Nonsurgical Permanent Contraception for Women
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Conference Synopsis
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SESSION 1: Welcome and Overview

Welcome to Oregon National Primate Research Center/Oregon Health and Science University:
Nancy Haigwood, PhD and Dan Dorsa, PhD

Center director Dr. Haigwood welcomed everyone. She expressed gratitude to the Bill & Melinda Gates Foundation for helping to build the infrastructure that makes the research at the Oregon National Primate Research Center (ONPRC) possible. She then introduced Dr. Dorsa, vice president for research. He expressed welcome to everyone, especially the representatives of the Gates foundation. From the perspective of Oregon Health and Science University (OHSU), this research conference is a good fit. There is a science base here and an existing collaboration between researchers at the primate center and the department of obstetrics and gynecology. The primate center also has existing collaborations with industry, foundation partners, AIDS research, and the Gates foundation, among others. He thanked everyone for attending.

Welcome and meeting objectives: Jeff Jensen, MD, MPH and Kirsten Vogelsong

Dr. Jensen thanked everyone involved in planning this meeting and thanked all the attendees and presenters, as well as the advisory board. He then posed the question, why OHSU for this conference and this proposed research center? OHSU is an internationally recognized leader in Contraception research. The Women’s Health Research Unit at OHSU is a site for foundation- and industry-sponsored clinical research in contraception and a National Institute of Child Health and Human Development (NICHD) Contraception Clinical Trial Network research site, established in 2003. A fellowship in family planning was started at OHSU the same year. In 2007, OHSU/ONPRC was awarded a NICHD-funded U-54 Contraceptive Development Research Center grant for basic research in female contraception. The ONPRC campus was established in 1960 and is one of eight national primate research centers. Its core grant is in year 53.

The reason for this meeting is to discuss permanent contraception. We are seeking to develop a nonsurgical method with voluntary initiation that has adequate counseling materials, is available in low-resource settings, has a low cost, and has no need for surgical facilities. Research into this area has been ongoing since the 1970s. As the traditional nomenclature (sterilization) carries a stigma and implies coercion, the research community has adopted the term “permanent contraception.” With high-level support from OHSU/ONPRC, Dr. Jensen proposes to develop a research center for permanent contraception at ONPRC, in consultation with the Bill & Melinda Gates foundation and supported by a planning grant from that foundation. The task set for this meeting is not simply to create a research center, but to develop a rich and robust research agenda. The long-term goal for the center is to develop
a low-cost, nonsurgical approach to permanent female contraception, in alignment with the Gates Global Program to improve human health by preventing unintended pregnancy.

Dr. Jensen concluded his remarks by stating the objectives of the meeting. First, to present strategies for nonsurgical permanent contraception, evaluating both opportunities and limitations, in order to prioritize the research agenda for the first year of the proposed center; second, to introduce and receive feedback on the plans for infrastructure improvements and scientific support at the proposed center; and third, to discuss the mechanism for solicitation of outside research proposals.

Kirsten Vogelsong from the Bill & Melinda Gates foundation then welcomed everyone. She thanked Dr. Jensen and Keri Brown for planning the meeting. The Bill & Melinda Gates Foundation has developed a revised strategy for family planning—development of new methods, lowering the cost of production of existing methods, and improving distribution and uptake of products in developing countries. The focus of the foundation is on the needs of women in low-resource settings (i.e., sub-Saharan Africa, southeast Asia). They want to identify groups of women who do not have access to contraception and identify the barriers preventing this access. What is needed are highly effective methods that are long-acting (more than 10 years). Half of women with unmet contraceptive needs in developing countries are women who do not want more children. There is an increasing demand for permanent contraception as the “youth boom” ages; the population of women aged 30–49 years is increasing. Surgical methods, however, are provider-dependent and pose risks. There are shortcomings in the nonsurgical methods currently available, and many of these methods are invasive. There is a burden on health systems and a burden on women (i.e., procedural risks, time investment). We want to develop highly effective methods that are long-lasting, with minimal touchpoints required prior to menopause. Improved side-effect profiles are also desirable.

The Use of Permanent Contraception and Unmet Need: Carolyn Westhoff, MD, MSc

Completely in accord with the title and spirit of this meeting, Planned Parenthood Federation of American has already adopted the term “permanent contraception” and eliminated “sterilization” from its national manual of medical standards and guidelines. Global unmet need for contraception is primarily concentrated in sub-Saharan Africa and Southeast Asia, but the issues around permanent contraception are relevant to United States (US) women and men as well. Referencing a book by Ian Dowbiggin (The Sterilization Movement and Global Fertility in the Twentieth Century), Dr Westhoff described that sterilization programs were initially motivated by eugenic values. Until the mid-twentieth century, population control in Europe and elsewhere involved coercion and little consent. After World War II, compulsory sterilization was reevaluated, and found to be morally unacceptable. Voluntarily sterilization began to gain acceptance and popularity in the 1960s–1970s. There are now substantial consent requirements, waiting periods, and paperwork. In the 1970s a national consent form and consent process was created; this form is intended for women using public funds; however, several states require the same consent process for all women seeking sterilization.

In US hospitals, 31–48% of women desiring postpartum tubal ligation do not receive the procedure. That is, nearly half of women who go through counseling and sign the appropriate papers still don’t undergo the procedure they want; particularly if they deliver vaginally (80% of women who want tubal ligation after vaginal delivery do not get it). Nighttime and weekend delivery also decreases a
woman’s chances of accomplishing the procedure... Women undergoing cesarean delivery are most likely to have the procedure they asked for. Half of births in the US are paid for by Medicaid; many of these women don’t have access to interval procedures, as their insurance expires after 60 days. One reason for the elaborate consent process is to limit post-procedure regret - Young age is most predictive of regret after sterilization.

From 1970 to 1979, most sterilizations performed in the US were inpatient procedures; the annual number of procedures performed increased from 200 000 to 700 000 during these years. Since 1980, the advent of laparoscopy has caused outpatient procedures to increase and inpatient procedures have almost halved, but the total number has remained similar (702 000 in 197; 643 000 in 2006). We lack robust national data to accurately measure outpatient procedures, particularly cases performed in outpatient surgery centers, but we do have data for 2006: there were 351 000 inpatient procedures and 292 000 inpatient procedures. No good national data enumerate and describe what types of laparoscopic procedures are being performed. Surveys are still limited for office-based permanent contraception procedures. Essure was approved in 2002 and has sold 750 000 devices worldwide since that time. It is regarded as an easier procedure than laparoscopy, both from the medical and the patient perspective, and is becoming increasingly popular. However, it has lower efficacy than laparoscopic tubal procedures.

Half of reproductive-aged women rely on permanent methods- either tubal procedures or vasectomy; the rate of tubal procedures per 1000 unsterilized women per year varies between 14.9 and 12.2. The U.S. rates vary according to race, ethnicity and geography, with higher rates of permanent contraception among Hispanic and African American women. This disparity has raised the question of possible coercion or perhaps limited access to alternative methods. White males are much more likely to have vasectomies than men of other ethnicities. The South has higher rates of permanent contraception, while the West has the lowest.

We know that tubal ligation confers a lower risk of ovarian cancer. The mechanism for this is unknown; one theory is that carcinogenesis begins in the tube and spreads to the ovary. The Society of Gynecologic Oncology (SGO) recommends considering salpingectomy at the time of hysterectomy or other pelvic surgery and in lieu of tubal ligation; an area of active research at present. SGO recommends that pathologists look at representative sections of the tubes, any suspicious lesions, and the fimbriae in their entirety.

Finally, a look at the use of permanent contraception in developing regions. In Latin America and the Caribbean 28% of married women aged 15–49 years are not using contraception and 30% use permanent contraception. In sub-Saharan Africa, 77% of women are not using contraception and 2% use permanent contraception. In Asia, excluding China, 44% of women do not use contraception and 20% use permanent methods. These figures indicate that higher rates of female permanent contraception (at the population level) are associated with lower levels of unmet need.
SESSION 2: Lessons from the Ongoing Quinacrine Experience

A brief history of Quinacrine sterilization: Jack Lippes, MD

The stimulus for quinacrine sterilization (QS) was the use of quinacrine effectively treating pleural effusions. When the effusion is drained from the chest cavity and replaced by quinacrine, the instilled quinacrine stimulates production of fibrous tissue causing the visceral pleura and the parietal pleura to adhere. This prevents a reoccurrence of a pleural effusion. Dr. Jaime Zipper suspected that when quinacrine is placed in the uterine cavity, a similar effect might ensue and close the lumen of human oviduct (fallopian tube). This turned out to be true.

Dr. Zipper’s initial approach was to instill a slurry of quinacrine. The 1500-mg slurry was discovered to have significant toxicity. This technique was subsequently refined to deliver seven slowly dissolving pellets of quinacrine into the uterus. Each pellet contained 36 mg of quinacrine, for a total of 252 mg, which was placed at the fundus of the uterine cavity. Generally, two administrations are required. The first quinacrine administration is performed after menses and the second about 28 days later. A 2-mm scar results in the isthmic portion of the tube. It is not known but suspected that patient positioning after insertion of the pellets might improve results. For example, placing the patient in the Trendelenburg position with hips elevated by two pillows might result in quinacrine following gravity, thus being maintained at the uterine fundus, closer to the tubal ostia.

The history of quinacrine is long. Over a 68-year period, more than 100 million people have taken quinacrine to prevent and treat malaria. Many took quinacrine on a daily basis for as long as 10 years and at 100 mg a day; this totals an exposure of 365 000 mg. Compare this exposure to the total of 504 mg of quinacrine placed in the uterus in split doses of 252 mg for QS. During World War II, some 3 to 4 million allied soldiers serving in the south Pacific took quinacrine daily for malaria prophylaxis. In the estimated 175 000 women worldwide who have undergone QS, there has been no increase in the ectopic rate, no deaths, and no adverse events requiring hospitalization or surgery.

Among the advantages of quinacrine as a sterilizing agent is its simplicity. The fact that with QS there is no need for general anesthesia and there is no need for the presence of a physician. Indeed, we know that nurses and nurse practitioners have performed many of the QS procedures. Another advantage is that oviductal scar can be seen on ultrasound. Sonographic examinations of the uterus have been done and the height of the endometrium is evaluated for possible hyperplasia/neoplasia. Dr. Claudia Ferreria in Belo Horizonte, Brazil, provided QS to 624 women. Each patient was sonographically examined after QS. The endometrium appeared normal within two weeks to one month of QS placement. This rapid endometrial recovery reveals that quinacrine does not produce chronic inflammation.

Human immunodeficiency virus (HIV)-positive women have a strong motivation to prevent pregnancy. Women do not want to chance transmitting HIV to their offspring. Surgeons may be reluctant to perform tubal ligations in these women, fearing they may be stuck inadvertently with a needle and acquire HIV. In the same study in Brazil where 624 patients received QS, 92 of the 624 were HIV-positive. A total of 15 pregnancies occurred in this series (2.4%), but no pregnancies occurred in the HIV-positive women.

There is still much we do not know about QS. Is there an adjuvant that will increase efficacy, such as a muscle relaxant that by dilating the smooth muscles of the oviduct will allow more quinacrine to enter the lumen of the oviduct? Is a single dose of quinacrine (with an adjuvant) sufficient to sclerose the oviduct? When is it advisable to instill a third dose? Will nonsteroidal anti-inflammatory drugs inhibit the epithelial reaction, preventing sclerosis? What position should the patient assume after QS to attain optimum efficacy? What is the role of ultrasound? Can sterilization with quinacrine be reversed? These
questions would undoubtedly need to be answered for any compound instilled into the uterine cavity to provide permanent contraception.

The efficacy of QS is expected to improve over time. This has been the pattern with many new medical technologies. For example, there have been steady improvements in laparoscopic sterilization. We have advanced from single-burn techniques, to rings and clips, to multiple-burn techniques. When the original bipolar single burn was replaced with multiple oviductal burns, the failure rate dropped from 54.3 per 1000 to 3.2 per 1000 procedures.

In Vietnam, where 50,000 QS cases were performed, the cost of the drug plus the inserters was US$ 1.06. Today, in Asia, an office visit might cost one dollar. It would be $125 in the United States (US). A laparoscopic tubal ligation in the US costs upward of $4000, including general anesthesia, and hospital and surgical fees. Essure, another outpatient permanent sterilization technique, costs $950 for two devices (one for each tube). But with the usual hysterosalpingogram (HSG) plus office visits, the Essure method may cost $1844.

Of all the studies about QS, the most important is epidemiologic. Such epidemiologic data has been accumulated and analyzed by the Degge Group, under the supervision of Dr. Judith Jones. This was a retrospective cohort analysis in Vietnam. A total of 10,503 women who had chosen QS were compared with a cohort of 9203 women who had used an intrauterine device (IUD). In this analysis, twelve cancers were found in the QS patients and eight were found in the matched cohorts. Considering this data and using the Fisher exact test, $p = 0.6562$; using Chi square, $p = 0.7063$; confidence limits are 0.49 to 3.71; the odds ratio is 1.3. The difference between the QS group and the IUD cohort is not statistically significant. Therefore, the conclusion from the data of the epidemiologic study is that quinacrine sterilization is not associated with an increased risk of cancer. When we are in possession of both animal and human data, the animal data becomes superfluous.

We know so much about QS. The cost is low. There is no need for anesthesia. Acceptability is high. Scars are visible on ultrasound. QS can be performed by nonphysicians. Why can’t well-informed women have QS as an option?

**Quinacrine Sterilization: opportunities for improvement (the ongoing quinacrine experience):**
Steve Mumford, DrPH

The transcervical quinacrine slurry was studied beginning in 1969, but it was abandoned because of toxicity. A 1977 study proved that quinacrine pellets could be used to achieve tubal occlusion. International clinical trials were instituted in the 1980s, and a 1993 Lancet publication demonstrated both safety and efficacy in more than 30,000 women. A phase 1 study in the US, begun in 2000, was completed and published in 2003. The International Services Assistance Fund (ISAF) began planning for phase 3 trials, and their application was accepted by the Food and Drug Administration (FDA) in 2006. However, a clinical hold was placed in 2007, two days before product was set to ship to the 36 participating institutions. The FDA rationale for the clinical hold was a two-year study, conducted by another sponsor, showing carcinogenesis in rats. To consider lifting the hold, the FDA required a large epidemiology study showing no association between QS and reproductive-tract cancers. ISAF contracted a large, international, retrospective study of women who had undergone QS in Vietnam, China, and Chile.
In 2008, the World Health Organization (WHO) conducted a technical consultation on QS safety, specifically evaluating the results of the rat study. This consultation raised the following questions: Was this carcinogenesis caused by chronic inflammation leading to tumor formation, a direct effect of quinacrine, or a combination? What was the mechanism of carcinogenesis in these rats? Is this mode of action relevant to women?

ISAF enlisted the help of experts to assess and evaluate the rat findings. These experts included a veterinarian pathologist, toxicologist, genetic toxicologist, immunotoxicologist, biostatistician, and reproductive toxicologist.

The rat study used two dose administrations, 21 days apart; there were six dose groups and the observation period was two years. 10/10 mg/kg was identified as the highest acceptable dose, but up to 70/350 mg/kg was used in the rat study. There were no true controls used, although two groups received a methylcellulose formulation. Of the rats in the two highest-dose groups, 30% died soon after quinacrine administration. The control groups had 2% tumor incidence, with chronic inflammation seen in 20%. The two highest-dose groups had 29% tumor incidence and 56% chronic inflammation. The 10/10-mg group had 6% tumor incidence, with chronic inflammation in 18%. The 70/70 mg/kg group had 18% and 62%, respectively. A highly significant correlation \((p = .002)\) was seen between the presence of chronic inflammation and malignancy in the treatment groups. These toxic effects were NOT seen in human women treated with QS.

