VII WORLD CONGRESS ON FERTILITY AND STERILITY

Tokyo/Kyoto 

17-25 October, 1971

MAIN SESSIONS (FREE COMMUNICATIONS)

VII WORLD CONGRESS ON FERTILITY AND STERILITY

17-25 October, 1971, Tokyo/Kyoto

ABSTRACTS OF MAIN SESSIONS
(FREE COMMUNICATIONS)

Editors
R. KLEINMAN
London
V. R. PICKLES
Cardiff

EXCERPTA MEDICA

INTERNATIONAL FEDERATION OF FERTILITY SOCIETIES

President:	Dr. H. DE WATTEVILLE (Switzerland)
Vice-President:	DR. A. CAMPOS DA PAZ (Brazil)
Secretary-General:	DR. G. I. M. SWYER (United Kingdom)
Associate Secretaries:	DR. R. PALMER (France) DR. A. F. MENDIZABAL (Argentina)
Treasurer:	DR. A. INGELMAN-SUNDBERG (Sweden)

VII WORLD CONGRESS ON FERTILITY AND STERILITY

ORGANIZING COMMITTEE

Former President:	DR. K. ANDO (deceased)
Honorary Presidents:	DR. K. OGINO DR. A. TAKAHASHI DR. T. HIROSE (deceased) DR. Z. YAMANE
President:	DR. T. HASEGAWA
Vice-Presidents:	DR. H. FUJIMORI DR. T. ICHIKAWA DR. Y. NISHIKAWA
Secretary-General:	DR. M. HAYASHI
Chairman of the Scientific Program Committee:	DR. T. KOBAYASHI
Treasurer:	DR. S. MATSUMOTO
President of the XVIth Annual Meeting of the Japanese Society on Fertility and Sterility:	DR. Y. NOTAKE

SCIENTIFIC PROGRAM COMMITTEE

Chairman: DR. I. HALBRECHT (Israel)

Members:

DR. A. ALVAREZ-BRAVO (Mexico)
DR. S. J. BEHRMAN (U.S.A.)
DR. R. CALDEYRO-BARCIA (Uruguay)
DR. A. CAMPOS DA PAZ (Brazil)
DR. E. M. COUTINHO (Brazil)
DR. H. DE WATTEVILLE (Switzerland)
DR. E. DICZFALUSY (Sweden)
DR. R. G. EDWARDS (United Kingdom)
DR. J. FÉRIN (Belgium)
DR. H. FUJIMORI (Japan)
DR. L. GEDDA (Italy)
DR. E. HAFEZ (U.S.A.)
DR. T. HASEGAWA (Japan)
DR. M. HAYASHI (Japan)
DR. T. ICHIKAWA (Japan)
DR. A. INGELMAN-SUNDBERG (Sweden)
DR. C. L. JHAVERI (India)
DR. T. KOBAYASHI (Japan)
DR. R. E. MANCINI (Argentina)
DR. L. MASTROIANNI (U.S.A.)
DR. A. F. MENDIZABAL (Argentina)
DR. Y. NISHIKAWA (Japan)
DR. R. PALMER (France)
DR. M. ROLAND (U.S.A.)
DR. S. S. RAO (India)
DR. G. SEEGER-JONES (U.S.A.)
DR. S. SEGAL (U.S.A.)
DR. K. SEMM (Germany)
DR. A. SOUTHAM (U.S.A.)
DR. H. C. TAYLOR (U.S.A.)
DR. S. VAN RENSBURG (South Africa)
DR. B. B. WEINSTEIN (U.S.A.)

INDEX OF AUTHORS

Figures refer to abstract numbers

CONTENTS

MAIN SESSIONS (FREE COMMUNICATIONS)

MAIN SESSIONS

(Free Communications)

Chapter 1

Sperm, semen

(Abstracts 1—29)

1. Organ culture of testis

Y. KUMAMOTO, *Sapporo, Japan*

The author studied the organ culture of human and rat testis. Spermatids and spermatocytes degenerated after 2 wk. But, even after 2-mth culture, the Sertoli cells and spermatogonia were still alive in the tissue. There was no notable difference in the cell survival period of human and rat testis tissue.

