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Short communication

CROSSREACTIVITY OF BOAR SPERM MONOCLONAL ANTIBODIES WITH HUMAN SPERMATOZOA

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Monoclonal antibodies against the head (H mabs) and tail (Tmabs) of boar spermatozoa were produced. Spermatozoa from boar, stallion, bull, human, ram, goat and rabbit were independently incubated with the monoclonal antibodies and later stained by immunofluorescence method. There were positive reactions of the monoclonal antibodies with the boar and human spermatozoa. There was no reaction with spermatozoa of all the other species. The crossreactivity observed between the sperm antibodies of boar and human spermatozoa may open up research efforts on non-steroidal contraceptives in humans.

Key Words: *Boar spermatozoa antibodies human cross- reactivity.*

INTRODUCTION

Monoclonal antibodies had been used as probes for biological structures (Kohler and Milstein, 1975, Lee et. al., 1984) especially to study the reproductive system in many areas of research such as cell biology, endocrinology, immunogenetics and pathology (Bellve and Moss, 1983). Monoclonal antibodies had been produced against sperm in the baboon (Isahakia, 1986), mouse (Saling et al, 1985), rat (Gaunt et al, 1983) rabbit (Naz et al, 1983) and boar (Fayemi and Joo, 1990). These monoclonal antibodies were specific for different parts of the sperm cell.

There had been clamour for human population control in the last few decades and various methods, surgical and non-surgical had been used for this purpose. These methods include vasectomy and the use of condom in the male and implants, intrauterine device and steroidal contraceptives in the female. These methods especially steroidal contraceptives have been noted to have some unfavourable side effects like pulmonary embolism and cerebral thromhosis (Tatum 1985).

Since sperm antibodies had been associated with infertility in human males (Paul et al, 1983, Haas et al, 1980) and females (Menge et al. 1982, Clarke et. al., 1995)), It is believed that sperm antigens or antibodies can be useful for contraception and thereby side-tract the side effects of steroidal contraception (Rieder and Coupey, 1999, Shetty et. al., 1999). Boar semen can be easily collected in large volume and therefore can be of commercial value if found useful in contraception.

The objective of this study was to check whether monoclonal antibodies to boar sperm can specifically bind to the surface antigens of the human sperm and it is believed that if this is achievable it may thereafter block the fertilization capacity or reduce motility of the sperm cells thereby becoming a useful contraceptive device.

MATERIALS AND METHODS

Preparation of Antigen: Monoclonal antibodies to boar spermatozoa were prepared as described below:

Semen collected from four adult boars and pooled into a flask was centrifuged at 1200g for 5 minutes to separate the spermatozoa from the seminal plasma. The spermatozoa were then washed three times in 0.005M phosphate buffered saline (PBS) and resuspended at a concentration of 1 x 10^9 cells/ml. The cells were then sonicated over a period of one minute with sonicator model W380 (Heat Systems Ultrasonic Inc.) and mixed with equal volume of either complete or incomplete Freund's adjuvant.

Immunization of Mice: Mice of the BALB/C strains were injected intraperitonially with 2ml of the mixture of sonicated sperm cells and complete adjuvant on day 0, mixture of sperm cells with incomplete adjuvant on day 14 and sonicated cells alone on day 24 and the spleens removed, minced and the spleen cells suspended in RPMI-1640 {Gibco}, 48 hours after the last injection.

Fusion of Cells: The spleen cell suspension in RPMI-1640 (Gibco) was put into 50ml sterile tube and myeloma cells Ag8.653 were harvested into another 50ml sterile tube. The two preparations

were washed three times with serum-less RPMI 1640 at 4^0C.

The cells were counted and mixed in a spleen cells to Ag8.653 ratio of 2:1. The cell mixture was washed by centrifugation in serum - free RPMI 1640 at 500g for 7 minutes, aspirating the supernatant, loosening the pellet and slowly adding polyethylene glycol (PEG) over a period of 45 seconds. The PEG was prepared by weighing 20gm, PEG 4000 which was autoclaved in 100ml bottle before adding 28ml sterile Dubelcco's phosphate buffered saline (DPBS) containing 15% Diethylsulfoxide (DMSO). The mixture was diluted by dropwise addition of 20ml serum-free RPMI 1640 at 37°C before filling the tube to 50ml with the RPMI 1640. This was then centrifuged at 500g for 10minutes and resuspended in sterilized Hypoxanthine and Thymidine (HT) medium prepared by dissolving 272.2mg hypoxanthine (Sigma) and 7.75mg thymidine (Sigma) in 20mls distilled water at pH8.1, at 37°C.