Twenty-five percent of all human malignancies result from chronic inflammation, and this association is well documented in rodents as well. Chronic inflammation in the reproductive tract is rarely seen in two-year rat studies, so why was there carcinogenesis in this study? The necropsy data showed nonrepairable endometrial destruction with subsequent chronic inflammation, cytokine production with accompanying oxidative stress, and tumor initiation and progression. In short, the rats were not able to recover from the toxic effects of excessively high doses of quinacrine. A major shortcoming of the rat study was that the concept of the maximum tolerated dose (MTD) was not used. Instead, a nonstandard concept, the maximum feasible dose, was employed—this concept is only supposed to be used when the MTD is not attainable. Data were available from a prechronic study; the MTD could therefore have been determined but was not. In addition, the rats were treated with Quinacrine slurry rather than pellets, although it was already known that the slurry was unacceptably toxic in human women. Methylcellulose, a known tissue irritant, was used as the control. The quinacrine doses produced tumors that are never seen in women. The rat uteri had chronic inflammation and necrosis present after two years, while, at the prescribed dose, human uteri are known to heal within two weeks to one month of quinacrine administration. The correlation between chronic inflammation and malignancy in the rat study was not statistically analyzed by the study’s sponsor, Family Health International 360 (FHI 360). Quinacrine was not carcinogenic at the 10-mg dose, a dose that did not produce chronic inflammation; thus, inflammation appears to be the carcinogenic factor, not the medication itself. Chronic inflammation is not seen in women receiving QS.

What do we see in women? The FDA required an epidemiology study to show just this. Many existing studies exist, but all were too small to produce definitive results. A retrospective study of women in Vietnam, Chile, and China was conducted. Safety was evaluated in 10 503 Vietnamese women who underwent QS and were compared with IUD receivers, with an average follow-up time of 16 years. The malignancy rate was 0.07 per 1000 women for QS and 0.05 per 1000 for the IUD. X-ray exposure
and age at procedure were associated with reproductive-tract malignancy, but the QS procedure was not ($p = 0.492$). The efficacy of QS was evaluated in China, where 3372 QS users were compared with 3466 women who underwent tubal ligation. The QS failure rate was 0.7%, equivalent to tubal ligation, in the clinics with the best-trained and most experienced clinicians. The overall efficacy of QS in the China cohort was 97.9%, with an average follow-up period of 8.1 years.

The findings in the rat study are not relevant to the human experience. The efficacy of QS is a function of training and repetition and is comparable to that of surgical methods. Quinacrine’s mechanism of action in humans is unique to our species. The procedure is safe, effective, affordable, and accessible. There are rare allergic reactions but no other human safety issues in 175 000 cases. Furthermore, the medication is not carcinogenic in rats if the MTD is not exceeded. The efficacy is acceptable, but needs to be (and could be) improved through solid clinician training.

**Discussion:** moderator, David Sokal, MD

*Why did FHI 360 halt its research into QS?* FHI 360 had planned to make a “Go-No Go” decision after receiving the results of the rat study and reviewing the overall advantages and disadvantages of the quinacrine method. Based partly on the expected difficulty of addressing the results of the rat study, but also on the relatively low effectiveness of the quinacrine method, FHI 360 decided that it would be more productive to shift its focus to other methods of long-term or permanent contraception.

*What other data does the FDA have on quinacrine?* The FDA did receive data from a one-year neonatal mouse study that used a dose over the MTD, but no increase in malignancies was found. Quinacrine does not appear to be genotoxic in the neonatal mouse.

*Where does quinacrine research currently stand?* The pellets are not in circulation, as production was stopped when the FDA placed the clinical hold on phase 3 trials. There is demand for pellets, but they are not currently being produced and the original supply has run out.

In its current form, QS requires more than 1 dose, and the success rate appears to be training/provider dependent. A lot was learned from the Vietnamese study, and the failure rate was actually reduced by two-thirds with fundal placement of the pellets. In China, the best efficacy was seen in the clinic that had the best-trained clinicians. Both training and pellet placement are key. There have been no studies using a lower dose since the importance of fundal placement was realized, but perhaps 100 mg is sufficient (as opposed to the 250 mg dose used over 2 applications). Large studies are needed. Treatment failures are infrequent, but there is no answer yet as to why they occur. If this is answered, perhaps the technique can be modified to reduce the failure rate.

The previous human studies were done in Vietnam and China, where patients are more “trackable” than in the US—patients don’t move around as much, and the health officials know the patients and their histories. This helps reassure us that the cancer rates were not higher than reported due to patient death preventing follow-up.

There were previous experiments using an IUD designed with Quinacrine pledgets at the ends; unfortunately, there was no improvement in tubal closure rates as the quinacrine concentrations were
not high enough. Quinacrine needs to be quickly released, but not too quickly. The 250-mg pellets are designed for 30-minute release; the release from the IUD pledgets was too slow. If release is too quick, however, as in the 1500-mg slurry dose, full-thickness ulceration of the uterine wall occurs. Other toxicities (unspecified) also contributed to the demise of the quinacrine slurry.

What role does chronic inflammation play in the rat-study results? In the rat control groups, nondegradable methylcellulose (which would not ordinarily cause chronic inflammation in the gut or on the skin), was trapped, causing a chronic stimulus. However, stimulating material does not need to remain in situ for chronic inflammation to occur. When significant tissue damage is not repaired, chronic inflammation is stimulated. The inflammation from quinacrine, occurring in one or both horns of the rat uterus, results not from uterine fibrosis but from the fact that the epithelial cells are denuded and only the stem cells remain, leaving no opportunity for uterine repair.

What do we see in rats exposed to quinacrine? The rat neoplasms were of stem-cell origin rather than epithelial origin. In the animals that died early, the quinacrine caused full-thickness ulceration of the uterus—it ate through the uterine wall and quinacrine was found in the animals’ abdominal cavities.

What other sclerosing agents might be used for tubal sterilization? This discussion was deferred to the upcoming session on the mechanism of tubal sclerosis and fibrosis. It was noted that the discovery of Quinacrine’s efficacy as a tubal sclerosing agent was serendipitous. Other compounds, such as talc, were tried, but quinacrine was the only one that worked in the uterine cavity. It is not known whether other antimalarials, such as chloroquine, produce sclerosis in the uterine tubes.

Is quinacrine too controversial to move forward with? Future research on any sclerosing agent must be done in the proper order. The QS investigator team feels that the human safety data strongly support additional trials in women in the USA. There is also a possibility of understanding ways to optimize delivery to make a single treatment approach feasible.
SESSION 3: Polidocanol Foam Sclerotherapy

Polidocanol foam for permanent contraception: Initial results from nonhuman primate studies: Jeff Jensen, MD, MPH

We believe that existing chemical entities can be adapted to create highly effective and safe agents for nonsurgical female permanent contraception. Careful preclinical evaluation and program development are needed to demonstrate the feasibility of human studies. The usual pattern of product development and subsequent clinical trials is typically geared toward United States (US)/European Union (EU) use. The development of new methods of permanent contraception should follow this careful path, defining the clinical applications and avoiding the problems associated with product introduction in low-resource settings prior to rigorous safety and efficacy trials.

Common sclerosing agents for cosmetic use (e.g., for varicose veins), such as sodium tetradecyl sulfate and Polidocanol, have low complication rates. Polidocanol is a synthetic long-chain fatty acid. Injection is painless, and intradermal use does not result in necrosis. It has a high safety index in other species. It is approved in the US as a solution (0.5%, 1.5%) and as 1% foam for intravenous injection. The fatty acid forms small micelles that interact with lipid membranes, causing disruption of those membranes. Experiments using liquid polidocanol for intrauterine administration were not successful, but polidocanol foam (PF) bubbles are more reactive, as the bubbles displace water and have more contact with lipid membranes, creating a broader area of tissue damage. A small hysterosalpingogram (HSG) catheter (less than 3 mm) is used to enter the uterine cavity, but placement at the fundus is not required. The catheter balloon is inflated past the internal os, and the medication is mixed with saline to generate foam that fills the uterine cavity and exits through the tubes. The technique is thought to be safe for nonclinicians or lower-level providers to perform, as the skill level needed is similar to that required for intrauterine device (IUD) placement.

The intratubal use of PF has been studied in rhesus macaques after confirming tubal patency with laparoscopy. Control monkeys had no treatment; 5% PF and (inert) methylcellulose foam were administered to separate treatment groups, and the animals were reassessed after 30 days. The treatment was repeated if the tubes were still patent. The reproductive tracts were removed for histologic evaluation. All untreated and methylcellulose-treated animals showed normal tubal histology. The PF group needed two or three treatments to achieve complete occlusion, which eventually occurred in four out of five subjects. The occluded tubes showed hyalinization and loss of epithelium on pathology. However, 67% of animals showed some evidence of tubal de-epithelialization and occlusion at one to two weeks after a single treatment. There was no evidence of long-term damage to the vagina, cervix, or endometrium. There were no adhesions in the peritoneal cavity, even though the foam was seen on laparoscopy to be bubbling out of the tubes. The damage-and-repair effect seen in the tubes is likely central to all sclerosing methods. This understanding is key for research moving forward.

The rhesus macaque has a colliculum in the cervical canal, making uterine-cavity entry through the internal os challenging. The baboon cervix is straight, and therefore much easier to cannulate, so work was transferred to this model. Studies were conducted with 1%, 3%, and 5% PF concentrations. HSG, rather than chromopertubation, was performed to assess tubal patency. The medication was administered with ultrasound guidance rather than laparoscopic guidance. Animals were reassessed at 30–60 days. All controls had normal tubal histology. PF alone, as a single treatment, appeared to cause abnormal histology in many animals but did not appear to have a permanent occlusive effect. Notably,
the addition of intramuscular depo medroxyprogesterone acetate (DMPA) resulted in 100% tubal occlusion in the 5% PF group after a single treatment, with de-epithelialization and hyalinization of the intramural portion of the tube highly suggestive of a permanent effect. This was not observed with DMPA alone or in the untreated group. The 1% PF group with DMPA showed histologic changes at two months suggestive of damage, but the tubal lumen was slightly preserved, suggesting that re-proliferation and repair was underway. To evaluate the effect of hypogonadism alone (without a progestin), depo leuprolide was coadministered to some PF groups. The effect was not as robust as that seen with DMPA, with total occlusion seen in only 33% of subjects. It is possible that the longer duration of action, or a direct effect on the progesterone receptor, seen with DMPA inhibits re-epithelialization more effectively than hypogonadism alone.

In another set of experiments, doxycycline 100 mg was given alone and as a cosclerosant with 3% PF. Abnormal histology was seen in one of three animals in the former group and two of two animals in the latter group. No animals experienced complete occlusion of the fallopian tube.

With all sclerosing methods, the key going forward is to figure out how to favor scar formation and discourage recanalization and re-epithelialization of the tube. Given that it is hard to draw conclusions with these small numbers, transcervical PF does seem capable of causing tubal epithelial damage in the nonhuman primate model. The effect is dose-dependent, with 5% PF superior to lower doses. Coadministration of PF and DMPA appears to favor occlusion and scar formation over re-proliferation; a single 5% PF treatment followed by DMPA resulted in bilateral occlusion in five of five animals. GnRH agonists also appear to have an occlusion-promoting effect, but not to the extent seen with DMPA. The use of cosclerosants such as doxycycline needs more study. More research is also needed on the optimum concentration of PF, length of tissue exposure, toxicity, duration of effect, other cosclerosants, and the ideal delivery system. An improved delivery system would need to be usable in low-resource settings and should improve safety by reducing potential vascular uptake. One example of a modification that could reduce endometrial vascular uptake would be a more occlusive balloon that covers most of the endometrial surface while allowing delivery of the sclerosant to the cornual region. This would reduce the dose of PF needed by providing targeted delivery.

A baboon contraception experiment is currently in progress. Both PF-treated (5% and 3%) and untreated control females are being socially housed with a fertile male. Preliminary results are expected by autumn of 2014.

**Optimization strategies for intrauterine delivery of foam (Characteristics of polidocanol foam for nonsurgical permanent female contraception):** Jian Guo, Ph.D.

In order to develop a simple, safe, low-cost PF-delivery system, we need to identify the ideal foam characteristics for optimum delivery and maximum sclerosing effect. Early data show some ways to achieve this through maximizing foam stability and uniformity.

The polidocanol molecule has a both a hydrophobic and a hydrophilic chain. It is a nonionic surface active agent (surfactant) that can reduce water surface tension. As surfactant concentrations increase in water, a critical micelle concentration (CMC) is reached at which micelles spontaneously

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form and interact with cell membranes, causing damage. Dispersion of gas into a surfactant solution produces foam. Surfactants with lower CMCs tend to foam easily. PF foam has a low density and a large surface area. The foam is compressible, flowable, shapable (adopting the shape of its container), elastic, and behaves like a gas, liquid, and sometimes like a solid. The advantages of using PF over polidocanol liquid include a larger contact surface area, a longer surface contact time, a lower dosage required, and enhanced interactions with cells.

The anatomy of the fallopian tube is different from that of the veins in which PF is known to be effective, an important factor to be considered in tubal foam sclerotherapy. The diameter of the fallopian tube falls between that of spider- and varicose veins. In addition, the surface area of the tube is much larger given its convoluted surface, and the various portions of the tube demonstrate varying anatomy. Finally, the tubes are filled with mucus rather than blood, providing a very different environment in which the foam needs to work.

Potential issues to investigate are the effects of foam stability, uniformity, and bubble size on PF efficacy; embolism potential; cell stress in the nontarget area; and the inherent properties of the gas used to generate the foam. Foam stability is key. Lowering surface tension, increasing viscosity, and improving the uniformity of bubble size and shape will all provide better foam stability.

Different filters for the foam–delivery system yield different bubble sizes and bubble stability. Filters with 5-µm circular pores generate more uniform and stable foam compared with irregular filter holes. When coadministered with PF, benzalkonium chloride (a cationic surface active agent with a hydrophobic tail and hydrophilic head) has a desirable effect on bubble size and stability. A stabilizing agent may therefore play a key role in optimum foam administration.

The preliminary data show that inexpensive filters improve the uniformity and stability of PF bubbles. Pore shape and filter size affect foam characteristics. Further preclinical research should be conducted to investigate the effects of filter hydrophilicity/hydrophobicity, the synergic effects of other sclerosants, whether pretreatment of the tube is necessary, and the optimal gas (O2 vs. CO2) to be used in order to develop a simple, safe, low-cost, and effective formulation for in vivo testing.

Perceptions of nonsurgical permanent contraception among potential users and clinicians in Oregon and India: Jennifer Aengst, PhD

Data from an ongoing study were discussed; data analysis is 50% complete.

Globally, permanent techniques account for the majority of contraception use. There are many new methods in development, but little understanding of how women and men feel about these methods. Qualitative research was conducted in Portland, OR and Sevagram, India (central India), its surrounding villages, and in Delhi. The research included semistructured interviews, conducted in both high- and low-resource settings and in different medical contexts. Subjects included married or partnered women (aged 18–45 years), mothers-in-law, married or partnered men, obstetrics and gynecology providers, and health advocates. The women interviewed in India were younger than those in Oregon. The interviews attempted to elucidate the perceptions of permanent contraception and
surgery, the level of interest in nonsurgical permanent contraception, the parameters of contraceptive
decision-making, and participants’ responses to new technology. Of particular interest were the
influence of family members (e.g., mothers-in-law) and societal gender norms. Another area of interest
was how opinions on coercion, choice, and rights influence attitudes. There was interest in assessing
what providers and women think about bleeding and other side effects and in determining their
definition of surgery.

Historical and political factors come into play. In India, there are government incentives for
permanent contraception, although vasectomy is more highly incentivized than female sterilization. In
India, “sterilization” is the typical term for female permanent contraception, often simply referred to
simply as “the operation.”

Contraceptive decision making is shaped by previous method use. In India, there is a strong
influence of family dynamics, with conflict avoidance a key concept. There were different attitudes
toward surgery, with both fear of anesthesia and reassurance by the use of anesthesia expressed.
Participants’ attitude toward bleeding depended on their previous contraceptive use, their definition of
spotting vs. bleeding, and their tolerance of bleeding.

