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Immunomagnetic separation and flow cytometry of rabbit sperms

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The rabbit sperms after incubation with an anti-FITC monoclonal antibody against SRY epitope of the HY antigen, are exposed to a defined magnetic field. Polymerised super magnetic nanoparticles bound to Y sperm cells are adherent to the walls of separation columns (positive selection). The suspension remains a fraction of sperms with X chromosomes (negative selection) to be used for insemination. Y sperms bound to the particles undergoing super magnetic vital separation techniques and also used for insemination. Stillborn young rabbits, respectively, dead rabbit under the age of three weeks, tissue samples are taken to detect of sex by SRY-PCR technique. For immunomagnetic separation was used the MidiMACS Magnetic cell sorting. Positive selection of sperms containing the Y chromosome was achieved at a concentration: 0.86 μ l FITC anti-HY TCR antibodies/7 x 10⁶/ml-14x10⁶/ml sperm. Using of the anti-HY monoclonal antibody and the artificial insemination of females by positively selected sperms was achieved a shift of sex young rabbits in favor of males in the ratio 63.63% ♂♂: ♀♀ 36.36 %, it is the statistically significant difference to expected sex ratio 50:50, (χ^2 (0,05)= 5,55+). Negative selection of sperms containing the X chromosome is achieved at a concentration: 1.0 μ l FITC anti-HY TCR antibodies/7 x 10⁶/ml-14x10⁶/ml sperm. Using of the anti-HY monoclonal antibody and the artificial insemination of females by negative selected sperms has been made a shift of sex young rabbits in favour of females in a ratio of 75% ♀♀ : 25% ♂♂, it is a statistically significant difference in expected sex ratio 50:50, (χ^2 (0,001)= 12,50+++). The FACS Calibur flow cytometric analysis give data that only a small fraction of rabbit sperm cells express receptor H-Y TCR (0.24% - 0.53%). This work was supported by the Slovak Research and Development Agency under the contract No. APVV-16-0067 and APVV-0044-12.

Biography

Vladimir Parkanyi is a research scientist at the National Agricultural and Food Centre Nitra in the Slovak Republic. He is interested in the area of animal genetics, cytogenetics, molecular biology and reproductive physiology. In 1990 he worked in Cytogenetics laboratory at the Department of Animal Breeding and Genetics of the Swedish University Agricultural Sciences, Uppsala, Sweden. In 1992/1993 he participated in DFG project in Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere, Forschungsbereich Molekularbiologie, Dummerstorf, Germany. He participated in some educational and as adviser of PhD. study program at Universities in Bratislava and Nitra. His publication activity concentrates on original papers indexing in international databases, articles for breeders from genetics and reproduction course of study. He was the coordinator and is the solver of research project activities nowadays, too.

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