RINGKASAN

Analisis Pengaruh Vitrifikasi terhadap Viabilitas
dan Struktur Oosit Sapi

Sri Wahjuningsih

Penelitian eksperimental laboratoris telah dilakukan melalui pemanfaatan oosit sapi dari ovarium yang diperoleh dari Rumah Potong Hewan. Tujuan penelitian adalah mengkaji fungsi Etilen Glikol (EG) sebagai krioprotektan dalam mempertahankan kualitas oosit selama proses vitrifikasi.

Penelitian ini terdiri dari 3 tahap penelitian : tahap 1, evaluasi morfologi, viabilitas dan tingkat fertilisasi oosit setelah vitrifikasi, tahap 2 adalah analisis fungsi reseptor primer spermatozoa oosit setelah vitrifikasi menggunakan tehnik dot bloting dan tahap 3 adalah analisis mikrotubulus oosit setelah vitrifikasi menggunakan teknik imunohistokimia.

Metode penelitian yang digunakan adalah : (1) rancangan penelitian acak lengkap pola faktorial 6X3 untuk evaluasi morfologi, viabilitas dan tingkat fertilisasi. Faktor pertama adalah konsentrasi Etilen glikol (0, 10,20,30,40,50 %) dan faktor kedua adalah lama paparan (1,3, 5 menit); (2) analisis deskriptif untuk mengetahui fungsi reseptor primer oosit menggunakan teknik dot blot serta struktur sitoskeleton oosit berbasis mikrotubulus. Data dianalisis secara deskriptif dan anova menggunakan pogram SPSS

Penelitian tahap 1, evaluasi morfologi, viabilitas dan tingkat fertilisasi oosit setelah vitrifikasi. Hasil penelitian menunjukkan bahwa konsentrasi etilen glikol dan lama paparan berpengaruh terhadap morfologi, viabilitas serta tingkat fertilisasi ($p<0,01$). Persentase oosit dengan morfologi normal dan oosit hidup tertinggi serta tingkat fertilisasi adalah vitrifikasi menggunakan 30 % EG dan lama paparan 3 menit, masing-masing sebesar 67.75 %, 65.06 % dan 61.05 Konsentrasi EG 30 % merupakan konsentrasi optimum untuk melindungi oosit pada proses vitrifikasi

Penelitian tahap 2 adalah analisis fungsi reseptor primer spermatozoa oosit setelah vitrifikasi. Uji spesifisitas secara kualitatif dilakukan berdasarkan metode dot blot, dihasilkan data berupa noda biru keunguan yang merupakan hasil reaksi spesifik antara antigen (oosit kontrol dan oosit hasil vitrifikasi) dengan antibodi (anti *b*ZP3) dengan gradasi warna yang tergantung pada variasi konsentrasi krioprotektan EG. Antibodi *b*ZP3 mampu mengenali dengan baik ZP3 dari oosit tanpa vitrifikasi dibandingkan ZP3 oosit yang mengalami vitrifikasi. Pada dosis EG tertentu dapat menurunkan imunoreaktifitas inti protein dari molekul *b*ZP3 terhadap anti-*b*ZP3. Namun pada dosis EG 30 % masih mempunyai reaksi positif seperti pada oosit tanpa vitrifikasi

Penelitian tahap 3 adalah analisis sitoskeleton berbasis mikrotubulus oosit menggunakan metode imunohistokimia. Hasil pengamatan diperoleh gambar yang menunjukkan adanya perbedaan struktur sitoskeleton oosit kontrol dan oosit hasil vitrifikasi. Pada oosit kontrol struktur sitoskeleton berbasis mikrotubul tampak seperti benang-benang berwarna kecoklatan. Hasil penelitian menunjukkan bahwa setelah proses vitrifikasi struktur sitoskeleton mengalami perubahan, kecuali pada konsentrasi EG 30 % struktur sitoskeleton masih dapat dipertahankan.

Kesimpulan dari penelitian ini adalah perubahan morfologi, viabilitas dan tingkat fertilisasi akibat hilangnya lingkungan hidrofobik dalam proses vitrifikasi dapat mengalami proteksi dengan bantuan EG. Namun demikian untuk menjaga fungsi oosit, struktur sitoskeleton berbasis mikrotubulus harus dapat dipertahankan. EG sebesar 30% terbukti dapat mencegah kerusakan lebih lanjut sitoskeleton. Oosit hasil vitrifikasi mampu melakukan fertilisasi *in vitro,* yang berarti *ZP3* sebagai reseptor primer spermatozoa pada oosit masih berfungsi, walaupun secara keseluruhan protein zona pelusida gagal melakukan *blocking polyspermia*.

