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Quick Start, Body Weight, Hormonal Contraception, and Black Box Medroxyprogesterone Acetate

Ann J. Davis, MD, Gynecologist, Department of Pediatric and Adolescent Gynecology, Tufts-New England Medical Center, addressed three topics relating to oral contraception, beginning with the “Quick Start” approach to hormonal oral contraceptives, followed by the effect of weight on hormonal contraceptive effectiveness, and the black box warning for depot medroxyprogesterone acetate.

Women currently are instructed to begin taking oral contraceptive pills at the end of a menstrual cycle. However, this raises concern that the time gap between the patient being given the pills and the date she’s scheduled to begin taking them may result in some women failing to take them at all. A new approach to oral contraception allows the patient to take the first pill immediately upon receiving the prescription (Quick Start), rather than waiting until her next menstrual cycle.

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Dr. Davis cited a study by Westhoff et al. that evaluated the continuation rate with the Quick Start approach (Contraception. 2002;66:141-145). “They found that women using Quick Start were more likely to continue oral contraceptive use through the second pack of pills,” she said. Among women in the control group (who started taking oral contraceptive pills at the end of a menstrual cycle), close to 5% did not initiate use of the oral contraceptive.

One concern that has been raised is the possibility for breakthrough bleeding when initiation of oral contraception is not cycle dependent. Dr. Davis cited another study by Westhoff, which looked at the bleeding patterns in women using the Quick Start approach (Fertil Steril. 2003;79:322-329). The study included 113 women who were assigned to either conventional start or Quick Start. “There was no difference in the unscheduled bleeding pattern between the two groups,” said Dr. Davis.

“The Quick Start approach is currently off label, but it is something that many clinicians are now considering,” said Dr. Davis.

Body Weight and Hormonal Contraception

The next issue Dr. Davis addressed was the possible effect of body weight on the efficacy of oral contraceptives. This question arose from a retrospective study of 2,822 women by Holt et al., which found that women in the highest (fourth) quartile of weight (greater than 70.5 kg) had increased risk for contraceptive failure (RR=1.6), compared to women in the first quartile (less than 56.5 kg) (Obstet Gynecol. 2002;99:820-827).

Dr. Davis noted that the typical failure rate for oral contraceptives is 3 to 8 per 100 woman-years. In the study, the failure rates among women in the first quartile versus those in the fourth quartile were 3.6 versus 5.6. “This is still a very low rate,” she said. Women who discontinue an oral contraceptive are likely to use a barrier method or no method at all. “These are both likely to have much higher failure rates,” she said. “So even if you believe the findings in this study (and there is debate), discontinuing oral contraceptives in heavy women based on this study is not warranted.”

The study also looked at ethinyl estradiol dosages in relation to body weight. Among the heavy women, those who used less than 35 mcg of ethinyl estradiol had an increased relative risk of contraceptive failure compared to those using less than 50 mcg. Dr. Davis again emphasized that the failure rates

This program was supported by an unrestricted educational grant from Wyeth Pharmaceuticals.
were still relatively low and do not justify discontinuing oral contraceptives in overweight women.

**Black Box Warning on Medroxyprogesterone Acetate**

The third issue Dr. Davis discussed was the black box warning that the FDA added to depot-medroxyprogesterone acetate (DMPA) in November 2004. The FDA stated that women who use DMPA may experience significant loss of bone mineral density and that the loss of bone mineral density increases with duration of use and may not be completely reversible. They also stated that the effect of adolescent or early adulthood use of DMPA on peak bone mass and future risk of osteoporotic fracture is unknown.

Dr. Davis added that a letter sent to healthcare providers from the pharmaceutical manufacturer advised using DMPA for no more than two years and to use it only if other birth control methods are inadequate. “This has led to some physicians declining to refill a prescription for DMPA beyond two years of use,” said Dr. Davis. She expressed concern about this because she has observed a reduction in unintended pregnancies with use of this agent.

*In fact, bone mineral density was actually higher in those who discontinued DMPA compared to non-users,* said Dr. Davis.

She continued with a review of the literature on the effects of DMPA on bone. A study by Cundey et al. in 1994 found that women on DMPA regained their bone mass after discontinuation (*Br Med J*. 1994;308:247-248). A more recent study, by Scholes et al., found that at three-year follow-up there was no difference in the bone mineral density in adult women who had used DMPA in the past compared to non-users (*Epidemiology*. 2002;13:581-587). Dr. Davis noted that there are also data showing that DMPA has not been linked to menopausal osteoporosis or fractures.

The black box warning was supported by a prospective cohort study of 370 adolescent girls (mean age of 15), who received no oral contraceptives, a 20 mcg oral contraceptive pill, or DMPA (Cromer BA et al. *J Adolesc Health*. 2004;35(6):434-41). Over 12 months, there were increases in spine and hip bone mineral density among the girls on no contraceptive and those taking the oral contraceptive. Among the girls using DMPA, there was a decline in bone mineral density, particularly in the hip. This was considered to be a troubling finding because adolescence is the time when bone accretion is at its highest.

Nevertheless, Dr. Davis believes the decision to add the black box warning may have been premature. She described a population-based prospective cohort study of close to 200 adolescent girls by Scholes et al. (*Arch Pediatr Adolesc Med*. 2005;159:139-144). The study included both DMPA users and non-users of any contraceptive method. The study found that girls who used DMPA did have a reduction in bone mineral density. However, the investigators also found that girls who discontinued DMPA regained bone mineral density. Their bone mineral density was at least as high as the non-users for all anatomic sites at 12 months follow-up. “In fact, bone mineral density was actually higher in those who discontinued DMPA compared to non-users,” said Dr. Davis (see Table 1).

