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Many of you will have noted that several years ago, the Department named Residents’ Research Day in honor of one of our past Chairs, Dr. Charles Lee Buxton. What follows is a brief summary of the events that led to Dr. Buxton’s notoriety as an advocate for family planning services in the United States.

This past June marked the 42nd anniversary of what may be considered as the landmark case leading to legal access to family planning services in the United States. On June 7, 1965, the Supreme Court overturned the convictions of Estelle Griswold, the Executive Director of the Planned Parenthood League of Connecticut, and Charles Lee Buxton, the Chair of Ob/Gyn here at Yale University. Both were arrested for defying a Connecticut statute by opening a clinic in New Haven that provided birth control services to married couples.

Below are more details leading up to this historic decision affirming the basic right to privacy in this country as we reflect on the actions of these courageous individuals.

**ACCESS TO CONTRACEPTIVES TARGET OF COMSTOCK ACT**

Anthony Comstock, an anti-obscenity crusader intent on legislating public morality in the United States, identified birth control as a major target. On March 3, 1873, Congress passed the “Act for the Suppression of Trade in, and Circulation of, Obscene Literature and Articles for Immoral Use,” later known as the Comstock Act. It became a federal offense to distribute birth control through the mail or across state lines. As late as 1960, birth control in the United States was still regulated by such laws that arose in the 19th century. Thirty states had laws on the books prohibiting or restricting the sale and/or use of contraception.

Immediately after enactment of the federal Comstock Act, states enacted versions of their own. Connecticut had one of the most draconian — it prohibited use as well as distribution or possession of contraceptives and did not make an exception to save a woman’s life. In Connecticut, married couples could be arrested for using birth control in their own homes.

**FAMILY PLANNING ADVOCATES TURN TO THE COURTS FOR RELIEF**

In 1938, in a case involving Margaret Sanger, U.S. Court of Appeals Judge August Hand lifted the federal ban blocking physicians from importing birth control materials and effectively ended the use of the Comstock Act to target birth control information and devices.

However, many state laws placing limits on use of contraception were still on the books. When efforts to alter these laws failed, family planning advocates turned to the federal courts. These efforts resulted in legal access to contraceptives for physicians when used for saving lives or protecting the health of patients. Frustrated by limited success in the federal courts, the Planned Parenthood League of Connecticut began efforts to seek constitutional relief. Plaintiffs were selected for eight lawsuits. While these attempts failed, the Supreme Court decision in Poe v. Ullman (1961) provided a road map for a winnable case. In Poe, the high court dismissed the claim of a doctor and his patients that the Connecticut law denied their Fourteenth Amendment due process rights, claiming that the plaintiff lacked standing to sue because the law had not been enforced in many years.

On November 1, 1961, in defiance of the Connecticut Comstock statute, the Planned Parenthood League of Connecticut (PPLC) opened a birth control clinic to directly challenge the law. PPLC Executive Director Estelle Griswold and Medical Director Charles Lee Buxton, M.D., chairman of the Yale School of Medicine Department of Obstetrics and Gynecology, opened the clinic in New Haven, Connecticut, and served crowds of women before being arrested by the police on November 10, 1961.

The clinic gave information, instruction, and medical
advice to married couples about preventing conception. They examined the wives and prescribed the best contraceptive device or material for them. Fees were usually charged, although some couples were provided complimentary medical care.

Griswold and Buxton were convicted of providing contraceptive information to married couples. On appeal, the Supreme Court overturned the convictions, ruling 7-2 in favor of Griswold and deciding that the Connecticut law was unconstitutional.

**U.S. SUPREME COURT ISSUES LANDMARK DECISION IN GRISWOLD V. CONNECTICUT**

Justice William O. Douglas’s majority opinion identified ways in which the First, Third, Fourth, and Fifth Amendments to the Constitution provided degrees of privacy. Further, Douglas maintained that married people’s right of privacy predated the Bill of Rights and laws invading this right were unconstitutional on their face. Douglas held that the rights people have are more than what can be read in the explicit language of the Constitutional text. Citing a number of earlier cases, he emphasized how the Court had established a justified precedent for protecting marital and family relationships from government interference without strong justification.

A concurring opinion by Justice Arthur Goldberg, joined by Justices Earl Warren and William Brennan, located the right to marital privacy in the rarely invoked Ninth Amendment, which states that the fact that a particular right is not mentioned in the U.S. Constitution does not mean that it can be curtailed by government.
EDITOR’S NOTE

I have taken the editor’s prerogative to ask some of our illustrious grand rounds presenters to prepare their talks as manuscripts for all our alumni, as I thought that these topics would prove interesting to all. In what I hope will become a tradition in this journal, the first paper is from our visiting professor for residents’ day in June. Emre Seli is of course one of our very accomplished former residents and reproductive endocrinology fellows, who presented an exciting update on our fertility preservation program and potential developments in the field. Martha Hickey spent a semester here working with Charly Lockwood and Hugh Taylor on endometrial biology, but as an expert in menopause management from Australia, she was asked to update us on therapy for menopausal breast cancer survivors. Denny Sakkas joined us several years ago to run the IVF lab, and is a major factor in our highly successful statistics; Denny addressed us on the often-overlooked paternal factors in reproduction. Laura MacIsaac visited with Susan Richman who has started our program in family planning, and gave a very enthusiastic overview of intrauterine contraception.

We will continue to try to bring you highlights of our most interesting grand rounds. In other sections, besides our residents’ presentations, we would like to share with you highlights of current research from the department, bringing you abstracts of papers our faculty presented or will present to the SGI and SMFM this year.
ABSTRACT

Behaviors that chronically activate the hypothalamic-pituitary-adrenal (HPA) axis and/or suppress the hypothalamic-pituitary-thyroidal (HPT) axis disrupt the hypothalamic-pituitary-gonadal axis in women and men. Individuals with stress-induced anovulation typically engage in a combination of behaviors that concomitantly heighten psychogenic stress and increase energy demand. Although it is not widely recognized clinically, functional forms of hypothalamic hypogonadism are more than an isolated disruption of GnRH drive and reproductive compromise. Indeed, women with functional hypothalamic amenorrhea display a constellation of neuroendocrine aberrations that reflect allostatic adjustments to chronic stress. Given these considerations, we have suggested that complete neuroendocrine recovery involves more than reproductive recovery. Hormone replacement strategies have limited benefit because they do not ameliorate allostatic endocrine adjustments, particularly the activation of the adrenal and the suppression of the thyroidal axes. Indeed, the rationale for the use of sex steroid replacement is based on the erroneous assumption that functional forms of hypothalamic hypogonadism represent only or primarily an alteration in the hypothalamic-pituitary-gonadal axis. Potential health consequences of functional hypothalamic amenorrhea, often termed stress-induced anovulation, may include an increased risk of cardiovascular disease, osteoporosis, depression, other psychiatric conditions, and dementia. Although fertility can be restored with exogenous administration of gonadotropins or pulsatile GnRH, fertility management alone will not permit recovery of the adrenal and thyroidal axes. Initiating pregnancy with exogenous means without reversing the hormonal milieu induced by chronic stress may increase the likelihood of poor obstetrical, fetal, or neonatal outcomes. In contrast, behavioral and psychological interventions that address problematic behaviors and attitudes, such as cognitive behavior therapy, have the potential to permit resumption of full ovarian function along with recovery of the adrenal, thyroidal, and other neuroendocrine aberrations. Full endocrine recovery potentially offers better individual, maternal, and child health.

KEY WORDS
Stress, Amenorrhea, Anovulation, Hypothyroidism, Allostasis

CLINICAL AND PHYSIOLOGICAL BACKGROUND

Our expanding scientific knowledge has permitted us to refine our conceptualization of the bidirectional interaction between behaviors and reproductive function (Figure 1) and to devise and test holistic interventions intended to mitigate acute and chronic health burden. Because we can monitor the endocrine responses to thoughts, feelings, and behaviors, we now can specify how attitudes...
and behaviors gate hypothalamic-pituitary-gonadal and other neuroendocrine function.

Gonadal function depends directly upon secretion from the hypothalamus of gonadotropin-releasing hormone (GnRH). If a marked decline in endogenous pulsatile GnRH secretion occurs, pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) falls accordingly. In women, compromised folliculogenesis with luteal insufficiency or anovulation may result. Decreased GnRH pulsatility has been shown to be a common cause of anovulation and amenorrhea (1). Decrements in central GnRH-LH/FSH drive exist on a continuum, however, and may vary from day to day (2). Because of this potential variability in GnRH secretion, ovarian compromise exists as a spectrum and may manifest as amenorrhea, polymenorrhea, oligomenorrhea, or luteal phase deficiency with a preserved menstrual interval and luteal length. Clinically, the decreased ovarian function can be occult or obvious. In men, decreased central GnRH drive may cause oligoasthenozoospermia. Typically, gonadal compromise in men is clinically occult unless fertility is sought and the compromise is sufficiently significant to cause infertility. However, severe hypothalamic hypogonadism in men may present as decreased libido, diminished muscle mass, or altered hair growth.

The most common cause of reduced GnRH drive is functional; that is, it is not due to identifiable organic causes such as hypothalamic tumors or pituitary adenomas. Functional hypothalamic hypogonadism can be defined as a common and theoretically reversible form of gonadal compromise in which psychophysiological and behavioral responses to life events activate central neuroregulator networks to effect metabolic mobilization and reproductive compromise manifested as suppression of GnRH drive (3). The GnRH pulse generator refers to a network of GnRH neurons diffusely distributed in the medial basal hypothalamus. Most, but not all, GnRH axons project to the median eminence, allowing pulses of GnRH to be released into the portal vasculature. GnRH neurons communicate with one another via synapses. Although GnRH neurons are endogenously pulsatile, their activity must be synchronized by GnRH-to-GnRH synapses for the GnRH bolus released into the portal vasculature to be of sufficient magnitude to trigger pituitary release of LH and FSH. The GnRH pulse generator is active during fetal and neonatal life (4) and then is inhibited or desynchronized by poorly defined central processes until the onset of puberty (5-7). Puberty and ovulation are centrally driven processes that can be achieved by providing exogenous pulses of GnRH at a frequency of one pulse every 60 to 90 minutes (8-10) or by stimulating the dormant GnRH neuronal network with excitatory amino acids (11).

The mechanisms that modulate the activity of the GnRH neuronal network or GnRH pulse generator are largely unknown. GnRH neurons have been demonstrated to receive synapses from neurons that contain GnRH, the endogenous opioid peptide β-endorphin (12), NPY (13), and catecholamines (14-16). Other factors that modulate the frequency or activity of the GnRH pulse generator are thought to exert their effects indirectly by acting through the neuronal systems that have direct synaptic connections or by interacting with glial cells that interpose between synapses. For instance, progesterone slows the frequency of pulsatile GnRH release by increasing hypothalamic opioidergic tone. Peripheral substances may gain access to the GnRH neuronal network via specialized neurovascular cells that line the fenestrated blood-brain barrier at the level of the hypothalamus and median eminence (17). It appears that the brain-gut axis communicates the metabolic state of an individual to the hypothalamic GnRH neurons, but the exact nature of these mechanisms remains to be better elucidated (18).
Quantifying GnRH drive is tedious. In humans, GnRH pulsatile secretion can be inferred only from the pattern of LH secretion in the circulation. Blood samples must be obtained via an indwelling intravenous catheter at intervals of 10 to 15 minutes for durations of 12 to 24 hours. Even so, inherent limitations exist in estimating GnRH secretion from peripheral LH patterns. Given these considerations, it is not surprising that documentation of the role of disturbed central GnRH drive as a cause of reduced ovarian function was accomplished only relatively recently (19).

Technical limitations also plague recognition and quantification of stress and metabolic mobilization. The accuracy of psychometric inventories for assessing and quantifying stress, mood, and cognitive patterns is inherently constrained by reporting biases, while biochemical and biophysical indices of stress and metabolic mobilization are technically cumbersome and expensive to collect. Neuroimaging techniques hold promise for understanding how stress signals are transduced within the brain, but technical issues limit investigative and clinical use.

Two primary systems that mediate the stress response are the hypothalamic corticotrophin-releasing hormone (CRH) and the locus coruleus-noradrenergic (LC-NE) neuronal networks and their respective effector systems, the pituitary-adrenal axis and the autonomic pathways (20). While innumerable animal studies have demonstrated that activation of the HPA axis by a variety of stressful paradigms induces reproductive compromise, only a few studies have elucidated the mechanisms mediating the disruption of GnRH drive. Direct evidence exists for CRH, β-endorphin, dopamine, and vasopressin (12,14,21-28). Recently, neuropeptide Y has been implicated as a neuromodulatory link between metabolic deficits induced by diet and exercise and reduced GnRH drive (29). A key inhibitory neurotransmitter system in the brain utilizes γ-aminobutyric acid (GABA). GABA opens the same potassium channels in neurons of the medio-basal hypothalamus as μ-opioid receptor agonists such as β-endorphin. GABA inhibited GnRH gene expression in rats (30) and suppressed pubertal GnRH increase in juvenile female rhesus monkeys (31), but the role of GABA in human hypothalamic hypogonadism remains unclear. There are many metabolic signals that also might communicate the metabolic status of an individual to the hypothalamus. The exact role of these signals in functional hypothalamic hypogonadism also remains to be elucidated. A partial list of putative neuromodulators of GnRH activity or synchronicity is shown in Table 1.

**Table 1: Putative modulators of GnRH drive**

- CRH
- Opioids
- Adrenergic
- GABA
- Dopamine
- Serotonin
- Immune
- Metabolic signals
- Sex steroids
- Gonadal peptides
- Growth factors
- Gial cells
- GnRH
- Other

**PATHOGENESIS OF FHA**

The best biochemical evidence supporting the concept that stress impairs ovarian function in women is the consistent demonstration that women with hypogonadotropic hypogonadism not due to defined organic conditions have higher cortisol levels than eumenorrheic, ovulatory women (3,32-33). There is direct evidence that this relationship holds in women with athletic amenorrhea as well (34,35). Loucks et al. observed an inverse relationship between the degree of ovarian compromise and the increase in circulating 24-h cortisol levels (34). When compared with eumenorrheic but sedentary women, eumenorrheic athletes had less luteal progesterone secretion as evidenced by lower urinary levels of pregnanediol-glucuronide, fewer LH pulses in a day, and higher cortisol levels. Furthermore, amenorrheic athletes who were anovulatory had the fewest LH pulses in a day and the highest cortisol levels.