In both Portland and India, there was general support for both concepts; permanent
contraception in general and nonsurgical permanent contraception in particular. However, this support
depended on a number of factors, such as the participant’s age and number of children. The concepts of
certainty and regret were also raised. More women in Oregon felt that it was their partner’s “turn” to
accept responsibility for contraception after childbearing was complete. In both settings, participants
were interested in new methods, but took a “wait and see” approach as they had concerns about safety,
efficacy, and side effects of new technology. Gendered social norms also came into play (sex
preferences for children, men’s attitudes toward vasectomy).

The key themes identified were trust (in doctors, method, presence of confirmation test); risk
(health, social/reputation/stigma, later regret); and ethical concerns (easy availability of a method might
generate the potential for misuse, coercion is possible). Both patients and physicians in both countries
want doctors only to administer these methods. Indian physicians were more open to other providers
eventually performing these procedures.

Future research is needed on the effect of religion on attitudes toward “cutting,” surgery, and
sterilization; exploring the difference between spotting and bleeding in patient perception; and how
disparities in education and rural/urban distinctions affect attitudes.

Discussion: moderator, Diana Blithe, PhD

Is there a difference between the use of sclerosing agents for blockade of veins and for tubal
occlusion? Vascular surgeons are able to sclerose veins more effectively than we are able to sclerose the
fallopian tubes because of the anatomy of the respective structures. In addition, vascular surgeons can
occlude the distal end of the tube and restrict flow, and can do repeated treatments with small amounts
of material (3–10 mL). There are different potential mechanisms of action for vessel- versus fallopian
tube sclerosis. Vessel epithelial cells have preformed granules with proinflammatory components readily
available for release. It is possible that a higher medication concentration is needed in the tube, as it
lacks these preformed granules. Furthermore, damage is probably needed at both the epithelial and subepithelial layers of the tube for a fibrosis response to occur. The injury inflicted needs to be adequate to obtain that response—this is harder to achieve in the tube than in blood vessels.

**How much foam is used?** Regardless of the foam concentration, the goal is to completely fill the tube with medication. The limiting factor for safety issues is the amount of medication that enters the vascular space, not the amount in the uterine or abdominal cavities. The maximum dose administered in historical studies with monkeys is up to 80 mL of foam in a single treatment; in current studies with baboons, we are limiting foam to 20 mL. The maximum Food and Drug Administration (FDA)-approved dose for vein occlusion (Varithena®) is 18 mL per treatment, but this is only a 1% foam.

**What is the stability of PF?** PF foam is fairly stable and does not require refrigeration. With more stable bubbles, foam stays active in the intramural portion of the tube for longer periods of time. There is better safety with small bubbles, as they dissipate faster if absorbed into the intravascular space. It is possible that smaller bubbles can deliver more medication for the same dose because of their increased surface area compared with larger bubbles.

**What is the site of action in the tube?** Similar to quinacrine, the tubal damage and occlusion caused by PF is limited to the intramural portion of the tube. There is an absence of histologic findings in other portions of the tube after PF administration. This could be related to the complexity of tube, as it is more convoluted in its distal portions. In addition, the natural compression of the intramural portion of the tube by the myometrium may be a factor.

**How does DMPA increase the success of PF treatment?** There is a dramatic effect of DMPA on the success of PF. We need to study how the long-term effect of progesterone may “shut down” the tubal repair mechanisms, allowing damage. There is hormonal regulation of tubal epithelial proliferation, and the effects of the absence of progesterone withdrawal need to be investigated, as do possible apoptotic effects. DMPA’s effect of thickening cervical mucus may also come into play, if a similar action occurs with tubal fluid. As a benefit to women, DMPA also provides bridge contraception while tubal occlusion is developing.

Dr. Sitruk-Ware suggested caution with the development of an approach that would be a combination product, as this would complicate the regulatory approval process. However, the use of DMPA could be entirely within labeling as a contraceptive and would not therefore require separate approval. Doxycycline is approved, but its use for tubal occlusion and its coadministration with PF would require additional study.

**Have other sclerosing agents or hypothermia been studied?** We have not yet looked into hypothermia activation and/or the use of mitomycin C as a sclerosing agent, both of which are used in the treatment of bladder cancer. The fact that other agents exist demonstrates the potential for future research. The potential of methylcyanoacrylate to create tubal occlusion was investigated more than 30 years ago, but these studies were stopped. The delivery system was an issue, as adhesion occurred rapidly, but perhaps better delivery could be achieved now; there is no current research into this topic. It was pointed out that methylcyanoacrylate has been shown to be genotoxic.

**What are the perceptions of an incision-free method of PC?** In the acceptability study, women in Portland were not terribly concerned with privacy (from their spouse and family) regarding healthcare
choices, stating that “I make my own decisions.” However, small subpopulations (Latinas, Catholics) did say that privacy was an issue. Indian women were more concerned about conflict in the family. Even when pressed, they were not as clear on yes/no answers regarding privacy issues, deferring instead to family conflict issues. Women living in their husband’s household were very concerned with not disrupting family relationships.

_Females in the polidocanol foam (PF) contraception study have received depo medroxyprogesterone acetate (DMPA). Could this affect the results of the contraception study?_ Male baboons use both visual (sex-skin color) and olfactory cues (the latter being critical) to time copulation. The concern was raised that the use of DMPA would impact effectiveness trials, as the medication makes female baboons look sex-skin pregnant and males might not mate with them. In any contraception study, it is important to ensure that mating behavior is not affected and that animals are of proven fertility. DMPA provides about three months of reversible contraceptive activity. At therapeutic levels, DMPA causes ovarian- and sex-skin cycle suppression. While mating will generally not occur during DMPA suppression, once ovarian activity resumes sex-skin changes also return to normal along with normal mating behavior. It is fairly straightforward for experienced animal care technicians to observe sex-skin changes and the presence of a semen plug as evidence of mating.
SESSION 4: Evaluation of Tubal Patency and Verification of Occlusion

Nonsurgical tubal occlusion in rabbits and women: Amy Thurmond, MD

Transcervical access to the fallopian tubes has intrigued clinicians for 160 years—both for tubal sterilization and treatment of proximal occlusion. In the 1970s and 1980s, there were experiments with Ovabloc, a hysteroscopically inserted silicone plug that never made it to the marketplace.

The rabbit model is theoretically ideal for testing contraceptive methods, as rabbits are reflex ovulators (ovulating upon coitus). However, they have a small, bony introitus; the vagina is big and connected to the bladder; and there is a tiny double-cervix. The use of hysteroscopy for cannulating the cervix, combined with fluoroscopy for guiding cannulation of the fallopian tube, has made rabbit transcervical contraceptive research much easier.

Many agents have been investigated for tubal occlusion. Ethanol, used as a sclerosant, causes occlusion on day 2, but the tubes recanalize by day 30. Ethobloc, an ethanol sodium amidotrizoate sclerosant more viscous than ethanol, causes occlusion by day 2 that persists to day 30 but also results in a horrible inflammatory reaction. Collagen glue appears promising on day 2 but by day 30 has ended up in the adjacent vessels and lymphatics. Hydrogel, a polymer liquid that behaves like a solid, shows occlusion on both day 2 and day 30; however, long-term tubal inflammation and foreign-body reactions occur. An inert metal coil has a 100% contraception rate when it stays in place, but expulsion is a problem. This latter method has since been modified into the current Essure design by adding Dacron fibers to the outside of the coil. In 2003, the Food and Drug Administration (FDA) approved the device for hysteroscopic placement, but it is also possible to place Essure under fluoroscopic guidance.

Dr. Thurmond expressed doubt that the pressure from hysterosalpingography (HSG) can “blow out” the scar from QS. Even with catheter probing, she has not been able to open the fibrotic, gritty tubal scar.

Fallopian tube: the challenges in imaging and determining patency: Amy Thurmond, MD

The first HSG was performed in 1910 and continues to be a common test to this day. In 1920, gas insufflation was first used to determine tubal patency; unfortunately, some patients died, presumably from intravasation and air embolism.

Apparent blockage of a tube by HSG or insufflation, when the tube is subsequently determined to be patent, has been long recognized and attributed to “spasm” in the intramural portion of the tube. Clinicians have tried antispasmodics: glucagon, terbutaline, verapamil, and intracavitary lidocaine, without reliable success. Dr. Thurmond has found that turning the patient over into the prone position works best—whether this simply a factor of time or of positioning is unclear.

When contemplating replacing HSG with a potentially more accessible and affordable method of evaluating the fallopian tubes, one must consider the risks of not having accurate images of the tube. For example, if there is a fallopian tube that is patent but dilated from distal peritubal adhesions, pelvic inflammatory disease (PID) can result when stagnant tubal contents are “blown out” into the peritoneal...
cavity. This will be visible at the time of HSG and can be prophylactically treated with antibiotics but would not be apparent if insufflation or ultrasound were being used for diagnosis. Similarly, intravasation of material into the peritubal veins and lymphatics would be recognized at the time of HSG, and the injection halted, but would likely not be recognized if insufflation or ultrasound were being used. Data indicate that venous intravasation occurs about 5% of the time, even in anatomically normal women.

Ultrasound is unfortunately unreliable at visualizing the fallopian tube. It is difficult to see extraperitoneal structures on transabdominal ultrasound, and even more difficult with transvaginal ultrasound. Echogenic bowel gas can confuse the picture.

**Imaging the tube with ultrasound during foam sclerotherapy in the baboon model:** Cassondra Bauer, MS, DVM

In order to conduct transcervical tubal sclerotherapy in baboons, the animals are sedated with ketamine, and then isoflurane is given via face mask. Baseline blood work and uterine ultrasounds are performed. The procedure room for baboons requires both abdominal ultrasound and digital x-ray equipment. While fluoroscopy would be ideal, digital films provide a low-cost alternative with HSG. A series of films is performed after some aliquots of contrast are administered to confirm tubal patency, then foam is administered under transabdominal ultrasound guidance. Since the small foam bubbles are highly echogenic, it is possible to monitor the flow of foam during treatment. The goal is to see flow of foam from the uterine cavity into both tubes on ultrasonography. Here, Dr. Bauer presented a number of ultrasound images showing the flow of foam through the uterine cavity and tubes.

Ultrasound also has demonstrated that foam can enter the subendometrial blood vessels in some animals. This can result in serious adverse effects (such as air embolism) that are dose-dependent, similar to those observed with other intrauterine procedures such as hysteroscopy.

It is not always possible to see foam exiting from the tubes; it becomes more difficult to see once the amount of intrauterine foam increases. Retroflexed or abnormally positioned uteri can be challenging, but the procedure can still be completed. Having digital radiography nearby is very useful.

**Verification of tubal occlusion using uterine pressure:** Jeff Jensen, MD, MPH

According to the FDA-approved labeling on the Essure transcervical sterilization system, interval HSG is required to confirm tubal occlusion. Verification is not routinely done for other surgical permanent contraceptive techniques. The question for development of nonsurgical permanent contraceptive methods is whether verification is needed for a procedure that is greater than 99% effective? HSG requires skill, is limited in availability, and may show false occlusion depending on cycle timing and hormonal therapy. Our experience in the baboon suggests that observed tubal patency is affected by hormonal status. In humans, the HSG procedure is typically done during the follicular phase, in part to prevent exposure of an early pregnancy; therefore, less is known about the human effects of hormone exposure on tubal patency.
Uterine filling pressure is easy to measure, and a variety of low-cost monitors are available. Theoretically, occluded tubes should yield a high uterine filling pressure, while patent tubes should yield a low pressure. Baboons examined during the proliferative phase and mid-cycle typically have low pressure and a high rate of observed tubal patency. This starts to change during the luteal phase, with many animals showing high pressure and apparent tubal occlusion. Baboons given depo medroxyprogesterone acetate (DMPA) for more than 7 days demonstrate high baseline pressure and functional tubal occlusion. Lupron administration results in low pressure and high patency. To follow up on these observations, we have obtained approval for human studies to evaluate the effects of the follicular phase, luteal phase, and the administration of DMPA or oral contraceptives on tubal patency.

Our hypothesis is that uterine filling pressure should remain consistent when woman are evaluated under the same hormonal conditions (i.e., the same phase of the menstrual cycle). In other words, if a woman is evaluated in the follicular phase and has low pressure and patent tubes, this would be true in a subsequent examination at the same cycle stage. If that same woman underwent a successful tubal occlusion procedure, the uterine pressure should be increased when examined in the follicular phase. Low pressure would indicate the need for retreatment or formal HSG evaluation. It is less clear-cut what to do when a woman presents with a baseline high pressure. Post-procedure high pressure would not be useful in this situation. Indeed, low pressure after an occlusion procedure may or may not indicate that the tubes are still open, as this finding could be due to vascular uptake of the filling solution. These women would need to undergo HSG evaluation to confirm success or failure of the occlusion procedure.

Dr. Jensen proposed both a baboon and human study. In baboons, uterine pressure would be evaluated before and after placement of polidocanol foam (PF), with HSG as the gold standard for determining tubal patency. In human women, the uterine filling pressure would be assessed following Essure, with HSG examination as the gold standard. One could then determine a cutoff-pressure value above which all HSGs show occlusion. This could allow the simpler filling pressure test to be used as a first-line evaluation, potentially obviating the need for HSG in many women.

Discussion: moderator, Alison Edelman, MD, MPH

*How can PF be safely administered to the fallopian tubes?* Vascular uptake of intrauterine material is not necessarily associated with high pressure during HSG or foam administration. Baboons can show lots of vascular uptake under low pressure, and no uptake under high pressure. There is a safe range and a lethal dose for intravascular PF. The lethal dose of intravascular air is about 2 mL/kg. For delivery of intrauterine agents, technology should exclude as much of the uterine cavity as possible to minimize the risk of vascular uptake. The appropriate dose (and the maximum dose) to get foam all the way through the intramural tube needs to be determined. As the goal of foam administration is to fill the tubes, and not the uterine cavity, it is possible that only a small volume (1-2mL) is needed. We don’t know, however, whether a greater amount of foam is needed to cause adequate tubal damage. A possible target is 5–10 mL per side, a non-life threatening dose even if all was delivered into a vein. It would be advantageous to have an intrauterine catheter with a balloon that covered most of the cavity but had a small opening on each side to aid in the delivery of foam to the cornual region only. A system
that occludes most of the uterine cavity in most women will deliver the bulk of the foam to the cornual region, and make administration easier, obviating the need for specialized equipment or expertise. Biotechnology innovation is needed. Pulsatile pressure to get solution into “high pressure” tubes has also been tried, but research is needed to know whether this is a reliable technique.

**What happens to PF when it enters the vasculature?** The average foam bubble size is 60–70 microns, with smaller bubbles less likely to cause emboli. It is known that administration of CO₂ improves dissipation of the bubbles, lowering the risk of embolus, but CO₂ also makes the bubbles less stable and can compromise treatment efficacy. Moreover, air is cheap and readily available, obviating the need for gas canisters and other equipment. For a system of tubal occlusion verification, Dr. Jensen stated he would use saline rather than air or foam for administration. The risk of embolus from foam administration is dose-dependent; for this reason, there is a limit of 18 mL set on intravascular foam with the approved Varithea™ product of 1% PF in humans. We need to know if a higher concentration of intrauterine foam is safe and something we can move forward with.

**What is the risk of PID with HSG?** In anatomically normal women, there is less than a 1% risk of PID after HSG. This risk is high, however, in women with abnormal tubes; these women need prophylactic antibiotics. In developing countries, tuberculosis of the tubes is also a potential risk. Clinicians need to be prepared for potential complications if they are administering tubal-filling agents to women whose tubes have not previously been visualized. All baboons in these studies received prophylactic antibiotics. Dr. Jensen noted that since most women in the target high fertility regions will likely access permanent contraception after proven fertility, the risk of undiagnosed pelvic infection is likely lower than that observed in a population of women undergoing HSG for an infertility work-up.