“We have the opportunity to affect two very important consequences of sexual activity for women: one is an unintended pregnancy and the other is a sexually transmitted infection,” began Kurt Barnhart, MD, MSCE, Associate Director, Division of Reproductive Endocrinology and Infertility, and Director of Reproductive Research, University of Pennsylvania. As many as one in two Americans will contract a sexually transmitted infection at some point in their life. For women, these infections are often asymptomatic, but they can result in serious complications. Dr. Barnhart discussed new methods to prevent transmission of sexually transmitted infections (STIs), including microbicides, which are in various stages of development.

Dr. Barnhart explained that a microbicide is any substance that can reduce the risk of a sexually transmitted infection when applied to the vagina pro-
Dr. Barnhart discussed new methods to prevent transmission of sexually transmitted infections (STIs), including microbicides, which are in various stages of development.

phylactically. “We’re talking about empowering a woman to protect herself because she can decide whether it is used,” said Dr. Barnhart. Some of the agents being investigated also may prevent pregnancy, while others protect against sexually transmitted disease only. These include HIV infection, gonorrhea and chlamydia.

“The microbicide compounds under investigation may protect both partners, but I am focusing on preventing infection of an asymptomatic or HIV-negative woman from an infected partner,” said Dr. Barnhart. “If we can develop a workable, easily-used microbicide, it will have a dramatic public health impact,” he continued.

There are several proposed mechanisms for microbicides, including killing or immobilizing pathogens before they reach the female genital tract, forming a barrier between pathogens and vaginal tissue, inhibiting viral attachment or fusion of the virus to white blood cells (WBCs), preventing pathogens such as HIV from replicating even if they have entered the WBCs, and boosting the innate vaginal immune system. “Ultimately, there will probably be a combination of these mechanisms,” said Dr. Barnhart.

He described the experience with the microbicide nonoxynol-9, which currently is used as a spermicide for contraception but once was thought possibly to prevent sexually transmitted disease. Dr. Barnhart cited several studies, which produced conflicting findings, including a decrease in HIV transmission of 25%, no effect on HIV transmission, and an increase in HIV infection. A meta-analysis of the studies concluded that “nonoxynol-9 probably is not harmful, but it certainly is not beneficial as a microbicide,” he said.

He continued with a discussion of the microbicides currently under development. Buffer gel is applied vaginally and works by keeping the vaginal pH low. The acid environment is toxic to both pathogens (gonorrhea, chlamydia, and HIV) and sperm. Buffer gel has just finished Phase III trials and the results will be released in the spring of 2006. Lactobacillus also keeps the pH low and maintains vaginal health. Therefore, suppositories that replenish lactobacillus are under development.

Agents that inhibit viral attachment are under investigation. These include sulphonated compounds that surround the HIV molecule, preventing it from presenting to a receptor. Agents far along in development are the topical vaginal products Carraguard® (PC-515) and PRO-2000® (polynaphthalene sulphonate). Products also are being tested that work as nucleoside reverse transcriptase inhibitors, inhibiting the HIV receptor or stopping the enzymes that allow HIV to replicate and divide in the cell. Examples are UC-781 and tenofovir. Cyanovirin-N is a small protein that binds to the HIV receptor and almost completely blocks it.

“There are about 28 of these products in development, and 14 of them are in relatively advanced trials,” said Dr. Barnhart.

In conclusion, he stated: “There is a future in microbicides. They could save lives and have a major impact on the HIV and STI epidemic, especially for women who can’t or won’t use condoms, or who have partners who refuse to wear them.”

Emergency Contraception: Where are We in 2005?

Emergency contraception is an important adjunct to contraceptive care because no contraceptive method is perfect, particularly among women who use barrier methods,” said Jeffrey T. Jensen, MD, MPH, Leon Speroff Professor of Obstetrics & Gynecology, School of Medicine, Oregon Health Sciences University. The available emergency contraception product is a levonorgestrel-containing preparation (Plan B®), which is safe because it lacks estrogen. It has been FDA-approved since July 1999.

“Of course, not all women who experience an episode of unprotected intercourse will get pregnant,” said Dr. Jensen. About 8 in 100 women having sex in mid-cycle would be expected to become pregnant without emergency contraception; with emergency contraception that is reduced to 1 in 100.

“One of the main controversies surrounding emergency contraception relates to the mechanism of action,” said Dr. Jensen. He emphasized that there is no evidence of post-fertilization effects. Instead, the evidence indicates that emergency contraception inhibits or delays ovulation. Emergency contraception with levonorgestrel does appear to shorten the luteal phase in non-pregnancy cycles, he continued, but there is no evidence that this effect on the endometrium is...
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harmful for an embryo that might implant.

Achieving the proper dosing for emergency contraception can be difficult. Dr. Jensen noted that the initial dose should be taken as soon as possible after unprotected intercourse, with a 0.75 mg dose that is repeated in 12 hours. “There have been two large randomized controlled trials that have documented no change in efficacy if the product is taken as a single dose (1.5 mg),” he said (von Hertzen H et al. *Lancet*. 2002;360:1803-1810; Ngai SW et al. *Hum Reprod*. 2005;20:307-311). To improve compliance and efficacy, he suggested allowing patients to take both pills in a single dose, although this is an off-label recommendation.

In terms of patient education, it’s important to counsel patients that when using levonorgestrel they might experience an earlier than usual onset of menses.

Within 72 hours, we expect a reduction in pregnancy of about 80%; beyond 72 hours, this goes down to 60%,” he said.

