids from the recombinant plasmid was verified using western immunoblotting. The titter of VDAC3 polyclonal antibody was determined by ELISA. The effect of VDAC3 antibodies against sperm qualities namely motility and viability was assessed using standard sperm analyses approved by the WHO.

Results. Western immunoblotting using Rabbit Anti Human VDAC3, produced a single band with size of ~ 16 kDa. No visible band was detected when anti-His (C-term) antibody was used in the analyses. Spectophotometric ELISA showed that the titer of VDAC3 polyclonal antibodies, derived from rabbits, polyclonal antibody increased better than the pre-immune. Analyses of VDAC3 polyclonal antibody against human sperm showed an increase in the number of sperm to move significant at 30 minutes ($p < 0.05$) and 60 minutes ($p < 0.05$), as well as an increase in sperm significant travel time at the time of 0-30 minutes ($p < 0.05$) after treatment. Polyclonal antibodies VDAC3 also significantly affect the percentage of sperm viability ($p < 0.05$).

Conclusion. Polyclonal antibody anti-VDAC3 was successfully produced via immunization of the pure recombinant VDAC3. Polyclonal antibody anti-recombinant protein Voltage Dependent Anion Channel-3 (VDAC3) may decrease human sperm motility and viability in vitro significantly.</i>