2. Morphogenesis of fetal gonads in mammals

K. BASRUR, *Guelph, Ontario, Canada*

Histochemical and ultrastructural studies were carried out on the gonadal cells of human and bovine fetuses of karyotypically confirmed sex, to determine the sequential changes during the onset of morphological and functional dimorphism in developing gonads.

17 human fetuses ranging in age from 6 to 21 wk post fertilization and 25 bovine fetuses ranging from approximately 6 to 24 wk were included in this study. Presence of hydroxysteroid dehydrogenase enzymes indicative of steroidogenesis was detectable in human fetuses of both sexes just over 6 wk old. Fetal human testes revealed a progressive increase in steroid producing cells between 7 and 10 wk while fetal ovaries of corresponding age showed an increase in number and size of the germ cells, some of which were in meiosis in 8-wk-old fetuses. Steroidogenesis in bovine testes was detected in 6- to 8-wk-old fetuses while it was not detectable at all in any of the bovine fetal ovaries included in this study. Synchronized meiosis in clusters of germ cells connected by intercellular bridges was detectable in bovine ovaries at the same gestational stage as in humans. The retarded steroidogenesis and precocious meiosis of bovine fetal ovaries may be causally related to the sterility generally noted in the genetic female born twin to a male in cattle while such heterosexual twins in humans develop normally.

3. Biochemical studies on human seminal plasma

R. NATH, *Chandigarh, India*

Human seminal plasma (412) has been analysed for sperm count, motility, protein, fructose and PPD-oxidase activity. Acid phosphatase and lactate dehydrogenase have also been estimated in 42 and 52 samples, respectively.

The sperm count showed 48% samples with 60,000,000/ml. Dead sperm (18%) was only noticed when sperm density was low (1,000,000-20,000,000/ml). Low motility (< 30%) was noticed in 72-80% of semen samples with a sperm density below 40,000,000/ml. There was a wide variation in the total range of the content of protein (1.2-7.6 g/100 ml), fructose and PPD-oxidase (0-90 U/10 mg protein). The average values (mean $\pm$ SE) however showed a good correlation and narrow range of variation, *i.e.* protein (3.4 $\pm$ 0.10 to 4.5 $\pm$ 0.12 g/100 ml), fructose (214 $\pm$ 0.16 to 303 $\pm$ 16 mg/100 ml) and PPD-oxidase (13 $\pm$ 1.4 to 15 $\pm$ 0.5 U/10 mg protein). In 10% of azoospermic semen and 8% of normospermic semen, high PPD-oxidase values (> 40 U) were obtained. It has been suggested that this may be due to either an inflammatory process or some infection.

The above investigations indicate that PPD-oxidase may be of value as a diagnostic aid in distinguishing the cases of sterility due to infection or to other acute pathology.

4. Separation of a substance containing ribulose in human seminal fluid

S. NAGAOKA, M. HAYASHI, H. AMANO and I. YANAGISAWA, *Tokyo, Japan*

From the standpoint of comparative biochemistry, human seminal fluid was studied in order to examine whether it has the substance containing ribulose which has been recently found in human follicular fluid.

Isolation by ion exchange column chromatography was carried out as described previously (Amano *et al.* (1969): In: *Abstracts, II Annual Meeting of the S.S.R.*). A substance containing pentose was found to be eluated in tubes 12 to 17; this was identical with that in human follicular fluid. This eluate was kept in a cold room until precipitation occurred by adding 15 volumes of cold acetone (Yanagisawa *et al.* (1964): In: *Abstracts, VI International Congress of Biochemistry*, p. 189). The reprecipitant was found to be a single component when examined with the analytical ultracentrifuge by means of synthetic boundary cells (59430 rpm). Sedimentation constant was 0.8. The sugar component in the reprecipitant was identified as ribulose by paper chromatography, color reaction, absorption spectra of reaction products in the orcinol and enzymatic analysis. Amino acids were detected in the acid hydrolysate of the reprecipitant; these were lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine and leucine.

