Aktivasi maturasi oosit mencit in vitro melalui regulasi ca2 intraseluler = in vitro activation of mice oocyte maturation through intracellular ca2 regulation

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Abstrak

Latar belakang: Pasien dengan total oosit immatur atau rendah jumlah oosit matur yang diperoleh dari proses ovum pick up OPU pada siklus berulang cenderung tidak dapat ditangani dengan kultur in-vitro atau in vitro maturasi IVM . Sejauh ini, pasien dengan riwayat rendah/kegagalan total maturasi yang kembali mengulang siklus in vitro fertilisasi, hanya ditangani dengan merubah protokol stimulasi untuk merubah respon ovarium dengan hasil yang belum memuaskan. Jalur pensinyalan Ca2 diketahui berperan penting dalam proses maturasi oosit. Karenanya, penelitian ini bertujuan untuk mengetahui apakah modifikasi regulasi Ca2 intraseluler oosit yang tetap immatur paska kultur in-vitro dengan aktivasi A23187 mampu menjadi solusinya. Metode: Oosit immatur dikoleksi dengan metode diseksi ovarium dan dilanjutkan kultur maturasi secara grup selama 20-24 jam berdasarkan status sel kumulus dengan atau tanpa sel kumulus . Oosit yang tetap immatur paska kultur maturasi, dibagi secara acak kedalam kelompok kontrol dan perlakukan aktivasi dengan CaI A23187 untuk mendorong maturasi. Proses aktivasi dilakukan selama 30 menit, kemudian dilanjutkan kultur maturasi kembali. Setelah 20-24 jam kultur, dilakukan evaluasi maturasi paska aktivasi dengan melihat ekstruksi badan polar I. Untuk memperoleh gambaran perubahan level Ca2 selama proses aktivasi, dilakukan pengukuran intensitas pendaran oosit immatur terlabel pewarna berfluoresen Fura-Red yang mampu berikatan dengan kalsium bebas intrasel menggunakan confocal laser scanning microscope CLSM pada panjang gelombang 405 dan 488nm. Hasil penelitian: Aktivasi oosit immatur dengan CaI A23187 secara bermakna meningkatkan jumlah maturasi dibandingkan dengan kelompok kontrol P<hr />Background Patients with total immature or high number of immatured oocyte obtained from repeated cycles of ovum pick up OPU are unlikely to be treated only with extended in vitro culture or in vitro maturation IVM . As known, patients with high percentage of immature failure history repeating in in vitro fertilization cycle are treated only by changing the stimulation protocol to change the ovarian response with unsatisfactory results. The Ca2 signaling is known to play an important role in oocyte maturation. Therefore, the aim of this study was to determine whether the modification of intracellular Ca2 of oocytes failed to resume meiosis even following subsequent in vitro culture could reach metaphase II after Calcium Ionophore A23187 activation.Method Immature oocytes were collected by ovarian dissection method and continued with group maturation culture for 20 24 hours based on cumulus cell status intact and without cumulus cells . Oocytes shows immature resistant after in vitro culture were randomly allocated to control and treatment groups. Oocyte activation group was exposed to A23187 solution for 30 minutes and then washed extensively. Maturation was evaluated 20 24 hours after CaI A23187 exposure by observing the first polar body extrusion. To identify Ca2 response during activation, Ca2 imaging was conducted using confocal laser scanning microscope CLSM . Oocytes were loaded to 10 M L of the ratiometric Ca2 sensitive dye Fura Red acetoxymethyl AM ester. Fluorescent measurement were made with filter that provided excitation at wavelengths of 405 and 488nm. Result Activation of resistant immature oocytes with CaI A23187 significantly increased number of maturation compared with the control group p