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Immunological Control of Reproduction, with Special Reference to Zona Antigen and H-Y Antibody

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繁殖의 免疫学的 制御, 特히 透明帶抗原과 H－Y抗体를 中心으로

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I. INTRODUCTION

The immune system of mammals is extreamly effective in disposing of foreign materials. Reproductive processes at various steps fail to destroy foreign material when it might be expected to do so. Firstly, during copulation, spermatozoa deposited within the female genital tract are not destroyed at least untill fertilization. Secondly, embroys or fetuses are not rejected, although they are foreign materials to the mother. If they do so, fertilization and pregnancy will be disturbed.

In the first part of my presentation, I will discuss the inhibitory effect of antibody to zona pellucida on fertilization to develop an contraceptive vaccine in human. Second, from the practical view of control of reproduction in farm animals, I will focus on the possibility of sex control by using H-Y antibody.

II. ZONA ANTIGEN

1. The Role of Zona Pellucida

Fertilization is initiated in mammals when sperm first interact with egg's zona pellucida surrounding the plasma membrane. As shown in this slide, zona pellucida plays a critical role in the early stages of the fertilization such as sperm recongnition and the prevention of polyspermy. After fertilization, zona plays an important role for the transit of embryos through the oviduct in normal pregnancy. In the rabbit, it is also necessary around implantation. Zona pellucida originates from the oocytes itself and starts to be produced at the stage of primordial follicles. The ground substance of the zona is mucopolysaccharide. Recent biochemical studies revealed the presence of three major components with avarage molecular weights of 60,000, 70,000 and 92,000 in the pig, and 83,000, 120,000, 200,000 in the mouse.

2. Zona Antibody

As shown in the slide, the hetero immune antibody to zona pellucida has been produced in the mouse, human, hamster, pig and cattle.

The zona antibody is detected by the various techniques; formation of the precipitate on zona(Slide 4), indirect immunofluorescence (slide 5,6), immunodiffusion test or immunoelectrophoresis, blockage to zona digestion by enzymes, inhibition of fertilization in vitro and in vivo and radioimmunoassay.

This table shows the effecf of passive immunization with anti-zona serum on fertilization in the mouse, pig and cattle. In all these species, fertilization was completely or significantly inhibited after passive immunization. Antiserum or control serum was injected intraperitoneally or intramuscularly to females at the time of PMSG injection or 1 to 6 days before mating.

This figure shows the effect of zona antibody to inhibit fertilization lasted 25 to 30 days in the mouse. A precipitate of different

intensity on the zona pellucida was observed under dark field illumination in the eggs recovered 5 to 30 days after passive immunization of females with antiserum. The ovulation was not affected by such treatment because failure of ovulation and a decrease of number of eggs were not observed. Such treatment, however, did not cause permanet sterility since four mice which were passive immunized with antibody 60 to 74 days before mating produced normal litter sizes.

Zona antibody has no species-specificity. The cross reaction of antisera with zonae of different species was determined at first by indirect immunofluorescence. As shown in the slide, each anti-zona serum strongly reacted with zonae from respective species. Each antiserum reacted to some extent with zonae of all other species tested.

Moreover, zona antibody inhibits fertilization in different species. As shown in the slide, passive immunization with anti-pig zona serum significantly or completely inhibited fertilization in the cow, sheep, rabbit, rat and mouse. It will be concluded that the pig and other mammalian zona pellucida has cross reactive antigens responsible for fertilization. It is believed that receptors of spermatozoa exist on the zona surface of mammalian eggs and they are species-specific. Our data mentioned above does not agree with this hypothesis. However, the antiserum which we used is multivalent one, so it might form a cross-linked lattice with complementary antigens and such cross-linkages could block the receptors of sperm through a secondary steric effect. In the next experiment, we examined the effect of bivalent and univalent zona antibody on fertilization.

IgG fraction from γ-globulin of antiserum and control serum was prepared by DEAE cellulose chromatography and univalent fragment(Fab) was obtained after digestion with papain. Bivalent Fab fraction was obtained by digestion of IgG with pepsin. As shown in the slide, IgG and bivalent Fab fractions of rabbit anti-mouse zona pellucida antiserum inhibited the fertilizability of mouse eggs in vitro but univalent Fab fraction did not inhibit. However, the treatment with anti-rabbit IgG serum, as a second antibody, inhibited the fertilizability of eggs pretreated with antiserum Fab. It is concluded that the inhibition of fertilization by bivalent zona antibody depends on the possibility that the antibody are directed against the zona pellucida as a whole and not specially against the receptor for the sperm. We consider that the antigens of zonae other than sperm receptors have high immunogenicity and the antibodies to them are not species-specific, thus inhibiting fertilization in other species. This suggests that it may be possible to use pig and cow zonae pellucidae, which are available in large quantities from a slaughterhouse for immunocontraception in human. It has been recently reported that active immunization of monkey with pig zona materials inhibited a pregnancy.