An inverse relationship holds between HPA axis activation, independent of the life events or behaviors that initiate or sustain this activation, and suppression of the hypothalamic GnRH drive to the ovary, as evidenced by marked reduction in the 24-h LH pulse frequency in women with functional forms of hypothalamic amenorrhea (3,32,33,36). Other hypothalamic outputs also are altered in women with
functional hypothalamic amenorrhea (FHA). Given the neuroanatomical integration of the hypothalamus, this is predictable. The purpose of the hypothalamus is to generate an "endocrine action plan" to preserve the organism in the face of challenge. Part of the action plan involves metabolic mobilization. However, metabolic mobilization involves more than an increase in cortisol secretion. In FHA, the HPT axis differs from that of eumenorrheic women in that TSH is not increased in response to decrements in thyroxine and thyronine. This pattern indicates an altered hypothalamic set point akin to what is seen in hospitalized patients who develop what is referred to as "sick euthyroid syndrome" (3). In athletic women, a similar alteration in the HPT was seen only in those who had compromised ovarian function (37). The secretory patterns of growth hormone, prolactin, and melatonin also differed in FHA from those in eumenorrheic women (3,38). The constellation of neuroendocrine aberrations that accompany FHA strongly suggests that central neurotransmission has been altered so as to allow homeostatic hypothalamic responses and, when chronic, a new allostatic state. The aim of these compensatory adjustments is to equip the individual to cope with actual and perceived challenge. In this context, then, the hypothalamus links the external environment, the internal milieu, and gonadal function.

The process of recovering from the allostatic state of FHA is less well documented. FHA is theoretically reversible. We first showed that women in the process of spontaneously recovering from FHA displayed cortisol levels comparable to those of eumenorrheic women before there was complete recovery of GnRH drive (36). Further, a marked increase in TSH occurred before increases in thyroxine and thyronine. We then utilized cognitive behavior therapy to catalyze reproductive recovery and showed that reproductive recovery did not require weight gain. This leaves open the question as to whether reproductive recovery is accompanied by full metabolic recovery. To date, there is limited data suggesting that hypothalamic recovery involves a sequence of interlinked adjustments starting with HPA restoration, return of GnRH pulsatility and reproductive recovery, and lastly remission of hypothalamic hypothyroidism.

The characteristic hypothalamic alterations associated with FHA only become problematic when ongoing challenges elicit a chronic (allostatic) rather than acute (homeostatic) response. The long-term consequences of persistent HPA activation have been studied in animal models and hippocampal neuron loss has been documented (39). Further, persistent stress has been linked to acute and chronic health burden in humans (40). For instance, recent data obtained in women with weight-restored anorexia nervosa who remained amenorrheic indicated that exogenous sex steroid replacement was unable to stimulate appropriate bone accretion (41, 42). Hormone exposure alone fails to compensate for the catabolic state induced by ongoing metabolic derangements such as increased cortisol exposure, altered growth hormone action, or hypothalamic hypothyroidism. Because of the concomitant endocrine and metabolic disturbances, hypothalamic hypogonadism must be regarded as a condition deserving clinical attention even when fertility is not an immediate goal.

The central neuromodulators responsible for the initiation and maintenance of the disruption of GnRH are difficult to identify in humans. First, the factors that initiate the disruption may differ from neuroregulators that maintain the disruption. To study the initiating factors, one would need to intensely monitor populations at risk for the development of hypothalamic hypogonadism or try to induce hypothalamic hypogonadism in a nonhuman primate model. Once a chronic hypogonadal state had been reached, it theoretically would be possible to identify the agents that maintain this disruption by administering antagonists that cross the brain-blood barrier, by performing lumbar punctures and obtaining cerebrospinal fluid, or by performing neuroimaging studies with an appropriate ligand. To date, efforts to identify these neuromodulators in humans have yielded inconsistent results. Thus, naloxone, an opioidergic blocker, increased LH pulse frequency or levels in some, but not all, women with FHA (43, 44). Also, infusion of metoclopramide, a dopamine receptor blocker, to women with FHA accelerated LH pulse frequency in our laboratory, while that of eumenorrheic women remained constant (45). To explore the hypothesis that the reduction in GnRH drive was maintained by CRH, vasopressin, β-endorphin, or a combination of these factors, we performed lumbar punctures to obtain rostral cerebrospinal fluid in women with FHA and those with eumenorrhea. This approach has revealed increased CRH in subjects with depression and anorexia nervosa, but we found that CRH levels were identical in women.
with FHA and eumenorrhea (46). Vasopressin levels were similar, and surprisingly, $\beta$-endorphin levels were lower in women with FHA. These data argue against the suspected role for CRH or opioids in the maintenance of FHA and suggest that other factors may play a role once FHA has been established. Recently, one group reported that a single 2mg dose of alprazolam, a GABA receptor agonist, decreased cortisol levels and increased LH pulse frequency from 0.8 to 2.0 pulses/8h in women with stress-related anovulation, while its administration decreased LH pulse frequency in eumenorrheic women in the follicular phase (47). We recently showed, using a monkey model, that stress-sensitive monkeys with reproductive compromise displayed altered prolactin and cortisol responses to the serotonergic agonist, fenfluramine, indicating an underlying reduction in serotonergic tone (48). Further, stress-sensitive monkeys demonstrated increased cortisol secretion when behaviorally challenged, underscoring the notion that “stressfulness” is more of a characteristic of the individual than the stimulus. Other data suggest a possible indirect role for GABA neurons in the stress-induced neuromodulation of GnRH pulsatility. More recently, we have been interested in how metabolic challenge and energy deficits may alter the brain and induce vulnerability, including reproductive compromise, to subsequent psychogenic stressors. It appears that undernutrition heightens reactivity to subsequent psychosocial challenge, but the neural mechanisms mediating this relationship are not clear. Not surprisingly, the neurochemistry of stress and FHA is far from simple, and firm conclusions are not possible at present.

**BEHAVIORAL CAUSES OF STRESS-INDUCED ANOVULATION**

The variables that activate the adrenal axis and suppress the thyroidal axis while leading to reproductive quiescence are not always readily identifiable. It has been suggested that different stressors and behaviors elicit somewhat different central mechanisms, such that the signals that alter hypothalamic function are specific to the type of stressor. Indeed, this variability may well explain in part why the neurochemistry of stress is so complex. Psychosocial dilemmas are seen as activating those central pathways subserving perception, whereas exercise and weight loss are generally viewed as disturbing metabolic regulation. Although it seems logical that there is specificity in the neural or peripheral cascades that mediate the response to specific stressors, we have no method for clearly differentiating psychogenic from metabolic stress. Psychogenic stress has a metabolic cost and metabolic stressors, such as food restriction and excessive exercise, are often initiated to cope with psychogenic stress. However, until proven otherwise, the safest assumption is that stress comes in flavors and that some individuals are more sensitive than others to the same stressor or set of stressors.

The notion that stress comes in neurochemical flavors is supported by animal studies suggesting that there are subtle but distinct differences in the neuroendocrine responses to different stress paradigms (49). Further, neuroendocrine and metabolic responses to acute exercise were greater in men whose HPA axis did not suppress when they were given dexamethasone before the exercise challenge (50). These data indicate that the degree of HPA activation potentiates the neuroendocrine and metabolic responses to subsequent challenge. Conversely, Altemus et al. showed that lactating women are hyporesponsive to exercise challenge (51). Taken together, these data buttress the notion that responses to a given stressor are gated not only by the stressor type, but also by the organism’s preexisting hormonal and metabolic state. Thus, some individuals are more, and some less, reactive to similar stressors. Even the same individual may vary in terms of stress sensitivity, depending on prevalent nutrition and psychosocial status. Obviously, emotional valence and expectation also determine the extent to which psychosocial variables serve as psychogenic stressors. Our preliminary investigations in women who developed FHA unrelated to weight loss, excessive exercise, and definable psychiatric disorders indicated that a primary factor that distinguished women with FHA from those with definable causes of anovulation and those who were ovulatory was the presence of unrealistic expectations (52). Fioroni (53) also found the women with FHA, as compared to eumenorrheic women or those with other causes of anovulation, held more negative attributions about recent life events. Kirschbaum (54) found that men who did not habituate when exposed to repeated psychogenic challenge viewed themselves as less attractive, had lower self-esteem, and reported being in a depressed mood more often. Apparently, unachievable ambitions or other cognitive distortions create vulnerability to
life’s inevitable challenges and likely heighten responsivity to metabolic stressors such as exercise or food restriction. Potentially, the converse is true. Metabolic mobilization may augment reactivity to psychogenic stressors.

There can be no doubt that weight loss and exercise serve as metabolic stressors. In monkeys trained to run, it was shown that caloric supplementation reversed the anovulation induced by training (55). Interestingly, the monkeys did not spontaneously develop a compensatory increase in appetite and had to be “bribed” with colorful candy to consume more calories. On the other hand, modest dietary restriction accompanied by small amounts of exercise greatly increased the proportion of monkeys who become anovulatory when presented with social stress. A prospective study of unselected women demonstrated that exercise and weight loss caused anovulation (56). Likely, sufficient exercise and weight loss, independent of psychogenic stress, can alter metabolism to the point that GnRH pulsatility is disrupted. Loucks and Thurma (57) recently quantified the amount of energy restriction needed to impact GnRH drive by studying eumenorrheic women in the follicular phase. Energy balance was achieved by providing 45 kcal/kg of lean body mass (LBM) per day. Graded daily energy deficits of 10, 25, and 35 kcal/kg were then experimentally induced for five days. An energy deficit of 33% had no impact on LH pulse frequency whereas an energy deficit of about 75% induced a decline in LH pulse frequency of about 40%. The induction of an energy deficit resulted in a graded increase in the 24-hour cortisol level. At an energy availability of 10 kcal/kg of LBM (75% deficit), the mean 24-hour cortisol was increased by about 30%, which is the amount of increase typically seen in women with FHA. Much like the stress-sensitive monkeys, women whose luteal phase progesterone levels were lowest at the initiation of the energy restriction showed the greatest response to the imposed metabolic challenge. In most real-life situations, except for extreme circumstances such as war or famine, metabolic deficits are not imposed, but rather initiated by individuals in response to self-imposed expectations. Most women with FHA, when carefully evaluated, display more than one trait, state, or behavior capable of activating stress response cascades or inducing a mild metabolic deficit, but most do not have a profound metabolic deficit that alone would explain the reduction in central GnRH drive. Many of the behaviors, such as exercise, that independently suppress central reproductive drive but only at more extreme levels, may be initiated as coping responses to psychosocial dilemmas. Because of the synergism between metabolic and psychogenic stressors, a combination of multiple, small magnitude, mixed stressors may be potentially more disruptive of reproductive function than a single large stressor limited to one category.

To better understand how a combination of seemingly minor psychogenic and metabolic stressors might synergistically disrupt GnRH drive as demonstrated in our monkey model (58), we compared endocrine responses to submaximal exercise in women with FHA to those in eumenorrhea (59). Women with FHA displayed a larger increase in cortisol than ovulatory eumenorrheic women (EW) in response to exercise. Further, glucose responses between the two groups were divergent in that women with FHA showed a 10% decrease in glucose and EW only a 3% increase. Interestingly, these two groups did not differ at baseline with regard to cortisol or glucose levels. The decrement in glucose seen in FHA but not EW suggests latent metabolic imbalance and indicates that women with FHA are unable to meet the energetic demands of ongoing activities. Further, it is likely that the drop in glucose activates the HPA axis and is at least partly responsible for the sustained hypercortisolemia characteristically seen in FHA. Since metabolic signals modulate GnRH pulsatility, exercise-induced metabolic imbalance also likely contributes to ongoing reproductive suppression. These results also reveal why the endocrine effects of a stressor, such as exercise, depend on the pre-existing allostatic state of the individual.

TREATMENT CONSIDERATIONS

In women, functional hypothalamic hypogonadism may not be recognized unless the menstrual interval is markedly short, long, irregular, or absent. Likewise, luteal phase insufficiency due to decreased hypothalamic drive may not be noted unless infertility results. Even then, it is notoriously difficult to document unless it is recurrent. Based on the foregoing concepts, the more clinically evident the ovarian compromise, the greater is the hypothalamic challenge and the more profound are the associated adrenal and thyroid derangements and sex steroid deprivation.
If a woman with functional hypothalamic hypogonadism is seeking to become pregnant, ovulation induction can be accomplished technically with exogenous administration of pulsatile GnRH therapy (9, 10) or gonadotropins. The obvious advantage of exogenous GnRH therapy is that it diminishes the risk of ovarian hyperstimulation and multiple gestation associated with gonadotropins. Clomiphene citrate can be tried, but it may not work because it has a hypothalamic site of action and the hypothalamus is already not responding to decreased sex steroid secretion. There also is some concern that ovulation induction may place women with FHA at risk for premature labor and intrauterine growth retardation (60). The parenting skills of women with FHA may be impaired because they are already overwhelmed and stressed prior to pregnancy and delivery and thus their children may be at risk for poor psychosocial development (61). Further, a recent study showed that children born to mothers with clinically occult hypothyroidism due to autoimmune thyroiditis had a mean full-scale intelligence quotient that was seven points lower than the control population (62). The women with clinically silent hypothyroidism had a 30% reduction in thyroxine, which is roughly what is observed in women with FHA. It is important to remember that maternal thyroxine is the only source of fetal thyroxine in the first trimester and the predominant fetal source in the second and third trimesters. Because the fetal brain requires an appropriate amount of thyroxine for neurogenesis, even small deficits in thyroxine may induce neurodevelopmental deficits. Increased maternal cortisol may also have independent effects upon fetal neurodevelopment and organogenesis. Recent evidence showed that severe stress such as that associated with the unexpected death of a child increased the risk of congenital anomalies of the cranial neural crest eight-fold (63). Further, stress and its endocrine concommitants have been implicated as a cause of preterm delivery. It is not known if the endocrine concomitants associated with FHA pose a similar risk, but this is clearly a potential hazard if ovulation induction is undertaken before amelioration of the allostatic changes in the adrenal and thyroidal axes.

A popular approach to a woman with FHA who is not seeking immediately to become pregnant is to offer her hormone replacement. This approach is based on the presumption that sex steroid deprivation is the primary therapeutic issue. There are inherent limitations with this approach, however. First, data indicate that exogenous sex steroid exposure does not fully promote bone accretion or cardioprotection in the presence of ongoing metabolic derangements (41, 42). Second, the ongoing insults to the brain from chronic amplification of stress cascades go unchecked. Further, estrogen therapy does not correct hypothalamic hypothyroidism (64). In short, hormone therapy may mask potentially deleterious processes that are unlikely to be ameliorated by hormone exposure alone. It is critical to remember that FHA is more than a disorder of reduced GnRH secretion.

Hormone therapy per se is unlikely to be harmful, but more than hormone administration is needed. The stress process needs to be interrupted. Although psychopharmacologic approaches have not been well studied, they probably could be used on an interim basis in special circumstances. The study of Judd suggested that a short course of alprazolam might be effective in reducing HPA activation and permitting hypothalamic-pituitary-ovarian recovery (47). However, this approach would not be the best recommendation in a woman hoping to conceive because of the risk of fetal exposure to benzodiazepines. The optimal intervention is to reverse the stress process so that the hypothalamus recovers and gonadal function resumes. An integral goal of the treatment plan for women with functional hypothalamic hypogonadism is to help them identify and ameliorate sources of psychogenic and metabolic stress and to provide emotional support while coping mechanisms other than dieting or exercising are learned. Nonpharmacologic interventions such as stress management, relaxation training, or psychoeducation empower individuals by fostering self-care and competency. In this regard, nonpharmacologic therapies have the potential to produce long-term benefits upon psychological and thereby physical health. Behavioral therapies acknowledge the wisdom of the body and recognize that functional hypothalamic amenorrhea represents an endocrine adaptation that can be reversed with appropriate psychogenic and behavioral modifications.

