Session 27. Sterility: diagnosis and treatment

155. Intrauterine insemination of mice with spermatozoa coincubated with ureplasma urealyticum and its sterile filtrate - pregnancy rate and litter size

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Ureaplasma urealyticum have been suspected of causing a variety of reproductive disorders and infertility in men. Decreased fertilization rates in mice in vitro and in vivo caused by u. realyticum are well known. The present study was designed to examine the effect of the coincubation of spermatozoa with two stains of u. realyticum of the sterile filtrate of those on consecutive pregnancy rate and litter size. Spermatozoa of 8 - 12 weeks old male mice (CB6 F1) were released from the vas deferens directly in HAM's F10 and allowed to capiciate for 30 min. Subsequently the spermatozoa were coincubated with u. realyticum (10^1, 10^3 cfu/ml), the sterile filtrate of u.urealyticum or HAM's F10 (control group). Before insemination the spermatozoa were washed twice with HAM's F10 to eliminate the germs in germs in suspension. 8 - 12 weeks old female mice (CB6 F1) were super-ovulated by intraperitoneal injection injection of 4 i.u. PMSG followed 47 h later by 4 i.u. hCG. Another 10 - 13 h later the female mice were anesthetized, the body wall was opened by a small incision and intrauterine insemination took place by injecting 15 ul of the coincubated spermatozoa into each uterus horn, using a sterile glass micropipet. 17 days after insemination the animals were killed by cervical dislocation and the uterine horns were examined for pregnancies. The pregnancy rate of the control group was 39.3%, while coincubation with bacteria or sterile filtrate decreased pregnancy rate to 14.3 - 28.6%. In the same manner the litter size worsened from 11.3 embryos/mouse (control group) to 2.0 - 9.1 embryos/mouse. Coincubation of spermatozoa with u. realyticum or its sterile filtrate results in lower pregnancy rate and decreased litter size - compared with the control group.

156. Dinamic: an expert computer system for the diagnosis and management of the infertile couple.

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The methods and definitions used in both male and female infertility work-up are greatly variable among centres, resulting in poor standardization and unacceptable confusion.

Based on the analysis of over 8000 couples, which were systematically investigated using a strict protocol and flow-charts, World Health Organization (HRP) has developed a system for the simplified management of the infertile couple. The resulting definitions, forms and flow-charts were incorporated into a computer programme (DINAMIC) which is an expert system suggesting standard diagnosis, serves as a database, and has major educational and clinical implications.

The programme uses Thoroughbred Basic, runs on any IBM or compatible machine under MS-DOS, is extremely user-friendly and facilitates patient management. Data input on history taking, physical examination, laboratory tests, and additional investigations is continuously checked against acceptable limits, previous inputs and clinical logic. The programme also imposes particular examinations without which no diagnosis can be made; it combines information about both partners to accept particular diagnostic categories, and it explains why a particular diagnosis is acceptable or rejected. The programme generates a structured summary report, permits extensive statistical data analysis, and allows for data-exportation to other programmes such as statistical packages or word-processors.

157. Environmental toxins in cervical mucus can negatively affect sperm-mucus interaction

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Various environmental polutants such as hydrocarbons can be found in human genital fluids, e.g. follicular fluid and seminal plasma. In human cervical mucus we could observe a 10 - 20 fold higher accumulation of different chlorinated hydrocarbons (PCB, HCH, HCB, DDT) compared to follicular fluid and seminal plasma. Furthermore, some of our in vitro experiments show a negative time and dose dependent effect of different chlorinated hydrocarbons, especially PCB, on important sperm functions, e.g. motility, membrane integrity, in concentrations between 1-1000 ng/ml. In this study, the penetration capacity and longevity of human spermatozoa in human cervical mucus was correlated to the concentrations of various chlorinated hydrocarbons in the mucus. 30 samples of cervical mucus were analyzed according to a modified method proposed by the senate commission of the DFG (German Research Society)for residue analysis; (mean, range) DDT derivates (5-130) µg/kg, PCB 54 (32-98) µg/kg. The sperm mucus interaction was investigated using a modified Kremer capillary test, and normozoospermic semen samples (Sperm suspension: 20 Mill/ml in Ham's F10). Furthermore, sperm antibodies in cervical mucus were determined (Biotec, F.R.G.).

Although there were great differences between samples in regard to penetration depth in cervical mucus and longevity of the spermatozoa, the penetration depth as a parameter of a rapid onset of sperm damage was not affected by chlorinated hydrocarbons. The longevity of spermatozoa in cervical mucus was determined after 4-8, 20-24 and 48 hours. At 20-24 and 48 hours a significant dose dependent (total buden of chlorinated hydrocarbons, PCB) reduction of sperm motility in cervical mucus could be observed.