5. Ornithine cycle and spermatogenesis

J. TANIMURA, *Osaka, Japan*

The free arginine and bound arginine content of normal and subnormal semen were determined by Pilsum's modification of Sakaguchi's reaction. A positive correlation was observed between the sperm count and the bound arginine content, and between sperm motility and the free arginine content. From these findings it may be suggested that arginine may have a certain physiological significance in spermatogenesis and may be closely related to sperm motility.

Patients, mainly with oligozoospermia, were treated with drugs which were supposed to participate in the ornithine cycle. The groups of patients each treated with citrulline, ATP, or arginine showed a tendency towards an increased sperm count with an improved motility rate after administration. The group of patients treated with aspartic acid showed a tendency to a decrease in their sperm count and motility rate. All the patients with oligozoospermia treated with ornithine showed a decreased sperm count and a lowered motility rate. Some of the patients with a normal count had no change or increase in their sperm count after administration, some had a temporary reduction which was improved by continued medication, and the rest had a continuous reduction after administration.

6. Deoxyribonucleoprotein changes during spermiogenesis

N. R. RINGERTZ, *Stockholm, Sweden*

Condensation of chromatin during differentiation of spermatids into spermatozoa in bulls is associated with drastic changes in the physical and chemical properties of the deoxyribonucleoprotein complex (DNP).

By applying quantitative cytochemical and biophysical methods, *e.g.* microspectrophotometry, microfluorimetry and autoradiography on individual cells, the author has demonstrated a decrease in the number of free DNA-PO_4 groups capable of binding cationic dyes, an increased stability of DNA to heat denaturation and acid hydrolysis and a decreased capacity of DNP to bind ^{3}H-actinomycin D. These changes in the properties of the DNA component in DNP are paralleled by modifications in the properties of the protein part: the basicity of the DNA-bound proteins increases as measured by the alkaline bromphenol blue method and an increase in protein-bound arginine (Sakaguchi reaction).

It appears therefore that also in higher species the somatic histones are replaced by more basic and arginine-rich proteins. It is possible that the DNP-changes described form the molecular basis for the condensation of chromatin and the inactivation of the genome. Certain forms of infertility in bulls have been found to be associated with defective or incomplete DNP-changes.

7. Studies on fibrinolytic activity in human semen and cervical mucus

H. OKAZAKI and Y. HATA, *Morioka, Japan*

Fibrinolytic activity in the human semen and the cervical mucus has been studied by means

of the fibrin plate method, and the results were as follows: A scanty amount of plasmin and plasminogen was found in the semen, which contained a considerable amount of proactivator and activator, showing their highest values at the presumed time of ovulation. In the cervical mucus after the postcoital test (Huhner), plasmin activity and proactivator value were increased as compared with those of the cervical mucus alone. The 1:1 mixture of semen and cervical mucus in the Miller-Kurzrok positive cases showed a marked acceleration in the fibrinolytic activity above the sum of each activity of the semen and the cervical mucus separately, while the plasmin value in the Miller-Kurzrok negative cases was low.

From the above-mentioned findings it may be surmised that fibrinolysis increases the liquidity of the cervical mucus and its fibrinolytic activity accelerated by mixing with the semen promotes the liquefaction of the cervical mucus as well as the motility of the spermatozoa. Thus the penetration of the spermatozoa through the cervical mucus may be facilitated.

8. Sperm plasma proteins and sperm fertilizing ability

S. D. DINULOVIĆ, *Belgrade, Yugoslavia*

Proteins of seminal plasma were separated into 9-11 compounds, whose relative percentage varied, subject to sample, by electrophoresis in starch gel which was preliminarily hydrolysed. It was also found that the pre-albuminal group is composed of 1 to 2 components and that the group which in blood serum would correspond to the globulin group, is composed of important and less important sub-groups (α-, β-, and γ-globulin region) as well as non-migrating proteins.

A gel in a form of transparent film whose values are quantitatively determined in densitometric or in automatic registers, has been obtained by a special method of enlightening and plastification of gels. Comparison of values of total protein of seminal plasma in normal and pathological spermogram and blood serum as well as the reciprocal relation of some components on 113 patients were carried out by this method.

No quantitative relation has been established between total protein in blood and seminal plasma, while some components represent more reflection of the physio-pathological state of accessory glands. On the basis of those indicators, in distinction from some amino acids, fertilizing ability of sperm cannot be completely determined.

