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Abramson Cancer Center of the University of Pennsylvania

The Reproductive Needs of the Cancer Patient

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THE SCOPE OF THE PROBLEM

In the USA there are 480,000 deaths per year due to cancer. This represents 21% of all deaths in the USA. However 30,000 patients per year survive after chemotherapy for cancer. One in 1000 persons aged 20 years or less will have been cured of cancer. In fact 5% of all cancer occurs in individuals less then 35 years of age and 50,000 new cases per year will affect persons during their reproductive years. Most of these will be treated with one or several of the 700,000 compounds which have been screened as possible anti-neoplastic agents. Until 1945 there was only one compound known to be anti-neoplastic, Nitrogen Mustard. Today there are more then 50 chemotherapeutic agents. Chemotherapy is used primarily to treat non operable or metastatic malignancies. The most common use of adjuvant chemotherapy is for the treatment of breast cancer.

Very little attention has been given to the reproductive and endocrine needs of these patients but I believe that the extraordinary advances in reproductive techniques will transform the quality of care for these patients. I'd like to share with you some of the exciting new developments taking place in this area. On Monday, November 17 1994, at the last AFS meeting in San Antonio there were 5 presentations that pointed clearly towards the future and confirmed to me that there is hope for these patients. Dr. Zahng from Beijing University presented a paper entitled; "Extra Corporeal Development of Human Fetal Ova". One square mm of ovarian tissue from 4 week old fetuses was cultured In Vitro for 36-42 hours. The primordial oocytes were denuded of their cumulus and then observed until extrusion of their first polar body. Seven days later 25% of these oocytes had extruded their polar body and were surrounded by zona. If it was possible to harvest immature oocytes from patients prior to chemotherapy or radiation, it could be possible to hope for reproductive potential for these patients. However, it would be necessary to cryopreserve these oocytes until a time when the patient is cured. Gook DA et al (Human Reproduction 9(4):684-91,1994) have shown a 73% oocyte survival rate after cryopreservation of human oocytes and a fertilization rate in these previously cryopreserved oocytes equal to that in their IVF program. However, there are justified concerns regarding chromosomal abnormalities in cryopreserved oocytes and this is why the paper by Park et al presented also in San Antonio is of such importance. These researchers from South Korea performed chromosomal analysis of 122 human oocytes, obtained from non stimulated ovaries in patients undergoing tubal ligations. They proceeded to compare chromosomal preparations from fresh and cryopreserved oocytes. Using FISH techniques they confirmed a much higher rate of chromosomal abnormalities in oocytes which had been frozen then in those which had not been frozen. This, and other experiments, point to the need to proceed with caution in this area and probably to the need to perform pre-embryonic genetic studies in embryos obtained from previously frozen oocytes. Also, given the high rate of failure to implant in IVF procedures it is important to improve our In Vitro culture techniques so that embryos may develop further in the laboratory and blastomeres can be genetically studied prior to uterine implantation. Because of the need to attain these two objectives, that is, embryo biopsy and implantation enhancement, the Norfolk group, presented their results using coculture systems, using specialized Vero cells and monkey oocytes thus proving that by using sophisticated coculture techniques they were able to increase the number of oocytes reaching the blastocyst stage. Numerous advances were also presented to deal with the best method to use sperm from men who had stored samples for later use. Because most men who store their sperm prior to cancer therapy have semen samples of poor quality, any techniques attempting to improve sperm micromanipulation is of special relevance to our discussion. At the present time there is no clear consensus as to what is the most efficient use of these few and often compromised samples. Should we use insemination techniques or should we proceed directly to ART techniques with or without gamete micromanipulation? Asada et al from Eastern Virginia Medical School proved that ICSI did not damage the meiotic spindle of hamster eggs provided that the sperm is micro-injected as far away as possible from the first polar body and Sherin, et al from Genetics and IVF Institute of Fairfax showed a 34% survival rate of oocytes after ICSI and a 27% pregnancy rate per embryo transfer. These are encouraging

results for patients who have poor quality samples. Silber, et al from St. Luke's Hospital, in St. Louis, in conjunction with the group from Belgium, went one step further and reported on the results of inseminating 240 eggs by ICSI using sperm recovered from testicular biopsies and obtaining an outstanding 31% pregnancy rate (n=16). If that was not enough, a very intriguing paper from Japan was presented by Sofkitis describing the technique for inseminating oocytes with round spermatids nuclei and proving development to 4 cell stage and pregnancy in rabbits.