158. Influence of heavy metals on sperm-mucus interaction in vitro

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Although exposure to a variety of heavy metals has been associated with reduced fertility in females, leading to miscarriages and abortions, little work has been done on the effects of potentially toxic chemicals on the male reproductive tract. Therefore the influence of cadmium, copper and mercury on the semen quality and sperm function was studied in 30 different semen samples in each of the test series. Semen was obtained in hospital after 5 days of sexual abstinence, the initial progressive sperm motility ranged from 15% - 60% with a median of 40%. To evaluate sperm functional capacity, the in vitro sperm-cervical mucus penetration test (SCMPT) was used as described previously (Eggert-Kruse et al., Fertil. Steril 51:317, 1989) taking into account the penetration distance, the number of penetrated spermatozoa and the quality and duration of motility. As a penetration medium estrogenized human cervical mucus (CM) of excellent quality was used. To aliquots of 90 µl of each semen specimen were added aliquots of either 10 µl of cadmiumchloride ($CdCL_2$) (concentrations varying from 50 µMolar (M) to 10 mM), copper sulphate (CuSO4) (conc. from 100 nM to 10mM) or mercurychloride ($HgCl_2$) (conc. from 5 µM to 500 µM). To fill the reservoirs of the penetration meters each of the solutions (in phosphate-buffered saline (PBS), pH 7.4 or aqua bidest (A)) was tested in six different concentrations. Untreated semen and semen with PBS or A only served as a control in each penetration test. The SCMPT was evaluated after 30 minutes, 2 h and 6 h incubation at 37°C. With regard to CdCl2, CuSO4 and $HgCl_2$ in all solutions, the quantitative and qualitative penetration of spermatozoa in CM fell sharply during this period, compared with the controls. The effect of the heavy metals was concentration depending and the measurements were highly reproducible. Semen samples with initially reduced functional capacity of spermatozoa were more affected by these chemicals, in particular with regard to the motility grade in CM.

The results of this investigation demonstrate that a) some heavy metals (cadmium, copper and mercury) have a deleterious effect on human sperm in vitro and b) the in vitro sperm-cervical mucus penetration test (SCMPT) using hormonally standardized human CM, is an excellent biological model to study the influence of toxic chemicals on the functional properties of spermatozoa.

159. Perlaparoscopic endotubal sample collection for the detection of silent chlamydia colonization: a study on 100 infertile patients

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Since November 1987 we have prospectively evaluated by laparoscopy with tubal perfusion 100 patients with primary (79 patients) or secondary (21 patients) infertility. All patients were asymptomatic at the time of hospital admission, and at laparoscopy no patient had signs of acute salpingitis.

During laparoscopy samples were collected from the urethra, the cervical canal, the cul-de-sac peritoneal fluid and the endometrium. By means of a specially designed instrument, a sterile cotton tipped swab was introduced into the distal end of both tubes to obtain an endotubal sample. Chlamydia trachomatis (C.t.) was isolated in 5-iodo-2-deoxyuridine treated McCoy cell culture.

C.t. was isolated from at least one site in 18 patients (18%). In 14 patients C.t. was isolated only from the upper genital tract; in 7 patients the tube was the only site from which C.t. could be isolated.

Our results demonstrate that it is necessary to obtain an upper genital tract culture, including an endotubal specimen, for an accurate diagnosis of C.t. infection, and that a cervical culture alone is inadequate.

160. Prediction of ovulation by a quantitative and automated, 30 minutes urinary luteinizing hormone assay. Comparison with an LH detection test.

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Prediction of ovulation is useful in timing an insemination and necessary for oocyte aspiration in a spontaneous cycle. Quantitative or semi-quantitative measurements of urinary LH have been found superior to all other methods used to predict ovulation.

The measurement of LH by the most rapid radioimmunoassays takes at least 3 to 4 hours. This rather long time is a limiting factor to repeated measurements during the same day in the same patient. The purpose of the present study is to evaluate the possibility of adapting to urine samples a rapid and fully automated serum LH assay and to see if urinary LH peaks can be detected in order to predict ovulation.

To this end, 32 spontaneously ovulating women (38 cycles) who requested an artificial insemination were studied by daily serum oestradiol (E2), daily ultrasonography (US) and by 3 times a days urinary LH determinations starting on day 10 of their menstrual cycle. These patients were followed until US signs of follicular rupture were recorded.

In all patients a clear cut LH peak lasted between 12 and 15hr and was followed in 35 cycles (no US available for 3) by follicular rupture which occurred 9 to 51 hr later. The data were grouped according to the time at which the LH peak occurred at day 0. Neither the peak value of LH nor the E2 levels on day 1 were different among the groups.

However, the patients experiencing an LH peak between 3 and 7 am on day O had significantly lower levels of E2 on day 0 as compared to those having their LH peak between 10 and 12 pm. This is due to the fact that the women with an LH peak between 3 and 7 am had already decreasing E2 levels (from day 1 to day 0) whereas those who experienced the LH peak between 10 - 12 pm had still increasing E2 levels in the morning of day 0.

It is concluded that the 30 minutes IMX urinary LH assay is a reliable, rapid and easily acceptable method of ovulation prediction.