Dr. Jensen noted that the emergency contraception product is most effective if taken within 72 hours of unprotected intercourse. “Within 72 hours, we expect a reduction in pregnancy of about 80%; beyond 72 hours, this goes down to 60%,” he said (see Figure 1). Because it is important that emergency contraception be administered as soon as possible, Dr. Jensen and many other gynecologists have recommended advance-of-need prescriptions for emergency contraception to reduce the barrier to access. When prescribing emergency contraception, “you have to make sure the pharmacy has the product and that the pharmacist is willing to dispense the product,” he said.

There are data showing that women receiving an advance prescription are more likely to use emergency contraception when they perceive the need for it, and that they use other methods of contraception equally well (Glasier A et al. *N Engl J Med*. 1998;339:1-4; Raine T et al. *Obstet Gynecol*. 2000;96:1-7). In addition, they are not more likely to have unprotected sex, which is a concern that has been raised about advance prescriptions.

“Levonorgestrel emergency contraception is a great advance in the health and welfare of women,” said Dr. Jensen, adding that “being able to obtain the product quickly when the need arises results in the greatest reduction in pregnancy risk and the lowest risk to the woman.” For these reasons, an application was submitted to the FDA to make Plan B® available over the counter.

On December 16, 2003, the Reproductive Health Drugs and Non-Prescription Drug Advisory Committees met and voted overwhelmingly to approve the over-the-counter availability of Plan B®. Nonetheless, on May 6, 2004, the FDA issued a non-approval letter. Ostensibly, the decision was based primarily on lack of data in younger women. The manufacturer submitted a reapplication for over-the-counter status for women over the age of 16 and prescription-only status for women less than 15 years of age. “The FDA missed two self-imposed deadlines for making a decision on this application: January 2005 and September 2005,” said Dr. Jensen.

In late August 2005, the FDA indicated that an additional open comment period of 60 days was needed because of the difficulty of having a drug be proposed as prescription-only for one segment of the population and over-the-counter for another.

Because of this decision, Dr. Susan F. Wood, Assistant FDA Commissioner for Women’s Health resigned, as did Dr. Frank Davidoff, a clinician-scientist on the Non-Prescription Drug Committee, both citing reasons relating to their objections to the FDA’s rejection of the committee’s recommendation. On October 11, 2005, the Government Accountability Office issued a report on the initial FDA decision, stating that the decision to reject over-the-counter status was “highly unusual and was made with both atypical involvement from top agency officials, and may have been made months before formally being announced.”

Dr. Jensen concluded: “Emergency contraception pills are safe and effective and there’s strong evidence supporting off-label use of a single dose of levonorgestrel (1.5 mg) for emergency contraception. The efficacy is improved when taken as soon as possible after unprotected sex. There may be an earlier onset of menses.”

![Figure 1. Emergency Contraception: Effect of Delay in Treatment after Intercourse](image-url)
Embryo Quality and Endocrine Profiles

Anders Nyboe Andersen, MD, Professor, Copenhagen University Hospital, discussed embryo quality and endocrine profiles.

Citing two randomized trials Dr. Andersen said that, “large multicenter trials, which we base much of our knowledge on, provide us with scant information on embryo quality in relation to the variables that we can control, which are endogenous and exogenous FSH and LH activity.” He noted that oocyte number poorly predicts implantation rate, and that pregnancy rates are the result of embryo quality and uterine receptivity.

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Dr. Andersen discussed the Menotrophin vs. Recombinant FSH in vitro Fertilisation Trial (MERiT), the results of which were presented at the 2005 Annual Meeting of the European Society of Human Reproduction and Embryology (21st ESHRE. June, 2005. Abstract i19 (O-054)). This was a randomized, open-label, assessor-blinded, parallel group, multinational study in which 731 patients underwent controlled ovarian hyperstimulation and IVF following GnRH agonist downregulation in a long protocol. Half of the patients received highly purified menotrophin (HP-hMG), and the other half received rFSH. All patients received identical type and dose of concomitant GnRH agonist, hCG, and progesterone. Transfer was on day 3 of one to two embryos of minimum quality (≥4 cells, no cleavage arrest, ≤20% fragmentation). The primary endpoint was ongoing pregnancy rate (transvaginal ultrasound showing at least one intrauterine viable fetus 10 to 11 weeks after embryo transfer).

Oocyte complexes were assessed at the time of retrieval. Fertilization was assessed at 20 hours. Cleavage and embryo morphology were assessed at 28 hours, 44 hours, and 68 hours. Parameters of embryo quality included stage of cleavage, degree of fragmentation, localization of fragments, blastomere uniformity, cytoplasmic appearance, and visible signs of multinucleation. A “top quality” embryo was defined as 4 or 5 cells (day 2), ≥7 cells (day 3), ≤20% fragmentation (day 3), equally sized blastomeres (day 3), and no sign of multinucleation (ever). Also defined for purposes of the study were “normally developed” embryos and “transferable” embryos.

The two treatment groups had approximately equal rates of oocyte retrieval (95% in the HP-hMG group and 94% in the rFSH group). The number of oocytes retrieved was significantly higher for the rFSH group compared to the HP-hMG group (11.8±5.7 vs. 10.0±5.4), but the fertilization rates were the same (51.6% for HP-hMG; 52.5% for rFSH).