The most frequent malignancies that bring these patients to consult with the reproductive endocrinologist are Hodgkin's, Lymphomas, Leukemias, breast cancer and testicular cancer. Their most common questions are related to the effects of surgery, radiation therapy and or chemotherapy on their future reproductive potential. We discuss with them and often with their parents the alternatives before treatment. At present the only viable option is to offer sperm or zygote cryopreservation. Hopefully, as mentioned earlier, oocyte cryopreservation may soon be another option. Another concern of these patients is the role of hormone replacement therapy, or the possible use of GnRH agonists in an effort to protect the gonads during chemotherapy. Other important but unresolved issues have to do with the effects of chemotherapeutic agents or radiation on the reproductive organs themselves. There is very little information available on these subjects and counseling is difficult. We can share with them for instance that cancer treatments increase the risk of early menopause and that this complication is related to therapy dosage and age of the patient at the time of treatment. Other better known facts are that the long-term effects of cancer and its treatment in men are oligospermia or azoospermia and ovulatory disorders including amenorrhea in women. The reversibility of some agents have been published and traditional chemotherapeutic combination therapy (CVP, MVPP, ABVD) has shown reversibility rates from 1 in 129 to 13 in 26. Whenever possible in young persons the less toxic agents must be chosen, for example it is known that AABVD is much less toxic then MOOP.

During a 5 year period from 1979 to 1983 all patients in Denmark with metastatic non- seminomatous and extragonadal germ cell cancer were treated with 6 cycles of cisplatin, vinblastine, and bleomycin (PVB). Thirty-nine patients referred to the Finsen Institute accepted a follow-up examination of side-effects 3.5 - 9 years after chemotherapy. Renal toxicity, pulmonary toxicity and neurotoxicity were the most pronounced long-term side-effects. Nearly all patients had a peripheral sensory neuropathy probably caused by axonal degeneration and PVB treatment caused low sperm counts and a subclinical Leydig cell dysfunction in the majority of patients. Azoospermia was observed in 27% of the patients.

Anti-neoplastic drugs interfere with cell mechanisms involved in cell growth affecting RNA and DNA synthesis and or function. These drugs include Alkalating agents, anti- neoplastic antibiotics, alkaloids and anti-metabolites. Treatment of cancer with multiple-drug chemotherapy regimens or radiation therapy can cause either temporary azoospermia of various durations or permanent azoospermia in young men. To identify which drugs in which doses contribute to long-term or permanent azoospermia, semen analyses were done on patients with Ewing and soft tissue sarcomas before, during, and after treatment with either CYADIC (cyclophosphamide, doxorubicin, and dacarbazine), or CYVADIC (vincristine added to CYADIC). Some patients also received other drugs or radiation therapy. From pre-treatment levels that were similar to those of control subjects, sperm production declined to azoospermia within 4 months of treatment. Sperm production returned in some patients after treatment; 40% of men recovered to normospermic levels by 5 years after treatment. Few patients showed continued recovery of sperm production after that time. The cumulative dose of cyclophosphamide was the most significant determinant of recovery to normospermic levels; approximately 70% of those who had received doses less than 7.5 g/m2 (median, 4.1 g/m2) recovered, but only 10% recovered when doses exceeded 7.5 g/m2. Thus, a risk of permanent sterility is associated with the use of the CYADIC and CYVADIC regimens in young men, especially when the cumulative dose of cyclophosphamide is greater than 7.5 mg/m2.

Radiation and chemotherapy reduce sperm count and cause infertility in males. In the mouse, rat, and human, the differentiating spermatogonia are the most sensitive to killing by cytotoxic agents, resulting in short-term azoospermia. Stem spermatogonia are also killed by some agents. In the mouse, sperm production gradually recovers from surviving stem cells without a lag period. In the rat, however, surviving stem cells may remain as A spermatogonia for a long time without initiating differentiation. In humans, there may be a long period of azoospermia; the time at which recovery or sperm production is initiated appears to be related to the degree of stem cell killing. Knowledge of the mechanisms regulating spermatogonial proliferation and differentiation could lead to ways to minimize the duration of azoospermia following treatment. Treatment of lymphomas with combination chemotherapy with or without radiation therapy (XRT) can result in long-term or permanent azoospermia. Semen analyses of lymphoma patients were performed before, during, and after treatment with cyclophosphamide, doxorubicin, vincristine, Prednisone, and bleomycin (CHOP- Bleo) chemotherapy. Some of the patients also received other drugs or radiation therapy. Although no patients were azoospermic before treatment, all were rendered azoospermic during treatment. Following the completion of treatment, the fraction of patients whose sperm counts recovered increased gradually over 5 years and plateaued by 7 years, with two thirds of the men achieving normospermic levels. Scattered gonadal radiation dose and cumulative cyclophosphamide dose were found to be independently significant determinants of recovery. Pelvic XRT and cumulative cyclophosphamide dosages greater than 9.5 g/m2 are associated with a high risk of permanent sterility in lymphoma patients treated with the CHOP-Bleo regime.