3. Controceptive Vaccine

To develop contraceptive vaccine, it is necessary to purify zona antigen. The purifications of porcine zonae were biochemically aimed by several workers, but only partially purified materials were obtained. Isolation of a single pure antigen from these materials proved difficult and a large amount of monoclonal antibody(Moab) to an individual antigen was essential for this purpose. So we started to produce Moab to pig zonae pellucidae in collaboration with Professor Isojima. As shown in the slide, 5 hibridomas which produce monoclonal antibodies to pig zona were established. Moab from G10G5 stained only pig zona under immunofluorescence. Moabs from C6H1, D3H4, G10F9 stained zonae of pig, human, hamster, rat and mice. Moabs from B11C8 and G10F9 strongly blocked pig sperm binding to pig zonae. It is further necessary to purify the

antibody. As shown in the slide, epidermal cells are collected from tails of Balb/c male and female mice. Packed epidermal cells, serially diluted antiserum and complement are incubated for 45 min at 37°C. Guinea pig or rabbit serum absorbed with agarose is used as a complement source. After addition of trypan blue to the mixture, stained and unstained cells are counted and sera positive only to male cells are selected. As a second step, positive sera are absorbed with male and female Balb/c spleen cells to check the specificity. Sera which are positive to male cells after absorption with female cells and negative after absorption with male cells are considered to be specific H-Y antisera.

Here, I showed some our data on the production of H-Y antiserum. When we injected male spleen cells into female C57BL mice at an interval of one week, none of 25 mice produced specific antibody. Situation was same when spleen cells were injected with Freund's adjuvants. This is not suprising since a lot of scientists tried to produce H-Y antibody, but a few people could obtain specific antibody. When we injected spleen cells at an interval of 2 to 3 days, we could obtain one specific antibody out of 15 mice. When we used spermatozoa for antigen, none of 14 mice produced antibody. Crichton and Cohen reported that none of monoclonal antibodies to C57BL spermatozoa produced in females was able to discriminate between male and female cells. This suggests spermatozoa is not a suitable antigen to produce H-Y antibody.

In this slide, one serum sample of a good H-Y antibody is shown. A broken horizontal line shows the complement control killing levels, around 30%, this level is quite high compared with other immunological reactions. Fig. a shows the reaction before absorption. The serum is cytotoxic to male epidermal cells but not to female cells. Fig. b shows the reaction after absorption. The cytotoxic effect to male cells disappeared after absorption with male cells but retained after absorption with female cells.

3. Effect of H-Y Antibody on Spermotozoa

This slide shows the effect of H-Y antibody on spermatozoa. Bennett and Boyse inseminated a group of C57BL female mice with sperm suspensions exposed to H-Y antiserum and complement. As you can see, only a small decrease in the frequency of male progeny was observed in antibody treated group (45.4% vs 53.3%). Thus, separation of X-bearing spermatozoa by using H-Y antibody seems to be unpractical method.

4. H-Y Antiserum and embryo

In 1976, Krco and Goldberg reported that H-Y antigen was expressed in male embryos at the 8-cell stages. When they treated 8-cell embryos with unabsorbed H-Y antiserum, half of them was degenerated. The cytotoxic effect of antiserum was completely lost after absorption with male cells, but it was only slightly reduced after absorption with female cells. Recently, white et al. reported the same results in a large scale. They cultured 8-cell mouse embryos with five different media for 24-30hrs in 25 μl drops. The culture media were complement and antiserum, none, complement, normal mouse serum, complement and normal mouse serum. As you can see, 48% of embryos were affected when they were cultured in medium including complement and antiserum. In other groups, only 4 to 11% embryos were effected.

We examined whether zona pellucida of embryos should be removed or not at cytotoxicity test. Mouse embryos with or without zona were treated with H-Y antiserum and complement, then degeneration of blastomeres was examined under phase contrast microscope. As you can see zona free embryos are more sencitive to antiserum.

White et al. transferred unaffected embryos

after incubation with different media. They obtained 58 young out of 420 embryos cultured with complement and antiserum, and 86.2% of them was female. The proportions of female young after transfer of embryos treated with other media were close to 50%. These results demonstrate that female animals can not be expected in this method.

More recently, they reported exciting results by using monoclonal H-Y antibody. They first cultured mouse morulae and blastocysts with monoclonal antibody, washed and then treated with FITC-labelled goat anti-mouse IgG serum as a second antibody. Following washing, embryos were examined under the fluorescence microscope. They treated 350 embryos in this manner and observed specific fluorescence in 55% of embryos. Then, they transferred 156 embryos fluoresing and 98 embryos non-fluoresing. As you can see, 78% of young were male in fluorescing group and 83% of young were female in non-fluoresing group. However, there are some problems associated with embryo sexing by H-Y antibody. Firstly as I mentioned earlier, the production of specific H-Y antibody is quite difficult. Secondly, the proportion of young is usually low after transfer or embryos treated with antiserum and complement or second antibody. Thirdly, the sex of 20 to 30% of young after transfer of sexed embryos are the opposite one. Inspite of these problems, the results shows a possibility for providing a means of determining the sex of embryos prior to embryo transfer in farm animals. Since H-Y antigen is common and cross reactive among mammals, the success experiments in mouse can be directly applied to farm animals. Actually, we can see a news in Nature published in January 1983 entitled "Techniques for sexing embryos now possible."