Purifikasi antibodi poliklonal anti VDAC3 (voltage dependent anion channel 3) rekombinan dan evaluasi pengaruhnya terhadap sperma manusia = Purification of antibody anti VDAC3 voltage dependent anion channel 3 recombinant and evaluation of effect to human sperm / Putri Rahayu Ratri

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Abstrak

Latar Belakang. Voltage Dependent Anion Channel3 (VDAC3) merupakan salah satu protein yang terdapat pada sperma. Pada pengembangan imunokontrasepsi, protein tersebut dapat dijadikan antigen potensial dalam menurunkan fungsi sperma. Beberapa penelitian menunjukan bahwa anti-VDAC dapat mengganggu fungsi normal dan meningkatkan abnormalitas morfologi sperma. Imunisasi protein VDAC3 rekombinan terhadap kelinci diharapkan dapat memicu terbentuknya antibodi poliklonal anti-VDAC3. Antibodi tersebut kemudian dipurifikasi dan dievaluasi kemampuannya dalam mengikat antigen VDAC3 yang terdapat pada sperma manusia melalui pengamatan motilitas, viabilitas, integritas membran, dan integritas akrosom.

Metode. Protein rekombinan VDAC3 diproduksi dengan cara mengkultur bakteri E.coli BL21 yang mengandung konstruksi vektor rekombinan. Protein rekombinan tersebut diimunisasikan pada kelinci kemudian diambil antiserumnya. Antibodi VDAC3 yang terkandung dalam antiserum dipurifikasi dengan metode kromatografi afinitas matriks sepharose protein A kemudian konsentrasinya diukur dengan metode ELISA dan dianalisis kemurniannya secara kualitatif dengan SDS-PAGE. Antibodi VDAC3 tersebut kemudian diuji kemampuannya terhadap sperma manusia normal dengan parameter: motilitas, kecepatan pergerakan, mortalitas (menggunakan metode pewarnaan Eosin Y), integritas membran ekor (menggunakan metode HOST), dan integritas akrosom (menggunakan metode FITC-PNA).

Hasil. Kultur biakan E. coli dengan penambahan IPTG menunjukkan konsentrasi protein terlarut lebih tinggi yaitu 2,051 mg/ml dibandingkan dengan tanpa IPTG yaitu 1,528 mg/ml. Imunisasi protein VDAC3 rekombinan terhadap kelinci menghasilkan antiserum yang mengandung antibodi antiVDAC3 dengan titer tertinggi yaitu 3,504 pada kelinci B. Hasil SDS-PAGE antibodi yang dimurnikan menunjukan dua pita yang mendekati ukuran ~ 55 kDa dan ~25 kDa. Analisis statistik dari pengaruh antibodi VDAC3 murni terhadap motilitas, kecepatan gerakan, mortalitas, integritas membran ekor dan integritas akrosom sperma menunjukkan perbedaan yang signifikan (p <0,05) dengan kontrol preimun serum.
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ABSTRACT
Background. Voltage Dependent Anion Channel 3 (VDAC 3) is one of the proteins found in sperm. On the development of immunocontraception, this protein can be used as potential antigens to reduce normal function of sperm. Several studies have shown that anti-VDAC can reduce normal function of sperm and also increase the morphological abnormalities of sperm. VDAC3 recombinant protein immunization to rabbit is expected to produce of polyclonal antibodies anti-VDAC3. Then, the antibodies are purified and evaluated its ability to bind antigen VDAC3 contained in human sperma by observation of motility, sperm

speed, viability, membrane integrity, and the integrity of the acrosome.

Method. VDAC3 recombinant protein produced by culturing of E. coli BL21 that contains the construction of recombinant vector. This recombinant protein immunized to rabbits and then the antiserum taken from blood sample. VDAC3 antibodies that contained in antiserum purified by affinity chromatography matrix protein A Sepharose then the concentration was measured by ELISA and the purity analyzed by SDS-PAGE. The ability of VDAC3 antibodies were then tested to normal human sperma with parameters are: sperm motility, sperm speed, mortality (eosin Y staining method), membrane integrity (using HOST), and the integrity of the acrosome (using FITC-PNA).

Results. Culture of E. coli with addition of IPTG showed a higher concentrations (2,051 mg/ml) than without IPTG (1,528 mg/ml). Immunization of protein VDAC3 recombinant to rabbit produce antiserum that contains antibodies with the highest titers 3,504 in rabbit B. SDS-PAGE analysis two protein fragmen with size of ~ 55 kDa and ~ 25 kDa. Statistical analysis of the effect of pure VDAC3 antibodies to motility, sperm speed, mortality, membrane integrity, and the integrity of the acrosome showed a significant difference ($p < 0.05$) with control preimun serum.