Individual agents within each of the major classes of antibiotics have been shown to have significant adverse effects on spermatogenesis or spermatozoal function in mammals. For humans, infertility or significant alterations in semen parameters have been well documented for the nitrofurans and for patients on sulfasalazine. Other commonly used antibiotics, such as minocycline, have been shown to be toxic to sperm at any concentration. Until further information is available, clinicians must keep in mind that treatment with antibiotics may adversely affect the fertility potential of men. It is possible that some classes of antibiotic agents, such as the penicillins or the quinolones, may have minimal effects on male fertility. Further investigation is needed into the relative toxicity of antibiotics and the mechanisms by which antibiotics affect spermatogenesis and spermatozoal function. {8}

The effects of chemotherapy on the chromosomes in sperm are very poorly understood and very few studies have addressed this question. In one of such studies four hundred fifty sperm complements from eight controls were analyzed. A conservative estimate of aneuploidy was 1.8% with a hyperhaploid rate of 0.9% (4/450). The overall frequency of structural aberrations was 8.9% (40/450). The proportion of X-bearing (47.5%) and Y-bearing (52.5%) sperm did not differ significantly. Sperm complements were analyzed from a cancer patient 9 months after polychemotherapy (n = 63) and from a patient being treated with Imurek (azathioprine) (n = 30). There was no significant increase in the incidence of numerical and structural chromosome aberrations in the sperm of either patient. The percentages of X-bearing and Y-bearing sperm were not significantly different from the expected 50%.

CRYOPRESERVATION - THE PRESENT REPRODUCTIVE ENDOCRINOLOGY

The ability of freezing living cells has open new possibilities for the process of reproduction. In general, cells stand well the process of freezing at low temperatures but the dangerous temperatures are between -15 and -60C that occurs during the process of freezing and during the process of thawing. At very low T (-130 to -196C) biologic activity stops. At -5C cells remain unfrozen between the surrounding medium. At -5 and -15C ice forms but the cell contents remain unfrozen because the cell membranes prevents the growth of ice crystals in the cytoplasm. The super cooled water in the cell has a higher chemical potential then the outside frozen solution and in response to this difference of potential water flows out of the cell osmotically and freezes externally. The subsequent changes in the cell depend on the cooling velocity. If cooling is slow the cell loses water rapidly by exosmosis to concentrate the intracellular solutes and thus maintain the chemical potential of the intracellular water in equilibrium with the extra cellular water. The cell dehydrates but does not freeze intracellularly. If the cell cools too rapidly and are not able to lose water fast enough to maintain the equilibrium, it freezes intracellularly.

Survival of mammalian cells during freezing requires the presence of cryoprotective substances such as glycerol or DMSO (Dimethyl Sulfoxyde). The discovery that compounds like glycerol could prevent slow freezing damage was totally fortuit. Sperm had been mixed with glycerol rather then sucrose and that compound have prevented the damage which was common when they had used sucrose in their experiments. The exact mechanism of the damage due to slow freezing is not understood but it is thought to be due to the increase in extra cellular electrolyte content and then the inability of the cells to shrink appropriately to the extent required to maintain the osmotic pressure without disturbing the cellular membrane. The cell may also be damaged at the time of warming and if intracellular crystals did form at the time of freezing there is a good chance that these crystals may damage the cell at the time of warming although some cells may be rescued by rapid warming. On the other hand slow freezing might have preclude intracellular crystal formation and the cells may do better at the time of warming.

MICROMANIPULATION OF OOCYTES AND SPERM

Avenues of improving the ability of culturing and maturing oocytes from the stage of oogonia would permit the cryopreservation of ovarian tissue and the posterior utilization of in vitro cultures systems to provide oocytes at a future time when the patient might recovered of her disease. This also would have the advantage of no need of hormone manipulation in an attempt to recruit oocytes to be harvested at a time when the disease is active and such a course may be totally impossible. A review of the cellular cycle is relevant at this point. The cell cycle has two phases; interphase and mitosis. In interphase the nucleus remains intact as the cell grows and its chromosomes replicate. In mitosis the nucleus divides in two. First the membrane surrounding the nucleus breaks and forms the mitotic spindle to which the previously duplicated chromosomes attach. Then the spindle breaks in two so that each cell daughter will have equal number of chromosomes.