Embryo cleavage is an early parameter of successful fertilization in vitro. Dr. Andersen noted that after administration of HP-hMG, 17.3% of oocytes exhibited early cleavage compared to 14.9% after rFSH, but the difference was not significant. The proportions of embryos classified as top quality embryos resulting from each treatment were 11.3% with HP-hMG and 9.0% with rFSH in the local assessment group (p<0.05). The percentages of embryos with four or more cells at day 2 were 33% in the HP-hMG group compared to 29.7% in the rFSH group (not significant). The percentages of embryos with seven or more cells at day 3 were 21.9% vs. 20.2% (not significant). There were 6% more embryos with less than 20% fragmentation in the HP-hMG group both at day 2 and day 3 (p<0.001). There were no differences in relation to blas-
Cytogenetic Determinants of Embryo Quality

David L. Keefe, MD, the James M. Ingram Professor and Chair, Department of Obstetrics and Gynecology, University of South Florida College of Medicine described the effects of age-related meiotic nondisjunction on embryo quality and presented theories of why nondisjunction occurs during meiosis in older women.

For women age 38 and older undergoing IVF, there is a marked decrease in the chance of getting pregnant, and an increase in miscarriages among those who do become pregnant.

“Virtually all 23 pairs of chromosomes can undergo nondisjunction,” said Dr. Keefe, adding that “the vast majority are embryo-lethal, and some are even oocyte-lethal.” Aneuploidy also is associated with poor embryo quality and poor outcome.

Turning to the mechanisms of embryonic aneuploidy, Dr. Keefe explained that using genetic and cytogenetic approaches, researchers have found nondisjunction of both homologs and chromatids. “Chromatids are supposed to stick together during the first cycle of meiosis, but sometimes they separate prematurely,” he said. He explored how age can increase the chances of nondisjunction.

Possibilities include spindle abnormalities, mitochondrial DNA mutations, increased reactive oxygen, decreased chromosome, late exit from the production line, and telomere shortening.

Dr. Keefe explained the rationale for the production line theory. During the narrow window of oogenesis, as oogonia replicate mitotically, some exit to meiosis early while others exit to meiosis late. The first eggs that a woman ovulates at puberty are the first ones to have exited mitosis, and the last eggs she ovulates are the last to have exited. To explain how this could affect nondisjunction, Dr. Keefe referred to telomeres, which are the ends of chromosomes composed of repetitive sequences of DNA. Telomeres encode no genes, but rather explain how this could affect nondisjunction.

Dr. Keefe explained that telomeres shorten with age. Every time a cell replicates it loses a small piece of telomeric
DNA. DNA replication is asymmetrical; the polymerase synthesizing one-strand copies in an uninterrupted, continuous fashion, while replication of the other strand occurs episodically, leaving an unreplicated, single-stranded overhang, which gets excised. This mechanism of telomere loss with each round of DNA replication is called replicative senescence.

“Chromatids are supposed to stick together during the first cycle of meiosis, but sometimes they separate prematurely,” he said.

Only a few cell types, such as stem cells and cancer cells, have the enzyme telomerase, which can add telomeric DNA back to the chromosome ends. “Eggs and early preimplantation embryos do not have appreciable amounts of telomerase activity,” said Dr. Keefe.

“So oocytes and early embryos acquire their telomeric length early during oogenesis,” he continued. Telomerase activity starts up again only late during preimplantation development, at the blastocyte stage.

Reactive oxygen, formed as a by-product of metabolism in every living cell, shortens telomeres through a second mechanism. The telomeres’ guanine-rich sequence makes them especially susceptible to oxidative damage. Intracellular excision repair mechanisms can aggressively shorten telomeres damaged by reactive oxygen, even without cell replication. “Even cells that don’t replicate, such as oocytes, can undergo telomere shortening by this mechanism,” said Dr. Keefe.

Dr. Keefe described research on mice from his laboratory that demonstrated the importance of telomeric length to reproductive aging. His group studied mice whose telomeres had been shortened genetically or pharmacologically, so they approximated the short length of telomeres from older women. Merely shortening telomeres recapitulated all the stigmata of reproductive aging in women, including abnormal spindles, reduced numbers of chiasmata, fragmented and arrested embryos and cytogenetic abnormalities.

In follow-up studies on human eggs, Dr. Keefe and his colleagues found that telomere length predicted embryo fragmentation and provided a better predictor of IVF outcome than the conventional biomarkers of reproductive aging, FSH and chronologic age. “Women who got pregnant had significantly longer telomeres,” Dr. Keefe said.

These findings support a telomere theory of reproductive aging: “The eggs that ovulate early in the life of the woman have gone through fewer cell cycles during fetal oogenesis, and therefore experienced less telomere loss via replicative senescence during fetal life. Eggs from older women have been through more cell cycles during fetal oogenesis, and therefore started development with shorter telomeres. Telomere shortening, by late exit from the production line, produces the ‘first hit’ of reproductive aging.

Telomeres of eggs ovulating from older women are shorter, which predisposes them to apoptosis, fragmentation, cell cycle arrest, and nondisjunction.”

Dr. Keefe concluded that the telomere theory of reproductive aging unifies existing theories of reproductive aging. “This is how a production line in the formation of oocytes during fetal life can impact the fate of those same oocytes 40 years later. Short telomeres endow them with fewer chiasmata, abnormal spindles, and increased susceptibility to apoptosis, cell cycle arrest, and nondisjunction; and even low levels of mitochondrial DNA can affect the process, by spilling reactive oxygen and further shortening the telomeres.”

Molecular Determinants of Embryo Quality

Robert F. Casper, MD, Professor, Division of Reproductive Sciences, Department of Obstetrics and Gynecology, The University of Toronto, discussed mitochondrial and reactive oxygen species and suggested some strategies to improve embryo quality.