9. The metal (sulphide silver method) pattern of human spermatozoa

K. BOSTRÖM, *Lund, Sweden*

The sulphide silver method is regarded as the most sensitive for histochemical demonstration of metals. Treatment of tissue with hydrogen sulphide precipitates metals as sulphides. In the further so-called physical development these metal sulphides act like 'germs'. The resulting silver precipitates denote the localization of substances precipitable with sulphide ions.

Human seminal spermatozoa were treated by the sulphide silver method. Silver precipitates were found in the head, middle piece and tail. In the middle piece of the spermatozoa silver deposits were arranged in transverse or oblique bands. The number of bands varied as well as their arrangement. The spermatozoa were classified as regular or irregular according to the appearance of the pattern of the middle piece. Spermatozoa whose middle piece showed 5-9 transverse or oblique, fairly regularly arranged bands were regarded as normal. It seems possible that the band-shaped pattern in the middle piece is identical with the spiral structure previously discussed by many light-microscopists and considered to be of mitochondrial origin.

The sulphide silver pattern was regular in, on the average, 58% of all normally outlined spermatozoa. The sulphide silver method thus offers a new possibility of differentiating spermatozoa. A significant correlation was found between the percentage of spermatozoa with a regular sulphide silver pattern and the percentage of motile spermatozoa.

10. Actions of ions and drugs involved in muscular contractility as they affect the motility of human spermatozoa

A. BONDANI and E. AZPEITIA, *Mexico, D.F., Mexico*

The detailed mechanism of spermatozoa motility is unknown. However, it has some biochemical similarities with muscular contraction.

The migration distance of human spermatozoa was measured at different intervals in capillary tubes filled with several solutions. Spermatozoal respiration was quantified by a Clark's electrode. Ca^{++} was found indispensable for spermatozoal motility since EDTA (5×10^{-3} M) inhibited the spermatozoal motion (SM) and this inhibition was counteracted by the addition of Ca^{++} (5×10^{-3} M). Ca^{++} and Mg^{++} in high concentrations (1×10^{-1} M) decreased SM but the same concentrations of Na^{+} and K^{+} failed to do so. Caffeine and ouabain (5×10^{-2} M) did not alter SM, but tetracaine (1×10^{-3} M), procaine (1×10^{-2} M) and quinidine (5×10^{-3} M) decreased it without any change in respiration. This effect was potentiated by Ca (5×10^{-3} M). The active form of these compounds is the non-ionized form. Since benzocaine (2×10^{-3} M) was inactive, the molecular moiety responsible for these effects appears to be the tertiary amine.

The results indicate that drugs which have been shown to alter muscular contraction also influence spermatozoal motility by a possible common mechanism of action, *i.e.* an alteration of the state of intracellular Ca^{++}.

11. Acetylcholinesterase activity in vertebrate spermatozoa

N. R. KALLA, *Chandigarh, India*

Human, bull, rat, parrot and frog spermatozoa fixed in formalin and formal-calcium acetate were studied for acetylcholinesterase activity. In all the species activity was localized in the middle piece of the spermatozoa. Human and parrot spermatozoa gave a very faint reaction. Spermatozoa from cadmium chloride treated rat testis did not give any reaction. Acetylcholinesterase activity was considerably reduced in stored bull and human spermatozoa.

None of the forms indicate the localization of acetylcholinesterase activity in the head of the spermatozoa. However, in mechanically separated spermatozoa acetylcholinesterase activity is seen at the base of the head. Localization of phospholipids and choline-containing phospholipids in the middle piece of the spermatozoa suggest that acetylcholinesterase is concerned with the metabolism of the choline-containing lipids.

Phospholipid metabolism and acetylcholinesterase activity in α-chlorohydrin treated rat testis will also be presented to throw some light on the *modus operandi* of α-chlorohydrin as a male sterilant.

12. Prostate function and fertility

B. FREDRICSSON, *Stockholm, Sweden*

In 122 infertile couples the hysbands were examined with respect to prostate function as shown by clinical evaluation and seminal acid phosphatase activity. Pregnancies were recorded by follow-up.