During sexual reproduction meiosis occurs so that the diploid number of chromosomes of the egg and the sperm becomes haploid. This process of halving is accomplished by 2 rapid chromosomal segregation. In the male the precursor divides symmetrically so that the end result is 4 identical sperm. In females the precursor divides asymmetrically resulting in one large egg and 3 small cells that are discarded. Cells must pace their division so that they should not enter their mitosis or meiosis until the chromosomes are replicated failure to do so may result in cells

that lack a chromosome also the cells should not divide until the cell volume has doubled. The integration of these functions is accomplished by certain substances such as the MPF or Maturation Promoting Factor which is not activated until cdc genes produce a cdc 2 protein kinase that transfers phosphates from ATP to proteins and therefore cell growth. The cdc2 protein is one of the 2 parts of the MPF the second is the cyclins.

In meiosis there is a first arrest in the germinal cells just before birth and this arrest is maintained up to the graafian follicle stage and there is no resumption of meiosis until the follicular cells interact with gonadotropins, steroids and other intrafollicular growth factors. This first interruption of meiosis is easily observed in immature oocytes because they display a prominent nucleus called the Germinal vesicle. The oocyte will not undergo further maturation until this GV undergoes a breakdown (GVB) and extrudes the first polar body and progresses to the second meiotic arrest at the metaphase II stage. This period of maturation between prophase I to metaphase II is the period of oocyte maturation. In vivo the oocyte does not progress and continues GV breakdown because the influence of follicular inhibiting factors specifically cAMP. Once this inhibiting factor is removed the Maturation Promoting Factor (MPF) becomes active and the oocyte progresses from G2 to M. The failure to fertilize eggs in vitro may be countered by micromanipulation of gametes to place selected spermatozoa underneath the zona pellucida of the egg or directly into the egg, thereby improving chances of fertilization and production of viable embryos. Tucker et al (Am J Obstet Gynec 169,1993) analyzed their clinical data for assisted fertilization. Retrospective analysis of 85 cycles (73 couples) of in vitro fertilization and embryo transfer in which micromanipulation for assisted fertilization was used to overcome either severe male factor infertility or idiopathic failure to fertilize, was performed in 60 cycles where only embryos from under zona insemination were available for uterine transfer, 15 singleton and two twin pregnancies occurred (28.3% viable pregnancy rate per transfer, 14.1% embryonic implantation). In 14 of these cycles embryos arose only after repeated under zona insemination adding more spermatozoa; this accounted for four of the singleton and one of the twin pregnancies (38.5% pregnancy rate, 22.2% embryonic implantation). No embryos arose from partial zona dissection performed in five cycles on sibling eggs. Direct egg injection of a single spermatozoon into 105 eggs gave an 88.6% egg survival and 32.3% fertilization. Overall, a 24.7% (21/85) viable pregnancy rate per cycle initiated occurred when only embryos from assisted fertilization were available. This strongly indicates that assisted fertilization made a real contribution in cases where either insufficient spermatozoa were available for conventional insemination or in cases where previous fertilization failure had arisen.

Fertilization failure is a serious problem in human in vitro fertilization (IVF) programs specially when sperm is of poor quality. When two hundred and ninety-four IVF cycles performed at the National Taiwan University Hospital from July 1989 to June 1991 were retrospectively analyzed, thirty-seven (13%) of the 294 cycles were observed to have fertilization failure. The incidence of fertilization failure in male factor patients was significantly higher ($p < 0.05$) than in others. Patients with oligo-asthenospermia tended to have a higher rate of fertilization failure than patients with oligospermia or asthenospermia alone. In non-male factor patients, a smaller number of oocytes and mature oocytes was found in patients with fertilization failure than in patients achieving fertilization. These results suggest that severe oligo-asthenospermia patients with repeated fertilization failure should be candidates for micromanipulation of gametes in subsequent IVF trials.