In older women, oocyte and embryo quality decrease and there is increased embryo fragmentation and delayed embryo development, which also is true for mice. There is an increase in the cell death index in embryos that develop to blastocysts in older mice compared to younger mice. “Embryo fragmentation is often associated with programmed cell death and apoptosis,” said Dr. Casper.

Programmed cell death is dependent on the interactions of a number of gene products that repress or activate cellular self-destruction. The Bcl-2 gene family—
Mitochondrial DNA is especially sensitive to damage through aging,” said Dr. Casper.

Mitochondria contain a circular genome (16.5 kb in length, coding for 13 proteins, 2 ribosomal RNAs, and 22 transfer RNAs). These proteins and RNAs function in energy production, generation of reactive oxygen species, and regulation of apoptosis. Mitochondrial DNA (mtDNA) mutations increase with age.

“Mitochondrial DNA is especially sensitive to damage through aging,” said Dr. Casper. This is because of a lack of protective histones, efficient DNA repair enzymes, and no introns. As a result, mtDNA mutates 17 times faster than nuclear DNA and there is an exponential increase in mtDNA mutations with aging. This results in decreased efficiency of mitochondrial electron transport, and subsequently decreased energy production and increased production of reactive oxygen species, which can have detrimental effects on oocyte metabolism and cause increased susceptibility to programmed cell death and cytogenic abnormalities. Embryo fragmentation and arrest occur in about 50% of IVF embryos, which may increase as women age.

Dr. Casper explained that a process of oocyte cytoplasm transfer was attempted to prevent embryo fragmentation, and it was found to produce successful pregnancies in several patients who had consistently fragmented oocytes (Cohen J et al. Mol Hum Reprod. 1998; 4:269-280). Potential drawbacks of this technique include the possibility that genomic DNA could be aspirated with cytoplasm, which could integrate into the embryonic genome with production of a transgenic infant.

“Mitochondrial DNA is especially sensitive to damage through aging,” said Dr. Casper.

Mitochondrial injection into these mice prevented fragmentation in the mouse oocytes where there were originally abnormal mitochondria,” said Dr. Casper.

“We believe that mitochondria are the critical factors responsible for the success of ooplasm transfer,” said Dr. Casper. Therefore, he and his colleagues tested the theory that injecting healthy mitochondria into aged oocytes might have beneficial effects, including improved energy production, addition of anti-cell death gene products, prevention of apoptosis, heteroplasmy with added normal mtDNA, and prevention of mitochondrial disease in subsequent generations. He tested this hypothesis in the FVB and AKR strains of mice, for which about 60% of oocytes undergo apoptosis by 24 hours in vitro, compared to B6C3F1 mice. The FVB oocytes had normal DNA integrity but abnormal mitochondria.

Dr. Casper and his colleagues took mitochondrial preparations from mouse embryonic stem cells or human leukemia cells and microinjected them into the cytoplasm of the oocytes and zygotes of the two strains of mice. “When the embryonic stem cell-enriched fractions of mitochondria were injected, FVB oocyte fragmentation was decreased by about 40%,” said Dr. Casper. In the AKR oocytes, containing DNA damage, injectable mitochondria increased the fragmentation rate.

“Mitochondrial injection into these mice prevented fragmentation in the mouse oocytes where there were originally abnormal mitochondria,” said Dr. Casper, adding that “oocytes that had DNA damage couldn’t be rescued.” Mitochondrial injection also accelerates preimplantation embryo development.

The key issue, then, is whether it's possible to prevent human embryo fragmentation by injecting healthy young mitochondria into oocytes. “Could we prevent mitochondrial disease by creating mitochondrial heteroplasmy (the coexistence of two genetically distinct mtDNA genomes),” posited Dr. Casper. Ooplasm injection is currently banned by the FDA in the U.S., so it is not clinically possible to do mitochondrial injection.

To circumvent the issue of changing the genome by injecting mitochondria, Dr. Casper’s group proposed microinjecting recombinant mitochondria proteins of the Bcl family to improve the embryo without altering the fetal genome. This protein will get deleted out over subsequent cell divisions.

He presented preliminary results from injecting recombinant analog of Bcl-x (which is available commercially) into ICR zygotes cultured in human tubal fluid medium (HTF). (ICR mice are a hardy outbred stock developed for their good reproductive performance and fast growth rate.) There was a 30% to 40% increase in blastocyst formation rate compared to the control embryos. At day 4, there was increased cell number and reduced cell death in zygotes injected with Bcl-x protein.

Injection of recombinant mitochondrial protein increases blastocyst formation, decreases cell death index, and increases cell number in murine blastocysts.”

Dr. Casper concluded: “Injection of recombinant mitochondrial protein increases blastocyst formation, decreases cell death index, and increases cell number in murine blastocysts. It is easy to manufacture and store compared to mitochondrial preparations and does not change the embryonic genome. This may be an optimal treatment to improve embryo quality in the future.”
Beyond Estrogen: Changing Approaches to the Management of Menopausal Symptoms

At a symposium held during the Conjoint Annual Meeting of the American Society for Reproductive Medicine (ASRM) and the Canadian Fertility and Andrology Society (CFAS), a panel of experts discussed issues in the medical management of menopausal women, including new treatment options for menopausal symptoms, the impact of postmenopausal treatment on mammographic findings, treatment options for osteoporosis prevention, and sexual health.