Given these considerations, we recently studied whether cognitive behavior therapy aimed at ameliorating problematic attitudes and behaviors would permit reproductive recovery in normal weight women with FHA (65). Women with FHA were randomized to observation versus cognitive behavior therapy (CBT). CBT consisted of 16 visits
with a physician, therapist, or nutritionist over 20 weeks. The two groups were followed for return of menses for up to eight weeks following the intervention. Regardless of menstrual pattern, estradiol and progesterone levels were monitored at weekly intervals for four weeks before and after observation versus CBT. About 88% of those who underwent CBT had evidence of ovulation compared with only 25% of those who were observed. Figure 2 illustrates the recovery of sex steroid secretion in a woman treated with CBT who showed ovarian recovery and in a woman randomized to observation who did not have recovery of ovarian function. Interestingly, when it occurred, ovarian recovery was not associated with significant weight gain. This does not mean that subjects did not alter food intake or energy expenditure, however.

**SUMMARY**

Functional hypothalamic hypogonadism is a clinical example of how attitudes, moods, and behaviors can have endocrine consequences and cause clinically evident reproductive compromise. Although a link between brain states and gonadal function has long been hypothesized, only recently have we been able to specify some of the mechanisms mediating this relationship. This understanding not only has concrete clinical implications, but it also expands our appreciation of what it means to be healthy. Health truly depends upon developing healthy attitudes and healthy behaviors. Misattributions, negative images of self and others, unrealistic expectations, and emotional disharmony can cause neuroendocrine havoc. We must seek to develop healthy mindsets that permit us to meet life’s innumerable challenges without overwhelm-
ing our coping mechanisms and without activating a chronic stress response. If we expect adversity and learn to cope well with it, then we likely will have more than just good reproductive functioning.

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**REFERENCES**


OTHER SELECTED GRAND ROUNDS PRESENTATIONS

Fertility Preservation in Cancer Survivors

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ABSTRACT

Seventy-five percent of cancer survivors report wanting to have children in the future. However, many common cancer therapies are toxic to the ovaries and testes. For this reason, a number of fertility preservation strategies have been developed, including gamete and embryo cryopreservation, ovarian tissue cryopreservation, and GnRH agonist co-treatment.

INTRODUCTION

Cancer is not uncommon among younger women. In the United States, approximately 600,000 women are diagnosed with cancer every year, and 10% of these women are under the age of 40 (1). Ninety percent of teenage girls and young women diagnosed with cancer will survive (2), and it is estimated that by 2010, one in 250 adults will be a cancer survivor (3).

The treatment required for most of the common cancer types occurring in younger women may involve removal of the reproductive organs and/or cytotoxic treatment that could partially or definitively affect reproductive function. Therefore, women diagnosed with cancer prior to or during their reproductive period often have to deal not only with the uncertainty of long-term survival, but also with the partial or total loss of fertility as a result of cancer treatment. In fact, there is ample evidence that women with cancer are highly interested in the topic of fertility preservation in their treatment regimens. In recent surveys, 75% of patients with cancer stated that they wanted children in the future, 80% felt that their cancer experience would make them better parents, 67% wanted a child even if they were to die young, and fewer than 10% stated that they would choose adoption due to a possible increased risk for their own child with treatment (4, 5).

Nonetheless, there remains a sharp divide between patient interest and caregiver education on the topic of fertility preservation. Only 60% of survivors diagnosed with cancer in young adulthood recall discussing cancer-related infertility. Furthermore, in a survey of 162 oncologists in two major cancer centers, 90% agreed that all men whose fertility could be impaired should be offered sperm banking; however, 50% stated that they never or rarely addressed this topic with eligible patients (4, 5). In a survey of 697 women diagnosed with breast cancer before the age of 40, 72% of respondents stated that they had discussed infertility with their physician, 17% had consulted an infertility specialist, but only 55% were satisfied that their concerns about childbearing were addressed (6).

In this chapter, we will describe both the established and experimental strategies for fertility preservation in women with malignancies. Most available options may also be applicable to women who face gonadotoxic treatment due to other
non-malignant disorders (such as systemic lupus erythematosus).

ESTABLISHED TREATMENT OPTIONS

Embryo cryopreservation

Currently, the only widely available option for fertility preservation in female patients that need chemo- and/or radiotherapy is the cryopreservation of fertilized oocytes and embryos. Cryopreservation of embryos involves an initial exposure to cryoprotectants, cooling to subzero temperatures, and storage. The embryos then may be thawed on demand, with a return to physiological conditions.

The methods involved in embryo cryopreservation and their success rates have been well established. Reported survival rates per thawed embryo range between 35% and 90%, with implantation rates between 8% and 30% (7-12). In the United States, approximately 16,000 assisted reproductive technology (ART) cycles using frozen non-donor embryos are performed yearly with a pregnancy rate of 25% per transfer, compared to a 35% pregnancy rate in cycles using fresh non-donor embryos (13). It is noteworthy that the effects of different types of malignancies upon reproductive potential are not yet known, and these statistics may not predict the outcome in women undergoing embryo cryopreservation for fertility preservation due to malignancy.

Despite well-defined success rates, embryo cryopreservation has a few critical pitfalls. First, it requires that the patient has a male partner or uses donor sperm to fertilize retrieved eggs. Second, ovarian stimulation precedes oocyte retrieval for in vitro fertilization (IVF), necessitating a delay in the initiation of chemo- or radiotherapy that may not be acceptable. Third, the high serum estrogen concentrations associated with ovarian stimulation may be contraindicated in women with estrogen-sensitive malignancies. However, given the success of ovarian stimulation protocols including letrozole in leading to successful term birth (14, 15), their combination with embryo cryopreservation is also likely to be successful.

Alternative strategies for ovarian stimulation in women with estrogen-sensitive malignancies

Ovarian stimulation protocols lead to an expansion of the pool of growing follicles and thus an increase in serum estrogen. Recently, new strategies for ovarian stimulation prior to IVF have been investigated for women with breast cancer, with the aim of retrieving more oocytes than would be available in a natural cycle, but without causing a significant increase in serum estrogen. Women with breast cancer constitute a special group due to the six-week hiatus between surgery and chemotherapy in most treatment protocols, which typically allows sufficient time for ovarian stimulation and egg retrieval.

Oktay et al. first used tamoxifen to stimulate follicle growth for IVF in 12 women with breast cancer (16). Using a dose of 40mg to 60mg daily, beginning on day 2 or 3 of the menstrual cycle, they obtained a higher number of oocytes and embryos per cycle compared to a retrospective control group consisting of breast cancer patients attempting natural cycle (unstimulated) IVF (16). However, mean peak estradiol level in the tamoxifen group was significantly higher than in natural cycle IVF patients. Following this initial study, Oktay et al. reported better stimulation and embryo development using a combination of FSH with tamoxifen or the aromatase inhibitor letrozole (17). While they obtained a similar number of mature oocytes and embryos per cycle compared to a retrospective control group consisting of breast cancer patients attempting natural cycle IVF patients. Following this initial study, Oktay et al. reported better stimulation and embryo development using a combination of FSH with tamoxifen or the aromatase inhibitor letrozole (17). While they obtained a similar number of mature oocytes and embryos per cycle in both groups, the mean peak estradiol was significantly lower in the letrozole group. Interestingly, combination of anastrozole with FSH does not seem to prevent the rise in serum estradiol (18).

EXPERIMENTAL STRATEGIES

Oocyte cryopreservation

The cryopreservation of oocytes avoids the need for sperm and thus is applicable to a larger group of patients compared to embryo cryopreservation. In addition, oocyte cryopreservation may circum-
vent ethical or legal considerations associated with embryo freezing. Moreover, it also has considerable advantages compared to ovarian tissue cryopreservation, at least in the short term. However, although the first human live birth from cryopreserved oocytes was reported more than twenty years ago (19), success rates in assisted reproductive technologies using frozen oocytes have lagged behind those using frozen embryos, most likely as a result of the biochemical and physical properties of the oocyte.

Due to the low efficiency of oocyte maturation in vitro, mature MII oocytes are most commonly used for cryopreservation. MII oocytes are among the largest cells in the human body and contain the very delicate meiotic spindle. As their cytoplasm contains a high proportion of water in comparison to other cells, damage due to ice crystal formation was an initial hurdle to oocyte viability after frozen storage. Recent protocols that include dehydration of the oocytes before or during the cooling procedure have reduced ice crystal formation and have led to much improved clinical outcomes.

Cryopreservation of mature oocytes has also been shown to cause hardening of the zona pellucida, resulting in adverse effects on fertilization (20). Significant improvement in the fertilization of cryopreserved oocytes has been achieved with the use of intracytoplasmic sperm injection (ICSI) (21, 22), likely due to avoiding the effects of zona hardening.

The two most common freezing protocols used are “Slow Cooling” and “Vitrification.” In the last two years, each protocol has shown increasing promise and, as a result, oocyte cryopreservation is becoming more routine and less experimental.

**Slow cooling procedure**

The first protocol used to freeze oocytes was based on a slow cooling and rapid thawing method that had already been applied successfully for the cryopreservation of embryos. Since then, much progress has been made, mostly in the optimization of cryoprotectant concentration and exposure time. Recent pooled reports on outcomes with this protocol have suggested a 47% oocyte survival rate, with a subsequent 52% fertilization rate, leading to an overall pregnancy rate of 1.9% per thawed oocyte (23).

**Vitrification**

Vitrification may be defined as a physical process in which a highly concentrated solution of cryoprotectants solidifies during cooling without the formation of ice crystals (24). Vitrification has certain advantages over freezing because it avoids the damage caused by intracellular ice formation and the osmotic effects caused by extracellular ice formation. Pregnancy outcomes to date have been similar to those seen with the slow cooling protocol, with a 68.4% survival rate per oocyte, a 48.5% fertilization rate, and a 2.0% pregnancy rate per vitrified oocyte (23).

**Ovarian tissue cryopreservation**

Cryopreservation of primordial follicles within ovarian tissue has several potential advantages over both embryo and oocyte freezing. Hundreds of primordial follicles containing immature oocytes may be cryopreserved without the necessity for ovarian stimulation and delay in initiating cancer treatment. Moreover, primordial follicles are significantly less susceptible to cryo-injury compared to both mature and immature oocytes due to their smaller size, slower metabolic rates, and the absence of zona pellucida. Two approaches to ovarian tissue cryopreservation are currently being investigated: cryopreservation of ovarian cortical tissue and cryopreservation of whole ovaries.

**Cryopreservation of ovarian cortical tissue**

The outer cortical layer of the ovary contains most of the primordial follicles. Therefore, it is conceivable to cryopreserve pieces of ovarian cortical tissue. The ovarian cortex is removed via laparoscopy or laparotomy and cut into strips of tissue 1mm to 3mm in thickness and less than or equal to 1cm² in total area in order to ensure adequate penetration of cryoprotectants (25). It is necessary to analyze a piece of the cortical tissue to confirm the presence of follicles and the absence of malignant metastasis (12, 26). Once the ovarian tissue is cryopreserved, future options include transplantation of the tissue back to the donor (autotransplantation) or to nude mice (xenotransplantation), or culture of the follicles in vitro.

Autotransplantation studies using animal models
have resulted in the return of ovarian function as well as pregnancies and live births (27-29). Two different surgical approaches have been used in humans for transplantation: orthotopic (pelvic) or heterotopic. Orthotopic transplantation places ovarian tissue at close proximity to the infundibulo-pelvic ligament with the hope that natural pregnancy may occur. Heterotopic transplantation is an alternative approach in which cryopreserved ovarian tissue is transplanted to a site outside of the pelvis. Transplantation to a heterotopic site such as the forearm (25, 30) or abdomen (31) is technically easier and imposes fewer surgery-associated risks compared to orthotopic transplantation. IVF-ET is absolutely necessary in order to achieve pregnancy. In 2001, Oktay et al. were first to report return of ovarian endocrine function with the development of a dominant follicle and resumption of menstrual cycles in two women using this approach (31). In one case, after stimulation with human menopausal gonadotropin, they performed percutaneous oocyte retrieval from the forearm; however, fertilization was not achieved (31). More recently, Oktay et al. were able to restore ovarian function in a woman previously treated for breast cancer by transplanting the cryopreserved ovarian tissue beneath the abdominal skin. They performed eight cycles of controlled ovarian stimulation using a combination of recombinant FSH and human menopausal gonadotropins for stimulation. A total of 20 oocytes were retrieved, of which one fertilized normally, but pregnancy did not occur (31).

A significant source of concern associated with autotransplantation is the risk of transmission of metastatic cancer cells. Xenotransplantation of cryopreserved ovarian tissue into nude mice eliminates the possibility of cancer cell transmission and relapse as the oocytes are retrieved from the host animal. Another advantage is the possible application in women in whom hormonal stimulation is contraindicated. However, possible transmission of zoonoses to humans is a serious concern, and this method is unlikely to be clinically available in the near future.

**Cryopreservation of whole ovaries**

Animal studies suggest that fresh whole ovaries can be successfully transplanted. Although the duration of subsequent ovarian function has initially been limited, mostly due to ischemia resulting from thrombosis (32), the use of microsurgical techniques has led to improvements in graft survival (32-36). In addition, careful dissection of ovarian vessels during ovariectomy and perfusion of the ovary with cryoprotectants through these vessels improved tissue survival and led to similar rates of follicular viability and apoptosis compared to ovarian cortical strips (34).

Bedaiwy and colleagues (37) investigated the immediate post-thawing injury to the ovary that was cryopreserved as a whole with its vascular pedicle or as cortical strips. Bilateral oophorectomy was performed in two women (46 and 44 years old) undergoing hysterectomy. In both patients, one of the harvested ovaries was sectioned and cryopreserved as ovarian cortical strips. The other ovary was cryopreserved intact with its vascular pedicle. After thawing seven days later, the overall viability of the primordial follicles was 75%-78% in intact cryopreserved-thawed ovaries and 81%-83% in ovarian cortical strips. Comparable primordial follicle counts, and absence of features of necrosis or apoptotic markers, led them to conclude that cryopreservation injury is not associated with significant follicular damage.

While these results are encouraging, definitive restoration of fertility resulting from the transplantation of a cryopreserved-thawed whole human ovary remains to be demonstrated. This technique does carry potentially increased risk of returning metastatic disease to the patient, compared to the handling of oocytes or even cortical strips.