**How does DMPA interact with the findings at HSG and in improving the results with PF?** DMPA has a glucocorticoid-like effect, increasing vascular permeability and water retention. This could result in the high uterine filling pressure seen in DMPA-treated baboons, but this effect might not necessarily be an issue with all progestins, so that needs to be tested. In an earlier session, it was stated that co-administering DMPA with PF leads to higher tubal-occlusion success rates. During this session, it was noted that giving DMPA more than 7 days prior to measuring filling pressure leads to high readings. The protocol is now to give DMPA on the day of treatment rather than before. The effect of DMPA definitely on uterine pressure appears to be time-dependent. This raises the question whether giving “bridging” DMPA prior to Essure, as is commonly done, compromises the success of Essure placement? Or perhaps it improves occlusion rates because it apparently has an effect favoring collagen deposition and opposing re-proliferation.

**Does chronic inflammation play a role in the success of Essure? Can inflammation also cause side effects, or increase cancer risk?** We know that the success of Essure depends on generating a prolonged acute inflammatory reaction. Is there a small subset of women for whom that inflammation does not resolve, and is this predictable? Women sometimes have pain after Essure, but it’s hard to determine whether the pain is truly due to the device, inflammation, or some other issue. Some women, however, probably do feel pain from the implants. There is no indication that the implants result in chronic inflammation or pre-malignant changes.
SESSION 5: Biology of Tubal Damage and Repair

Biology of tubal occlusion: lessons learned from the development of Essure, Adiana, and from endometrial ablation: Charles Carignan, MD

For many years, the function of the fallopian tube was poorly understood, but we now know that it is not just a passive organ. It is active, with many functions related to reproduction. We know that the reaction to polidocanol foam (PF) is only seen in the intramural portion of the tube; it is not seen in the isthmus, ampulla, infundibulum, or fimbria. The walls of the tube need to be in contact for occlusion to take place; therefore, the narrower portion of the tube is more reactive.

The tubes are constantly in motion. The fimbria ovarica contracts to pull the fimbriae closer to the ovary. Beating cilia cells capture and move the ovum, and there are muscular contractions of the tube. Prostaglandins, progesterone, oxytocin, and human chorionic gonadotropin (HCG) all impact tubal contractility. To prevent conception, one can block the sperm entering the tube or the transport of the oocyte, or one can suppress muscular and/or ciliary action. Products that address multiple targets are more successful.

We know that the tube can recanalize after fimbriectomy. Surgical methods that involve ligation and resection of a more central portion of the tube are most effective. Tubal clips can cause necrosis if too tight, or they can fall or pop off. The Ovabloc device, a silicone tubal plug, seemed promising, but the tube actually dilated around the device (a concept later used in the development of tissue expanders). The Essure device spans the intramural portion of the tube so it does not get pushed out in either direction; however, pregnancy can occur when the device is not well placed (well-placed devices have resulted in zero pregnancies thus far).

After Essure placement, muscle cells embed within the device and neovascularization occurs. At 13 weeks after placement, there is no epithelium left. This is crucial, as an intact epithelium means that no fibrosis can occur. Pressure from the device causes epithelial necrosis and induces a fibrotic response. The initial acute inflammation decreases, but chronic inflammation peaks by 4 weeks and is still present at 16 weeks—some is still present at 5 years. This could be what is responsible for keeping the tube from recanalizing. While some changes within the tube are time-dependent, the critical epithelial disruption starts and stays high. There is actually a low granulation tissue response. Loose fibrosis starts early and continues; dense fibrosis with type 2 collagen increases over time. There is an initial vascular response that wanes, and then waxes again after 4 weeks as neovascularization occurs. The overall tissue response to the device is high, yet the tube is completely normal 5 mm past the device. Essure is sometimes used prophylactically with in vitro fertilization to block turbulent flow from the tubes. Pain studies have not borne out any definite associations, but the potential to reverse the procedure is an issue. This is why Adiana was developed.

Adiana uses radiofrequency energy to denude a portion of the intramural epithelium, and then a small insert is placed to induce the necessary fibrotic reaction. Unfortunately, the insert is not radiopaque and so it can be difficult to verify that the implant is correctly placed on follow-up HSG.

Successful tubal occlusion devices must render the tube completely nonfunctional. However, any inflammation induced must not be severe enough to lead to sarcoma development. It is vital to obliterate the epithelium to render the cilia inactive. It is also important to incorporate a mechanism
for preventing transport of the egg to the uterus. We want to develop devices that are durable, safe, and effective.

**Mechanism of action of quinacrine in the human fallopian tube:** Roger Growe

The mechanism of action of quinacrine in the fallopian tube has not been previously described. A plausible theory is that its mechanism is quite similar to that of *Neisseria gonorrhoea*. Both result in an immune reaction and repair processes that resolve tissue damage into a collagenous plug that occludes the tube.

The fallopian tube has resident macrophages and inflammatory cells. When quinacrine disperses into the lumen, its positively charged molecules bind to acetylcholine receptors on the epithelial cells and act as a noncompetitive inhibitor or antagonist; these bound receptors should disrupt cell adhesion, similar to other antagonists that cause the cells to shrink and round up, interfering with the intercellular and cell-matrix adhesions. In gonorrhea infection, this loss of cell adhesion is the signal event in causing acute inflammation. The disrupted epithelium allows immune cells to migrate through the intercellular gaps. Proinflammatory cytokines (tumor necrosis factor alpha [TNFα], interleukin [IL]-1) are expressed from the epithelial cells and induce epithelial-cell sloughing. Cell death occurs by apoptosis. Superoxides are released from inflammatory cells. The acute proinflammatory and profibrotic response leads to increased tissue damage; the subsequent repair process creates the collagenous plug that occludes the lumen.

The progression of events with quinacrine and gonorrhea is very similar. There is a loss of cell adhesion and detachment of living cells. Tissue damage results in an intense inflammatory response, and the repair process results in a collagenous plug. This common pathway might help define predictable biological targets, reliable organ- and animal models, strategies for screening new drug candidates, and more effective, lower-dose application methods. This research into gonorrhea’s effects suggests an alternative to traditional sclerosing agents that have demonstrated a wide variety of mechanisms of action. A new list of potential agents can be created from acetylcholine-receptor antagonists that demonstrate the potential to control cell adhesion in the epithelium of the fallopian tube without additional, unwanted, toxicity. We can then develop more effective and lower-dose application methods. Perhaps only 25 mg of quinacrine is needed, rather than the 250 mg dose that has been used previously. Future developments will hopefully help to break down the economic and political barriers to effective permanent contraception.

**Molecular and cellular events that lead to fibrotic occlusion of the fallopian tube?** Mike Luster, Ph.D.

Innate immunity involves vascular dilation, increased vascular permeability, and infiltration of neutrophils and macrophages. There are varying degrees of severity, dependent on the extent of tissue injury, and the reaction can be acute or chronic.

Pattern-recognition receptors (PRRs) make up part of the innate immune system of the female reproductive tract. They include toll-like receptors (TLRs) and nod-like receptors (NLRs). PRRs bind to
components of pathogens and cell debris and, based upon the receptor(s) activated, produce various
types of cytokines and inflammatory mediators. TLRs and NLRs also can respond to anthropogenic
materials such as asbestos. Quinacrine and chloroquine affect PRRs by specifically reducing endoplasmic
TLRs; this mechanism is believed to be responsible for the efficacy of these medications against certain
autoimmune diseases.

The nine TLRs work through four adaptor proteins. These proteins control the type of cytokine
produced and therefore regulate the type of inflammatory response. Different parts of the female
reproductive tract have different TLRs and therefore different responses to exogenous agents. TLRs are
highly regulated by hormones (estrogen/progesterone) and are site-specific and life-cycle specific. Why
is this important? The types of cytokines and other mediators produced in response to TLR activation
control macrophage polarization. There are (in general) four types of macrophages defined by the
mediators they release: M1 macrophages produce proinflammatory mediators (e.g., TNFα and IL-1),
which results in classical inflammation; M2a macrophages produce cytokines involved in allergies and
resistance to parasites; M2b macrophages are involved in immunoregulation; and M2c macrophages
produce fibrotic mediators responsible for matrix deposition and tissue remodeling. This last type of
response is the one desired in tubal sclerosis, rather than persistence of the classical M1-type response.

Understanding why and how different materials elicit these different inflammatory pathways in
the female reproductive tract can help identify materials that will initiate tissue remodeling and fibrosis
in a specific manner. Producing this specific response will avoid causing acute or, even worse, chronic
inflammation.

Polidocanol foam treatment in nonhuman primates: histologic features: Ov Slayden, Ph.D.

We know, so far, that the occlusion caused by PF is in the intramural portion of the tube. We see
the accumulation of extracellular matrix (ECM) and the obliteration of the tubal epithelium, but it is hard
to figure out from the resulting pathology what exactly the treatment is doing and why it requires
multiple treatments unless depo medroxyprogesterone acetate (DMPA) is added.

Experiments have been conducted to characterize the reproductive tract epithelium after
exposure to PF. Rhesus macaques were given 5% PF during the follicular phase, with tubal filling
visualized on ultrasound. Controls were conducted with both methylcellulose foam and no foam.
Animals were evaluated 24 hours after and 7 days after PF administration.

At 24 hours after PF treatment, glands in the control tubes stained positive for estrogen
receptors and Ki-67 (indicating proliferating cells); these were healthy cells. In PF-treated cells, the
damaging effect in the functionalis zone of the tubal wall was seen to be dependent on the distance
from the luminal surface; the basalis zone still contained proliferating cells. PF damage was seen in the
intramural tube, but not in the isthmus or ampulla. In the zone around the administration surface,
oviductal glycoprotein was compromised, as were estrogen and progesterone receptors. Seven days
later (no menstruation yet), there was still visible damage at the luminal surface and an absence of
estrogen receptors in the functionalis zone. The muscular layer, however, was still making estrogen and
progesterone receptors. The isthmus and ampulla remained healthy.
The researchers were surprised by the number of proliferating cells seen after treatment. Almost every damaged cell stained positive for Ki-67, which is unusual for an oviduct sample. There appears to be an estrogen-independent wound healing process. Those animals that still had estrogen-receptor staining did not have much Ki-67, indicating that less damage resulted in less proliferation. We typically think of estrogen as a proliferative hormone and progesterone as the hormone that blocks that effect, but these proliferating cells were low on both estrogen and progesterone receptors. Cell proliferation in the ampulla is typically seen at the end of the menstrual cycle, when progesterone withdrawal occurs; this proliferation falls off as hormone levels increase again. Therefore, any effect of DMPA on proliferation should be a suppressive effect.

There is an ongoing addition to the above experiment: treating animals with bromodeoxycytidine (BrdU) 48 hours after PF treatment for 4 days to evaluate for tissue proliferation. The preliminary results show that less than 20% of cells from control animals exhibit positive staining for proliferating cells, while greater than 90% of PF-treated cells stain positive. This indicates an attempt at tubal recovery—the researchers are interested in whether administration of DMPA will change that.

PF results in mucosal damage to the upper zone of the tubal wall, sparing the basalis zone, at 24 hours. The loss of estrogen and progesterone receptors lasts for at least 1 week, indicating long-term disruption and sustained, lasting damage. One mechanism for tubal recovery may be prompt re-epithelialization with recovery of hormone receptors. Therefore, agents that block cell proliferation (such as DMPA) could be good combination therapy with PF.

Discussion deferred to after session 6.
**SESSION 6: Natural Inflammation and Tubal Occlusion**

**Pathogenesis of fallopian tube damage caused by Chlamydia infections:** Louise Hafner, PhD

*Chlamydia trachomatis* infection of the genital tract is the cause of the most common bacterial sexually transmitted disease (STD) worldwide, with 4–5 million new cases annually. About 70% of infections are asymptomatic, and the condition may lead to pelvic inflammatory disease (PID), tubal pregnancy, and chronic pelvic pain. Tubal-factor infertility accounts for 36% of female infertility; chronic persistence of chlamydia is a major cause, as the disease can result in tubal scarring and occlusion.

Chlamydia is an obligate intracellular, gram negative organism. There is an intricate exchange between the host and the bacterium, and persistent infection can result from inadequate clearance. The developmental cycle involves an infectious, extracellular elementary body (EB) and non-infectious, metabolically active intracellular reticulate body (RB). The RB typically redifferentiates back into an EB and is released from the infected cell. However, when stressed, the RB goes into a persistence phase (survival mode), which may result in chronic infection.

The host displays both cellular and humoral immunity to chlamydia. There is an inflammatory response, with infected epithelial cells releasing cytokines that then recruit neutrophils, macrophages, dendritic cells, and lymphocytes. The inflammation is intense and chronic, leading to epithelial-cell proliferation and tubal scarring, with subsequent tubal occlusion and infertility. Note that it is the immune response to infection, not the chlamydial organism itself, which is responsible for the tubal damage. Epithelial cells and macrophages are the primary targets of the microinflammatory environment induced by chlamydia, with disruption of the mucosal surface and loss of microvilli and cilia. What is the mechanism? Chlamydia can target caspsases and matrix metalloproteinases (MMPs) that are involved in apoptosis, inflammation, and fibrosis. Cytokines can lead to generation of nitric oxide, causing permanent tubal damage. There may also be multiple positive feedback loops between the inflammatory stimulation of the EBs and the reactions of the immune cells. There are likely several concurrent mechanisms of action causing the tubal damage associated with chlamydia.

Proinflammatory cytokine genes and Type-1 interferon genes are activated through toll-like receptor (TLR) 2 and TLR4. Chlamydial heat shock proteins (cHSPs) are recognized by TLR4, and TLR2 may be responsible for the long-acting immune effects generated by *C. trachomatis* in the fallopian tube. Tubal epithelial cells release interleukin (IL)-1β in response to both ectopic pregnancy and *C. trachomatis* infection—blocking this cytokine has the potential to prevent tubal damage, as it is known to initiate chlamydial tubal injury and to be profibrotic.

cHSP60 is a target for B and T cells and is implicated in apoptosis of cervical epithelial cells. Both cHSP60 and cHSP10 are involved in the pathogenesis of persistent infection. It is thought that this is mediated through chronic inflammation and the prevention of apoptosis by the presence of intracellular chlamydial bodies. Inflammation and the presence of IL-17 are associated with chronic pathology in animal models. IL-17 in human cervical cells may be involved in protection or pathogenesis – more experimentation is needed to elucidate its role.

The presence of antibodies to cHSP60 indicates persistent infection in vitro. The effectiveness of the antibody response, however, is debated. Cell-mediated immunity is critical to clearing the infection. If untreated, chlamydia is usually chronic and can be recurrent. Multiple infections result in worse
sequelae. It is possible for chlamydial infections to resolve without tubal damage, but damage can occur with a cell-mediated response that is either poor or too strong.

Host genetic factors are certainly involved, because not everyone with chlamydia develops scarring sequelae. Some individuals have genetic variations that predispose to an aberrant immune response and a higher risk of tubal pathology.

If chlamydia’s immunopathology could be controlled, it might yield a method of inducing fibrosis and therefore permanent contraception. It is not known whether this fibrosis could subsequently be reversed.

**Inflammation in the uterine environment: lessons learned from studying endometriosis and implantation: Asgi Fazleabas, Ph.D.**

Endometriosis is widespread, affecting 176 million women worldwide, with an average 7-year diagnostic delay. It can lead to infertility and poor pregnancy outcomes. Endometriosis can cause significant pelvic pain not correlated with the extent of disease. Pain leads to loss of productivity and decreased quality of life. Endometriosis is an estrogen-dependent inflammatory condition that may contribute to deficient immunity within the uterine cavity and in its ectopic sites.

Baboons menstruate and can develop spontaneous endometriosis. Endometriosis can also be induced by inoculating the baboon peritoneal cavity with menstrual tissue—disease is seen within 1 month that appears visually identical to the spontaneous condition. The advantage of this model is that inoculated animals can be investigated at regular time intervals. Unfortunately, pain cannot easily be investigated in baboons.

