Identifikasi sumber, mekanisme, potensi penggunaan DNA bebas medium kultur embrio dan model kecerdasan buatan sebagai modalitas seleksi genetik embrio non-invasif = Identification of sources, mechanisms, and potential applications of cell-Free DNA in embryo culture media and artificial intelligence models as non-invasive embryo ploidy modalities

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Abstrak

Latar Belakang: DNA bebas dalam medium kultur embrio dan kemajuan pemodelan berbasis kecerdasan buatan berpotensi menjadi modalitas uji genetik yang non-invasif. Saat ini, tidak diketahui apakah DNA tersebut dilepaskan oleh sel embrio euploid atau aneuploid, sehingga melemahkan dasar keilmuan penggunaannya. Penelitian ini bertujuan untuk mengetahui sel sumber embrio pelepas DNA bebas dalam medium kultur, validasi potensi klinis penggunaan DNA bebas untuk skrining status ploidi embrio pasien Fertilisasi In-Vitro (FIV), dan konstruksi model pembelajaran mendalam menggunakan gambar embrio tersegmentasi untuk deteksi status ploidi embrio.
Metode: Penelitian ini terbagi dalam dua desain penelitian yaitu eksperimental in-vitro menggunakan embrio hewan model dan observasi kohort menggunakan 28 sampel medium kultur embrio dari 21 pasien program FIV di Klinik Morula IVF Jakarta, periode September 2022–Januari 2023. Konstruksi model pembelajaran mendalam menggunakan gambar embrio pasien FIV yang menjalani program bayi tabung periode Januari 2021– Juni 2023. Deteksi sel embrio sumber pelepas DNA bebas dilakukan dengan memapar salah satu embrio (galur DDY atau C57BL) dengan reversin untuk memperoleh blastomer pembawa sel-sel aneuploid. Embrio kontrol dikultur bersamaan tanpa reversin sebagai pembawa sel-sel blastomer euploid. Agregasi membentuk embrio mosaik dilakukan antara embrio pembawa blastomer aneuploid (perlakuan) dan embrio pembawa blastomer euploid (kontrol). Polimorfisme gen GABRA2 antara galur DDY (alel wildtype) dan C57BL (alel delesi) mejadi alel target yang dikuantifikasi dengan metode qPCR. Empat jenis sampel dibuat sebagai berikut: medium kultur tanpa embrio, medium kultur embrio agregasi tanpa pemaparan reversin, rev-DDY, rev-C57BL. Embrio mosaik diwarnai dengan marka apoptosis untuk deteksi mekanisme pelepasan DNA bebas. Analisis status ploidi embrio menggunakan medium kultur embrio pasien FIV dilakukan dengan metode sekuensing. Konstruksi model pembelajaran mendalam menggunakan gambar embrio tersegmentasi yang dipotong urut selama 10 jam sebelum proses biopsi. Variabel yang diamati dalam penelitian adalah konsentrasi alel delesi dan wildtype gen GABRA2, jumlah sel terwarnai marka apoptosis, Pada sampel medium kultur embrio manusia, keberhasilan amplifikasi dan interpretasi hasil sekuensing, serta tingkat kesesuaian uji antara DNA bebas dengan biopsi trofoblas dianalisis. Kemampuan prediksi model berbasis kecerdasan buatan dinilai dengan akurasi dan loss. Analisis data penelitian dan konstruksi model pembelajaran mendalam menggunakan perangkat lunak SPPS versi 21, OpenEpi. pyhton.
Hasil: Sebanyak 0,08 ng/reaksi alel wildtype ditemukan pada embrio mosaik dengan pemaparan reversin pada blastomer embrio DDY (rev-DDY, pembawa sel-sel aneuploid) dan 0,01 ng/reaksi alel delesi ditemukan pada embrio mosaik dengan pemaparan reversin pada blastomer embrio C57BL (rev-C57BL,