**Ovarian transposition**

Transposition of the ovaries (oophoropexy) outside of the pelvis to protect them from pelvic radiation was initially described in 1958 (38). The procedure is indicated in patients diagnosed with malignancies that require pelvic radiation, but not removal of the ovaries, as part of their treatment. The most common indications are Hodgkin’s disease, cervical and vaginal cancer and pelvic sarcomas. Initially, the procedure was performed through a laparotomy incision. More recently, oophoropexy has been described laparoscopically (39). During the last four decades, several reports have documented different degrees of ovarian function and ability to conceive a pregnancy after radiation treatment. The
procedure has been successful in 16% to 90% of the reported cases (12, 40, 41). The variations are likely due to the inability to calculate and prevent scatter radiation, different doses of radiation utilized, and concomitant use of chemotherapy (12).

**Gonadotropin-releasing hormone agonist co-treatment**

Based on the postulated role of gonadal suppression in the preservation of testicular function in men receiving chemotherapy, and the belief that the fertility of pre-pubertal girls is not affected by gonadotoxic treatment, the effect of gonadotropin-releasing hormone agonist (GnRHa) treatment in preserving fertility by creating a pre-pubertal hormonal environment has been investigated (12). Animal studies have shown a protective role for GnRHa treatment against chemotherapy-induced gonadal damage (42, 43). Ataya et al. demonstrated that loss of primordial follicles in response to cyclophosphamide chemotherapy was significantly less in rhesus monkeys receiving GnRHa co-treatment compared to those receiving chemotherapy alone (44). Interestingly, they did not find GnRHa co-treatment to be effective in protecting against radiotherapy-induced gonadal damage (45).

Following encouraging findings in animal models, non-randomized studies with short-term follow-up have suggested a protective role for GnRHa co-treatment (46-50). However, these studies have been criticized for their lack of randomization and the use of ovarian failure as an endpoint, which may not reflect the decrease in primordial follicle count in response to chemotherapy in young women (12). At present, despite encouraging reports, the benefits and long-term effects of GnRHa co-treatment are unclear, and a consensus regarding the effectiveness of ovarian suppression is lacking.

**SUMMARY**

Fertility preservation in females diagnosed with cancer has become an important area of investigation due to increasing cancer survival rates combined with delayed childbearing. Alternative treatment strategies for early-stage gynecologic cancers have recently been studied with promising results for both survival and fertility preservation.

In addition to embryo cryopreservation, encouraging findings have recently been reported using oocyte cryopreservation, ovarian cryopreservation, and GnRHa co-treatment with chemotherapy. In addition to the possibilities for surgical management, female patients today have a wide range of options for fertility preservation that should be discussed prior to undergoing gonadotoxic therapy.

**RECOMMENDED READINGS**

REFERENCES


How Can Menopausal Symptoms Be Safely and Effectively Treated Following Breast Cancer?

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DEFINITION

Menopause is the permanent cessation of menstruation resulting from loss of ovarian activity. Natural menopause is diagnosed following twelve months of amenorrhea, which is not due to a pathological cause. Menopause can also be induced by surgery, chemotherapy and radiotherapy.

INTRODUCTION

Breast cancer is one of the most common causes of morbidity and mortality in women of middle years in the Western world (www.asco.org). There has been a reduction in the annual death rate from breast cancer in almost all Western countries, which may be due to increasing use of population breast screening and use of adjuvant systemic therapy. As a result, more women are becoming long-term breast cancer survivors.

A common side effect of breast cancer treatment is the earlier onset of menopause or menopausal symptoms. Many women who are premenopausal at diagnosis will develop premature menopause resulting from chemotherapy, endocrine therapy, bilateral oophorectomy or ovarian radiation and may experience severe and long-lasting menopausal symptoms. All endocrine therapy will generally induce menopausal symptoms, often for the duration of its use. This can occur in women at any age, regardless of menopausal status. Premenopausal women who are older at the time of chemotherapy are more likely to experience chemotherapy-induced ovarian failure leading to permanent menopause (1). Those who do not have ovarian failure may subsequently go through menopause at a younger age. Younger patients require a higher cumulative dosage of chemotherapy to induce ovarian failure and may have temporary menopausal symptoms. However, it is not possible to reliably predict whether an individual woman will go through menopause as a result of her chemotherapy treatment.

A significant number of peri- and postmenopausal women are taking hormone replacement therapy (HRT) when they are diagnosed with breast cancer. Cessation of HRT commonly leads to a return of menopausal symptoms. Recurrence of menopausal symptoms and premature menopause in breast cancer patients can have significant negative impact on quality of life, body image, sexual function and self-esteem (2). The clinical challenge lies in providing safe and effective therapy to these patients. The safety of conventional hormonal treatments for menopausal symptoms has not been established following breast cancer. Data from randomized controlled trials suggest that use of conventional HRT following breast cancer may lead to a three-fold increased risk of breast cancer recurrence (3). Furthermore, many women will have hormone receptor positive tumors, and endocrine therapy aims to reduce circulating estrogen and/or suppress ovarian activity. It is possible that HRT may undermine the efficacy of these treatments.
Hence, there is an urgent need to provide some alternative form of treatment to alleviate menopausal symptoms in breast cancer patients.

TREATMENT OPTIONS

Menopausal symptoms are variable in nature and severity and each woman requires careful individual assessment. This should include an assessment of the likely cause, nature, frequency, severity of symptoms, and their impact on quality of life. For some women, reassurance that her symptoms are normal and are likely to reduce over time, combined with practical suggestions for minimizing their impact, is sufficient management.

Available treatment interventions fall into two main groups:

[1] Synthetic hormonal compounds

Tibolone has weak estrogenic, progestogenic and androgenic actions. It shows similar efficacy to HRT in reducing hot flushes and vaginal dryness and may improve sexual function more effectively than HRT (4). It improves bone density but its impact on fracture rates is not known. Unwanted side effects of tibolone include a reduction in circulating high-density lipoproteins, uterine bleeding, body pain and headache (5). The safety of tibolone in women with a prior history of breast cancer has not been confirmed following the premature termination of a large randomized controlled trial (the LIBERATE study: www.organon.com).

[2] Non-hormonal therapies

A variety of non-hormonal therapies aimed at reducing vasomotor symptoms or urogenital atrophy have been subjected to randomized controlled trials. The most common vasomotor symptom is hot flushes. However, the basic physiology underlying hot flushes is poorly understood. It is hypothesized that reduced estrogen levels cause an induction of noradrenergic activity, leading to a feeling of warmth, heat loss and sweating. Because the placebo effect is profound and prolonged in treatments for hot flushes, it is essential that new therapies be tested for adequate durations (at least 12 weeks) in randomized placebo-controlled trials (www.fda.gov).

(A) Phytoestrogens: are plant derivatives either in the form of isoflavones found in soy products or lignans found in flaxseeds. They exhibit both estrogenic and antiestrogenic effects, depending on their concentration. Although some studies have shown small benefits in hot flushes from using phytoestrogens, a recent meta analysis failed to demonstrate any benefit over placebo (6). Their safety in women with a history of breast cancer is unknown.

(B) Black cohosh (Cimicifuga racemosa): was traditionally used by native North Americans for treating a range of menstrual problems. It appears to act by competing for the estrogen receptor, and binds to gamma amino butyric acid (GABA), serotonin and dopamine receptors. Again, results have been conflicting, with some studies finding black cohosh mildly effective in decreasing severity and frequency of hot flushes, genitourinary symptoms and mood disturbances in a few studies. However, a systematic review showed no overall benefit of black cohosh over placebo (7). Black cohosh has recently been associated with several episodes of hepatotoxicity and liver failure and now requires a warning label in the UK (www.mhra.gov.uk).

(C) Neuroendocrine agents

1. Clonidine is a central alpha-adrenergic agonist, found to be moderately more effective than placebo in treating hot flushes. Some studies have also shown improved quality of life in breast cancer patients treated with clonidine for hot flushes. Studies of clonidine are generally of poor quality and of insufficient duration to demonstrate a clinically significant benefit for this treatment. In breast cancer patients, clonidine was inferior to venlafaxine in reducing the frequency and severity of hot flashes (8). There are no safety data regarding clonidine and breast cancer.
2. Selective serotonin and noradrenaline reuptake inhibitors (SSRI/SNRI). Over the menopause transition, serum serotonin levels fall and monoamine oxidase activity increases. Venlafaxine is an SNRI, which has demonstrated short-term (up to 6 weeks) superiority over placebo in reducing hot flushes (9). Side effects of venlafaxine are largely dose related and include nausea, constipation, dry mouth and decreased appetite. Other SSRIs have included paroxetine, fluoxetine and citalopram, which have also demonstrated short-term efficacy in reducing hot flushes (6) although their safety in some breast cancer patients has been questioned due to their potential interaction with the metabolism of tamoxifen.

The GABA analogue gabapentin may be more effective than SSRI/SNRI and is well tolerated by most women though side effects may include somnolence, dry mouth, heart palpitations and dizziness. Gabapentin effectively reduced hot flushes for at least 12 weeks in randomized placebo-controlled trials of women, some of who have had a personal history of breast cancer. Gabapentin is the only non-hormonal agent to have shown equal efficacy with estrogen in reducing the frequency and severity of hot flushes in inadequately powered randomized controlled trials (Reddy 2006).

TREATMENT OF UROGENITAL ATROPHY AND RELATED SYMPTOMS

Lack of estrogen results in vaginal atrophy, urinary frequency, dysuria and incontinence. In breast cancer patients urogenital atrophy is relatively uncommon in tamoxifen users, due to the estrogenic effects of tamoxifen in the vagina. Urogenital atrophy is more common in those using aromatase inhibitors. Topical estrogens are more effective than non-hormonal preparations in the treatment of urogenital atrophy (11). They have been widely used following breast cancer but recent reports that topical estradiol may increase circulating estrogen levels in women using aromatase inhibitors have raised some concern (12). Topical estrone is also effective and may be a safer choice following breast cancer.

Sexual dysfunction is often multifactorial and the management of urogenital atrophy should include a discussion of relationship problems, loss of libido and depression. Physiotherapy and lubricants such as olive or almond oil may also be helpful for some women.

TREATMENT OF BONE LOSS

Osteoporosis is a common condition seen in older postmenopausal women. Endocrine therapy for breast cancer may increase the risk of osteoporosis and fracture (13). Abrupt withdrawal of circulating estrogens occurs following chemotherapy-induced ovarian failure, oophorectomy or gonadotrophin releasing hormone (GnRH) or following treatment with an aromatase inhibitor (AI) in the postmenopausal setting. Chemotherapy alone does not cause osteoporosis if not associated with ovarian failure.

Bone loss and increased fracture risk (especially non-hip fractures) are major concerns for long-term breast cancer survivors. Tamoxifen shows agonistic estrogenic activity in bone and reduces risk of fracture in postmenopausal but not in premenopausal women.

In postmenopausal women AIs have a deleterious effect on bone density and may increase the risk of fracture. A baseline Bone Mineral Density test to be repeated up to annually is advised. A bisphosphonate in conjunction with AI may mitigate the deleterious effect of AI on bone (14).

Lifestyle modifications to minimize bone loss, including diet, supplementary calcium, weight bearing exercise, stopping smoking and minimizing alcohol consumption, should be encouraged. Calcium and Vitamin D supplements should also be advised to any at-risk women.

CARDIOVASCULAR COMPLICATIONS

In women with early breast cancer, one of the major reported causes of deaths is cardiovascular disease. The American Heart Association guidelines stratify women in three groups based on their 10-year probability of having a coronary event. Universal recommendations for all groups are moderate exercise, cessation of smoking, body weight aimed at less than 25 BMI (body mass index), waist circumference less than 35 inches, limited intake of saturated fat and cholesterol, and dietary modification to include grains, fruits and vegetables and fish.

Some drugs used in the treatment of breast cancer can have adverse cardiovascular effects. Tamoxifen
increases the incidence of thromboembolic events and may increase triglyceride levels and lower low-density lipoproteins (LDLs) (15). AIs have less impact on lipid profile, but their long-term effects on cardiovascular disease are not yet known. Tibolone may also have an adverse effect on LDLs in some women.

CONCLUSION

Menopausal symptoms are common in women treated for breast cancer. Estrogen containing HRT is the most effective treatment for menopausal symptoms, but is not recommended following breast cancer, even for those with estrogen receptor negative disease. There is an urgent need for safe and effective non-hormonal treatments. Several non-hormonal treatments have been studied with varied results and outcomes. Lifestyle modifications are an integral part of treatment, especially for osteoporosis and cardiovascular diseases. Younger women who develop premature menopause often need psychological assessment and support. Topical estrogens are effective for vaginal atrophy but their safety in women using aromatase inhibitors is not established. Gabapentin and clonidine appear effective for hot flushes but have significant side effects. Several short studies support the efficacy of SNRIs and SSRIs but their medium- to long-term efficacy is not established. Very few studies have addressed effective non-hormonal treatments for symptoms other than hot flushes. Because of the complexity of these cases, management of menopausal symptoms following breast cancer is best conducted within a multidisciplinary environment and with individualized care (16).

GLOSSARY

Secondary amenorrhea – absence of menstruation for >3 months.

Early menopause – menopause at age younger than 45 years.

Menopause – the final menstrual period.

Osteoporosis – thinning of bones, increased risk of fracture.

Placebo – tablet without medication.

Premature menopause – menopause before age of 40 years.

Surgical menopause – menopause that occurs when a premenopausal woman has both of her ovaries removed.

Adjuvant treatment – treatment given after surgery to cancer patients in the form of chemotherapy, hormone therapy or radiotherapy.
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Intrauterine Contraception: The Evidence for Liberalized Use

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“Everything we can do to give women control over their bodies and their fertility enhances their health and also changes the world for the better.”
Malcolm Potts, Presidential Address, Annual Clinical Meeting of the American College of Obstetricians and Gynecologists, May 2, 2005

INTRAUTERINE CONTRACEPTION USE IN THE UNITED STATES AND WORLDWIDE

Intrauterine contraception (IUC) is the most widely used method of reversible fertility regulation in the world. Over 100 million women worldwide use it for contraception (1). IUC is undergoing a renaissance in the United States (US) due to recognition of its many benefits and safety. As more new devices and intrauterine hormonal systems are developed and introduced into the US market, IUC utilization will continue to expand. Liberalization of previously overly restrictive product labeling and medical protocols will encourage a new age of modern IUC use in the US.

UNMET NEED FOR CONTRACEPTION AND THE ROLE OF INTRAUTERINE CONTRACEPTION

The unmet need for contraception in the US and worldwide constitutes a public health crisis. The scope of this unmet need is evident: worldwide, an estimated 60 million unintended pregnancies occur annually. These unintended pregnancies result in an estimated 26 million births, 26 million abortions, and 8 million miscarriages worldwide (2). In the US, nearly half of all pregnancies, an estimated 3 million annually, are unintended, and nearly half of these end in abortion (3). Over half of unintended pregnancies are a result of contraceptive failure or misuse (3). Because the intrauterine device (IUD) rarely fails and is difficult to misuse, more widespread use of IUC could have a powerful effect in reducing unintended pregnancy in the US. Despite its popularity worldwide, IUC is just beginning to be appreciated by US clinicians, and perhaps more importantly, by US women. Female fellows of the American College of Obstetricians and Gynecologists indicated they would choose IUC as their first choice contraceptive method when childbearing was completed and as their second choice, after oral contraceptives, if desiring to space their children (7). US women who have the most knowledge to make their contraceptive decisions—obstetrician/gynecologists—choose IUC far more frequently than their patients do.