SUMMARY

Analysis of Vitrification Effect on Viability and Structure of Bovine Oocyte

Sri Wahjuningsih

A laboratory experimental research has been conducted using bovine oocytes from bovine ovaries collected from slaughter house. The objective of this research was to examine function of Etilen Glycol (EG) as cryoprotectant agent to preserve quality of oocytes during vitrification process.

Three research stages were conducted : stage 1, evaluation of morphology, viability dan fertilization rate during vitrification, Stage 2, analysis of sperm primary receptor in oocytes during vitrification using dot blot technique dan stage 3, cytoskeleton analysis based on microtubulus during vitrification by immunohistochemistry technique.

The method that used in this research were : (1) experimental design by randomized complete design 6 x 3 to know the effect of concentration of ethylene glycol (0, 10,20,30,40 and 50 %) and exposure time (1,3 and 5 minutes) to morphology, viability and fertilization rate. (2) descriptive, to know sperm primary receptor in oocytes during vitrification using dot blot technique and structure of cytoskeleton based on microtubulus. The data analyzed by descriptive and anova using SPSS program

Stage1, Evaluation of morphology, viability dan fertilization rate after vitrification. Concentration of EG and exposure time had significant different ($p<0,01$) to morphology, viability, and fertilization rate. The highest percentage of normal morphology, viability and fertilization rate were 67.75 %, 65.06 % and 61.05 % respectively. The optimal concentration of EG of these research was 30 %.

Stage 2. analysis of sperm primary receptor in oocytes during vitrification using dot blot technique and resulted blue dot indicated specific reaction of antigen (control oocytes and vitrified oocytes) wiith anti-*b*ZP3 and showed the gradation of colour depend on concentration of EG. Control oocytes reacted with anti-*b*ZP3 showed more positive that vitrified oocytes. This result showed that oocytes after vitrification changed the conformation of zona pellucida, except the 30% of EG gave the similar respon with control

Stage 3. Cytoskeleton analysis based on microtubulus during vitrification by immunohistochemistry technique. The structure cytoskeleton especially its microtubulus showed depolimerization of microtubulus comparing by control, but concentration of 30 % EG has a similar resullt with control.

It was concluded that structure and function changes of oocyte caused by decreasing hydrophobic environment in vitrification process can be protected by EG. Vitrified oocytes can be fertilized, it means Zona Pelusida-3 as a primarily sperm receptor still have a function,but zona pelucida protein failed to block the others sperm.

ABSTRACT

Analysis of Vitrification Effect on Viability and Structure of Bovine Oocyte

Sri Wahjuningsih

A laboratory experimental research has been conducted using *bovine* oocytes from bovine ovaries collected from slaughter house. The objective of this research was to examine function of Etilen Glycol (EG) as cryoprotectant agent to preserve quality of oocytes during vitrification process.

Three research stages were conducted : stage 1, evaluation of morphology, viability dan fertilization rate during vitrification, Stage 2, analysis of sperm primary receptor in oocytes during vitrification using dot blot method dan stage 3, cytoskeleton analysis based on microtubulus during vitrification by immunohistochemistry method.

Evaluation of morphology, viability and fertilization rate showed that exposure 30 % EG during 3 minutes was an optimal concentration that have a similar result with control. Based on dot blot method, this result showed that oocytes after vitrification changed the conformation of zona pellucida, except the 30% of EG gave the similar respon with control. Using immunohistochemistry technique, the structure cytoskeleton especially its microtubulus showed degradation of microtubulus comparing by control, but concentration of 30 % EG has a similar resullt with control.

Structure and function changes of oocyte caused by decreasing hydrophobic environment in vitrification process can be protected by EG. Vitrified oocytes can be fertilized, it means Zona Pelusida-3 as a primarily sperm receptor still have a function,but zona pelucida protein failed to block the others sperm.

Key words: *vitrification, EG, bovine oocytes, morphology, viability, cytoskeleton structure.*