Thesis Title Sex determination of bovine embryos using H-Y antibody

Name Mayuva Areekijseree

Degree Master of Science (Anatomy)

Thesis Supervisory Committee

Kanok Pavasuthipaisit, M.D.,Ph.D.

Prapee Sretarugsa, Ph.D.

Yindee Kitiyanant, D.V.M.,M.Sc.

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Abstract

The purpose of this study was to produce H-Y antibody from adult C57 BL/6 female mice, to test the titer and specificity of H-Y antibody by sperm cytotoxicity assay and to determine the sex of transferable stage of bovine embryos by using H-Y antibody.

In the present study, we have produced H-Y antibody from inbred C57 BL/6 female mice by injection with the male spleen cells of the same strain once a week for 3 weeks and booster by every 2 weeks. Serum was collected at maximum of once a month throughout the experiment. Both titer and specificity were determined by a sperm cytotoxicity assay. Sera containing high titer antibody and specificity were used for embryo sex identification by indirect immunofluorescent technique. Embryos were individually classified as either H-Y positive (male embryo) or H-Y negative (female embryo). Moreover, the alternation of sex ratio during the developmental stage of embryo were investigated. The sex of 192 bovine embryos produced by in vitro fertilization were immunofluorescently detected at Days-6, Day-7 and Day-8 of in vitro culture. The results showed that sex ratio (male : female) on Day-6 and Day-7 were significantly ($P<0.05$) shifted to the male (1:0.5 and 1.0:0.7), but the sex ratio on Day-8 embryos was significantly ($P<0.05$) shifted to the female (1.0:1.7). The total sex ratio was 1:0.9 which was not significantly different from 1:1.

The results indicated that H-Y antibody can be produced in adult C57 BL/6 female mice and sperm cytotoxicity assays have been used to quantify H-Y antibody activity and specificity. H-Y antibody can be used to determine the sex of transferable stage bovine embryos (morula, compact morula, early blastocyst and blastocyst stages). It is thus concluded that male bovine preimplantation embryos develop faster than female embryos. Embryos after exposed to H-Y antibody are viable. Therefore, it is a rapid, noninvasive and practical procedure for sexing bovine embryos.