The withdrawal of progesterone in the normal menstrual cycle induces inflammatory changes in the uterus, resulting in menstruation. Women with endometriosis may have progesterone resistance, leaving them prone to inflammation and compromising the innate immune system. Progesterone resistance is a progressive phenomenon, occurring over 6–9 months of disease progression in the baboon model and is a hallmark of the disease in women.

The inflammatory cascade is complex, but we will focus on a few key enzymes. Cyclooxygenase (COX)-1 is constitutive and physiologic, protecting the gastrointestinal system, while COX-2 is inducible and leads to prostaglandin release and inflammation. Staining for COX-2 progressively increases in both eutopic and ectopic endometriosis tissue in inoculated baboons. It is also highly expressed in endometriosis tissue in human women and is associated with estrogen biosynthesis. There is an associated increase in matrix metalloproteinase (MMP) 2 and MMP9, aromatase activity, and pelvic pain. This response is then exacerbated by resistance to progesterone. Regulatory T cells are suppressed by IL-6, which also inhibits transforming growth factor beta (TGFβ) and produces an inflammatory response. When regulatory T-cell numbers are high, the immune system is suppressed, creating a favorable environment for embryo implantation. When they are low, the patient is immunocompromised within the uterus and infertility can result. Normally, increased Notch-1 levels
lead to immunomodulation while decreased levels, seen in spontaneous baboon endometriosis, lead to inflammation.

Oviductal glycoprotein is aberrantly expressed in baboons with endometriosis. The gene is strong, directly regulated by estrogen and suppressed by progesterone. However, in the context of endometriosis, oviductal glycoprotein remains elevated during the luteal phase, compromising oviductal function and potentially contributing to infertility.

The baboon endometriosis model provides us with the opportunity for developing noninvasive diagnosis and treatment capabilities. It allows us to understand the underlying immune mechanisms that contribute to this disease.

Generating a localized inflammatory and wound healing response as a potential strategy for tubal occlusion: Jon Hennebold, PhD

Vaccine research is all about trying to induce an inflammatory response that promotes the generation of a protective immune response, but not all such responses are created equal. Which response leads to fibrosis and the deposition of extracellular matrix (ECM)? This is the critical question in developing an immune-mediated approach for tubal occlusion.

Adjuvants such as aluminum salts promote immune responses through their ability to provide a sustained release of antigen at vaccine sites (the depot effect); to stimulate the production of cytokines and chemokines; to elicit immune-cell recruitment; to activate inflammasomes; and to increase antigen uptake by and maturation of antigen presenting cells, which in turn travel to draining lymph nodes and present antigens to T and B cells.

Adjuvants play a key role in stimulating inflammatory responses, in part through the activation of TLRs. TLRs are critical for pathogen recognition and activate innate immunity by recognizing pathogen-associated molecular patterns (PAMPs) on infectious agents. Activation of toll-like receptors (TLRs) induces several cellular activities, including cytokine production. Cytokines are also produced in response to inflammasome activation; inflammasomes are stimulated when intracellular PAMPs are recognized by specific cytoplasmic protein complexes. Oviductal epithelial cells have been shown to possess both TLRs and inflammasomes and, thus, have the capacity to respond to inflammatory stimuli. TLR- and inflammasome pathways are activated in human chlamydial infection, leading to the production of IL1-β that directly damages the oviduct epithelium. Can adjuvants therefore be used to generate a localized inflammatory response in the oviduct through the induction of TLRs and/or inflammasomes, resulting in tissue damage, ECM remodeling and deposition, and, ultimately, tubal occlusion?

Once inflammation is induced, it must be directed down a pathway that promotes tubal occlusion, leading to fibrosis and ECM deposition, avoiding the pathway that promotes tissue repair and restoration. Inflammatory fibrosis can be a physiologic response. One example is the corpus albicans in the ovary, a collagen-rich, scar-like structure that develops from the regressed corpus luteum at the end
of the menstrual cycle. The corpus albicans persists over several cycles and is composed of ECM, primarily collagen. Preceding this collagen deposition, macrophages infiltrate the regressing corpus luteum at the end of the menstrual cycle after it stops secreting progesterone. The infiltration of macrophages is then followed by luteal TGFβ1 synthesis, a known inducer of ECM formation and promoter of collagen synthesis in a number of cell types and tissues.

Can we use TGFβ1 to actively promote tubal occlusion, in combination with an adjuvant to act as a TLR/inflammasome activator? We know that we can direct macrophages down certain pathways; we want to favor the development of profibrotic macrophages that produce TGFβ1 and thereby ECM deposition.

Discussion: moderator, Jeff Jensen, MD, MPH

How could adjuvants be adapted for use in permanent contraception? We know from our research into adjuvants with vaccines that it is both possible and safe to stimulate a local inflammatory response without causing development of systemic disease. The depot effect maintains the desired reaction in a localized area. The doses need to be scaled, however, to localize the response and avoid a systemic effect. The depot effect can be applied to the tube, using immobilized components that contact the tubal wall and create the desired effect.

How can proliferation be evaluated in the absence of estrogen and progesterone receptors after damage with a sclerosant? The question is whether proliferation can be reliably observed after depletion of estrogen and progesterone receptors. Are cells that are Ki-67-positive simply “carried through” from the pretreatment phase, or has actual proliferation occurred? Labelling allows us to determine that proliferation is actually hormone-independent. Hormonal stimulation, in particular the presence of estrogen, is not needed to promote proliferation in the oviduct and endometrium. Even castrated and spayed animals show tissue recovery. A local effect is desirable, as blocking cell proliferation globally would not be well tolerated.

Is there a common pathway that activates proliferation after epithelial damage? It is not known whether there is a common pathway by which components such as TGFβ and fibroblast growth factor activate cell proliferation. We see upregulation of individual growth factors, but the big picture is not known at this time. TGFβ is known to activate Ki76 in some cells but has the opposite effect in others. Even with this cell-type specific effect, one commonality is the induction of collagen deposition and ECM production.

What makes the intramural tube vulnerable to damage? The anatomy of the intramural tube is unique, with a small lumen surrounded by muscle; the distal tube is less restricted. This may increase the pressure of sclerosing agents in the lumen. We know from the field of surgery that opposing two
denuded surfaces will result in their fusion. Healing occurs “across” tissues with pressure applied and “along” tissues if there is no pressure. We need to sustain tissues in apposition long enough to get complete occlusion. Perhaps this is the mechanism of depo medroxyprogesterone acetate (DMPA)?

*It is possible to slowly administer cHSP into the tube?* This can theoretically be done, and the desired response (the specific inflammatory protein) can also be targeted. First, we need to create both a recombinant protein and a delivery system.

*How does DMPA influence the inflammatory pathway?* The typical effect of progesterone withdrawal, namely the induction of inflammation in the corpus luteum allowing for immune-cell influx, is not seen when antiprogestins are administered. DMPA has different effects on different local tissues. The interplay of cortisol with progesterone is not known, although both are considered to suppress inflammatory responses. DMPA does have activity at the glucocorticoid receptor. Cortisol alone, within a specific dose range, allows anti-inflammatory cytokines to be produced in macrophages and inhibits proinflammatory cytokines, but we don’t know how it acts in the oviduct. When monkeys are treated with gonadotropin releasing hormone (GnRH) antagonists and steroid production is blocked, giving back a progesterone-receptor agonist prevents immune-cell influx in the corpus luteum. Estrogen-receptor agonists are not able to block this response in luteal tissue. This experiment has not yet been attempted with cortisol.

The discussion ended with a consensus that areas of future research should include the use of natural tubal occlusion immune activators like cHSP and adjuvants as strategies to induce tubal damage. This was seen as a most promising approach.
SESSION 7: Magnetic Nanoparticles for Permanent Contraception

Magnetic nanoparticles: what they are and how they work: Everett Carpenter, PhD

What are magnetic nanoparticles (MNPs)? They are both natural (silica) and manmade (red stained glass is created by reduction of gold-salt nanoparticles). They are widely used in the electronic industry, so it is time for materials chemists to get with the program. Control is critical: it is possible to control particle size, composition, shape, aggregation, functionality, and composition. Iron is a favorable material for medical use—by manipulating the LaMer crystallization curve we can create biocompatible iron oxides. Materials can also be coated to make them biologically compatible. Magnetism can be used in medicine to control cellular interactions. MNPs can be used in various areas: drug delivery, magnetic resonance imaging, stem-cell tracking, cell isolation (separation/purification), proteomics, generating hyperthermia, diagnostics, and treatment (for example, magnetic iron particles can be used to treat anemia). The greatest potential is in therapeutic applications (hyperthermia and drug delivery).

Think of little bar magnets with north and south poles; they will align with a magnetic field. Saturation refers to the number of spins that have aligned. If the magnetic field is removed, nature will try to realign the particles. Resistance is the same as coercivity: it refers to the size of the field necessary to get the particles to randomize again—to overcome their innate tendency to align. When a magnetic field is removed, whatever particles remain aligned (how many spins) is referred to as remanence. We don’t, however, want a magnetized material in the body or in a cell because it will seek out another magnetic particle. The ideal is zero remanence at room temperature; this is referred to as a superparamagnet.

Coercivity (resistance to change) is size- and frequency dependent. Resistance comes out in the form of heat—this is referred to as magnetic hyperthermia. The heat is extremely localized and can be very intense. When particles are placed in tissue and exposed to a radiofrequency generator, they build up energy which is released in the form of heat. Heat can be released by either Brownian motion/relaxation (spinning) or Neel relaxation (vibration). The latter generates a tremendous amount of heat.

Magnetic particles can have different tissue effects depending on the amount of heat generated. Ablation takes place at a temperature increase of 50–80 degrees C and thermal sensitizing at a 10-degree increase. Heat can also be used to release packets of medication inside the body. To use ablation as an example: nanoparticles are injected into the region of a neoplasm and radiofrequency energy is applied, resulting in localized tumor destruction with minimal “collateral damage.” The radiation field can be finely localized so that, even if particles migrate outside the field, they will not heat up.

Practical considerations in the use of functionalized magnetic nanoparticles in biologic applications: Michael Schultz, PhD
When synthesizing MNPs, we want to control their properties for functionalization, and we want to purify them to nontoxic levels of unwanted constituents. Particles must also be stable throughout their desired application (e.g., heating), they must be prepared in biologically relevant media and conjugation buffers, and they must have low toxicity. Carboxymethylated polyvinyl alcohol, used for functionalization of Fe3O4 nanoparticles, allows the particles to meet stability requirements while being biodegradable and having low overall toxicity. Functionalized Fe3O4 nanoparticles are stable in phosphate buffered saline, saline, tissue culture medium, and various conjugation buffers. They are stable over the long term, freeze-thaw stable, and heat stable.

Crystallite size affects the crystallite’s heating when energy is applied. Larger particles heat up dramatically: a 15.2-nm crystallite heats up twice as fast as a 12-nm crystallite. Energy absorption also changes with particle size. The size of the crystallite is combined with the size of the particle for optical heating capability.

Radiofrequency heating of magnetic Fe3O4 nanoparticles allows for a greater amount of cell killing at a given temperature than does heating without nanoparticles. This is due to a combination of nanoheating effects and bulk-heating effects. Concentration is also key: the heating effect drops off at lower concentrations. Targeting ligands can be used to increase the concentration of particles at a region of interest; however, targeting ligands also need to be stable after conjugation with the particle. Ligand proteins that induce apoptosis can be useful for treatment, but some applications may require the a high percentage of the protein to remain functional after conjugation.

**Preliminary results with an antioviductal glycoprotein-linked magnetic nanoparticle in a xenograft model:** Ov Slayden, Ph.D.

Our goal is to target nanoparticles to the oviduct and heat them, causing epithelial-cell death and creating an opportunity for stromal cells to come together and cause tubal occlusion. So how do we get magnetic nanoparticles into the tube? Can they be injected systemically rather than placed transcervically?

Oviductal glycoprotein (OVGP)-1 is a naturally occurring mucin produced by the estrogen-stimulated tube. Its expression is limited to the oviduct, although it is also seen in some malignant ovarian epithelial tumors. The ant-OGP-MNP reagent is synthesized by linking MNPs to commercially available OVGP-1 antibody.

Tubal sections from rhesus macaque monkeys were grafted into immunodeficient severe combined immunodeficiency (SCID) mice for tissue amplification. The mice were also given an E2-releasing implant, and endometrial grafts were placed as control tissue. The grafts were allowed to grow for 2–4 weeks before treating with ant-OBG-MNP. Grafts were collected 24 hours after injection and subjected to immunohistochemistry. OVGP-1 was found in the oviduct grafts but not in the endometrial grafts. Injecting labeled ant-OVGP-MNP into graft tissue showed that, while the antibody initially spread out in the subcutaneous tissue, it localized to the oviduct tissue after 8 hours. There was a tiny bit of staining in the endometrial grafts.

The mice were then exposed to radiofrequency energy, with no apparent effect or tissue damage. Did we lose functionality in the process? Injection of ant-OBG-MNP at different concentrations
(2 µg/mL to 10 µg/mL) did not produce any staining on immunohistochemistry. However, once a magnet was used to pull down the magnetic nanoparticles into a pellet, injection of the pellet resulted in strong staining; injection of the supernatant resulted in weak staining. This meant that the original dose/concentration was way too low. At higher concentrations, the magnetic nanoparticles “stuck” to the surface of oviduct epithelial cells.

ant-OBG MNP localizes to oviductal epithelial cells, with specific binding to OVGP-1. Future studies are needed in different animal models. We also need to show that the reagent will get into the oviductal fluid. This can be ascertained by inject the reagent into the monkey oviduct, harvesting oviductal fluid, and using pull-down to show the reagent is getting to the target.

**Discussion:** moderator, Jeff Jensen, MD, MPH

*How can we use MNPs in tubal contraception?* We need to figure out the optimal size and concentration of the nanoparticles so that radiofrequency energy can be delivered with an antenna, preferably on the lower abdomen or in the vagina, rather than needing huge external coils to deliver the energy.

*Can the particles be superheated as they get to the fallopian tube, or does the tube need to be preloaded with subsequent energy application?* Nanoparticles are incredibly soluble, so keeping them localized to the tubal lumen after infusion could be problematic.

*Can we make larger particles that are less soluble and will stay in the tube?* Small particles are ideal for antibody localization studies, but larger particles might be more appropriate for treatment. It is possible that particles for intrauterine insertion don’t need to be antibody labelled. This would certainly be less expensive. So far, we have not seen any labelled particles taken up into the epithelial cells—they appear to stay in the tubal lumen.

*Will MNP heating cause pain?* We know that radiofrequency ablation of the endometrium causes cramping. We don’t know if tubal heating would cause pain in women. Nanometer heat, as opposed to whole-tissue heat, may be more appropriate to the fallopian tube as well as better tolerated. The transurethral antenna used in prostate treatment heats locally and also generates more extended tissue heat. This could potentially be used inside of the uterus.

*What role might OVGP play in MNP administration?* There are four known oviductal proteins upon which we could experiment. The advantage to OVGP-1 is that it is very specific to the oviduct, and sometimes seen in small amounts on the surface of the ovary. We could also consider experimenting with the other three proteins, or at the very least finding out more about them. OVGP is secreted into the tubal lumen and could act as a “sink” for any administered nanoparticle, preventing it from attaching to the epithelial cells. A huge amount of nanoparticles may need to be administered to avoid having them flushed out of the tube. This could be accomplished either with a larger size of particle or a higher concentration. Larger particles and/or a larger concentration of smaller particles will also make heating easier. We could also look for a more selective or more favorable protein target.
Preclinical study of transcervical delivered RISUG as female contraceptive in the Langur monkey: Nirmal Kumar Lohiya, FNASc, FAMS, FIAES

The global population is projected to reach 9 billion by the year 2050, with India predicted to overtake China as the most populous country in 2030. The ideal contraceptive would exhibit long-term safety and efficacy, be cost effective, as noninvasive as possible, and have a novel delivery method. At this conference, we are focusing on permanent methods for couples who have completed their family (limiting methods) as opposed to temporary spacing methods used to achieve gaps between children or to postpone childbearing. Many options are available, but we are looking for something better than the currently available methods: a single delivery system that is less invasive than current methods.