The husbands all claimed themselves to be healthy and denied symptoms from the urogenital tract. On massage of the prostate gland, however, material was obtained containing leucocytes in an amount indicating infection in 21% of the cases. Subnormal levels of seminal acid phosphatase were found in 25% of these. Of those without clinical signs of prostate affection, 23% had subnormal acid phosphatase levels.

The pregnancy rates were calculated for those 77 couples where no disorder could be found explaining the infertility. Among these the groups with symptom-free prostatitis and clinically unaffected prostates showed identical pregnancy rates.

Thus, as long as infections of the prostate do not give symptoms, they do not significantly affect prospective fertility.

13. Further studies in human prostatic maltase and its application

S. RAO, K. P. GUNAGA, A. R. SHETH and D. S. PARDANANI, *Bombay, India*

Previous work by the authors established that human semen contains maltase. Based on studies with split ejaculates, the prostate was considered to be the source of the enzyme. This assumption was demonstrated to be correct based on a large number of experiments carried out with human prostates. A bioassay procedure was later developed to estimate the androgenic potency of chemical compounds using maltase activity of the dorsolateral prostate of the rat as control. The assay was also employed to evaluate androgenic function in man.

Administration of testosterone enhanced the activity of maltase in semen with a con-

comitant increase in protein. The results confirmed our earlier postulate that androgenic function in the human male is reflected in the maltase activity present in seminal plasma.

These studies further indicate that maltase and phosphatase levels in prostatic secretion are significantly lowered in cases of benign hypertrophy of the prostate as compared with the normal.

14. The correlation between sperm-cell density, mobility and malformation rate and their clinical significance

H. J. HEITE, *Freiburg i. Br., F.R.G.*

On the basis of several thousand spermiograms, correlations were calculated between sperm-cell density, relative numbers of mobile sperm cells, malformation rate and other parameters. The clinical significance is explained.

15. The effect of gonadotropins and an androgen on the spermatogenesis of rats tested by continuous semen analysis

J. MAUSS and W. GERKMANN, *Essen, F.R.G.*

Wistar albino rats with their coagulating glands and seminal vesicles surgically removed were electroejaculated. The copulation plug was dissolved in 1% α-chymotrypsin to make counting of the spermatozoa possible. Heat exposure of both testes in a water bath (41 °C, 27 min) caused a decline of spermatozoa counts to nearly 0 in 30 days. Because of spontaneous regeneration of spermatogenesis, about 70 days later spermatozoa output reached the counts before heat exposure.

Groups of 5 animals were treated daily from the 16th to the 44th day after heat exposure of both testes with 1 mg mesterolone s.c., 500 IU PMS s.c., 30 IU HCG s.c. and 1 mg mesterolone together with 500 IU PMS s.c. Spermatozoa output was evaluated every 2nd or 3rd day.

This treatment could not alter the decline of spermatozoa output significantly after heat exposure of both testes in comparison to untreated control animals. Neither could it accelerate the spontaneous regeneration of spermatogenesis following the decline of spermatozoa counts. The group treated with 1 mg mesterolone together with 500 IU PMS s.c. even showed a significant inhibition of spermatozoa output through the spontaneous regeneration phase.

16. Semen and pregnancy responses in the treatment of oligospermia with varying dosages of gonadotrophins

J. A. EPSTEIN, A. J. SOBRERO and S. BICHACHO, *New York, N.Y., U.S.A.*

Husbands, in whom oligospermia was a significant factor in 60 couples' infertility problems, were treated with gonadotrophins in varying doses after physical examination, biochemical and hormonal evaluation, and 3 or more pretreatment semen analyses.

Group A consisted of 22 men who received 4000-5000 IU of human chorionic gonadotrophin (HCG) and 750 IU of pregnant mares' serum (PMS) twice a week for 6-10 wk; Group B comprised 8 men who received the same dose of HCG alone for 10-12 wk; Group C were 30 men who received 10,000 IU of HCG twice a week for 10 wk. Semen improvement and pregnancy incidence were analyzed in each group and were correlated with testicular biopsy findings, presence of varicoceles, and presence of associated fertility problems in the spouses.