Speakers

Edward Morris, MD
Norfolk and Norwich Hospital
Norwich, United Kingdom

John E. Buster, MD
Baylor College of Medicine
Houston, Texas

Roger A. Lobo, MD
Columbia University
New York, New York

Sheryl A. Kingsberg, PhD
MacDonald Women’s Hospital
Cleveland, Ohio

Managing Menopausal Symptoms in 2005

In light of the widely publicized results of the Women’s Health Initiative study, if a woman wishes to reconsider her options for postmenopausal treatment, she has two choices: either stay on hormone therapy or avoid hormone therapy,” began Edward Morris, MD, Consultant Gynecologist, Norfolk and Norwich Hospital, Norwich, United Kingdom. He discussed both options, beginning with hormone therapy.

Symptoms that justify use of hormone therapy include hot flashes, lower genital tract changes, psychological changes, memory changes, and decreased libido. Dr. Morris recommended starting hormone therapy any time symptoms become an issue for the patient.

If the patient wishes to use hormone therapy, she can use normal doses, low doses, or ultra-low doses. Dr. Morris noted that estrogen therapy, with or without progestogen, has been shown to significantly reduce hot flashes, according to a systematic review of 21 randomized controlled trials (MacLennan A et al. Cochrane Database. Syst Rev. 2001: CD002978). A study by Utian et al. found that both low and normal doses of estrogen (0.3, 0.45, and 0.625 mg) significantly reduced vasomotor symptoms (Fertil Steril. 2001:75:1065-1079).

Treatment options for urogenital atrophy include systemic therapies (estrogen, tibolone [not available in the U.S.], and phytoestrogens) or local therapies, including moisturization or vaginal estrogen (cream, tablets/pessaries, or vaginal rings). A meta-analysis of 58 studies, 10 of which were randomized, placebo-controlled trials, looking at varying routes and types of estrogen, found that, compared to placebo, estrogen improved dyspareunia, patient-reported symptoms, clinician assessment, and cytology (Cardozo L et al. Obstet Gynecol. 1998;92:722-727). There was greater clinical effect with local treatment compared to oral administration.

Another meta-analysis that looked at vaginal atrophy in postmenopausal women examined 16 studies of local therapy (creams, pessaries, tablets, and ring) (Suckling J et al. Cochrane Database. Syst Rev. 2003: CD001500). On patient assessment of dyspareunia, the ring was more effective than placebo, the tablet was more effective than both placebo and the ring, and estrogen cream was more effective than the vaginal moisturizer Replens. On patient assessment of vaginal dryness, the tablet was more effective than the ring, estrogen cream, and placebo; the cream was more effective than vaginal moisturizer.

“Vaginal administration of hormones appears to be effective for vaginal symptoms,” said Dr. Morris.

For women who wish to avoid hormone therapy, Dr. Morris advises stopping smoking and alcohol use and increasing exercise. In addition, many women choose alternatives to hormone therapy, such as homeopathic and herbal preparations or phytoestrogens. Tibolone can also be used as an alternative to estrogen/progestin therapy. Pharmaceutical alternatives for osteoporosis prevention include bisphosphonates and raloxifene.

Dr. Morris noted that estrogen therapy, with or without progestogen, has been shown to significantly reduce hot flashes, according to a systematic review of 21 randomized controlled trials.

Tibolone for menopausal symptoms. Studies in the United Kingdom (where tibolone is available) have shown that doses of 1.25, 2.5, and 5 mg reduced the frequency of hot flushes and sweating episodes compared with placebo (Landgren MB et al. Br J Obstet Gynaecol. 2002;109:1109-1114). The effect of tibolone on urogenital symptoms was studied by Rymer et al., who found significant reductions in vagi-

This program was supported by an unrestricted educational grant from Organon Pharmaceuticals Inc.

Dr. Morris next discussed non-hormone drug treatments for hot flushes. The data on clonidine are poor. Danazol has a weak effect. Gabapentin appears to have a good effect, but more data are needed. More data also are needed on the selective serotonin reuptake inhibitors (SSRIs). Mirtazapine appears to have a good effect, but, again, more data are needed. Progestins also have a good effect, but side effects rule it out for some patients.

Dr. Morris cited a randomized controlled trial of the SSRI paroxetine (12.5 mg and 25 mg), which reported a statistically significant reduction in hot flushes with both doses (Stearns V et al. JAMA. 2003;289(21):2827-2834). Fluoxetine (20 mg) and venlafaxine (75 and 150 mg) also have been shown to reduce hot flushes.

Dr. Morris presented a theory, proposed by Berendsen, as to how serotonin may influence menopausal symptoms. “5-hydroxytryptamine (5-HT) blood levels are 50% lower in menopausal women than in premenopausal controls,” he said. Hormone therapy (estrogen or progestrone) increases urinary excretion of 5-hydroxyindole acetic acid (5HIAA) and expression of tryptophan hydroxylase. Stimulating the 5-HT$_{2A}$ receptor in rats induces hyperthermia.

In humans, stimulation of the receptor produces hot sweats. Withdrawing estrogen upregulates the receptor. Giving hormone therapy downregulates the receptor. Giving a 5-HT$_{2A}$ receptor antagonist (mianserin, mirtazapine) to rats normalizes ovariectomy-induced tail temperature changes.

A hot flush begins with a stimulus (heat, coffee, alcohol, etc.), which results in an increased release of serotonin and stimulation of 5-HT$_{2A}$ hypothalamic receptors, which changes the setpoint temperature. To release body heat, peripheral vasodilation occurs, and thus the hot flush. In a hypoestrogenic state, there is a 50% reduction in 5-HT, which leads to increased 5-HT$_{2A}$ receptor sensitivity within the hypothalamus. “This thermoregulator cycle becomes more sensitive,” said Dr. Morris. The slightest stimulus can set off a hot flush. SSRIs eliminate the 50% reduction in 5-HT, which stops, or limits, some of the hyper-sensitivity of the system. Mirtazapine blocks the 5-HT$_{2A}$ receptor, which reduces the sensitivity of the response.