Reversible inhibition of sperm under guidance (RISUG) is an injectable intravasal male contraceptive, composed of 60 mg styrene maleic anhydride (SMA) dissolved in 120 µL dimethyl sulfoxide (DMSO). It is a nonsclerotic occlusive copolymer that lowers the pH in the vas deferens and generates a positive charge that disturbs the negative charge of the sperm head membrane, blocking passive transport of the spermatozoa. It is reversible and therefore useful as both a limiting and a spacing method. There are no significant side effects. While vasectomy takes several months for reliable efficacy, RISUG is effective after a single injection. RISUG is currently in phase 3 trials in men, but so far seems to be effective for more than 10 years (most studies have about 7 years of follow-up data). There is now a proposal to study this medication for hysteroscopic administration in female langur monkeys. The hope is to achieve tubal occlusion with a single dose.

Why the langur monkey? Their reproductive exocrine and endocrine profiles are close to those of humans, and they lack seasonality in their cycles. Females have a straight cervical canal and nontortuous fallopian tubes. The vaginal cytology and temperature are very predictive of the animal’s hormonal cycle status. Preclinical contraceptive studies have been done with RISUG. In males, there is a significant reduction in the number of spermatozoa at 30 days and 60 days, and those that remain are of abnormal morphology, without fertilizing ability. The medication is reversible, with a return of sperm to the fertilizing range. However, studies have shown exfoliation of the lumen of the vas deferens over 150 days.

The objectives of the proposed plan are to demonstrate the contraceptive efficacy of RISUG in inducing tubal occlusion, to determine the status of menstrual cyclicity and hormonal milieu following injection, and to evaluate the medication’s safety. There will be a control group of five animals and an experimental group of 15 animals, receiving a single dose into each tube. The compound will be administered by hysteroscopy with tubal cannulation, under sodium theopentone anesthesia. Parameters to be monitored will include cyclicity, toxicity, long-term contraceptive efficacy, oogenesis, and toxicity.

This nonsurgical transcervical approach has great potential. Dr. Lohiya welcomes any brainstorming as to a better delivery system, standardizing of the dose, combining RISUG with other molecules, or any other candidate molecules or methods.
The mechanical intratubal device (ITD) for permanent contraception: Leiguang Wang, MD

Tubal ligation was used in about 40% of fertile women in China in the 1970s, but its use has now decreased to less than 10% because it is invasive and associated with complications, pain, and acceptability issues. A “sticky tubal block”—phelol-atabrine paste—was used from 1976–1983 and achieved successful blockage in 85% of patients. Because of the high side-effect rate (fever, abdominal pain, lumbosacral pain) and the severe, nonreversible tissue adhesion it caused in the oviduct, its use was abandoned. Bismuth polyurethane was tested from 1993–1994, but it had a high failure rate. What is the ideal standard? A method that is safe, effective, simple, economic, and reversible. What are the design requirements of a tubal plug? It must conform to the morphology and biomechanical characteristics of the tube. To achieve this, we investigated the cyclical changes in oviductal and endometrial receptivity and the morphology and biomechanics of the tube. We then designed a silicone shape memory alloy intratubal device (ITD). The product development has proceeded through the proper testing channels (safety, animal preclinical, clinical).

Digital subtraction angiography was used to measure the interstitial portion of the tube in 100 women. The inner and outer openings of the intramural portion, the length, and the shape (horn shape) were determined. The silicone plug was designed to be about 20 mm in total length with expansions at both ends, and to be elastic, with memory function. The frame of the ITD was made of a nickel and titanium (Ni-Ti) shape memory wire and covered with a layer of silicone rubber. Biologic safety testing was conducted, and the device was found to have a very minor effect in the animal model. Goats showed no local tissue damage after up to 12 months of use. The implant was used in rabbits, with only one of 30 becoming pregnant; it was removed from 10 rabbits and all recovered fertility.

The device was surgically implanted and removed in rabbits, which is obviously not what we want for women. Human studies were designed for 12 months of follow-up. The device was tested in 36 healthy women, 26–40 years of age, with a history of childbearing. The women reported mild to moderate pain upon device insertion, but left the hospital within 2 hours. About 5.5 mm of the device was left protruding into the uterine cavity to facilitate later removal. At 1 day after placement, 16 women reported a small amount of bleeding, and 15 reported mild pain. At 1 week, three women had a small amount of bleeding, while one reported slight pain. At 1 month and 3 months, no symptoms were reported. At 12 months, all patients were doing well and there were no intrauterine pregnancies, ectopic pregnancies, bleeding, pain, or menstrual disorders. Three patients had migration of the device into the uterine cavity of more than 3 mm (evaluated by ultrasound and HSG). At 18 months, three patients had expelled the device—it is not known whether this was related to the prior HSG. One patient with an expelled device became pregnant at month 19. Over five years of use, four devices were expelled (11.11%), and the pregnancy rate was 2.78% (1 woman).

Because the efficacy is only 88.89% (although with a small sample size of 36 patients), the researchers are currently improving the shape and method of insertion for ongoing study. The preliminary human results and the rabbit results indicate that the contraception provided by the device may be reversible upon ITD removal, but none of the original study subjects have been willing to remove the devices to test this. Larger clinical trials are needed to determine the efficacy of the improved design and the potential for reversible contraception using this method. This ITD has no serious adverse reactions and does not interfere with endocrine function.
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**A novel approach to tubal cauterization: combining a low-cost endoscope and dual-intensity heating:** David Sokal, MD

EvoTech (evotechmed.com) is a small company, working in the social sector, focused on developing healthcare products for the bottom of the pyramid. EvoTech has developed a portable, lightweight (5 kg), low-cost endoscope system, the EvoCam, which requires only a 30-Watt power supply and has a projected retail price of about $2500. In comparison, a Stryker Endoscopy system weighs 100 kg, requires 300 W of power, and costs about $70 000. EvoTech received assistance in finalizing the design from a team at Ideo.org.

Using this endoscope to deliver dual-intensity heating to the fallopian tube could be an ideal method of providing permanent contraception to patients in low-resource areas at a more manageable cost. A thermal tissue insult delivered with dual intensity heating has two results: there is a zone of complete tissue ablation and a peripheral transition zone of partial injury. Within the complete ablation zone, there is both thermal fixation (direct denaturation and desiccation of cellular and extracellular tissue components) caused by a higher dose of thermal energy and coagulative necrosis (direct cellular injury, delayed secondary host responses) caused by a lower dose. Thermal fixation resists wound healing, while coagulative necrosis results in cellular autolysis and follows the classical pathway of wound healing, with lesion reabsorption and scar formation.

Applying dual intensity thermal injury to the fallopian tube should result in a zone of thermal fixation on the uterine side of the coagulative necrosis zone. This thermal fixation should prevent ingrowth of endometrial cells that could interfere with scar formation. This dual intensity thermal approach to fallopian tube occlusion was invented and patented by Russ Sampson at Sierra Technologies, a venture-based company that ran out of funding before finalizing development of a product (see US Patent application 2010/0217250 A1, published August 26, 2010). The appropriate thermal dose needs to be determined and confirmed in a primate model, in parallel with development of a delivery system using an EvoCam-like device.

This combination could result in an effective procedure that only requires a single treatment and that has a lower cost and a faster route to Food and Drug Administration (FDA) approval than a new drug. Another advantage of this method is that no implanted device is required.
**Discussion**: moderator, John Sciarra, MD, PhD

Dr. Sciarra started the discussion session with a reminder that we need to remember the past lest we be doomed to repeat it. The first approach to hysteroscopic sterilization with tubal thermal destruction was in the 1970s. Thousands of cases were performed with an enormous number of complications and even some deaths. The procedure was abandoned in favor of occlusive devices. Research with these revealed that, unless tissue ingrowth (e.g. collagen replacement) occurred, the device would not stay in place for very long; this was the basis for the design of the Essure device. China has been researching tubal injections of various compounds since 1970, but tubal occlusion rates were not 100%. US researchers felt that an 80–90% success rate was not high enough to further pursue these lines of research.

*Is there something special or different about thermal damage (versus chemical/immunological) as far as occlusion?* Tubal damage and subsequent repair is central to many of the modalities we are discussing. There are good data on healing after radiofrequency ablation and Adiana. The developers of the Adiana device researched the depth of destruction needed to create tubal occlusion, so we should be able to use their findings. Radiofrequency alone of the fallopian tube results in incomplete ablation and re-canalization. That is why the implant matrix was added to create tissue ingrowth. We also know that the endometrium does grow back after radiofrequency ablation. Some researchers looked at intrauterine placement of a fabric scaffold after ablation, but channels of endometrium grew through and there were problems with potential hematometra.

*Is hysteroscopic visualization necessary for dual-intensity heating of the tubes?* During his presentation, Dr. Sokal commented that he is not a surgeon and is therefore not sure if the dual-intensity heating method could be used without hysteroscopic visualization of the tubal ostia. Several participants confirmed that visualization is necessary for this kind of procedure. Doubt was also voiced about the likelihood of a fast-track approval for this technique.

*Is there a possibility of RISUG toxicity:* Styrene (a component of RISUG) has a metabolite that is a very potent carcinogen (DMSO) that will get absorbed very effectively, and SMA will stay in the tube for years, causing chronic inflammation? Dr. Lohiya stated that the possibility of RISUG toxicity has been extensively investigated. The quantity being used in RISUG is also being used in several other biomedical compounds and was thoroughly investigated before approval for phase 1 trials was granted. The 60 mg administered occupies a very small portion of the vas deferens. Comparisons with vasectomy suggest that RISUG is equally effective but has better reversibility. The possibility of a RISUG slurry was raised, but the medication only has good penetration as a soluble solution, not as a suspension.

*Why was work with methylcyanoacrylate abandoned?* Toxicity was a definite concern with methylcyanoacrylate in the 1980s. A tiny amount (0.2 mL) is able to glue the tubes together, but the delivery system was problematic and toxicity concerns derailed its potential as a permanent contraceptive method. Again, we need to go back and look at the past to avoid repeating mistakes made by our predecessors.

*Can nanotubes, rather than nanoparticles, be used for tubal occlusion procedures?* Nanotubes would allow for cross-binding of multiple ligands. Unfortunately, particles with an aspect ratio of a certain size have biocompatibility issues. Particles do have capability for multiple functionality—they can be made with multiple antibodies or ligands. Nanoparticles might be more likely to be approved if they
are directly placed into the uterus (via a spray or gel) rather than given as intravascular injections. It was pointed out that a lot of iron oxides are already approved for intravascular use as agents for magnetic resonance imaging and as treatments for anemia, so regulatory concerns might not be an issue. The problem with these particles lies in getting a sufficient concentration at the region of interest. Larger particles are perhaps needed, and these would be better placed by irrigating the tubes.

*Is the silicone ITD reversible, or should it be considered a permanent method?* The silicone tubal insert has the advantage of reversibility. However, there is some evidence that it causes minor tubal inflammation at 6–12 months of use. The device might perhaps create occlusion over time. Yet reversibility has been documented—pregnancy occurs when the devices fall out. There are no tissue samples to evaluate in these women to determine the amount or extent of any tubal damage.
SESSION 9: Novel Materials and Drug Delivery Technologies for Tubal Occlusion

Drug-eluting fibers: contraception and HIV inhibition: Kim A. Woodrow, Ph.D.

There is a need for multipurpose technology that can simultaneously prevent both unintended pregnancy and sexually transmitted infections, including human immunodeficiency virus (HIV). These multipurpose prevention technologies will take the form of combination drug products and need to be safe, acceptable, affordable, easy to use, and reversible.

There are a number of formulation challenges to delivering different drugs with different mechanisms of action, but often these combinations are needed for full potentiality. While a number of technologies exist for delivering drug combinations, such as oral and injectable dosage forms, only topical delivery systems have the benefit of being “on-demand” and realizing both short- and long-term prevention needs. These topical delivery systems include both physical and chemical barrier methods, alone and in combination (i.e., diaphragm with gel).

Electrospun fibers are several hundred nanometers to a few microns in diameter. They are able to accommodate multiple design specifications in a single device (strength, geometry, drug diversity). The fibers are created by accelerating a charged fluid polymer jet in an electric field, where the fluid electrohydrodynamic properties will dictate its nanoscale architecture. Fibers can be fabricated according to different specifications: drug-eluting fibers for topical delivery, drug-combination fibers, and asynchronous drug-release fibers. A blend of hydrophilic and hydrophobic fibers can be used to encapsulate agents with different physicochemical properties. Manufacturers can control the mechanical properties and device geometry to further expand the multifunctional capabilities of these medical fabrics.

Finished fabrics can be composed of fibers in a stacked, interwoven, or combined microarchitecture. The choice of microarchitecture is influenced by the physicochemical diversity of the drugs and their interactions. There are also constraints related to programming drug release. For example, some usage indications may require rapid drug action over 30–60 minutes for prevention of HIV and other sexually transmitted infections. Other usage indications may need to be active over a longer time frame and therefore require sustained drug delivery. Fibers that provide asynchronous release of different drugs can be realized by composite fabrics.

To date, drug-eluting fibers have been fabricated that will deliver diverse agents for contraception and HIV prevention. The materials have been observed to be safe and nontoxic to tissue explant models and have shown efficacy against HIV infection and sperm motility and viability, using in vitro assays. These materials are envisioned for use with tampon applicators, as films, or as cervical caps; there is also the potential to design fibers for uterine or direct cervical application. Fibers can be impregnated with tubal occlusion-promoting drugs that are released over days to weeks. Polidocanol, other sclerosing agents, and immunotherapeutic agents could all be delivered this way. The fibers can serve as both a chemical and a physical barrier to contraception while the occlusive agents take effect.

Nitinol wire delivery system for permanent and reversible contraception: Bob H. Katz
This presentation was not given by a clinician or academic, but rather a biomedical engineer: someone whose job it is to take technology from the academic realm to clinical applicability and then to the marketplace.

Nitinol is a shape memory metal alloy (SMA). Shape memory, seen in both metal alloys and polymers, is the ability to undergo deformation at a particular temperature, then to recover the original shape at a temperature above the transformation temperature. Superelasticity happens above the transformation temperature, when the material may exhibit 10–30 times the elasticity of normal metals and materials. Biomedical engineers are also interested in biocompatibility and biostability. Materials need a good “fatigue life,” the ability to flex and bend for years. Nitinol is a nickel-titanium alloy and is the most common SMA used in biomedical applications. It has all of the desired qualities: shape memory, superelasticity, biocompatibility, and biostability. It is used in cardiovascular stents, orthopedic materials, and contraceptive materials (Essure). The success of Essure prompted the conversation: how we do this procedure in an easier way, using fewer resources?

We want a product with a single deployment that will provide bilateral tubal occlusion. The product should be self-aligning to minimize the need for visualization of the tubal ostia, and it should have platform capability for drug delivery. The clinical prototype design is an intrauterine system consisting of a nitinol frame with polymer tips. The device is placed 1–2 cm from the fundus and is engineered to “climb” up the uterine wall, with the tips finding their way to the oviducts. These polymer tips can be engineered to deliver a variety of sclerosing agents, drugs, or medicinal products.

Hysterosalpingography (HSG) was performed at baseline (immediately after device placement) and again at 3 months. No tubal spillage was seen at either time. The system is highly visible on radiography. Although it has been shown to be stable at 3 months, both success and failure have been seen at 6 months, with the device slipping down the uterine cavity in some women.

This design was then manipulated to create a new-generation intrauterine device (IUD), using the same spring-element nitinol frame but adding copper to the arms and to the bottom of the stem. Just like the original design, this new-generation IUD springs up to the tubal ostia, but the oviducts are not occluded in this configuration. The copper is concentrated at key areas: the os and the ostia. While the traditional “copper T” IUD can get contorted in the uterus and can cause pain, irritation, and bleeding, the nitinol model is more conformable to the uterus. So far, there is a substantial difference in patient complaints of pain and bleeding.