Semen quality improved in 27% of the men in Groups A and B and in 20% in Group C. Pregnancy occurred in 5 of 8 (62.5%) of the A and B cases with improved semen, and in 8 of 30 men in these combined groups (27%). There were 9 pregnancies by the 30 men in Group C (30%) with 2 pregnancies having been produced from among the 6 cases with improvement in semen quality. 29 wives of the 60 couples had associated fertility problems, half of which were classified as minor.

17. Seasonal variation in the semen characters of buffalo

M. R. SHALASH, *Lusaka, Republic of Zambia*

The reproductive patterns of seasonal fluctuations in fertility of continuous breeders should be thoroughly understood for each species and class for an efficient breeding program.

Semen characters of buffalo according to the season of the year under Egyptian conditions were studied in 720 samples from 5 buffalo bulls, aged between 2.5 and 10 yr. The semen of a

normal buffalo bull is opaque, whitish or whitish-blue, and milky or milky-creamy in consistency. The average volume of ejaculate examined is 3.8 ± 1.4 ml. Variation in volume between ejaculates and seasons is highly significant. The motility of spermatozoa is usually high, the average percentage being 79.08 ± 6.91. Seasonal variation in the motility rate is highly significant. The average sperm concentration is $1898.33 \times 10^6 \pm 1164.55 \times 10^6$ sperms/cm^3, highly influenced by ejaculate and season. The average percentage of living sperm is 71.26 ± 5.75, the difference between ejaculates and seasons being highly significant. The presence of 6.06 ± 1.71% abnormal spermatozoa in buffalo semen is considered normal, but variation occurs between ejaculates and season. The pH of buffalo semen is 6.84 ± 0.4, which indicates its acidic nature. However, this varies with the seasons.

These results show that the physiological characters of buffalo semen are much as those reported by Mahmoud (1952), Hafez and Darwish (1956), and Oloufa *et al.* (1959). They also indicate that semen characters are much affected by the month and season of the year. The quality of semen is lower in summer. This can be attributed to variation in temperature, humidity, light and foodstuffs in different seasons.

18. Problems of spermatogenesis following testicular injury (clinical and experimental)

Y. MATSUMOTO and M. WAKU, *Tokyo, Japan*

The etiology, symptoms, histological findings and treatment of traumatic testis are well known, but very little is known about follow-up studies of ruptured testicles, especially with regard to spermatogenesis.

In the authors' clinic, there were 14 cases of traumatic rupture of the testicle during the past 10 yr. One wk to 18 mth following surgery, postoperative study of 5 out of 6 cases which were operated on by closure of the tunica albuginea was carried out. Physical examination revealed that softness and slight atrophy of the testicle were noted, with normal testicular sensation. 3 of the 5 cases were examined for sperm count and 1 case demonstrated normospermia 1 wk postoperatively, but motility decreased to less than 40%. The other 2 revealed oligozoospermia and azoospermia. In 1 case which showed azoospermia, follow-up biopsy was performed and demonstrated arrest of spermatogenesis, not only on the affected side, but also on the unaffected testicle. These conditions would suggest the presence of an immunological suppressive effect. On the other hand, histological studies of the experimentally ruptured testicles of rats and dogs were done at various times following the injury.

The histological findings showed that bleeding and tissue damage just after the injury, resulted in necrosis, edema and atrophy in both the seminiferous tubules and the interstitial tissue with Leydig cells.

19. Enzymohistochemical investigations on various testicular disorders

G. BREITENECKER, W. LUDVIK and G. LUNGLMAYR, *Vienna, Austria*

Enzymohistochemical investigations (determination of alkaline phosphatase, acid-phosphatase, ATPase, DPNH-diaphorase, succinic dehydrogenase, lactic dehydrogenase, glucose-6-phosphate dehydrogenase, 3 β-ol steroid dehydrogenase, primary and secondary alcohol dehydrogenase) were carried out on the testes of 30 infertile men showing various testicular disorders without hormonal alterations. Further studies were performed on experimentally damaged testes of Wistar rats.