Dr. Morris concluded: “Hormone therapy is still an option for treatment of menopausal symptoms. The alternatives are not yet well researched, and therefore regular review is essential.” He advised particularly watching SSRIs and serotonin norepinephrine reuptake inhibitors (SNRIs).

Mammograms and Menopause

John E. Buster, MD, Professor of Obstetrics and Gynecology, Baylor College of Medicine, discussed new technology for mammograms that measures breast mammographic percent density (MPD). Breast MPD can be measured with little variation among radiologists using standard screening mammography. A digital mammogram is ideal, but a film mammogram also can be used by scanning and digitizing it. Digital mammography takes an electronic image of the breast, allowing the data to be stored on a computer. Film mammography uses film to capture and display the image of the breast. An MPD score of 50% or higher is considered abnormal.

Factors that influence breast MPD that are not related to malignancy and must be considered include heredity, age, and obesity. “As a woman ages, breast density decreases, which makes it easier to see abnormalities because the density does not impair the image on the mammogram,” said Dr. Buster. Obesity also is associated with decreased breast MPD. “Overweight women are at increased risk for breast cancer, but their MPD is lower than it is for normal-weight women,” he said. Therefore, studies should control for body weight.

“Increased breast MPD is associated with an increase in breast lesions (e.g., atypical ductal hyperplasia),” said Dr. Buster.

Given these findings, it is recommended that a woman with a high breast MPD score probably should stop estrogen or estrogen-plus-progestrone therapy two weeks prior to a mammography. Tibolone does not need to be stopped prior to mammography.

Dr. Buster next raised the issue of whether an increase in MPD in response to hormone therapy increases risk for breast cancer, which is not known at this time.

“Breast MPD (as currently measured) is probably a useful marker for increased risk of benign breast disease and cancer,” concluded Dr. Buster, adding that “clearly, more data are needed.”
Making Choices in the Prevention of Osteoporosis: Evaluating Treatment Options

In women, peak bone mass occurs by the third decade of life and then drops throughout life, most abruptly at the time of menopause—largely due to estrogen deficiency,” began Roger A. Lobo, MD, Professor of Obstetrics and Gynecology, Columbia University.

He cited a meta-analysis of postmenopausal women that showed that estrogen, alendronate, risedronate, and raloxifene are beneficial for spine and hip bone mineral density (BMD). Calcitonin is also useful for spine BMD (Cranney A et al. *Endocr Rev*. 2002;23: 570-578). Dr. Lobo noted that tibolone is available in other countries and has an indication for osteoporosis. “It causes a reduction in bone resorption and therefore prevents osteoporosis,” said Dr. Lobo.

Another measure of the effectiveness of osteoporosis drugs is fracture risk reduction. Alendronate and risedronate reduce risk for both hip and spine fracture. “Calcitonin has some efficacy for vertebral fractures, but it is not very potent for prevention of all fractures,” said Dr. Lobo. Data from the WHI study show a reduction in all fractures (including hip fractures) with estrogen.

“When a patient has a bone fracture after age 50, we presume it’s osteoporotic or related to bone mass,” said Dr. Lobo. Nonetheless, according to a study by Elliot-Gibson, only 20% of women over age 50 who have fractures receive osteoporosis treatment (*Osteoporos Int*. 2004;15:767-778). Dr. Lobo listed the following factors that warrant osteoporosis treatment: prior osteoporotic-related fracture, any fracture after age 50, a prevalent vertebral fracture, BMD T score of ≤ -2.5, or T score -1 to -2.5 (osteopenia) plus another risk factor. Risk factors include a strong family history of osteoporosis, older age, low body weight, and family history of fracture.

Dr. Lobo emphasized that it is not just bone mineral density that determines fracture risk. He cited a study of 8,000 postmenopausal women in which 54% of hip fractures occurred in women with T scores that were nonosteoporotic (Wainwright SA et al. *J Clin Endocrinol Metab*. 2005;90:2787-2793).

### Case Studies

Dr. Lobo next presented case studies. The first was a 51-year-old woman with no prior fractures; she had been amenorrheic for one year and was experiencing hot flashes. Her mother had osteoporotic-related fractures. BMD T scores were -2.0 (spine) and -1.4 (total hip). She meets the criteria for treatment based on her BMD scores, but she also needs treatment for menopausal symptoms. Dr. Lobo suggested menopausal hormone therapy as a reasonable approach with this patient. If she has had a hysterectomy for nonmalignant reasons, estrogen alone is indicated.

### Table 1. Fracture Risk Score

<table>
<thead>
<tr>
<th>Feature</th>
<th>Point Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥65 years</td>
<td></td>
</tr>
<tr>
<td>Broken bones &gt;50 years</td>
<td>1</td>
</tr>
<tr>
<td>Maternal hip fracture &gt;50 years</td>
<td>1</td>
</tr>
<tr>
<td>Weight &lt;125 lbs.</td>
<td>1</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1</td>
</tr>
<tr>
<td>Need for arms to stand up</td>
<td>1</td>
</tr>
<tr>
<td>T-score</td>
<td></td>
</tr>
<tr>
<td>-1.0 to -2.0</td>
<td>1</td>
</tr>
<tr>
<td>-2.0 to -2.5</td>
<td>1</td>
</tr>
<tr>
<td>≤-2.5</td>
<td>1</td>
</tr>
</tbody>
</table>


### Figure 1. Clinical Vertebral and Non-Vertebral Fracture: 5-Year Risk

Study of Osteoporotic Fractures.