The final concentration was then adjusted to 1.5 x 10^5 spleen cells/ml. Peritoneal wash of one normal mouse using 100mls RPMI-1640 was added and mixed before dispensing into 96 well plates. Each well thus contained approximately 5 x 10^5 spleen cells. The plates were incubated in 10% CO_2 at 37oC. HAT, (Hypoxanthine Aminopterin Thymidine), prepared with 1ml HT + 1ml Aminopterin (Lederle) made up to 100ml in complete media which was a mixture of 100ml RPMI-1640, 20ml, Fetal calf serum (FCS), 2µm Glutamine, 50µm 2-mercaptoethanol, 1ml penicillin streptomycin and 2µm Fungizone, was added at 2 drops/well on Day 1.

The plates were observed with inverted microscope on Day 5 to check for myelona cell death and contamination. Copper sulphate solution was used to kill contaminated cells. On Day 14, 0.5ml supernatant was removed and discarded from each well and replaced with 0.5ml/well complete media. This process of using complete media to replace discarded supernatant was repeated on Days 17 to 21.

The plates were scored for hybridoma growth and the supernatant tested for antibody production on days 21 to 23. The monoclonal antibody production was tested by the indirect immunofluorescence technique as described for spermatozoa by Lee et al., 1984.

Two of the monoclonal antibodies, anti Head and anti-Tail were collected and stored at -20°C until ready for use.

Preparation of Sperm Cells of other Species

Semen was collected from ram, goat, buck, bull, boar and human. The method of collection for the ram, goat, buck and bull was by the use of artificial vagina, the boar by the gloved hand method, the stallion by the use of polythene nylon as condom and semen from human was donated by volunteers using condoms. The semen collected were kept at 37°C before washing in the laboratory. The semen samples from each species were pooled and centrifuged at 1200g for 5 minutes, resuspended each time in PBS and centrifuged three times. The cells were resuspended at a concentration of 1 x 10^6 cells/µl. The final resuspended sperm cells were used to make smears on clean glass slides. The slides were fixed in methanol for 5 minutes and then incubated with 1% bovine serum albumin (BSA, Sigma) for 2 hours before washing for 15 minutes in PBS.

Incubation with Boar Sperm Monoclonal antibodies

The two boar sperm monoclonal antibodies were applied separately on the sperm slides for each of the species and incubated for 24hrs at 4°C. The slides were washed in PBS for 15 minutes and then incubated with 1:600 FITC conjugated goat-anti-mouse 1gG for 1 hour at 37°C. The slides were later washed in PBS for 15minutes, mounted and observed under fluorescent microscope.

RESULTS

The two Mabs, H (anti-head) and T (anti-tail) as expected reacted with the porcine sperm, but also cross-reacted with the human spermatozoa. The staining of the H was more intense than T. There was no cross-reaction observed with the equine, bovine, ovine and caprine spermatozoa as there was no immunofluorescence observed after the staining in these species.

DISCUSSION

The results demonstrated cross reactivity of boar sperm monoclonal antibodies with human sperm. This suggests that boar sperm antigens and antibodies to them may be candidates for non-steroidal contraception in humans. Steroidal contraceptives have been reported to have possible relationship with the incidence of thromboembolic diseases, including pulmonary embolism and cerebral thrombosis, and cancer of the endometrium (Tatum, 1985). Attempts have been made to get alternative contraceptives that will be safer.

The anti-head mab will probably be useful in the blockade of fertilizing capacity of sperm by causing head to head (H-H) agglutination (Boettcher et. al., 1977) inactivation of acrosomal enzymes like hyahuronidase and acrosin which are involved in sperm penetration of the investment surrounding the egg (Bellve and O'Brien, 1983, Archibong et. al. 1995, Kadam, et. al. 1995). The anti-head antibody may also block binding sites of sperm to egg membranes thereby preventing fertilization (Menge, 1971, Clarke et. al., 1985, O'Rand and Porter, 1979, Castle et. al.

1997). The anti –Tail mab will affect fertility via the inhibition of sperm motility (Methur et. al., 1984, Mathur et. al., 1986). The crossreactivity if further investigated may open the gate for the use of porcine sperm cells or the monoclonal antibodies, when purified to be cheaper because the boar semen can be obtained in large quantity per ejaculate from a large population of swine animals. This will also be safer because it will be devoid of the side effects of steroidal contraceptives mentioned earlier.

In conclusion, it is believed that the porcine semen may become useful in human population control but more work needs to be done to guarantee safety from other unforeseen side effects.

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