161. Reduction of adhesion reformation following adhesiolysis at laparotomy by Interceed® (TC7): final report of the Interceed® (TC7) adhesion barrier study group

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Adhesion reformation following reproductive surgery occurs in the vast majority of women. A preliminary report by this study group of 74 women described reduction of adhesion reformation by the use of INTERCEED® (TC7), a resorbable, biocopatible barrier (Fertil Steril 51:933, 1989). We now present our final data from this prospective, randomized, controlled trial of INTERCEED® (TC7) use in 134 women, as assessed at early second-look laparoscopy (within 14 weeks) following adhesiolysis at laparotomy; All women had bilateral sidewall adhesions lysed at the initial procedure after recording the extend and type of adhesions present. Just prior to closure, the sidewalls were randomly assigned to placement or no placement of INTERCEED® (TC7); thus each woman served as her own control. Prior to replacement, meticulous hemostasis was observed. At the second look, the same sidewall areas were again scored fro incidence, extent, and severety of adhesions. Sixty-eight INTERCEED ® (TC7) treated sidewalls were totally free of adhesions, as compared to only 32 control sidewalls ($p < 0.0001$). Surgical treatment alone on the control side resulted in reduction of the sidewall area involved in adhesions from 9.2 ± 0.7 to 3.6 ± 0.4 cm^2, a decrease of 76%. Direct comparison thus demonstrates a significantly greater 36% reduction on the INTERCEED® (TC7) treated sidewall ($p < 0.0001$). A specific reduction in ovary to sidewall adhesions on the INTERCEED® (TC7) treated side was also noted ($p < 0.0001$). No complications attributable to INTERCEED® (TC7) were identified. We conclude that INTERCEED® (TC7), when used in combination with meticulous gynaecologic surgery, significantly reduced adhesion reformation to a greater extent than is achievable with state of the art gynaecologic surgery alone.

162. Spontaneous abortion risk in women with recurrent spontaneous abortion after normal first-trimester ultrasound examination

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Several studies have been conducted to ascertain the art of spontaneous abortion in pregnancies thought to be normal by ultrasound assessment in the first trimester. This rate has been reported to be 2 - 3 %. Epidemiological studies of women with a history of recurrent spontaneous abortion (RSA) indicate that the rate of abortion in subsequent pregnancies is higher than the rate in the first pregnancy. The aim of this study was to determine the spontaneous abortion (SA) rate in pregnancy thought to be normal by ultrasound assessment in the first trimester in women with RSA (≥ 2 previous SA).

After serological confirmation, each pregnancy was followed by serial ultrasound examinations every 1 - 2 weeks (transvaginally before 8 weeks and usually transabdominally thereafter). Spontaneous abortions were confirmed by ultrasound evidence of missed abortion or fetal demise before 20 completed weeks of pregnancy. Thereafter was defined as being ongoing.

Ninety-five patients were studied and 26 (27%) had spontaneous abortion. Fetal cardiac activity (FH) was defined at least once in 85 patients and 16 (19%) underwent spontaneous abortion. The risk of spontaneous abortion with respect to gestational age when FH was detected is shown below.

Gest. age (days)	36-42	43-49	50-56	57-63	64-70	71-77	≥78
Risk of SA (%)	21	16	15	13	8	3	0

Not until ≥ 71 days gestation does the risk become similar to that in the general population. A substantial risk of SA is present in women with RSA even when fetal cardiac activity is detected in early pregnancy.

Session 28. Endometrium implantation

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163. Culture and characterization of cells from endometrium and endometriosis

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Endometriosis, the occurrence of endometrial tissue in extra-uterine sites, is frequently associated with infertility and pain, although asymptomatic disease is a common finding at laparoscopy. The aetiology of endometriosis remains poorly understood. Some insight into this may be gained by elucidating the properties of cells isolated from endometriosis. We report an experimental protocol for the culture of cells from both endometrium and endometriosis.

Samples of endometriosis obtained at laparotomy and endometrium from patients undergoing gynaecological surgery were digested in collagenase. The resulting suspension was filtered through a fine mesh, separating the tissue into intact glands which were retained on the filter and a suspension of single stromal cells. Red blood cells were removed by centrifugation on Percoll. The isolated glandular and stromal fractions were cultured in a medium used by Smith and Kelly (1987) for glandular cells from human endometrium, consisting of a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12, buffered with Hepes and supplemented with glutamine, insulin, amino acids, antibiotics and the serum substitute Ultroser G.

Endometriotic cells had a similar morphology to stromal and epithelial cells derived from normal endometrium. In addition, the nature of cells in cultures of endometrium and endometriosis was further defined by the use of cytoskeleton component-specific monoclonal antibodies in indirect immunofluorescence. Endometrial stromal cells were found to contain vimentin, characteristic of mesodermal cells, while glandular epithelial cells contained cytokeratin. Such staining patterns were also observed