*Most vertebral fractures are asymptomatic.*

Beyond Estrogen: Changing Approaches to the Management of Menopausal Symptoms

Dr. Lobo described a scenario in which the same patient was not treated with hormones. Instead, she tried clonidine and an SSRI for symptoms. She has a prior history of breast cancer, and is on tamoxifen and an aromatase inhibitor. Tamoxifen is antiresorptive, causing a small reduction in BMD, while aromatase inhibitors can cause profound hypoestrogenism, which causes more bone loss.

At age 55, this patient now has BMD T scores of -2.2 (spine) and -1.7 (hip). “At this point, she would certainly warrant treatment, probably with a bisphosphonate,” said Dr. Lobo.

T scores alone may not be sufficient to predict future fractures. Fracture risk factors can be used along with T scores to predict five-year fracture probability (Black DM et al. Osteoporos Int. 2001; 12:519-528) (Table 1 and Figure 1).

Dr. Lobo presented two cases to illustrate how a fracture risk scoring system works. Mrs. L is a 61-year-old woman with osteoporosis (T score -2.8). Mrs. R is 64 years old with a T score of -1.7. There appears to be a greater need to treat Mrs. L than Mrs. R. However, Mrs. L is a 155-pound nonsmoker with no family or personal history of fracture. Mrs. R, on the other hand, smokes, weighs 124 pounds, has a history of humerus fracture at age 57, and has maternal history of hip fracture.

Using the scoring system, Mrs. L (with an osteoporotic T score) has a 10% to 15% chance of having a fracture within five years, whereas the risk for Mrs. R (with an osteopenic T score) is 20% to 25%. “DEXA scores just give you bone mineral density,” emphasized Dr. Lobo. “Bone strength and the integrity of bone can not yet be measured.”

Sexual Health and the Menopausal Woman: Addressing the Challenges

Sheryl A. Kingsberg, PhD, Associate Professor, Departments of Reproductive Biology and Psychiatry, MacDonald Women’s Hospital and Case Western Reserve University, addressed the challenges of assessing and treating female sexual disorders.

She began by pointing out barriers to effective physician/patient communication about sexual problems. For example, the Pfizer Global Study of Sexual Attitudes and Behaviors, of 26,000 adults aged 40 to 80, found that only 14% of U.S. respondents reported that their physician had asked them whether they had had sexual difficulties at any time in the last three years. In addition, patients are often reluctant to bring up sexual concerns. A poll of 500 adults over age 25, by Marwick, found that 71% felt the doctor would dismiss their concerns, 68% thought the doctor would be uncomfortable, and 76% said there was no medical treatment for their problem anyway (JAMA. 1999;281:2173-2174). Bachmann et al. studied 887 gynecologic outpatients and found that only 3% spontaneously offered sexual complaints, while 19% acknowledged a complaint if directly asked by the physician (Obstet Gynecol. 1989;73:425-427).

Physicians may not address their patients’ sexual concerns because of lack of training and inadequate knowledge or skills. Most U.S. medical schools offer less than 10 hours of education in human sexuality. In addition, physicians may not be aware of associated comorbid conditions. Common medical causes of hypoactive sexual desire disorder (HSDD) include thyroid disease, metabolic/nutritional disorders, depression, immunologic disorders, fatigue from medical disorders, and androgen insufficiency.

“Physicians also may believe that sexuality is relatively unimportant to quality of life in menopausal women,” said Dr. Kingsberg. “However, healthy sexual functioning can improve self-esteem and self-image, nurture relationships, and increase a woman’s motivation to adopt a healthier lifestyle.” In the Pfizer Global Health Survey, 63% of women responded that sex is very important.

Two additional barriers preventing physicians from addressing sexual concerns are time constraints and the fact that there are no FDA-approved treatments for female sexual dysfunction.

The DSM IV lists six female sexual dysfunctions: HSDD, sexual aversion disorder, female sexual arousal disorder, female orgasmic disorder, dyspareunia, and vaginismus. Menopause can have an impact on all of these, with the exception of sexual aversion disorder. Dr. Kingsberg focused on HSDD, which is most relevant to postmenopausal patients. HSDD is defined as persistently or recurrently deficient (or absent) sexual fantasies and desire for sexual activity that causes personal distress.

Dr. Kingsberg explained the components of sexual desire. The first is drive, “which is the biological piece of desire, the spontaneous sexual interest,” she said. Drive declines with age in both men and women, and it can be impacted by factors such as illness, depression, and treatment for depression (such as SSRIs). The second component of sexual desire is expectations, beliefs, and values; and the third component is motivation. “When assessing a woman with low desire, it’s important to address all three components,” said Dr. Kingsberg.

She continued with a description of the female sexual response cycle. For many postmenopausal women, desire for emotional intimacy is more likely to start the cycle than sexual drive. Sexual stimulation and the appropriate psychological mood then lead to arousal and desire, which trigger the sexual drive. Emotional and physical satisfaction continue to drive the cycle.

There are currently no FDA-approved medical treatments for HSDD in postmenopausal women, but several therapies are in Phase III clinical trials, including tibolone, topical testosterone patches and gels, and oral methyltestosterone.

In conclusion, Dr. Kingsberg encouraged physicians to include sexual health in the management of menopausal patients and to open more dialogue with patients about their sexual concerns.
American Society for Reproductive Medicine

would like to thank the following companies for their support of this publication:

Ferring Pharmaceuticals, Inc.
Organon Pharmaceuticals Inc.
Wyeth Pharmaceuticals