The studies of the human testes revealed evidence of a marked reduction of alkaline phosphatase reaction in the germ cells in cases of spermatogenetic arrest and severe hypospermatogenesis. In germ cell aplasia, no alkaline phosphatase activity was observed in the remaining Sertoli cells. The enzyme reactions in the Leydig cells were normal in all cases. Similar enzyme alterations were found in the testes of Wistar rats after experimental cryptorchidism and nitrofurantoin intoxication.

Pathogenetic mechanisms are discussed.

20. Kinetics of prespermatogenesis of the Wistar rat under pathological conditions

W. HILSCHER, *Dusseldorf, F.R.G.*

The effect of the alkylating drugs procarbazine and Myleran on pre- and postnatal 'prespermatogenesis' was studied in 4 groups of Wistar rats. In 2 groups of pregnant rats the cytostatic drugs were given on the 14th, 15th, 16th, 17th, 18th and 19th days of pregnancy by intraperitoneal injection in a single dose. The testes of the born male rats were studied on the

1st and 5th days after birth. In 2 other groups procarbazine or Myleran were given to immature male rats on the 1st, 2nd, 3rd, 4th and 5th days after birth. The testes of these animals were studied from the 1st up to the 50th day after birth.

It was observed that gonocytes are sensitive to both the cytostatic drugs, whereas the supporting cells are not so vulnerable. Also, Myleran is more toxic to the gonocytes than procarbazine. Death and degeneration of gonocytes occurred primarily as the cells were approaching their mitotic activity.

21. The effects of radiation (X-, γ-, neutron-rays) on male fertility in the mouse, domestic fowl and Drosophila

T. KASHIWABARA, R. TANAKA and C. STERN[1], *Tsuchiura, Japan and* [1]*Berkeley, Calif., U.S.A.*

The purpose of this report is to study male sterility after radiation of 3 different kinds, in regard to dose rate, total dose, days after irradiation and the sensitivity of the animal species. It is surprising that there was no temporary sterility of the mouse after the pile irradiation (JRR-1: neutron flux density about 20×10^{10} n/cm^2, γ-dose 900 R). The male fowl was temporarily sterile from the 1st to the 120th day after irradiation with ^{60}Co (1.500 R: 16.3 R/day), but not with 700 R of X-rays (100 R/5.6 min). In another experiment with male mice and domestic fowls, only the decline of fertility was observed.

The weight of the testes decreased by 30% during 30 days and by 60% in another 60 days after irradiation. In an extreme case the testicular weight of irradiated fowl (2600 R : 66 R/day) decreased by 90% over 40 days. In Drosophila, the testis size was reduced by about 30% after the 4th day of γ- or neutron-irradiation, but the X-ray irradiated testis was the same as the control.

It may be concluded that the reduction of the size of the irradiated testes is one of biodosimetry and the indicator of male fertility in these species.

22. Radiation-induced sterilization in the mature male Heteropneustes fossilis (Bloch)

S. K. RATHI, *Kota, Rajasthan, India*

Indian catfish, *Heteropneustes fossilis* (Bloch) were internally irradiated with 5 μc or 10 μc of radiophosphorus (^{32}P). Histological changes were studied at various intervals up to 8 wk.

There was no change in the control testes. But internally irradiated testes developed various radiolesions. The important radiopathological changes were pyknosis, karyolysis, fragmentation, vacuolation in the nuclei of resting cells and spermatogonial cells, cytoplasmic vacuolation, appearance of abnormal forms like giant cells, binuclear cells and multinuclear cells. Spermatocytes and spermatids also exhibited lesions. The infiltration of lymphocytes and fibroblasts led to phagocytosis. In early stages hyperemia, edema and hyalinization were observed. Fish treated with 5 μc were found completely devoid of germ cells after 8 wk, whereas 10 μc ^{32}P treated testes were completely sterilized after 6 wk.

It is concluded that sterilization may be due to the direct effect of radiation on testes and partly due to an indirect effect through the pituitary gland.

23. Temporary sterilization of a male dog by an estrogen

T. IMORI, R. SHIMIZU, S. NAKAMA, K. NOMURA, S. NIIYA, T. SAKURADA and H. MIYATA, *Osaka, Japan*

Hexestrol dicaprylate (H_8), a long-acting estrogen, was used in an attempt at temporary sterilization of an adult male dog. The method of a single injection of H_8 in oil into the muscle of the dog was taken up in each animal, and the doses, 0.05 to 2.0 mg/kg body weight were tried. The morphological and histological changes in the testis, and androgen content of the testis were examined after injection.