pembawa sel-sel aneuploid). Median jumlah sel embrio terwarnai marka apoptosis antara ketiga group embrio (agregasi kontrol, rev- DDY dan rev-C57BL) tidak berbeda bermakna (nilai p = 0,95 untuk pewarnaan late apoptosis (propidium iodide) dan p = 0,42 untuk early apoptosis (Ann-V) menandakan adanya proses koreksi sel pada kedua group embrio mosaik selama masa perkembangan pra-implantasi. Keberhasilan amplifikasi DNA bebas medium kultur embrio manusia dalah 100%, dengan nilai interpretasi 92,8% (26/28). Nilai kesesuaian DNA bebas dengan biopsi trofoblas adalah rendah sebesar 65,4% (17/26) dengan kesesuaian kromosom seks adalah 61,5% (16/26). Sepuluh dari 11 embrio XY pada biopsi trofoblas terdeteksi XX pada DNA bebas. Seluruh model pembelajaran mendalam mengalami peningkatan akurasi menggunakan gambar embrio tersegmentasi dengan algoritma InceptionV3 mencapai akurasi tertinggi sebesar 0,67 dengan nilai loss sebesar 1,4.
Kesimpulan: Sel embrio anueploid adalah sel sember pelepas DNA bebas medium kultur embrio pada embrio mosaik hewan coba mencit yang dilepaskan melalui mekanisme apoptosis. Embrio masik tersebut diperkirakan melakukan self-correction dengan mengeksklusi sel-sel aneploid untuk mempertahankan euploiditasnya. Rendahnya tingkat kesesuaian antara DNA bebas dengan biopsi trofoblas disebabbkan oleh adanya kontaminasi maternal yang ditandai dengan perubahaan koromosm seks yang signifikan. Penggunaan gambar blastosis tersegmentasi meningkatkan akurasi model prediksi pembelajaran mendalam.
.....Background: Cell-free DNA and advanced artificial intelligence-based modeling uphold the potential of a non-invasive approach to determining embryo ploidy status. The specific embryonic cells (whether euploid or aneuploid) that release cell-free DNA are largely unknown, causing a weak scientific basis for its use. This study aimed to identify the source of embryonic cells releasing cell-free DNA in culture media, validate the clinical potential of using cell-free DNA to screen embryo ploidy status in an in-vitro fertilization (IVF) program and develop a deep learning model using segmented embryo images to detect embryo ploidy status.
Materials and Methods: This study employed two research designs including an in-vitro experimental study using animal model embryos and an observational cohort study using 28 samples of spent embryo culture media from 21 patients undergoing IVF program at Morula IVF Clinic Jakarta (September 2022 to January 2023). A deep learning model was constructed using images of embryos from IVF patients who participated in IVF program from January 2021 to June 2023. Detection of the source embryonic cells releasing cell-free DNA was achieved by exposing embryos (DDY or C57BL strains) to reversine to induce the formation of blastomeres carrying aneuploid cells. Control embryos were cultured simultaneously without reversine to serve as the source of euploid blastomeres. Mosaic embryo aggregation was performed by combining embryos carrying aneuploid blastomeres (treatment) with those carrying euploid blastomeres (control). The GABRA2 gene polymorphism between the DDY strain (wildtype allele) and the C57BL strain (deletion allele) was the target allele quantified using qPCR. Four types of samples were prepared: culture medium without embryos, culture medium of aggregated embryos without reversine exposure, rev-DDY, and rev-C57BL. Mosaic embryos were stained with an apoptosis marker to detect the mechanism of cell-free DNA release. The ploidy status of embryos using spent embryo culture media from IVF patients was determined using sequencing methods. The deep learning model was constructed using segmented images of embryos captured over 10 hours before the biopsy process. The variables observed in the study included the concentration of deletion and wildtype alleles of the GABRA2 gene, the number of cells stained with apoptosis markers, the success rate of amplification and interpretation of sequencing results from human spent embryo culture medium samples, and the concordance rate between cell-free DNA and trophectoderm

biopsy analysis. The predictive ability of the artificial intelligence-based model was evaluated using accuracy and loss metrics. Data analysis and deep learning model construction were performed using SPSS version 21, OpenEpi, and Python.

Results: A total of 0.08 ng/reaction of the wildtype allele was detected in the culture media sample of mosaic embryos exposed to reversine in DDY embryo blastomeres (rev- DDY, carrying aneuploid cells), and 0.01 ng/reaction of the deletion allele was found in the sample exposed to reversine in C57BL embryo blastomeres (rev-C57BL, carrying aneuploid cells). The median number of embryonic cells stained with apoptosis markers among the three groups of embryos (control aggregation, rev-DDY, and rev-C57BL) did not differ significantly ($p = 0.95$ for late apoptosis staining with propidium iodide and $p = 0.42$ for early apoptosis with Annexin V), indicating the presence of cell correction processes in both groups of mosaic embryos during pre-implantation development. The success rate of cell-free DNA amplification in human spent embryo culture media was 100%, with an interpretability of 92.8% (26/28). The concordance between cell-free DNA and trophectoderm biopsy was low at 65.4% (17/26), with sex chromosome concordance at 61.5% (16/26). Ten out of eleven XY embryos from the trophectoderm biopsy were detected as XX in cell-free DNA analysis. All deep learning models showed improved accuracy using segmented embryo images with the InceptionV3 algorithm, achieving the highest accuracy of 0.67 with a loss of 1.4.

Conclusion: Aneuploid embryonic cells were identified as the source releasing cell-free DNA in culture media during embryo animal model experiments, releasing DNA through an apoptotic mechanism. These mosaic embryos were expected to activate embryonic cell correction mechanisms by excluding aneuploid cells to maintain their euploidy. The low concordance rate between cell-free DNA and trophectoderm biopsy was attributed to maternal contamination, as indicated by significant changes in sex chromosomes. The use of segmented blastocyst images improved the model's accuracy.