Session 10

BASIC RESEARCH

67 SPERM MORPHOLOGY AND VAGINAL SECRETIONS

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This study was designed to evaluate the morphological change in human spermatozoa when mixed with vaginal secretions at different times of the menstrual cycle. Fifty couples were studied. There was no evidence of vaginal infection and all semen samples were within the fertile range. Semen samples were collected twice: once at the mid-proliferative phase and the other at the ovulation time of the female.

Each semen sample at each period of time was divided into three portions: (1) was examined alone, and saved as the control; (2) was mixed with vaginal secretion obtained from the posterior fornix at mid-proliferative phase for 30 minutes prior to examination; and (3) was also mixed with vaginal secretion at the time of ovulation, for the same period prior to examination.

The specimens were prepared according to Hamdey New Technique. All were examined by electron microscopy. The percentage of abnormal forms was recorded as abnormal in the head, midpiece and tail.

68 CORRELATION BETWEEN TRACE ELEMENT OF THE HUMAN SPERM HEAD WITHIN THE FALLOPIAN TUBE AND TUBAL FLUIDS AT THE TIME OF OVULATION

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To demonstrate the dynamic event during the fertilization period, this study was designed to correlate the major trace element in the sperm head found in the fallopian tube during the ovulation period in patients selected for tubal sterilization at this phase, and compared with the same element in the tubal fluid.

Ten couples were selected for study. All had proven fertility. The couples were instructed to have intercourse 6-8 hours prior to the wife's tubal sterilization, preceded by 4 days' abstinence after the husband's semen was analyzed (all were within normal semen parameters).

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All samples were prepared according to Hamdey New Technique for electron microscope microprobe analysis. The average period of menstrual cycle was 28 (±2) days and ovulation period (according to 4 months' basal body temperature) was between days 13-15 of the cycle. Statistical analysis using the t test showed no significant difference in the measurement of Na, Mg, Cl, K, Ca and Cu, while there was significant difference in the measurement of P, S, Cd and Zn.

PROTAMINES, THE PROTEINS ESSENTIAL TO COMPACT THE SPERMATOZOAN NUCLEUS, AS TARGETS FOR MALE CONTRACEPTION

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To produce a functional spermatozoon, the DNA of the nucleus must be properly condensed. This is accomplished towards the end of spermatogenesis by replacing the histones, the structural proteins of the nucleus, with a class of small, highly basic proteins called protamines. The protamines serve to compact and package the DNA of the spermatozoon into a volume estimated to be required by the DNA alone. The resulting DNA-protamine complex serves to stabilize the spermatozoal DNA and provide rigidity for the head of the spermatozoon.

Although all mammalian spermatozoa contain one form of protamine, only the human and mouse have been shown to contain multiple protamine variants (hereafter designated P1 and P2) [Hecht, N.B., (1987), Mammalian Protamines and Their Expression. In Histones and Other Basic Proteins, (Stein, G. and Stein, J. eds.) CRC Press, Boca Raton, Fl., in press]. Using recombinant DNA techniques, we have isolated cDNAs (complementary DNAs) for the two mouse variants. Gene mapping studies of the human and mouse protamines reveal that a gene encoding protamine 1 is located on chromosome 16 in humans. In the mouse, protamine 1 is near the proximal end of chromosome 16 where it is tightly linked to the protamine 2 gene. The protein sequences predicted from DNA sequence analyses reveal the P1 and P2 protamines differ markedly in molecule size and amino acid sequence. Comparison of the human and mouse P1 and P2 variants reveal strongly conserved sequence domains in both proteins suggesting they share a common function in DNA binding and compaction. Moreover, all P1 protamines contain 50 amino acids, whereas the size of the P2 protamine varies. In the mouse, it is synthesized as a precursor molecule of 106 amino acids which is subsequently processed into its mature form of 63 amino acids in the sperm nucleus. In the human the P2 protamine is either 57 or 54 amino acids in length. The requirements for cleavage of the P2 protamine offers a promising intervention site to disrupt sperm formation.

Considering that condensation of the nucleus of the spermatozoon is essential for normal sperm formation, perturbation of the synthesis or processing of the protamines would produce non-functional spermatozoa. Such efforts offer a novel approach to male contraception. (Supported by N.I.H. grant GM 29224).

70 DOES THE MALE ANTIFERTILITY AGENT, TRIPTERYGIUM WILFORDII, INHIBIT RAT SPLEEN CELL-MEDIATED SHEEP RBC HEMOLYSIS?

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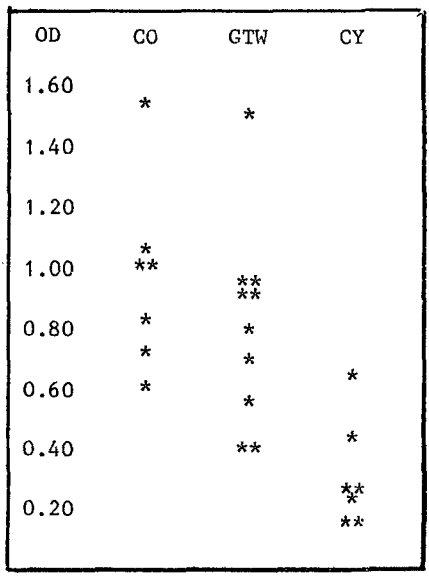
The multi-glycosides of Tripterygium wilfordii Hook.f. (GWT), a quasi-pure mixture extracted from the Chinese herb used for the treatment of rheumatoid arthritis and various skin diseases, was recently shown to possess a male antifertility effect both in rats (Qian S.Z. et al: Contraception 33: 105, 1986) and in humans (Qian S.Z. et al: Adv. Contracep. 2:253, 1986). Preliminary data suggested that the drug might have some immuno-suppressive activity; in these studies, a huge dose (30 mg/kg per day) of the mixture was administered parenterally to the animals; therefore, the results obtained seem to be inconclusive. The present study was designed to clarify whether an antifertility dose of GTW given orally could affect rat spleen cell-mediated sheep RBC hemolysis by means of the quantitative hemolysis spectrophotometry assay.

Adult male SD rats were divided into three groups, i.e., the GTW (n=10), the cyclo-phosphamide (CY, n=7) and the control (CO, n=7). In the GTW group, the drug was administered at a daily dose of 10 mg/kg for 8 weeks, a known antifertility dose for the rats. In the CY group, the vehicle was given for 7 weeks, followed by CY at a dose of 10 mg/kg per day for another week. To the control animals, only the vehicle was given for 8 weeks. All the drugs were administered through gastric gavage. At the end of dosing, fertility tests showed that the GTW group became infertile and both the CY and CO groups remained fertile, while the density and motility of spermatozoa in the cauda epididymis were significantly lower in the GTW than in the other 2 groups ($p<0.01$). The immune rat spleen cells, sheep RBC and 1:10 complement were incubated at 37°C for 1 hour in PBS (pH 7.2). The culture media were centrifuged and the optic density (OD) of the supernatent liquid read at 413 nm. The results indicated that the OD values of GTW [0.77 ± 0.30 (SD)] and CO (1.01 ± 0.36) groups were not significantly different from each other, while the OD value of CY group (0.35 ± 0.16) was significantly lower than those of CO ($p<0.01$) and GTW ($p<0.02$) groups. Moreover, it can be seen from the figure showing the distribution of the OD values that the data of the CO and GTW groups are largely overlapping, while the data of the CY group are essentially separated from those of the other 2 groups.



The authors believe that at the antifertility dose employed in the present experiment, GTW does not significantly inhibit the antibody-forming cells of the spleen.

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