ADDRESSING BARRIERS

Restrictive labeling has created unnecessary barriers to effective contraceptive provision (20) by re-
inforcing the safety and liability concerns that have prevented broader acceptance of IUC in the US. Restrictive labeling creates discomfort in clinicians who have to practice “off-label” and encourages the development of rigid medical protocols.

**RISK OF PELVIC INFLAMMATORY DISEASE**

Clinicians’ most deeply rooted fear about the IUD is an association between IUD use and the development of pelvic inflammatory disease (PID). A large body of evidence refutes this association and is summarized in a Cochrane review (24). In a US study in which over 2000 women were randomized to prophylactic antibiotics for IUD insertion, PID rates were not elevated above baseline rates (26). The most important conclusion from this study was that even during the vulnerable peri-insertion period—the possibly high-risk timeframe—the absolute rate of PID was 1/1000, approximating the background rate of PID. The two IUDs available on the market in the US do not appear to differ in PID risk, although data are conflicting. The LNG IUS has demonstrated a trend toward protection against PID. Biologic plausibility for PID protection lies in progestin’s effect of thickening cervical mucus and decidualizing the endometrium, creating a barrier to ascending infection. A protective benefit has not consistently reached statistical significance.

**RISK OF INFERTILITY**

Fear of infertility because of clinical or subclinical tubal infection or inflammation has been the main barrier to widespread use of IUC in young and/or nulliparous women. Although some initial reports from the 1980s suggested an increased risk of infertility in women who used IUDs, the heightened risk disappeared when controlling for number of sexual partners and STIs. Research consistently demonstrates no difference in fertility rates in multiparous women who discontinued various methods of contraception, including IUC (28,29). Further, fertility rates are not statistically different in women who discontinue IUC because of planning pregnancy compared with those who discontinue because of a complication (30,31). A recent follow-up prospective study examined time to pregnancy and need for fertility evaluations in women randomized to two investigational copper devices in Norway. There was no difference in these two fertility outcomes in women who had their IUD removed to become pregnant compared to women who discontinued the IUD because of problems (31). In the best-designed study examining the association between IUD use and infertility, 1895 women who had primary tubal infertility were compared with several control groups. Previous copper IUD use was not found to be associated with an increased risk of tubal occlusion in nulligravid women. Rather, presence of chlamydia antibodies, indicating past chlamydia infection, was associated with tubal occlusion (32). Prior STI exposure determined tubal patency and fertility, not past contraceptive choices.

**NULLIPARITY**

Once STIs were established as the causal agents for PID, the restriction of IUC in nulliparous women had no basis. Both safety and efficacy of IUC in nulliparous women have been established in clinical trials outside the US where IUC use in nulliparous women is both common and on-label (36,37,38). To address the increased expulsion rates and side effects observed in some studies of nulliparous women, new devices are being developed and tested that are uniquely designed for the nulliparous uterus (36,38). Efficacy and continuation rates of the LNG IUS are similar in nulliparous and parous women, regardless of age. In one study of nulliparous women randomized to the LNG IUS or oral contraceptive pills, continuation rates at one year were 80% for the LNG IUS versus 68% for the oral contraceptive group (38). In addition to the long-acting and excellent protection against unplanned pregnancy, many of the health benefits of the LNG IUS, namely the reduced menstrual blood loss and diminished dysmenorrhea, make this method particularly useful in young nulliparous women intending to postpone pregnancy. In other countries, the many restrictions in the US FDA package insert do not exist. Organizations that develop evidence-based practice guidelines, such as the WHO Committee for Medical Eligibility Criteria and the Faculty of Family Planning & Reproductive
INTRAUTERINE CONTRACEPTION USE IN WOMEN WHO HAVE MEDICAL PROBLEMS
As IUC becomes more accepted as an appropriate first choice method for many women throughout their reproductive lives, regardless of age or parity, it is also being studied in women who have complex medical conditions in whom IUC use was previously considered contraindicated, such as HIV/AIDS, lupus or other settings of relative immunosuppression. In 2003, the Expert Working Group for the WHO’s Reproductive Health and Research Department made changes to recommendations for IUD use in settings whereby HIV and other STIs are common. The main conclusions of the group were: (1) the IUD does not increase a woman’s chance of acquiring HIV; (2) the IUD does not increase HIV transmission to sexual partners; and (3) a woman can generally initiate IUC even if she is HIV-infected, at high risk of HIV infection, or has AIDS but is clinically well on anti-retroviral therapy (39–41). Many women who have medical problems have few contraceptive choices; now clinicians and women can feel more secure in using IUC in many of these complex medical scenarios whereby pregnancy can pose a life-threatening risk.

NON-CONTRACEPTIVE BENEFITS OF INTRAUTERINE CONTRACEPTION
The increasing list of non-contraceptive benefits of IUC, mostly with LNG IUS, will encourage further appreciation of IUC. Currently all non-contraceptive use of IUC is off-label in the US, but in many European countries therapeutic uses of LNG IUS are common and on-label.

ENDOMETRIAL CANCER PROTECTION
IUC with various devices has been associated with a decreased risk of endometrial cancer both across study designs and IUD types. Nine case controls and one large cohort study all show a relative risk of 0.4 to 0.6 of endometrial cancer in IUD users versus nonusers. In a systematic review, Hubacher and Grimes (42) rated the mostly II-2 evidence illustrating the IUD’s protective effect against endometrial cancer. In a recent cohort study of 2,037,883 woman-years of follow-up from 1989 to 1998 in China, ever-use of an IUD was associated with a decreased risk of endometrial cancer with an adjusted OR of 0.6 (95% CI 0.3–0.9) (43). Although IUD types are not specified in this large cohort, a combination of copper T IUDs and stainless steel rings were the most frequently used devices in this population. The mechanism of protection for copper and inert IUDs remains unclear. All IUDs create an inflammatory response in the uterus. Copper IUDs also release copper ions into the endometrial cavity, and the LNG IUS exposes the endometrium to a high local level of the potent progestogen, LNG. Any or all of these effects may reduce the risk of neoplasia. Although biologic plausibility supports the LNG protective effect against endometrial cancer, the LNG IUS has not been in widespread use long enough to document direct evidence of its impact on endometrial cancer risk. New evidence examines molecular markers for the mechanism by which LNG and other progestins protect against endometrial cancer (44). The LNG IUS may eventually be used for treatment of early endometrial cancers (44).

THERAPEUTIC INDICATIONS FOR THE LEVONORGESTREL INTRAUTERINE SYSTEM
In addition to providing top-tier contraception, the LNG IUS is being studied for various uses including treatment of idiopathic menorrhagia, menorrhagia and/or pain symptoms secondary to fibroids, endometriosis, adenomyosis, and hyperplasia. A recent comprehensive review examines the evidence for these therapeutic uses (45). The LNG IUS is already approved for treatment of menorrhagia in 102 countries and for hormonal protection of the endometrium during postmenopausal estrogen use in 93 countries. Two Cochrane reviews have evaluated medical treatment with LNG IUS compared with endometrial resection (46) and hysterectomy (47) for the treatment of menorrhagia. Satisfaction rates at one year were similar for the IUS and endometrial resection (47). Extrapolating from the therapeutic effects of the LNG for such conditions as menorrhagia, research is examining a possible role for the LNG IUS as a preventive and/or therapeutic agent...
for other gynecologic problems, such as dysmenorrhea, endometriosis, adenomyosis, infertility, and fibroids. Future devices will test intrauterine delivery of other steroid hormones, such as progestogen receptor modulators to prevent disease in high-risk women and to treat symptomatic women medically instead of surgically (48). The expanding use of the LNG IUS for prevention of and therapy for a large array of common gynecologic conditions may result in a decrease in surgery for benign gynecologic disorders, including sterilization procedures, myomectomy, and hysterectomy. These trends have already been observed in the UK, with expanding use of LNG IUS suspected as the cause (48).

**POSTPARTUM AND POSTABORTION INSERTION**

IUC can be initiated immediately postpartum and postabortion. Several advantages of insertion in these settings include the assurance that the patient is not pregnant, the high motivation to begin a contraceptive method, and diminished discomfort with insertion. Immediate postpartum insertion with copper T devices is common outside the US and, if performed within 10 minutes of delivery of the placenta, appears safe and effective (55,56). Neither bleeding nor infectious complications nor increased perforations have been associated with immediate insertion with copper devices (57). The main disadvantage of immediate postpartum insertion is increased expulsion. In one recent study, expulsion risk was 12.3% at one year after immediate post-placental insertion with the copper IUD (55). In older studies, insertion of an IUD after the immediate postpartum period and before four weeks postpartum was associated with more uterine perforations. IUDs should therefore be inserted either within 10 minutes of placental delivery or after four weeks postpartum (13,33). Immediate IUD insertion after spontaneous or induced abortion is both practical and safe (58). As with postpartum insertion, expulsion rates may be increased, depending on the duration of pregnancy at the time of abortion. IUC after early first trimester abortion has the same expulsion rate as interval insertion, no higher complication rates, and high continuation rates (59,60). When immediate postabortion studies include abortion up to 12 weeks gestational age (61), higher cumulative expulsion rates at one year of 7% to 12% have been observed. Randomized trials in the US are currently underway with both IUDs to better define the differences in expulsion rates in immediate versus delayed postpartum and postabortion insertion. The role of ultrasound guidance in assuring fundal placement in postpartum or postabortion insertion is also under investigation. Because the local effect of the LNG IUS on involution of the uterus is unknown, it is not recommended until some of these data become available (53). WHO medical eligibility criteria rates immediate insertion after an uncomplicated first trimester abortion a category 1 for both IUDs and a category 2 after a second trimester abortion only because of the increasing risk of expulsion after placement in larger uteri (56). Many medical protocols as well as product labeling package inserts (Para-Gard [33]) now support IUC initiation immediately following spontaneous or induced abortion, by both surgical methods or medication abortion (62).

**SUMMARY**

The US has an unacceptably high rate of unintended pregnancy. Many of these unintended pregnancies are due to method failures. Method failures are exceedingly rare with IUC; its effectiveness is equal to or better than sterilization—yet it is fully reversible with immediate return to fertility. Evidence continues to support broader IUC safety and acceptability in young and nulliparous women, and immediate insertion at the time of a pregnancy event—either immediately postpartum or immediately after emptying the uterus at the time of abortion or miscarriage. Broader use of IUC could dramatically lower US unintended pregnancy rates—and the evidence supports it.
REFERENCES


Paternal Effects on Reproductive Outcome

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INTRODUCTION

The impact an abnormal paternal genome may have on reproductive outcome is unquestionably less when compared to its female counterpart’s role. The egg’s importance has been well established, as shown by the success of donor oocyte programs. It can be estimated that in about 80% of cycles, egg quality plays the major driving force in respect to the chances of a patient achieving a pregnancy. In contrast, the influence of the human sperm on reproductive outcome has been less well characterized. The paternal importance, however, rises significantly with the increased use of intracytoplasmic sperm injection (ICSI), where the quality of spermatozoa is generally accepted to be poorer. In light of this, the safety of ICSI is being increasingly examined as concerns have arisen that an aberrant paternal inheritance can be derived at the chromosomal, epigenetic and nuclear DNA level (Figure 3).

THE IMPACT OF PATERNALLY INHERITED CHROMOSOMAL AND EPIGENETIC EFFECTS ON REPRODUCTIVE OUTCOME

The spermatozoa may provide a paternal influence at different levels. Based on chromosomal studies, paternal errors account for 5%-10% of autosomal trisomies, while maternal metaphase I errors are the predominant etiology. A paternal effect on sex chromosome trisomies is higher since 100% of 47, XYY and nearly 50% of 47, XXY are paternal in origin. The potentially increased risk of birth defects after ICSI has been of major concern and has been addressed in numerous studies. Overall the consensus appears to be that in comparison with the general population, there is a slight but significant increase in de novo sex chromosomal aneuploidy (0.6% versus 0.2%) and structural autosomal abnormalities (0.7% versus 0.04%), and an increased number of inherited (mostly from the father) structural aberrations.

Another area of concern is whether epigenetic effects are related to ICSI and the sperm. Epigenetics refers to the covalent modifications of DNA or core histones that regulate gene activity without altering DNA sequence. These disorders manifest themselves in what are known as imprinting disorders. Some recent publications have associated assisted reproductive treatments with a number of children who were affected by diseases caused by imprinting disorders, but a direct link to a paternal contribution is still speculative.

SPERM NUCLEAR DNA STRAND BREAKS AND REPRODUCTIVE OUTCOME

The presence of DNA breaks in ejaculated spermatozoa was reported in the early 90s; however, how they originate and their impact on reproductive outcome are still not completely understood. Reproductive parameters that could theoretically be affected by an increased presence of DNA strand breaks in ejaculated spermatozoa include fertilization, embryo development, and pregnancy rates. Investigation of the possible association between DNA strand breaks in spermatozoa and fertilization rates in patients undergoing ART have in general found no strong correlation between the DNA integrity of ejaculated spermatozoa and IVF and ICSI fertilization rates.

In contrast to fertilization, a negative correlation between the extent of nuclear DNA damage in ejaculated spermatozoa and embryo development after IVF and ICSI has been observed. In addition, pregnancy rates after IVF are believed to be
reduced in couples who have higher percentages of spermatozoa with DNA strand breaks; however, strict diagnostic values of assessment are not conclusive as it appears that when treated by IVF or ICSI, men with high levels of DNA-damaged sperm can still establish viable pregnancies. Finally, the percentage of sperm staining positive for DNA fragmentation is significantly increased in men whose wives suffered recurrent pregnancy loss.

The increasing number of publications in this field indicates that an increased fraction of sperm showing DNA damage is a negative trait that reduces the chances to father a child.

CONCLUSION

The incredible success of ICSI as a treatment technique also brought about fears that the quality of spermatozoa being used may lead to adverse reproductive outcomes. The advent of scores of healthy ICSI offspring has largely allayed these fears; however, there are still undercurrents of concern. It is clear that we still need to improve our current knowledge in relation to the DNA anomalies in spermatozoa, how to detect them more accurately and how they may relate to failed or abnormal reproductive outcomes. Finally, improvement in the detection and selection techniques of abnormal spermatozoa prior to choosing them for ICSI should alleviate the growing concerns over the safety of ICSI.

RELEVANT REVIEWS


OBJECTIVE
The endometrium undergoes an ordered process of differentiation leading to receptivity to embryonic implantation. Endometrial development is dependent on the ordered exposure to estrogen and progesterone. Homeobox genes act as regulators of embryonic morphogenesis and differentiation by assigning identity to cells along the developmental axis. In the adult, HOX genes are regulated by sex steroids and are essential for endometrial development and endometrial receptivity. Hox transcription factors are also important regulators of normal and malignant hematopoiesis and are expressed in specific compartments of the hematopoietic hierarchy.