SMA/nitinol frames have the potential to be successful platforms for permanent contraception and long-acting reversible contraception. The mechanisms involved may include mechanical occlusion, drug delivery, and tissue growth. There are many potential benefits to this system. Bilateral tubal occlusion is achieved with a single delivery, the device exhibits self-directed placement, and it is able to be placed with limited visualization. All of this makes it ideal for use in low-resource settings.

Discussion: moderator, Jon Hennebold, Ph.D.
**What are the potential uses of electrospun fibers?** Many current contraceptive methods have the drawback of precoital insertion, leading to failure of the method—not because it doesn’t work but because it isn’t used. Any potential fibers/fabrics and devices will need a longer lead time than 1 hour, or could potentially be designed for postcoital use (over a 12–24 hour period). User adherence will, of course, need to be worked into the design. For permanent contraception, hysteroscopy or other introductory methods could be used to deliver fibers that release alum, chlamydial heat shock protein (cHSP), or many other products. Researchers are currently working on polymeric and inorganic particles for drug delivery. There is a lot of diversity in what kind of products can be delivered using these fibers. Fibers can be used to coat many different kinds of devices.

**How would intravaginal or intrauterine fibers be designed?** There is the constraint of adherence and timing functions. Researchers are just starting to think about the mucosal/fiber interface and material adherence as it impacts the biologic response to the medications, and it is not clear yet how soon intrauterine fabrics might be available. Nonhuman primate models would be valuable for future research. Fibers can be “surface-functionalized” with proteins or adjuvants, then coated to make them mucoadhesive. The fibers would dissolve over time but would hopefully provide sustained release of medication while they last. Gel and foam demonstrate good delivery of chemical compounds, but electrospun fabrics can be loaded with high doses (60–80% of material weight). Even with the current 20%-loaded fibers, high doses can be delivered. The amount of drug is a small component of the delivery mass, so attention can be turned to engineering easy delivery methods.

**Can the kinetics of electrospun fibers be changed? For example, can an initial immune reaction be triggered, then “backed off”?** This is indeed possible with composite fabrics. One type of fiber can have an adjuvant to initiate a reaction, then, a secondary biologic compound could be released. Right now, drug release from stacked-fiber fabrics can be programmed out to 10–12 days. It is possible to have sustained release for very long intervals.

**How effective is the nitinol IUD?** The new-generation nitinol IUD is bulkier than the simple occlusion device, but it has less than half the amount of copper of the current standard IUD. It has demonstrated excellent contraceptive effectiveness. Perhaps this is because there are higher concentrations of copper in more specific areas. Chemical analysis and corrosion resistance data show that, when processed correctly, ion release of copper can be achieved with little to no nickel release. The products that can be delivered using the nitinol intruterine system seem to be limitless. Perhaps a woven material, impregnated with therapeutic agents, can be delivered using the nitinol device.

The audience was impressed by the ability of the nitinol device to find its way to the tubal ostia without the need for visualization. This device has high potential as a drug-delivery system for induction of permanent contraception.
SESSION 10: Other Strategies for Permanent Contraception

**Selective targeting of GnRH-II neurons to block ovulation:** Henryk Urbanski, Ph.D., D.Sc

We are now going to turn our focus to the reproductive neuroendocrine axis and how contraceptive methods that utilize it might be reversible and therefore more appealing to women.

Gonadotropin releasing hormone (GnRH) is released from the hypothalamus, stimulating the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary, and these go on to stimulate the production of estrogens, progesterone, and testosterone from the gonads. Feedback loops help to regulate this system.

Rhesus macaques are very similar to humans in their reproductive cycles. During the follicular phase, LH is held in check by estradiol until somehow, estradiol’s influence changes to positive and an LH surge occurs. This surge is the trigger for progesterone production and ovulation. The assumption has always been that GnRH neurons are relatively homogenous, releasing only one type of GnRH molecule. In other words, the same neurons are responsible for both the day-to-day pulsatile release of LH and the monthly surge. How then does the change from a negative estrogen response to a positive estrogen response take place? There are now data to suggest that these assumptions are incorrect. If there are different types of GnRH molecules and neurons, there is theoretically a way to develop a novel long-term reversible contraceptive that will block only the LH surge, not the day-to-day pulsatile release.

Different nonmammalian vertebrate species produce multiple forms of GnRH, with some species exhibiting more than one form. More importantly, there is evidence that humans and nonhuman primates express two distinct molecular forms of GnRH, both of which are highly effective at stimulating LH release. GnRH-I and GnRH-II are encoded on different chromosomes, and the neurons that secrete them have completely distinct locations in the hypothalamus. Furthermore, GnRH-I neurons respond to estrogen exclusively in a negative manner, while GnRH-II neurons respond to estrogen only in a positive manner. Taken together, these data suggest that different aspects of reproductive function in primates are orchestrated by two distinct populations of GnRH neurons. From a contraceptive perspective, inhibition of GnRH-II neurons (e.g., by silencing the GnRH-II gene) should block ovulation while maintaining the tonic pulsatile secretion of GnRH-I. Restoration of fertility would simply involve administration of any type of biologically active GnRH to induce an LH surge.

Mice and rats are not good models for research because, unlike primates, they do not express the second form of GnRH.

**Microchip drug delivery technology:** Robert Farra, BSME, SMME

The concept of the microchip as a programmable drug delivery implant was validated in 1999, technically validated in 2008, and clinically validated in 2011. There are currently 113 patents granted and 36 applications pending. Product development is ongoing.

How does the technology work? In the traditional use of microchips as electronic components, a silicon wafer is processed using photolithography techniques, and metal is either deposited or taken away to create the desired pattern and response. In the medical field, silicon wafers are processed to
create reservoirs where drug can be deposited; these reservoirs are then hermetically sealed for long-term stability. A metallic membrane covers the reservoir, and when an electric current is applied, the membrane behaves like an electric fuse—the membrane is activated (melts) and resolidifies at the perimeter of the reservoir opening, allowing the drug to be dispersed. Each reservoir opening is 100 microns in diameter: the size of a human hair. These reservoirs are programmable for on-demand release. They are also scalable as far as the number of reservoirs per chip and the number of membranes per reservoir. The chips are placed on an implanted device with an internal battery source, microprocessor, two-way radio, and clock chip. When the membrane is opened, interstitial fluid diffuses into the reservoir and reconstitutes the drug, which then diffuses back into the interstitial space.

The reservoirs do not need to have drugs inside. Metal traces can be deposited on the inside surfaces of the reservoir to make an electrochemical sensor (e.g., a glucose sensor) that is discrete and sealed until needed. Therapy can be delivered based on the real-time physiological response from the sensor. This “pharmacy on a chip” allows for remote monitoring of the desired parameters, facilitating long-term care of patients with chronic disease, children, or those who are at the end of life. Consistent and precise medication dosing using this technology could reduce healthcare costs.

Microchip delivery was validated in women with osteoporosis. The use of parathyroid hormone (PTH) for osteoporosis treatment traditionally has poor compliance because a daily injection is required (to be anabolic, PTH needs to be delivered in a pulsatile manner). A PTH-delivery chip was placed on a 3 x 5-cm implant. The drug delivered by chip was shown to be bioequivalent to that given by injection. The trial was a success: patients’ bone markers increased and the device functionality was confirmed by wireless communications. Safety was also confirmed: patients’ calcium levels were in the normal range, there was no remarkable histology, and patient acceptance was good.

For contraception, a product that meets the various and varying needs of women is highly desirable. Ideally, a contraceptive implant would be long-term, address permanent contraceptive needs, and feature on/off capability so that a woman would be able to start and stop hormone dosing according to her needs at the time. Such an implant has been developed for levonorgestrel. This implant is much smaller than the PTH implant, measuring 20 x 20 mm. There is a microchip on both sides, allowing for 200 months (16 years) of treatment, at 1 month per reservoir. The implant can be placed in the arm, buttock, or abdominal wall. It can be programmed by either the physician or the woman herself, and can be turned on and off wirelessly for family planning.

Consistent daily dosing may reduce the side effects associated with other long-term levonorgestrel products. The device could be modified to deliver both progestin and estrogen for cycle control. Theoretically, a woman could receive the implant and use it for several years, then turn it off to get pregnant. She could then turn on only the progesterone component while nursing, then turn both components back on at the desired time.

Discussion: moderator, Richard Stouffer, Ph.D.

How will the ovary respond to day-to-day pulsatile LH with no surge? We don’t know yet how the ovary will respond; this could end up as an analogous condition to polycystic ovarian syndrome. Yet the switch on/switch off concept for contraception is fascinating. The plan is to begin testing by
suppressing GnRH-II activity in monkeys and monitor the effect on the ovary and the monkey as a whole. We also need to keep in mind that this could result in unopposed estrogen stimulation of the endometrium. Progesterone supplementation may be needed.

Could the microchip technology help with that? It is possible that chips could be programmed to sense a rise in estradiol and subsequently release progestins; this would have the effect of blocking ovulation without leaving the endometrium exposed to excessive estrogen. More research is needed into measuring hormones in the subcutaneous space where the implant dwells. Assuming a sensor can be developed, and then it’s just a matter of setting up the feedback circuit. This will not make the device bigger—the size is dependent on the number of doses contained. The lifetime of a subcutaneous sensor, and how many sensors are required, needs to be determined.

What are the specifications of the drug-delivery chips? Each chip reservoir holds 1000 nL (1 µL). Aqueous drug solutions can be used, and solutions can also be lyophilized (freeze-dried). Researchers are also working on solid tableting of the reservoir drug. The chips are capable of differential release for different drugs. For example, with PTH, instantaneous release is desired but with levonorgestrel, the release might need to last for 30 days. Each dose is hermetically sealed and so there is no ingress of moisture or oxygen. This allows the medication to remain viable in the chip for many years of use. The drug does need to exhibit thermal stability. A crystalline form of the desired drug, with a melting temperature higher than body temperature, is best. The glass transition temperature of morphous (unordered, noncrystalline) materials can be manipulated to be higher than body temperature so that the materials do not become too viscous. Drug degradation profiles are taken into account when designing the implants.

Are the chips time-consuming to implant? Do they have side effects? Each implant takes 20–25 minutes for placement, including the administration of lidocaine and suturing. There have been no adverse events documented and no skin irritation; the implants appear to be completely biocompatible.

How expensive is chip technology? The current challenge lies in reducing the costs of this sort of technology for developing nations. In the field of computer electronics, integrated circuits have come way down in price since they were first introduced. The main costs incurred are in the regulatory process and in making the product traceable.
SESSION 11: Regulatory and implementation challenges for nonsurgical permanent contraception

Regulatory challenges for permanent contraception methods: Carol Danielson, DrPH, MS

Permanent contraception includes both surgical and nonsurgical options. The Food and Drug Administration (FDA) does not regulate the practice of medicine, but does regulate both the devices and drugs used and their labeling. The FDA Center for Device and Radiological Health (CDRH) evaluates the safety and effectiveness of devices, and the FDA Center for Drug Evaluation and Research (CDER) does the same for drugs. When approval is granted for permanent contraception devices and drugs, post-approval requirements are included. For example, the manufacturer of Essure was required to perform a 5-year follow-up to monitor pregnancies and adverse events, and to report histological data on explants. The FDA was also interested in the rate of successful placement at first attempt (how are practitioners doing?). Adiana had a 10-year follow-up set for pregnancy rates and subject comfort.

The FDA requires both safety and efficacy data for product approval. New drugs and devices can be benchmarked against existing data such as the United States (US) collaborative review of sterilization (CREST), or a current comparative study of failure rates using the Pearl index: the total number of months or cycles of exposure against the number of pregnancies.

Drug and device discovery and design is the first step, followed by preclinical research prior to submission of an investigational new drug (IND) or an investigational device (IDE). The development process continues with clinical studies (phase 1 for safety, phase 2 for dose, and phase 3 for confirmation of safety and efficacy), after which a new drug application (NDA) or premarket approval (PMA) form is submitted for approval. Phase 4 studies for long-term safety follow FDA approval.

There are lessons to be learned from the quinacrine sterilization (QS) experience, a journey that has been discussed at this conference several times. There were previous safety and efficacy data for quinacrine as an oral antimalarial treatment, and the medication was FDA-approved for this indication. Over 100 000 women were treated with PS before submission of the IND, and safety was confirmed in a phase 1 study. After a pivotal study, approval was anticipated with a shorter review time (10 months vs. 12 months) under the FDA’s 505 (b) (2) process, because quinacrine was a previously approved drug. However, a clinical hold was placed due to a study showing carcinogenesis in rats (CaBio), and the FDA required a human epidemiology study to consider removing the hold. A full carcinogenicity committee meeting was held. The committee acknowledged that the maximum tolerated dose (MTD) was exceeded in rats, but the study was deemed still valid. The human epidemiology data were submitted, but despite recognition by the FDA that the MTD was exceeded in the rat CaBio, and the human data showed no statistical increase in reproductive tumors, the clinical hold was continued. Assistance was requested from the FDA ombudsman, and a formal dispute appeal was undertaken. This appeal is currently going up the chain of command, with a 30-day decision clock at each level. The FDA has agreed, at the sponsor’s request, to commission three independent non-FDA reviews of the rat CaBio study to resolve the issues.

What have we learned, and what advice can we offer from our experience to assist developers of new drugs and devices for permanent contraception? First, be aware of the regulatory and political landscape; second, engage experts in specific fields to develop protocols (don’t rely on the FDA to tell you what your study needs to look like); third, be aware of safety concerns (yours and others’); fourth, partner with the agency when possible; and finally, move up the chain of command if necessary.
FHI360 experience with a permanent contraception advisory board: Karen Katz, MSc, MPA

Family Health International 360 (FHI 360) is a nonprofit company with a focus on increasing access to a range of contraceptive methods. They are involved in development, clinical trials, acceptability studies, and registration of methods. FHI 360 supported the initial development and testing of QS pellets but put its clinical research on hold due to concerns about a cancer cluster in QS patients Chile and the low efficacy of the method.

Separate from the FHI 360 research, a controversy surrounding QS stemmed from the large-scale introduction of the method in Vietnam (by individuals not associated with FHI 360) without approval from a regulatory authority and with no standard method of delivery. Concerns were raised about the possible risk of uterine cancers, the adequacy of informed consent, and the possibility of coercion and abuse; QS became a symbol of unethical treatment to some women’s groups. Distribution continued despite the protests and concerns, and the story was picked up by the media. The medication was ultimately banned in India and Chile, and its use was discontinued in Vietnam. The manufacturer eventually stopped producing the pellets.

With the possibility of new funding and the conclusion that the cancer cluster in Chile was not related to QS, FHI 360 renewed its research agenda on nonsurgical permanent contraception. As part of this agenda they took the unusual step of creating an advisory board incorporating women’s health advocates. They felt it was time to become more proactive, to develop communications and outreach strategies, to develop key messages, to update their website, and to reach out to women’s groups. The committee had seven members with backgrounds in biochemistry, law, medicine, social science, and family planning service delivery and policy. The board’s mandate was to review and comment on clinical and behavioral protocols, provide input on ethical issues, liaise with women’s groups and stakeholders, and advise on the eventual method of product introduction. Meetings of the board were held annually from 2001 to 2006, with discussion on research studies, statistics and study design, toxicology and pharmacology, research ethics, and product development. Results from the rat carcinogenicity study, combined with the high failure rates of QS, led FHI 360 to discontinue its involvement with the contraceptive method.