Over the past decade, In Vitro Fertilization (IVF) has become a routine and acceptable tool in the treatment of infertility. However, major limitations in solving certain infertility problems still remain. Male infertility is one area in which only a small fraction of patients have benefited from IVF. Union of male and female gametes, either in vivo or in vitro, requires sperm penetration through the cumulus oophorus and the zona pellucida. Failure of fertilization despite the increased number of spermatozoa introduced into the oocyte's vicinity by IVF, has been shown to be directly related to abnormalities in sperm cell morphology and motility. The improved technology for micromanipulation of gametes has made it possible to circumvent the oocyte barriers to sperm penetration, thereby greatly reducing the number of normal sperm cells needed to achieve fertilization. Three major micromanipulative strategies have been developed over the past five years. Advances in clinical micromanipulation of gametes and embryos, assisted fertilization and hatching. Several methods for micromanipulation of human gametes have been proposed to enhance fertilization in cases of male infertility. Of these methods, two have been successful in producing pregnancies and live births worldwide; these include partial zona dissection and subzonal sperm insertion. During the period between October 1989 and July 1991, 251 patients with male infertility due to impaired sperm function were treated with in vitro fertilization in conjunction with gamete micromanipulation at our center. Sixty pregnancies (24% per cycle, 42% per replacement) resulted in 144 patients. In another study, in an attempt to increase the incidence of implantation, the authors conducted three clinical trials of assisted hatching and selected assisted hatching. The combined results of the trials indicate a clinical pregnancy rate of 51% in the control group and 60% in the micromanipulated group (P less than 0.05). Various microsurgical procedures at the cellular and subcellular levels using laser non-touch techniques have been used for micromanipulation of human spermatozoa. This manipulation is aimed at increasing the fertilization rate following insemination with low-quality spermatozoa. Another intracellular application of the laser beams is the destruction of extra pronuclei in polyspermic fertilized human oocytes.

Two hundred and thirteen SZI cycles were performed at Sydney IVF in the 4 year period from September 1988 to September 1992, for extreme male factor patients with previous IVF failures or extremely low sperm numbers for whom SZI was the first option. A total of 138 embryo transfers were reported, producing 20 clinical pregnancies after performing SZI on 1899 oocytes. giving an overall pregnancy rate of 14.5% per embryo transfer or 9.4% per cycle.

IN SITU HYBRIDIZATION IN GENETIC STUDIES

Cytogenetic analyses is used for detection of chromosomal errors and also for cancer diagnosis. Such assays are time consuming with turn-around of 6 days to 3 weeks. Therefore other methods such as In situ hybridization to cell or chromosomes preparations to chromosome to chromosome specific DNA probes that are visualized by fluorescence. Assays can be used to count the number of copies of a specific chromosome (aneuploid detection), to identify unknown chromosomes (marker) and to identify chromosome translocations. In contrast with standard karyotypes that require cells in the metaphase stage. In situ Hybridization can be carried out in the interphase stage in which each chromosome occupies a discreet area in the nucleus. Since we have now DNA specific probes that have hybridized to an interphase nucleus a bright colored dot is generated for each copy of the chromosome present in that nucleus. Aneuploidies are detected by counting the number of dots per cell. For instance, in Downs Syndrome there will be 3 dots for chromosome 21. The exciting future developments of this technology will bring the possibility of analysis of multiple chromosomes each tagged with a different color and the possibility exists that in the future it might be possible to analyze the entire chromosome complement say from fetal sampling or embryo biopsies.

CANCER IN THE PREGNANT WOMAN

There are numerous questions to consider with regard to the situation of a women who develops cancer while pregnant. Does pregnancy affect the course of the disease? What are the risks for the fetus? Should the pregnancy be terminated? If the cancer is diagnosed prior to conception what kind of contraception is appropriate? Is pregnancy always contraindicated after cancer treatment?

In general cancer therapy during pregnancy consists of surgery, radiation or chemotherapy and the following generalities can be made:

1. Extra-abdominal surgery can be well tolerated and intra-abdominal surgery is necessary the ovaries can be removed after the 8th to the 10th week but if this done before, progesterone replacement is necessary.

2. Radiation may affect the fetus especially from the 8th to the 15th week. Ten to 49 rads results in a incidence of 3% of mental retardation. Ten to 49 rads results in mental retardation in one out of 5 pregnancies. Less then 5 rads is considered safe.

3. Chemotherapy is contraindicated during pregnancy and most oncologist would recommend to avoid pregnancy for at least 12 months after completion of the treatment There is no evidence that anti-neoplastic drugs have any effect on the fetus after the 10th week of pregnancy but there are no good long-term follow up studies It is thought that single agent therapy is less dangerous then combination regimes. Fetal wastage may be increased in women treated with chemotheraputic agents. Breast feeding is contraindicated.

DIFFERENT CANCERS AND PREGNANCY

Breast cancer and pregnancy

1 in 3 to 10,000 pregnancies may occur in breast cancer patients. Tendency to be diagnosed in more advanced stages because of the difficulties in breast examination. Pregnancy in itself may not aggravate the course of the disease. About 10% of post cancer patients become pregnant within 5 years and no well recognized negative effects have been described. Lactation is not contraindicated.

Genital/Cervical Cancer

1.3 in 1,000 - Invasive cancer 1 in 2000

RECOMMENDED READING

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