We have previously demonstrated that HOXA-10 is differentially expressed in the adult endometrium through the menstrual cycle. Here our objective was to determine the pattern of HOXA-10 gene expression in the peripheral leukocytes of ovulating, non-ovulating, postmenopausal and pregnant women. We hypothesized that this gene, which is directly regulated by sex steroids in the endometrium using two EREs, would be regulated by sex steroids in leukocytes as well.

METHODS
Peripheral blood samples were collected from male and female volunteers. Specimens were collected at different phases of the menstrual cycle in normally ovulating women, from women with anovulatory cycles, women with successful pregnancies, women undergoing spontaneous abortion and women with an ectopic pregnancy.

Leukocytes were isolated from peripheral whole blood. Leukocyte RNA was extracted and reverse transcriptase real time PCR was used to determine HOXA-10 expression level.

Additionally in vitro the U937 myelomonocytic cell line was treated with graded concentrations of estradiol. HOXA-10 RNA expression was determined and dose response curve generated.

RESULTS
HOXA-10 gene expression was detected in the leukocytes of normally ovulating, anovulatory, pregnant and postmenopausal females. HOXA-10 expression was normalized to that of beta actin. Normalized HOXA-10 gene expression was minimal in male volunteers (normalized HOXA-10 ratio 1.4). Peripheral leukocyte HOXA-10 gene expression was detected in the proliferative phase in normal cycling women (2.4 to 159.2), increased further in the secretory phase (172.4 to 333.1; P=0.0048) with a peak during ovulation (389.4). Patients with anovulation had lower levels of HOXA-10 expression (14.3 to 23.8; P=0.0068) than normal cycling women.

Subjects with successful pregnancies had significantly higher HOXA-10 gene expression starting at five weeks of gestational age (800.6) and further increased with pregnancy (4,096 at 26 weeks and 77,129 at 36 weeks). Three subjects with an ectopic pregnancy and three subjects who subsequently underwent a spontaneous abortion had lower HOXA-10 gene expression for gestational age (P=0.01). Postmenopausal females had the lowest HOXA-10 gene expression of the females sampled (8.1).

In U937 cells, a myelomonocytic cell line, HOXA-10 mRNA expression showed a dose responsive increase to increasing molar concentrations of
estradiol (1.9 with \(10^{-7}\) M E2, increasing to 277.2 with \(10^{-4}\) M E2).

**CONCLUSIONS**

HOX genes have previously been identified as markers of endometrial receptivity. Here we have identified the novel expression of the HOXA-10 gene in circulating human leukocytes. The expression was regulated by estradiol in vitro and also correlated with serum estradiol levels and with menstrual cycle phase in women. Peripheral leukocyte HOXA-10 expression also appears to correlate with successful pregnancy. HOXA-10 gene expression in the peripheral blood could provide a noninvasive way of monitoring target tissue response to sex steroids and to pregnancy. The endometrium is a well-characterized estrogen-responsive tissue while leukocyte response to sex steroids and their role in pregnancy is still poorly understood. A common response of both endometrium and leukocytes to estradiol that is mediated by HOXA-10 may play a role in the establishment and maintenance of pregnancy.
The Incidence of Postoperative Delirium in Geriatric Patients Undergoing Surgery for Suspected Gynecologic Malignancies

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OBJECTIVE

The incidence of postoperative delirium (PD) in the elderly ranges between 3% and 60% but has never been examined in gynecologic oncology. The goal of our study was to identify pre-, intra-, and postoperative risk factors associated with the development of PD.

METHODS

English-speaking women > 60 years old undergoing major surgery for suspected gynecologic malignancies were invited to participate. Enrolled patients were administered a pre- and postoperative Mini-Mental State Exam (MMSE), and the postoperative Confusion Assessment Method was used to diagnose PD. Pre-, intra-, and postoperative clinical, laboratory, and pathology parameters were collected and compared to identify risk factors in developing PD. Statistical analysis included the Pearson Chi-squared tests and multivariate logistic regression.

RESULTS

Eighteen (18) of a total of 103 patients (17.5%) developed PD. Univariate significant associations (p<0.05) were shown between the development of delirium: age, albumin level (pre- or postoperative), Charlson comorbidity index, performance status, prior dementia, level of education, number of pre- and postoperative medications, prolonged oxygen or foley catheter usage (>2 d), increased narcotic use (above standard PCA and oral regimens), postoperative transfusion, bed restriction, and change in MMSE scores (pre vs. post). Using multivariate logistic regression analysis, older patients (p=0.0002) on multiple medications (p=0.008) given additional narcotic doses (p<0.0001) were at highest risk for the development of delirium. Interestingly, intraoperative parameters (surgical and anesthesia time, EBL, time of day procedure performed) were not correlated with outcome.

CONCLUSIONS

PD is a common complication in older women undergoing major gynecologic surgery. Increased narcotics, age, and preoperative medications were strongly associated with the development of delirium. Prevention needs to focus on identifying patients at higher risk for POD based on preoperative parameters and eliminating known postoperative risk factors.
Neoadjuvant Chemotherapy Lessens Surgical Morbidity in Advanced Ovarian Cancer and May Be Associated with Improved Survival in Stage IV Disease

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OBJECTIVE
To compare the survival and peri-operative morbidities of patients with advanced epithelial ovarian cancer (EOC, stage IIIC and IV) who were treated with primary debulking surgery (PDS) followed by adjuvant platinum-based chemotherapy, or neoadjuvant chemotherapy followed by cytoreductive surgery (NAC).

METHODS
One hundred seventy-two (172) patients with advanced EOC diagnosed at YNHH (1998-2005) were retrospectively reviewed. One hundred nine (109) patients were treated with PDS and 63 patients were treated with NAC (37 received carboplatin/paclitaxel [CP], 26 received carboplatin/cyclophosphamide [CC]).

RESULTS
NAC patients had significantly less intraoperative blood loss, operating time, and units of transfusion, and shorter hospital stays (p < 0.05). Optimal cytoreduction was achieved in 95% of NAC patients, versus 71% of the PDS group (p < 0.001). Three patients in the NAC group (5%) versus 27 patients (25%) in the PDS group required aggressive surgery in addition to standard cytoreduction. Within the NAC group, overall survival (OS) is improved in patients who received CP compared to CC (83 vs. 26 months, p = 0.008). Patients with extra-abdominal disease who received CP as NAC had improved progression-free survival (PFS) and OS when compared to the PDS group with stage IV disease (15 vs. 9 months, p=0.015; 31 vs. 20 months, p = 0.032, respectively).

CONCLUSIONS
This study demonstrates that NAC is associated with less peri-operative morbidity, less need for further aggressive surgery, and similar survival. Additionally, in patients with extra-abdominal disease, NAC is associated with an improved PFS and OS. Therapy with platinum and taxane should be the treatment of choice in NAC.
OBJECTIVE

The purpose of this study was to evaluate the levels of adrenocorticotropin hormone (ACTH), cortisol, and heat shock protein-70 (HSP70) as markers for stress in non-pregnant women and mid-gestational gravidas seeking health care in New York City before and after 9/11/01.

METHODS

This was a nested cohort study of banked serum samples drawn from two populations of women of reproductive age seeking Ob/Gyn care in New York City from 2000 to 2001: (1) non-pregnant women eligible for IVF treatment and (2) pregnant women seeking prenatal care. The pre-9/11 samples were drawn in the year prior to 9/11/01; the post-9/11 samples were drawn within six months after the crisis. The pre-9/11 and post-9/11 pregnant samples had all been drawn at 20+1 weeks’ gestation and were matched for age, race, and parity. All pregnant samples were banked specimens drawn at random times during the day, and were originally used for a non-related research study. All of the pregnant samples were from women with known normal pregnancy outcomes; diabetes, preeclampsia, and IUGR were excluded. The non-pregnant samples were discarded serum specimens drawn at random times of day from potential IVF candidates with serum E2 levels<75 pg/mL and FSH serum levels<12.5 mIU/mL, respectively.

The serum samples were assayed for levels of ACTH and cortisol by a commercially available Immulite™ system. HSP70, an additional marker for stress, was measured by a commercially available ELISA. These results were compared in the patient populations before and after 9/11. T-tests were used for analysis. Statistical significance was considered when p<0.05.

RESULTS

In the pregnant subjects, there were no statistical differences in the mean values of randomly drawn ACTH and cortisol levels between the pre- and post-9/11 groups. However, the mean values of HSP70 were significantly lower in the post-9/11 group: 15.90 pg/ml (+/-8.9 SEM) in the post-9/11 group vs. 122.25 pg/ml (+/- 31.3 SEM) in the pre-9/11 group (p=0.0023). However, in the non-pregnant subjects, the randomly drawn mean serum ACTH, cortisol, and HSP70 levels were all significantly different pre- and post-9/11 (ACTH: pre-9/11=78.3 vs. 84.9 pg/ml, p<0.0031; cortisol: pre-9/11=61.2 vs. 49.4 ug/ml, p<0.0014; HSP70: pre-9/11=290.4 vs. post-911=581.6pg/ml).

CONCLUSIONS

Serum HSP70 levels were unexpectedly decreased in pregnant subjects after the stressful event of 9/11 in comparison to non-pregnant women whose HSP70 levels were elevated in response to the same psychologically stressful event. The unexpected decrease in HSP70 levels in response to stress may suggest that pregnancy conveys an altered response to external stressors.
Macrophage Migration Inhibitory Factor Expression in Ovarian Cancer

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OBJECTIVE

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that regulates both innate and adaptive immune responses. It is also thought to cause tumor progression, angiogenesis and tumor invasion in several cancers including gastric, prostate, breast, colon, brain, bladder, kidney, skin and lung-derived tumors. We evaluated the hypothesis that ovarian cancer patients have significantly higher levels of serum macrophage migration inhibitory factor.

METHODS

MIF levels were determined by ELISA in cell lysates and culture supernatant of a panel of epithelial ovarian cancer cell lines isolated from malignant ascites and immortalized normal ovarian surface epithelial cells (immortalized with telomerase) and in the serum of ovarian cancer patients (n=54) and age-matched healthy women (n=60). To determine the impact of Toll-like receptor-4 ligation on MIF levels, cells were treated for 48h with lipopolysaccharide.

RESULTS

Cancer cells, but not normal cells, secrete significant amounts of MIF (p=0.007). This correlates in vivo, where serum MIF levels are significantly higher in ovarian cancer patients (p<0.0013). Treatment of cancer cells with TLR-4 ligand lipopolysaccharide induced a significant increase in MIF secretion in cancer cell lines but not in immortalized ovarian surface epithelial cells (p=0.0007). ROC curve showed 2.1ng/ml as the cutoff to discriminate between healthy women and cancer patients, generating sensitivity and specificity of 77.8% and 53.3%, respectively.

CONCLUSIONS

MIF may play a role in the process of ovarian cancer formation and progression. The events leading to the induction of MIF expression and its contribution to ovarian cancer progression need to be studied further and may open new venues for targeted therapy. MIF may also be useful as a marker in screening for early detection of ovarian cancer.
Differential Cell-Specific Modulation of HOXA-10 by Estrogen and Sp1 Response Elements

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OBJECTIVE

HOX genes are highly evolutionarily conserved regulators of embryonic development. HOXA-10 also regulates differentiation of the adult reproductive tract and mammary gland in response to sex steroids. We recently identified two HOXA-10 estrogen response elements (EREs). Here we demonstrate that estrogen-responsive HOXA-10 expression is cell type specific.

METHODS

Ishikawa cells are a well-differentiated endometrial adenocarcinoma cell line in which we have previously characterized estrogen regulation of HOX genes (4,14-19). These cells were cultured in phenol red-free Eagle’s Minimum Essential Medium (MEM; Life Technologies, Inc., Gaithersburg, MD) containing 10% (v/v) charcoal stripped fetal bovine serum and supplemented with penicillin/streptomycin (100μg/ml), L-glutamine (2mM), and sodium pyruvate (1mM). MCF7 cells were also cultured in MEM containing 10% (v/v) charcoal stripped fetal bovine serum and supplemented with penicillin/streptomycin (100μg/ml), L-glutamine (2mM), and sodium pyruvate. Estrogen receptor status was verified by ELISA in both cell lines according to the manufacturer’s instruction (Abbot Laboratories, Weisbaden, Germany). Cells were grown in plastic flasks (75cm², Falcon, Franklin Lakes, NJ, USA) and maintained at 37°C in a humidified atmosphere (5% CO₂ in air). The 70%-80% confluent monolayers were maintained in serum-free media for 24 hours, and subsequently treated with E2 (17ß estradiol; 1 x 10⁻⁸M; Sigma).

RESULTS

The HOXA-10 EREs and a specificity protein 1 (Sp1) binding site differentially drive the cell-type-specific E2 response. In electrophoretic mobility shift assays, both estrogen receptor-α and -β bound both EREs but not the Sp1 site. In reporter assays, both EREs and the Sp1 site demonstrated estrogen responsiveness and tissue specificity; transiently transfected uterine Ishikawa cells or breast MCF-7 cells showed differential responses to E2 treatment. Each response element (Sp1, ERE1, and ERE2) drove distinct differential expression in each cell type. Sp1 protein was expressed in a menstrual-cycle stage-specific expression pattern in the endometrium, first expressed in perivascular cells.

CONCLUSIONS

Tissue specificity inherent to a regulatory element as well as differential cellular expression of transcription factors imparts differential tissue-specific estrogen responsiveness.
Ultrasound Evaluation of the Uterine Scar After Cesarean Section: A Study of Women Randomized to One- or Two-Layer Closure

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BACKGROUND

Cesarean delivery (CD) has become more common in women giving birth for the first time, with primary cesarean rates increasing from 21.2% in 1996 to 27.1% in 2003. Over the same interval, vaginal birth after cesarean section decreased from 28.3% to 10.6%. A low transverse incision is the preferred method of hysterotomy incision during cesarean delivery; however, controversy persists regarding the best technique for hysterotomy closure.

OBJECTIVE

The purpose of this study was to survey the uterine scar thickness by ultrasound during the first six weeks postpartum after one- or two-layer closure in women undergoing primary cesarean delivery.

METHODS

This was a randomized, blinded, prospective trial of uterine scar closure with sonographic follow-up. We enrolled 30 consecutive patients undergoing primary CD and randomly allocated them to either one- or two-layer closure of the hysterotomy incision. Ultrasound surveillance of the uterine scar was performed at baseline (prior to surgery), 48 hours, two weeks, and six weeks postpartum.

RESULTS

Patient compliance with the postpartum surveillance protocol was 90% and the uterine scar was visualized in 99% of attempted sonographic examinations. There were no differences between the groups at baseline or at 48 hours, two weeks, or six weeks post-op. For both closure techniques there was an initial five-to-six-fold increase in scar thickness at 48 hours postpartum (p<0.001) compared to the preoperative myometrial thickness, followed by a gradual decrease in the thickness of the scar. However, even at six weeks postpartum, the uterine wall at the site of the scar was significantly thicker than prior to surgery. Repeated measures ANOVA showed significant variation across time points starting either at baseline (p<0.001) or at 48 hours post-op (p<0.001) but this variation did not depend on closure type (p = 0.79 for all visits and p=0.81 beginning with the 48-hour post-op time point).