In cases of 1.0 and 2.0 mg/kg dose levels, spermatozoa in each seminiferous tubule disappeared within 2 to 3 wk, and this seemed to continue over 150 days. In 1 dog treated with 1.0 mg/kg, spermatozoa were not found in the testis on day 270, but were found on day 300. Testosterone and androstenedione were assayed in H_8-treated and non-treated dog's testicles by GLC. The content of both androgens decreased markedly within 48 hr after injection, and

undetectable amounts were assayed in the 72-hr samples. Up to 60 days after injection, no detectable amount of both androgens was assayed.

This estrogen may probably be safely used for temporary sterilization of male dogs for about 6 mth or a little longer, at the 1.0 to 2.0 mg/kg level.

24. Estrone, estradiol 17β and estriol in seminal fluid of man in normal and pathological conditions

R. SCHOLLER, J. GRENIER, J. CASTANIER and Y. TEA, *Paris, France*

Estrone, estradiol 17β, and estriol have been estimated in total sperm and seminal fluid in 100 normal men and subjects in whom a suspicion of infertility existed. The levels of these estrogens in the different groups are compared.

25. Effect of tetracycline on metabolism of human spermatozoa

G. S. BERNSTEIN and T. K. YU, *Los Angeles, Calif., U.S.A.*

Tetracycline has been used as a fluorescent marker for spermatozoa in studies of sperm capacitation and of sperm migration through the female reproductive tract. Since the influence of tetracycline-HCl on sperm metabolism has not been evaluated the authors have conducted studies of the effect of this antibiotic on washed human spermatozoa. The concentration of tetracycline-HCl was 200 μg per 10^8 sperm.

Tetracycline has an immediate inhibitory effect on oxygen uptake as measured with the oxygen electrode. This effect is transient and is not related to an inhibition of sperm motility. In contrast, seminal plasma rapidly oxidizes tetracycline. The antibiotic depresses sperm motility only after 6 to 12 hr of treatment. There is a progressive decrease in oxygen consumption and anerobic fructolysis as motility declines in the presence of tetracycline.

The results indicate that tetracycline does alter sperm metabolism. The most marked effect occurs after prolonged incubation and is associated with the inhibition of sperm motility.

26. Role for the cumulus cell in sperm capacitation

R. B. L. GWATKIN, *West Point, Pa., U.S.A.*

The manner in which sperm undergo capacitation as they pass through the cumulus oophorus was studied *in vitro*.

Epididymal sperm of the Golden Hamster were observed to attach to, and penetrate, the cumulus cells. This interaction, which capacitated the sperm, required the presence of a dialyzable heat-stable factor present in the cumulus matrix. Treatment of cumulus cells with trypsin or β-galactosidase had relatively little effect on sperm capacitation, but exposure of the cells to neuraminidase blocked capacitation completely. Electron microscopy showed that the sperm passed through the cytoplasm of the cumulus cell, losing their plasma and outer acrosomal membranes in the process.

Attachment of sperm to cumulus cells was also observed to occur *in vivo*. These experiments showed for the first time that the cumulus cell plays a key role in the capacitation process.

27. The acrosome of the human spermatozoon. Morphology and enzyme content

H. PEDERSEN and H. PAULUS, *Boston, Mass., U.S.A.*

The limiting membrane of the acrosome displays regional morphological differences. In the equatorial segment it has a 5-layered appearance due to a thin dense zone apposed to the inner aspect of the unit membrane. The same 5-layered appearance is seen in localized areas of the inner limiting membrane of the anterior segment of the acrosome, but most of this membrane and all the outer membrane of this segment has the 3-layered unit membrane structure. In freeze-etched preparations the surface of the acrosome displays a distinctive difference between the smooth equatorial segment and the slightly irregular, finely granulated anterior segment.

A method involving thorough washing and rapid freeze-thawing has been devised for isolation of the acrosome material, and as judged from electron microscopical control it