CONCLUSIONS

The process of uterine healing can be successfully monitored by ultrasound. Scar thickness diminishes progressively following both one- and two-layer closure but does not vary with mode of hysterotomy closure. The thickness of the uterine wall at the scar site remains increased even at six weeks after CD, suggesting that the process of uterine scar remodeling extends beyond the traditional postpartum period.
Yale Oral and Poster Presentations at the Society for Maternal-Fetal Medicine 2008 Annual Meeting

(Yale faculty are co-authors on an additional oral and three poster presentations with faculty from other institutions)

ORAL PRESENTATIONS

* The Soluble Form of the Receptor for Advanced Glycation End-Products (SRAGE), a Decoy for Rage, is Increased in the Serum Level of Women with Severe Preeclampsia. Emily Oliver, A. Dulay, G. Zhao, S. Jing, M. Cackovic, CS Buhimschi and Irina A. Buhimschi.


Chronic Nitric Oxide Inhibition in a Rat Model of Preeclampsia (PE) Leads to Urinary Proteomic Biomarker Signatures Similar to Humans. Irina A. Buhimschi, G. Zhao, V. Ravishankar, M. Bahtiyar, G. Saade and Catalin S. Buhimschi.

A Functional Role for the Novel Soluble Toll-Like Receptor-2 (STHR2) in Modulating Intra-Amniotic Immune Responses. Antonette T. Dulay, G. Zhao, E. Oliver, S. Jing, CS Buhimschi and Irina A. Buhimschi.


POSTER PRESENTATIONS

**Proteomic Profiling of Urine in Preeclampsia (PE) Identifies Biomarker Sets Which Predict Outcome and Differentiate This Condition from Other Hypertensive Disorders During Gestation. Irina A. Buhimschi, G. Zhao, E. Funai, I. Bernstein, G. Saade and Catalin S. Buhimschi.

* Award for Best Concurrent Session Oral Presentation
** Award for Best Poster Presentation


Effect of In Vitro Fertilization (IVF) on Prevalence of Congenital Heart Disease in the United States: A Single Institution Experience. Mert Ozan Bahtiyar, AT. Dulay, BP. Weeks, AH. Friedman, and JA. Copel


Evidence of Uncultivated Bacteria as Etiologic Agents of Intra-Amniotic Infection and Inflammation Leading to Preterm Birth (PTB). Yping Han, L. Zhang, P. Chung, L. Kirchner, IA. Buhimschi and Catalin S. Buhimschi.


Evidence for a Role of Inflammation in Control of Angiopoietin-1, Angiopoietin-2 and of Their Novel Antagonist Soluble TIE-2 in Pregnancies Complicated by Intra-Amniotic Inflammation. Catalin S. Buhimschi, V. Bhandari, G. Zhao, VA. Rosenberg, E. Zambrano, M. Cackovic, E. Funai and Irina A. Buhimschi.


**Effect of Two vs One Stitch on the Prevention of Preterm Birth in Women with Singleton Pregnancies Undergoing Elective Cervical Cerclage Placement.** Karljin Woesdregt, E. Norwitz, M. Cackovic, M. Paidas and Jessica Illuzzi.

**Fractional Excretion of Tumor Necrosis Factor-alpha (TNFα) in Women with Severe Preeclampsia.** Michael Cackovic, IA. Buhimschi, G. Zhao, G. Luo, S. Thung, ER. Norwitz, E. Funai and Catalin S. Buhimschi.

**Relationships of Maternal Serum Levels of Vascular Endothelial Growth Factor (VEGF) and Tensile Properties of the Cervix in a Rat Model of Chronic Hypoxia.** Anna K. Sfakianaki, IA. Buhimschi, V. Ravishankar, MO. Bahtiyar, AT. Dulay and Catalin S. Buhimschi.

**Effect of Thrombin on GnRH-I and GnRH Receptor (GnRHR) Gene Expression in Human Cytotrophoblast Cells.** Guoyang Luo, V. Snegovskikh, S. Guller, M. Rahman, E. Funai, Y. Ma, S. Thung and Errol R. Norwitz.


**Screening for Gestational Diabetes: Is a 130 mg/dl or 140 mg/dl Glucose Challenge Test Threshold More Cost Effective?** Stephen Thung, C. Pettker and Edmund Funai.


**OTHER PRESENTATIONS WITH YALE UNIVERSITY FACULTY AS CO-AUTHORS**

**Antenatal Screening for Cystic Fibrosis: Cost-Effectiveness by Ethnicity.** Sarah Little, William Grobman, Anthony Odibo, David Stamilio, Stephen Thung, and Aaron Caughey.

**Doppler and BPP as Predictors of Outcome in Severe IUGR Fetuses.** Erich Cosmi, Carlos Saccardi, Gianna Bogana, Edmund Funai, Vincenzo Berghella, and Giancarlo Mari.

**Doppler Carotidocography and Biophysical Profile Score in Idiopathic IUGR Fetuses and in IUGR Fetuses Because of Maternal Preeclampsia.** Erich Cosmi, Edmund Funai, Gianna Bogana, Carlo Saccardi, Vincenzo Berghella, and Giancarlo Mari.
Society for Gynecologic Investigation 2008 Annual Meeting Abstracts from Yale Full-Time Faculty

ORAL PRESENTATIONS


Spastin Confers Specificity to HOXA-10 Mediated Transcriptional Regulation in Reproductive Tissues. Gaurang S Daftary, Amy M Tetrault, and Hugh S Taylor.

A Factor VII/IgG1 Fc Immunocugjugate Molecule (Icon) Regresses Established Disease in a Mouse Model of Human Endometriosis. Graciela Krikun, Kevin Osteen, Kaylon Brunner-Tran, Zhiwei Hu, Frederick Schatz, William Konigsberg, Alan Garen, and Charles J Lockwood.

Progesterone and HOXA-10 Regulate GABA-A pi Receptor Expression, Membrane Translocation and Activation. Homayoun Sadeghi and Hugh S Taylor.

Identification and Characterization of miR-199a as Regulator of IKKβ Expression and Its Function in Ovarian Cancer Cells. Rui Chen, Ayesha B Alvero, Thomas Rutherford, and Gil Mor.

Racial Disparity in Genotype-Phenotype Interactions in Preterm Birth. Stephen J Fortunato, Digna R Velez, Errol R Norwitz, Charles J Lockwood, Scott M Williams, and Ramkumar Menon

CLIP-170 Functions Downstream of mTOR Kinase During Local Ovarian Responses to Stress. Niels Galjart and Joshua Johnson.


Identification and Characterization of Human Embryonic Poly(A) Binding Protein (ePAB). SA Pauli, O Guzeloglu-Kayisli, H Demir, MD Lalioti, D Sakkas, and Emre Seli.

POSTER PRESENTATIONS

HuR Expression Is Altered in Ectopic Endometrium. S Karipcin, T Altun, UA Kayisli, and Emre Seli.

Progestin Suppresses Thrombin-Enhanced Interleukin-6 Expression in Term Decidual Cells: Implications for Abruption-Induced Preterm Delivery. Edward Kuczynski, Lynn F Buchwalder, Frederick Schatz, and Charles J Lockwood.

* Recipient of the SGI President’s Presenter Award
** Recipient of the SGI President’s Presenter Award; The Trainee Plenary Session Selection

Rheological Study of Fetal Descending Aorta in Real-Time Using Multigate Spectral Doppler Analysis. Gabriele Urban, Patrizia Vergani, Stefano Ricci, Piero Tortoli, and Michael Paidas

3-Phosphoglycerate Dehydrogenase (PGDH) Is Regulated by HOXA-10 During Early Implantation. Danielle Vitiello, Hongling Du, Robert Pinard, and Hugh Taylor

Leukocyte HOXA-10 Expression Correlates with Endometrial Receptivity in Mice and Humans. Danielle Vitiello, Elena Ratner, Hongling Du, Robert Pinard, and Hugh Taylor

Expression of Myoferlin in Human Endometrium During the Menstrual Cycle. Pinar H Kodaman, Tugba Altun, William Murk, Umit Kayisli, Pascal N Bernatchez, and William C Sessa

Interleukin-1 Beta (IL-1β) Regulates IL-6 Signaling in Decidua-Implication in the Pathophysiology of Preeclampsia (PE). SJ Huang, CF Yen, CP Chen, F Schatz, and Charles J Lockwood


Differential Effects of Anti-Phospholipid Antibodies on First Trimester Trophoblast Cells. Vikki M Abrahams, Melissa J Costello, Shawna Joyce, Michael J Paidas, Larry W Chamley, and Jan J Brosens

Characterization of the Embryonic Poly(A)-Binding Protein During Oocyte Maturation. JK Friend, FB Bezirci, and Emre Seli.

Subcellular Localization and Functional Capacity of Follicle Stimulating Hormone Receptor (FSHR) Splice Variants Detected in Patients with High and Low Response to FSH. Dimitrios Zattas, Tsilya Gerasimova, Emre Seli, Denny Sakkas, and Maria D Lalioti.

ERK Expression and Activity in Human Myometrium and Leiomyoma. Tugba Altun, William Murk, Yesim H Uz, Sinem Karipcin, Umit A Kayisli, and Aydin Arici.

Non-Invasive Metabolomic Profiling of Human Embryo Culture Media Using 1H NMR Correlates with Pregnancy Outcome. E Seli, L Botros, D Sakkas, and DA Burns.

Activation of TLR-3 in the Trophoblast Is Associated with Preterm Delivery. Kaori Koga, Paulomi B Aldo, Bing Peng, Sara Fill, Ingrid Cardenas, and Gil Mor.


The Inflammatory Cytokines Interleukin-1β and TNF-α Increase G-CSF Expression in Term Decidual Cells. Felice Arcuri, Paolo Toti, Lynn F Buchwalder, Alessandra Casciaro, Marcella Cintorino, Frederick Schatz, and Charles J Lockwood.

Effect of Community-Based Group Prenatal Physical Activity on Pre-Eclampsia Rate. Ann F Cowlin, Robyn Brancato, Gil Mor, Daniel Zelterman, and Peggy DeZinno.


CPEB Is Expressed in Mouse and Human Oocytes and Early-Preimplantation Embryos Prior to Zygotic Genome Activation. IE Sasson, O Guzeloglu-Kayisli, S Uckac, and Emre Seli.


Duration of Intrapartum Group B Streptococcal (GBS) Prophylaxis and Concentration of Penicillin G (PCN) in Fetal Serum at Delivery. Emma L Barber, Guomao Zhao, Irina A Buhimschi, and Jessica L Illuzzi.


Thrombin Enhances Vascular Endothelial Growth Factor (VEGF) Expression in Term Decidua: Implications for Abruptio-Related Preterm Birth. Victoria V Snegovskikh, Lynn F Buchwalder, Rebecca Caze, Mizanur Rahman, Graciela Krikun, Frederick Schatz, Charles J Lockwood, and Errol R Norwitz.

Use of Laser Capture Microdissection (LCMD) to Measure Levels of Syncytial Fas Ligand Expression in Human Placenta. Seth Guller, Yula Y Ma, Vikki M Abrahams, and Gil Mor.

Enhanced Placental Villus Core IL-1β in Preeclampsia (PE) and Intrauterine Growth Restriction (IUGR): Evidence for Cell Type Specific-Inflammatory Response. Seth Guller, Irina A Buhimschi, Catalin S Buhimschi, and Yula Y Ma.


A Functional TLR-4-MyD88-NF-κB Pathway in Epithelial Ovarian Cancer Cells Induces a Pro-Tumor Phenotype in THP-1 Monocytic Cells. Ayesha B Alvero, Michele K Montagna, Paulomi B Aldo, and Gil Mor.


KSP Inhibitor (ARRY-520) as an Alternative for Paclitaxel in MyD88-Positive Epithelial Ovarian Cancer Cells. Ki H Kim, Ayesha B Alvero, Yanhua Xie, David Trollinger, and Gil Mor.

Eriocalyxin B Induces Apoptosis of Chemoresistant Ovarian Cancer Cells Through NF-κB Inhibition. Aliza Leiser, Han-Hsuan Fu, Rui Chen, Ayesha Alvero, Yung-Chi Cheng, and Gil Mor.

BPA and DES Exposure In Vitro and In Utero Increases EZH2, a Histone Methyltransferase Associated with Uterine and Breast Cancer. Jason G Bromer, Jie Wu, and Hugh S Taylor.


Impaired Gremlin Expression in Cumulus Cells May Underlie Reproductive Dysfunction in Young Women with Diminished Ovarian Reserve (DOR). Keri Greenseid, Sangita Jindal, Joshua Hurwitz, Nanette Santoro, and Lubna Pal.
Welcome to Our New Interns

We are pleased to introduce our 2007 interns. For the 2007 class we interviewed 70 applicants from approximately 344 applications. We ranked only those candidates who mixed high academic achievement, humanistic qualities and commitment with sparkling personalities, and we firmly believe that is what we got... peppy, positive and academically accomplished interns!

Congratulations to the following:

Hakan Cakmak – Istanbul Universitesi. Hakan was our preliminary resident during the 2006-07 academic year. He is an associate member of the American Society for Reproductive Medicine, Junior Fellow of the American College of Obstetricians and Gynecologists, and Resident Member of the American Institute of Ultrasound in Medicine. In 2005 he received the Wyeth President’s Presenter Award at the 52nd Annual Meeting of the Society for Gynecologic Investigation. Among his achievements, Hakan has authored 29 publications. His hobbies and interests include playing basketball and collecting coins and stamps. He is fluent in Turkish.

Amanda Carlson – University of Connecticut School of Medicine, undergraduate of Duke University, B.S. Biology with neuroscience concentration, Psychology, Chemistry minor. Amanda received the following awards: John Gibbons Medical Student Award (District I, American College of Obstetrics and Gynecology), 2006; University of Connecticut Auxiliary Scholarship, 2004, 2005 and the Dean’s Circle Scholarship, 2005. She is a member of the American College of Obstetrics and Gynecology, American Medical Association and the American Medical Student Association. Amanda was involved in numerous volunteer programs such as the South Park Women’s Clinic, a free clinic based in the South Park homeless shelter; UConn Pediatric Curriculum Committee; Obstetrics and Gynecology Scholars Group; Medical Students for Choice and Student National Medical Association, to name a few. One of her research projects included the analysis of the relationship between psychiatric treatment at a clubhouse-affiliated facility (versus an outpatient hospital setting) and subsequent mental health outcomes. For her senior honors thesis she genotyped hundreds of hyporesponsive mice and identified a mutation in TLR-4, an endotoxin receptor. This work allowed her to graduate with distinction from Duke University. Amanda’s hobbies and interests include travel, interior design, cooking, and music. She is also an NCAA Division I Fencing (Duke University) champion and an American Heart Association certified Basic Life Support Instructor.
Sarah Cross – University of Chicago Pritzker School of Medicine, undergraduate Swarthmore College, B.A. in Psychology. Sarah was the recipient of the following awards: Teaching Assistant, Clinical Pathophysiology Therapeutics Course; Voting Member, Pritzker School of Medicine Admissions Committee; Member, Gold Humanism Honor Society, University of Chicago Chapter; and Exceptional Performance, Junior Medicine Clerkship. She volunteered on numerous programs such as the Chicago Youth Programs, Maria Shelter Free Weekly Clinic, Committee on Admissions, University of Chicago, and Liquid Poets & Liquid Writers. Her research included authoring “Molecular genetics of the platelet serotonin system in first-degree relatives of patients with autistic disorder,” which received honorable mention for a scientific paper. Sarah authored five publications. Her hobbies and interests include poetry, painting, running, and cycling.

Charlene Hooper – Brown Medical School, graduate of Harvard School of Public Health, M.P.H. in Family and Community Health, undergraduate of Brown University, B.A. in Sociology. Charlene is a member of the American College of Obstetricians and Gynecologists, Student National Medical Association, and the American Medical Student Association. During medical school she was involved in numerous volunteer activities such as Dwight Morrow H.H. Alumni-Student Mentoring Network, Big Sisters Association of Boston, Minority Association of Premedical Students, and COSALUP Medical Clinic. Charlene was also involved in research and co-authored the paper “Predictors of Endoscopy in Minority Women,” which was published in the *Journal of the National Medical Association*. Among Charlene’s hobbies and interests are cooking, cake decorating, salsa and hip-hop dance, tennis, running Bible study, youth ministry, and international travel. She is also fluent in Spanish.

Ken-Yu Lin – Northwestern University - The Feinberg School of Medicine, graduate of Johns Hopkins University, Ph.D., Biochemistry, Cell, and Molecular Biology, undergraduate of Stanford University, B.S. Chemistry. Ken-Yu was a member of the Phi Beta Kappa Honors Society. His awards and accomplishments include the Johns Hopkins Pathology Young Investigator Day Award (2005 and 2004) and the Howard Hughes Predoctoral Fellowship (2000-2005). His volunteer experiences included the Patient Perspective Hospice Program, co-organizer of the bone marrow typing drive at Northwestern, and the Curriculum Review Committee. He was the author of eight publications. Among Ken-Yu’s hobbies are cooking, running, snowboarding, biking, and music. His interests are environmental issues, computers, science, and technology. Ken-Yu is fluent in Chinese and Taiwanese.
Daniel Paik – Boston University School of Medicine, graduate of Boston University School of Medicine, M.A., Medical Sciences, undergraduate of Dartmouth College, B.A. Classics and Biogenetics. Daniel was the recipient of the Atherton Greek Prize, Dartmouth College and the Class of 1939 Senior Scholars Program, Dartmouth College. His work experience included the Autism Research Foundation as a Research Coordinator and the New England Center for Children as a Level II Teacher. He was the co-author of “Differential effects of mitomycin C and doxorubicin on P-glycoprotein expression,” published in the *Biochemical Journal*. Daniel is a second-degree black belt instructor with OomYoung Doe Martial Arts, LLC.

Kieu Smith (Preliminary) – Case Western Reserve University School of Medicine, undergraduate of Smith College. Kieu is a member of the American College of Obstetrics and Gynecology and the Student National Medical Association. As part of Kieu’s professional experience, she participated in the Case Western School of Medicine Primary Care Track, spending one day a week seeing patients with her preceptor in her pediatric office, and she co-designed a research project looking at the psychologic effects of Alzheimer’s disease on patient caretakers. She also taught tenth grade biology at the South Bay Lutheran High School. Kieu’s research included performing biochemical research under the NIH minority fellowship. Among her hobbies and interests are rowing and kayaking, and she has recently taken up cycling. She is acquiring fluency in Spanish.
Our Fellowship and Residency Program Graduates

**Chris Pettker**

Chris completed his fellowship in MFM and joined the Yale faculty as an assistant professor in the MFM Section.

**Beth Rackow**

Beth completed her fellowship in REI, and has stayed on as a new assistant professor in REI.

**Lissa Magloire**

Lissa completed her MFM fellowship and has joined a perinatal practice in Texas.

**Michael Kelly**

Michael completed his Gynecologic Oncology fellowship and has gone on to join the faculty at the University of Colorado in Denver.
Chief Residents

*Rinki Agarwal*

Rinki has secured a Genetics fellowship at Memorial Sloan-Kettering Cancer Center in New York City.

*Leah Ahoya*

Leah has gone into private practice at Geisinger Medical Center, Danville, PA.

*Eric Hodgson*

Eric is one of our new MFM Fellows.

*June Hou*

June has gone on to fellowship training in Gynecologic Oncology at Albert Einstein College of Medicine in the Bronx.

*Ryan Martin*

Ryan is our new REI Fellow.

*Elena Ratner*

Elena is our new Gyn Onc Fellow.

*Shelley Saber*

Shelley has gone into private practice at Hackensack University Medical Center in Hackensack, NJ.
Newest Additions to the Yale Faculty

Beth Rackow, MD – Reproductive Endocrinology and Infertility

It is with great pleasure that we announce the addition of Dr. Beth Rackow to the faculty of the Division of Reproductive Endocrinology and Infertility in the Department of Obstetrics, Gynecology & Reproductive Sciences. Dr. Rackow joined the faculty as Assistant Professor in July 2007.

Dr. Rackow received a B.A. in Environmental Biology from Yale College. She earned her M.D. at the University of Connecticut School of Medicine. During medical school, Dr. Rackow received the CIBA Award for Community Service and the Connecticut Society of the American Board of Obstetricians-Gynecologists Award for excellence.

Dr. Rackow then completed her residency in Obstetrics & Gynecology at the Hospital of the University of Pennsylvania in Philadelphia, where she received the Excellence in Vaginal Surgery Award.

In 2004, Dr. Rackow returned to Yale for a fellowship in Reproductive Endocrinology and Infertility. During these past three years, Dr. Rackow published 10 peer-reviewed papers and book chapters. In 2006, Dr. Rackow presented her research entitled “Uterine leiomyomas affect molecular determinants of endometrial receptivity” at the American Society for Reproductive Medicine 2006 Annual Meeting, where it was selected as one of the top three presentations by reproductive endocrinology fellows. She has also played a significant role in resident education and was recently awarded our most coveted C. Lee Buxton Faculty Teaching Award.

Dr. Rackow is currently the ACOG District I Junior Fellow Immediate Past Chair, and she serves as a member of the Committee on Practice Bulletins – Gynecology. In 2006, Dr. Rackow was selected to attend ACOG’s Future Leaders in Obstetrics and Gynecology seminar.

Dr. Rackow’s clinical interests include reproductive surgery, uterine myomas, abnormal uterine bleeding, and Müllerian anomalies. Additionally, she has a particular interest and training in pediatric and adolescent gynecology. Dr. Rackow will continue to be involved in resident and medical student education as the Director of the Ob/Gyn Endoscopic Training Program and as the Director of the Sub-internship in REI.

Dr. Rackow will be available for appointments starting this July in the Department’s Long Wharf office.
Lubna Pal, MD, Reproductive Endocrinology and Infertility

It is with great pleasure that we announce the addition of Dr. Lubna Pal to the faculty of the Section of Reproductive Endocrinology and Infertility in the Department of Obstetrics, Gynecology & Reproductive Sciences.

After graduating from Dow Medical College in Pakistan, Dr. Pal received postgraduate training in the United Kingdom, followed by a Fellowship in Reproductive Endocrinology & Infertility at Massachusetts General Hospital, a residency in Obstetrics & Gynecology at Yale-New Haven Hospital, and a second Fellowship in Reproductive Endocrinology & Infertility at the Albert Einstein College of Medicine, Yeshiva University, New York. Following completion of her fellowship, Dr. Pal joined the faculty of the Albert Einstein College of Medicine, where she received an NIH Mentored Clinical Researcher Award and a master's degree in Clinical Research Methods. Her research has focused on multisystem implications of reproductive aging, with a specific focus on bone health. In addition to menopause, perimenopause and diminished ovarian reserve, Dr. Pal's clinical interests include reproductive endocrinopathies including PCOS, central reproductive disturbances attributable to hypothalamic and pituitary disorders, obesity-related reproductive dysfunction, and low bone density.

Dr. Pal is Board Certified in both Obstetrics & Gynecology and Reproductive Endocrinology & Infertility. She joins us as Director of the Reproductive Aging and Bone Health Program, and she is available for appointments immediately at the Yale Reproductive Endocrinology Long Wharf practice site in the areas of infertility, menopause, bone density, and reproductive endocrinology.

Chris Pettker, MD, Maternal-Fetal Medicine

It is with great pleasure that the Section of Maternal-Fetal Medicine announces the addition of Christian M. Pettker, M.D. to our faculty.

Dr. Pettker spent his undergraduate years at Princeton, where he received an A.B. in Architecture in 1995. He then went on to earn his M.D. at Columbia, where he remained as a resident in Obstetrics & Gynecology. He excelled at Columbia, and was selected as both Administrative Chief Resident and the recipient of the Arnold P. Gold Foundation Humanism and Excellence in Teaching Award of New York-Presbyterian Hospital.

Dr. Pettker then came to Yale as a Maternal-Fetal Medicine Fellow in 2004, and quickly made a positive impression. As a testament to his dedication to education, he received the Jose Asis Memorial Award for Teaching in 2005. He has been highly productive as a fellow, publishing more than 16 articles, chapters and letters during the past three years. In 2006, Dr. Pettker presented his research, "Value of placental microbial evaluation in diagnosing intra-amniotic infection," in a plenary session at the Society for Maternal Fetal Medicine's Annual Meeting. He has clearly proven that he is an outstanding clinician and educator, and has substantial promise as an investigator.

Dr. Pettker and his wife, Hesse Metcalf, a local attorney and teaching fellow at Yale Law School, reside in New Haven. They enjoy cycling (even biking to work in snowstorms) and performing desperately needed restoration to their cabin in Vermont.
Fond Farewells

**Antoni Duleba** moved back West with his family to take a faculty position at the University of California, Davis.

**Ervin Jones** has joined New England Fertility Institute in Stamford.

**Men-Jean Lee** has returned to New York, joining the faculty of the Mount Sinai School of Medicine in Manhattan.
PHOTO HIGHLIGHTS
FROM THE 2007 YALE OB/GYN RESIDENTS’ RESEARCH DAY
PHOTO HIGHLIGHTS FROM THE FIRST YOGS GATHERING AT ACOG IN SAN DIEGO, MAY 2007
NEWS ITEMS

PEOPLE

Dr. Carl Cassin is retiring after 43 years as a Yale community Ob/Gyn.

Dr. Paulo Rinaudo is the proud father of a baby girl.

Dr. Rinki Agarwal had identical twin girls in early December — mother and twins are doing well.

Dr. Erin Wolff is doing well with her baby, and she has been spending a bit of time in Colorado with her husband.

Dr. Steve Fleischman had his fourth child this year — his first son!

EDITORIAL COMMENT

Attention Yale Ob/Gyn Alumni!

I know that I always check any alumni magazine for professional and personal updates (yes, gossip) and we would love to present any news you might like to share with your Yale family. So please send us updates on careers, personal achievements, family — anything you think your friends would like to know. – MJM

SERVING THE YALE COMMUNITY

YOGS has been established to serve the Yale Ob/Gyn faculty, alumni, and anyone in Ob/Gyn or Reproductive Sciences with Yale roots.

This is your society, so we encourage you to join and contribute content. A membership form is available near the back of this Journal.

Here are a few things that YOGS has accomplished so far:

• The first issue of this Journal, obviously
• An ACOG ACM reception held in May 2007 (photos within)
• The first alumni conference, planned for April 2008 (invitation and directions mailed with this Journal)
• A reception at the SGI Annual Meeting in March 2008
• The second ACOG ACM Reception, to be held in May 2008

YALE PODCAST – WWW.YALE.EDU/OPA/PODCAST

You may still think of iTunes as a place to get, well, tunes, but your alma mater has other ideas. The Office of Public Affairs has launched an effort to put up “podcasts” — downloadable digital files — of talks by alumni, faculty, and others at the iTunes website. All are free, ranging from a reunion talk on the second Bush Administration by historian John Gaddis to a lecture by art historian Vincent Scully on architect Philip Johnson. Of course, our Department is well represented.
DO YOU HAVE NEWS TO SHARE WITH YOUR CLASSMATES?

Complete the following form (also available online at www.med.yale.edu/obgyn/yogs) and return to yogs@yale.edu — include address, phone, fax and email. Alternatively, you can mail it to:

Yale University School of Medicine
Department of Obstetrics, Gynecology & Reproductive Sciences
333 Cedar Street, PO Box 208063, Room 334 FMB
New Haven, CT 06520-8063
Attn: YOGS Coordinator

Name:        Year:
Street:        City:
State:        Zip:       Country:
Is this a new address (circle one)?       Yes       No
Tel:        Fax:       Email:

Tell us your news (marriage, birth, death, career milestones, honors, etc.):

If you are advising us of an alumni death, please provide us with the following:

Name of deceased:        Class year:
Approximate date of death:
Contact name and phone number:

Does the family of the deceased wish to continue receiving mail from YOGS (please circle)?       Yes       No

All submissions must be made in writing. Class notes may be edited for clarity and space. Due to limited space, the YOGS Journal cannot guarantee the publication of all items.

DONATIONS

Our department is constantly seeking philanthropic donations to help us fund our research and teaching missions. Examples of programs and initiatives constantly in need of support are the Lee Buxton Memorial Lecture, Resident Research Day, the Nathan Case Annual Lecture, Gynecologic Oncology’s Discovery to Cure Program, and a host of under-funded reproductive sciences research programs.

If you would like to make a donation to Yale Ob/Gyn, use the form below, or donate directly using our website at www.med.yale.edu/obgyn/yogs. If you are mailing, make checks payable to:

Yale University School of Medicine
Department of Obstetrics, Gynecology & Reproductive Sciences
333 Cedar Street, PO Box 208063, Room 334 FMB
New Haven, CT 06520-8063
Attn: YOGS Coordinator

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**Membership Dues** *(please check one)*

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- Lifetime Membership $1500    Amount Enclosed $___________

*(Dues already paid will be applied toward Lifetime Membership if selected)*

Please make checks payable to *Yale Obstetrical and Gynecological Society (YOGS)* or online at [www.med.yale.edu/obgyn/yogs](http://www.med.yale.edu/obgyn/yogs)

Mail to:

Department of Obstetrics, Gynecology & Reproductive Sciences
Yale University School of Medicine
333 Cedar Street, FMB 334
P. O. Box 208063
New Haven, CT 06520-8063
Attn: Susan Andranovich

Please note the alumni event will be held on Saturday, April 5, 2008 in New Haven, CT.
Yale Obstetrical and Gynecological Society

YOGS