The advantages of having such a committee lie in building trust, advocacy, contributions to research agenda, reassurance, and the bringing of different perspectives to the work. The board has helped explain complex science to the community and women’s groups. The different perspectives, however, can also be challenging. Other difficulties in creating and running such a group include the logistical issues involved in meeting, the need to explain technical content to nontechnical individuals, and the fact that the company cannot always carry out the board’s recommendations. There is a need to balance proprietary information with the responsibility to report back to communities. However, scientists can become too focused on research and not take into account the effects of the end product on people. Transparency and trust are essential. The early development of a communication strategy is key.
Implementation of contraceptive services for limiting births in low-resource settings: John Townsend, PhD

There is no doubt about the need for new methods of safe and effective contraception. In many low income countries in Asia and Latin America, unmet need is higher for limiting births than for spacing them; the exception to this is sub-Saharan Africa, where the unmet need for limiting is 9% and the total unmet need is 25%. In south Asia, the total unmet need is 15%, and unmet need for limiting births is 9% (about 30 million women). We also anticipate that there will be increased demand in the future as the very large youth cohort ages and achieves its desired smaller family size at an earlier age than previous generations.

It is important to recognize that unmet need is not the same as demand for permanent contraception. The former is affected by a lack of skilled providers and support staff, lack of equipment, poor referral networks, limited safe clinic space, and complications from low-quality procedures. There is poor social communication around sterilization. In some contexts in south Asia, there is a preference for abortion over contraception when the risk of pregnancy is perceived as low; in other contexts, we see a lack of engagement of men in supporting permanent contraception (lack of approval, dearth of information) and a belief in some cultures that sterilization is equivalent to mutilation. Globally, up to 20% of women report some form of regret after permanent contraceptive procedures, with 5% of women desiring reversal due to changing life circumstances such as the death of a child or spouse, divorce, or simply an interest in having another child after remarriage.

A global “demand generation” is driving the growth in the perceived need, from both the clients’ and providers’ experience, for more modern contraception methods; other drivers include more structural factors such as countries’ investment in infrastructure, government champions, community-based distribution, and pooled funding. Market factors also come into play: diversity of method choices, ranges in types of providers, market competition, user-centric designs (taking into account cultural concerns), price, and risk mitigation. Permanent contraception in sub-Saharan Africa, as well as in other developing regions, is largely provided by the public sector. What would it take to diversify the market? Task sharing is a possibility, but the experience with this in sub-Saharan Africa has been mixed. There is high turnover of staff combined with a low client load at many service delivery points that have already received investments in training, and the loss of skills among providers who do not perform these services on a regular basis is an issue of concern. On the issue of program design, the concept of rights-based delivery gives consumers a say in the design of the services. It takes into account availability, access, quality, informed consent, equity, nondiscrimination, accountability, and redress.

For future development, policies must reconsider the design, implementation, and financing of limiting contraceptive methods for both women and men. We need safer technologies that are able to be administered by a wide range of clinical providers. New methods must have equal efficacy to existing permanent alternatives and, at the same time, should be responsive to clients’ needs. Regulatory and risk issues are important considerations in developing, registering, and marketing new devices.

Discussion: moderator, Regine Sitruk-Ware, MD
**How does the FDA’s 505 (b) (2) process work?** This process, used to apply for a new application for a previously approved drug, actually makes the approval process easier. As long as exclusivity is exhausted and there is no patent protection on the medication, then applicants are able to bypass a lot of preclinical work. Often, a bridge study is required to show relative bioavailability, as long as the new indication provides less exposure than the reference indication. Information for the same drug can be taken from several labels. As far as combining 2 approved drugs, the patent and exclusivity requirements need to be met for both, and the applicant needs to show that neither drug alone is adequate to the purpose. The effects of the combination do need to be documented, and there can be no negative effect or interference. This 505 (b) (2) pathway has been used for many contraceptives that are off patent/exclusivity. The use of a 505 (b) (2) is not limited by route of administration, indication, or patient population. However, clinical studies will still need to be performed for the new parameter (e.g., route of administration). The 505 (b) (2) is not equivalent to an abbreviated new drug administration application (ANDA). Many 505 (b) (2) applications have been approved. Nitroglycerin is a good example: it was first used for angina, and a topical form is not available for anal fissures. It is now approved for cardiac use in topical, patch, and sublingual forms.

**Why did the FDA place a clinical hold on quinacrine when the negative human carcinogenesis data would seem to outweigh the animal data supporting carcinogenesis?** From the FDA’s perspective, the weight of evidence is used to make a risk-based assessment. Once a negative response is documented, it takes weight of evidence to reverse that. In contrast, when courts get involved, they look more at potential damage—a different frame of reference than weight of evidence.
SESSION 12.1: Proposal for a Research Center for Permanent Contraception at OHSU/ONPRC

**Overview of the Center Concept:** Jeff Jensen, MD, MPH

We are proposing to create a new research center for permanent contraception. Over the past year, a generous planning grant from the Bill & Melinda Gates Foundation has been used to assess the existing research capacity at Oregon Health and Science University (OHSU)/Oregon National Primate Research Center (ONPRC) and to determine the development in infrastructure, scientific support, and animal resources that would be needed at ONPRC to fully support collaborative research in the field. An external scientific advisory board has been assembled to facilitate planning and provide scientific direction. The goals for this conference are to review the plans for the proposed center and to generate and develop an initial research agenda. Following this meeting, a detailed funding proposal will be submitted to the foundation. In this session, the ONPRC committees on infrastructure, animal resources, and scientific support will present their findings and recommendations.

The proposed structure for the new center will be as follows: the Bill & Melinda Gates Foundation will provide grant support to OHSU/ONPRC to fund the Oregon Nonsurgical Permanent Contraception Center (ONPCC). OHSU/ONPRC will then administer these funds to develop the infrastructure of the center, providing scientific and technical support; there will also be direct funds available to extramural and intramural investigators for research projects. The scientific advisory board (SAB) will solicit and review research proposals and award grants to investigators. In other words, the center would have an operating budget to fund infrastructure, animal services, and scientific and administrative support, and a research budget that would fund projects approved by the SAB and the Gates Foundation.

Requests for research-grant funding during the first year of the proposed center will be solicited from participants at this experts’ meeting and reviewed by the advisory board. Solicitation of a proposal is not a guarantee of funding, and not all applications will be approved. Selected proposals will be recommended for inclusion in the application to the Gates Foundation for the proposed center. Not all proposals that are recommended for inclusion in the application will be funded. If the Center is funded, a formal request for a detailed research plan and budget will be requested from among the selected proposals, and project funding will be contingent on final review and approval by the SAB. We hope to fund 4 or 5 Pilot projects, and 2 or 3 Foundation projects during the first year.

If the proposed research center is funded, it will be necessary to establish a process for solicitation of proposals to maintain a pipeline of new research projects for subsequent years of funding. While we expect that ONPRC/OHSU investigators will generate many research proposals, we want to ensure that the best science is funded by the center. For this reason, it will be necessary to announce a call for research proposals early in the first year of the proposed center. Intramural and extramural investigators will compete for the available research dollars. The scientific advisory board will review and approve all applications for funding. To encourage collaboration, funded investigators will attend an annual meeting to discuss projects.

Three different research funding opportunities will be proposed. Pilot projects, up to $100,000, will fund early-phase research; these projects will be completed within one year. It is proposed that 3-5 pilot projects be funded each year. Foundational projects will receive up to $200,000 per year for up to two years; these projects will typically involve the use of nonhuman primates at ONPRC. The proposed
research center will offer a partial subsidy to the cost of the animal studies. Proof-of-concept projects will receive up to $350,000 per year for up to two years. Pilot projects may include laboratory-intensive research into key pathways, using existing tissues. These projects will use small numbers of animals; the use of nonhuman primates is encouraged, where appropriate and relevant, but not required. Pilot project funds may also be used to fund perception and acceptability studies, or bioengineering solutions for method delivery or verification of tubal occlusion. Foundational projects will generally use nonhuman primate models at ONPRC. These projects may involve in vivo testing of approaches identified in pilot projects, dose-finding studies, or refinement of equipment or delivery systems. Typically, these projects will involve short-term (up to six months) animal assignments, will involve comprehensive histological analyses, and will last up to two years. The proof-of-concept projects will also be funded over two years. These studies will generally involve a contraception study using either macaques (ONPRC) or baboons (Southwest National Primate Research Center [SNPRC]).

**Animal resources:** Ov Slayden, Ph.D.

The ONPRC has 5 species of nonhuman primates (4500 animals) and a long history of developing and maintaining research cores. Existing cores include obesity, contraception, and the Specialized Cooperative Centers Program in Reproduction and Infertility Research (female hyperandrogenism, diet, female reproductive health). Several investigators, both intramural and extramural) can take advantage of the resources in each core simultaneously. The use of nonhuman primates allows investigators to perform experiments that would not be possible in women (e.g., novel techniques, new chemical entities, tissue removal at discrete time points). However, if the research would be appropriate for human subjects, the use of nonhuman primates would not be preferred.

Rhesus macaques are the primary breeding colony at ONPRC. Their reproductive biology is well understood, including the use of assisted reproductive technology, and they have similar menstrual cycles to humans (but are seasonal breeders). However, vaginal swabbing for menses is needed to monitor cyclicity in group-housed animals. Cynomolgus macaques are successfully used for contraception studies at ONPRC, and there are investigators at the center currently using this model. These animals have the advantage over rhesus macaques of year-round cycling, but they also require vaginal swabbing to detect menses when group housed, and are a little smaller than the rhesus macaques. Baboons are a good model for endometriosis and studies that involve transcervical procedures. Their overt cycle-related sex-skin changes obviate the need for vaginal swabbing. Currently, there are few baboons at ONPRC and no breeding groups. However, our group has conducted experiments with baboons at Southwest National Primate Research Center, and are very familiar with this model.

Nonhuman primates are the best model for preclinical contraceptive research and development work, as they have menstrual cycles and uterine anatomy similar to women. Although less is known about the cycle of the fallopian tube compared with that of the endometrium, the tubal anatomy of nonhuman primates is the best model for women. Domestic species such as the cow, sheep, goat, and rabbit have a very different uterotubal junction and uterine anatomy. Specifically, they lack the intramural portion of the tube that is most vulnerable to damage from sclerosing agents. Nonhuman primates can also be used in research designs requiring surgical interventions that would not be
acceptable in humans. For example, it is possible to remove a portion of the reproductive tract after administration of a novel treatment, and then remove the remaining tissue at a second time point.

Anatomy dictates the kind of studies that can be performed in different nonhuman primate species. For example, the macaque model has a 50% failure rate for transcervical approaches. This is due to the presence of a cervical colliculum that creates a tortuous cervical canal. In contrast, transcervical procedures are relatively simple in baboons due to a straight cervical canal similar to that seen in women.

The animal resource committee considered that since most research ideas will likely require a transcervical approach, the best nonhuman model for these types of studies would be the baboon. Therefore, the recommendation was to expand the baboon resources at ONPRC. Providing a limited (i.e., 20–30 animals) colony of baboons for pilot- and foundation level projects would allow investigators to take advantage of the scientific, technical, and infrastructure support available at ONPRC. While we have developed a successful collaboration with SNRPC, repeated travel to work with their baboons for short-term projects presents logistical difficulties and complicates experimental design, particularly when repeat sampling is needed. The animal models needed for each study will depend on the focus of each project. When rhesus or cynomolgus macaques are appropriate, they will be recommended. An advantage of rhesus is that they are a supported species at ONPRC, and an established breeding program exists at the center. It would not be appropriate or cost-effective to establish a breeding colony for baboons at ONPRC, so animals would need to be obtained from other centers and transported to ONPRC as needed. We have investigated this capacity, and have determined that it is feasible to bring in groups of baboons in sufficient numbers to meet the anticipated demand of the proposed research center.

For proof-of-concept contraceptive studies, breeding groups of socially-housed females, both treated and controls will be established, and a fertile male will be introduced. This model has been successfully used in studies using cynomolgus macaques at ONPRC and baboons at SNPRC. It would not be appropriate to develop breeding harems of baboons at ONPRC for proof-of-concept studies, as this resource is well established at Southwest. In contrast to the intensive early-phase work, the travel needed to set up the contraceptive experiment is more limited. Therefore, it is most appropriate to recommend that contraceptive experiments that will use baboons be performed at SNPRC. If a Transcervical approach is not needed for the experiment, it would be possible to use the existing Nonhuman Primate Contraception Core cynomolgus macaques.

Scientific support: Jon Hennebold, Ph.D.

Dr. Hennebold introduced the members of this committee, noting that they provided expertise from reproductive and developmental sciences; neuroscience; the ONPRC collaborative resource program; obstetrics and gynecology; the ONPRC obesity resource program; and the diabetes, obesity, and metabolism division. The objective of the committee is recommended what kind of scientific and technical-support expertise is needed to ensure that the research objectives of the proposed center are met. The committee has assessed the need for dedicated staff scientists, support staff, and administrative personnel to support the center’s research activity. Two factors in determining the
The required level of scientific support are the animals needed (species and number) and the expertise required to manage and oversee different projects.

The committee recommended funding for four key personnel. A **scientific support manager** (PhD or DVM with postdoc training/significant expertise in reproductive physiology) will oversee individual projects at ONPRC and act as the primary liaison between the center and individual projects. This person will be involved with regulatory affairs (Institutional Animal Care and Use Committee compliance), coordinate and monitor day-to-day research activities, communicate with the division of Comparative Medicine to ensure the availability of animals and housing, and supervise the support staff. The **animal resource research assistant** (BS in a relevant field, experience with animal models) will conduct the daily activities and specific tasks of each research project. The **cell biology/histology research assistant** will work with the project principal investigators to ensure successful collection and processing of specimens. They will be responsible for microscopy, immunohistochemistry, and histology, verifying and confirming findings based on histologic and morphologic endpoints. The **administrative coordinator** is already in place at ONPCC: Keri Brown, who coordinated this conference. She will prepare reports and manuscripts, handle applications, and manage accounts and expenses.

In addition to these key personnel, the proposed research center will also have a **Principal Investigator** (Dr. Jensen). The PI will manage the overall center and provide oversight to the **scientific support manager** and to the **administrative coordinator**. The PI will also organize meeting s of the Scientific Advisory Board and an annual investigator meeting, solicit requests for research proposals from intramural investigators, communicate with extramural investigators, and produce and submit an annual progress report to the Foundation.

The scientific staff of the new center will work closely with the division of Comparative Medicine at ONPRC to ensure the health and well-being of the animals.

**Southwest National Primate Research Center: Cassondra Bauer, MS, DVM**

Dr. Bauer provided a presentation on baboon husbandry and housing. Baboons are larger and stronger than macaques, and require stronger cages for housing and transportation. There are currently 1500 baboons at SNPRC. An adult female weighs 14–18 kg (sex-skin changes can affect the weight), and a male weighs 25–35 kg. Females mature at 4–6 years of age, and males mature at 5–6 years of age. Females are bred when they are about 6 years old. The gestational period is 185 days, and premature infants born at 160–165 days tend to survive. Babies are weaned at 5–6 months and taken off their mothers at 9 months of age. Tattoos and neck-chain tags are used to identify the animals. Age groups are divided into Infant (up to 9 months), juvenile (9 months to 3–4 years), young adult (4–6 years), adult (6-16 years), and geriatric (over 16 years).

The appearance of the female’s sex skin is very characteristic of the reproductive-cycle stage. Baboons have a straight cervix and a single discoid placenta. Following delivery, they are able to conceive again on the first postpartum cycle. Single births are typical; twinning is possible but rare. Baboons are kept in gang cages, and harem breeding is employed. Vasectomized males are placed in groups of females to prevent fighting and maintain social order. Baboons have distinct social groups and a hierarchy structure.
The Southwest center has an on-site clinical pathology laboratory and necropsy suite, incubators, and a nursery. They have X-ray (digital, traditional, and fluoroscopy) capability and an ultrasound room, treatment/procedure rooms, and surgical suites.

**Infrastructure: Jeff Jensen, MD, MPH**

The infrastructure committee was tasked with developing a plan for capital investments needed for the proposed center. The rationale for facility upgrades is that space at ONPRC is limited and currently prioritized for National Institutes of Health (NIH) projects. Funding of infrastructure improvements would provide a core resource dedicated to Gates-funded ONPCC research.

Three major facility needs were identified. (1) Baboon housing: to provide the baboon resource suggested by the animal committee, facilities for housing and quarantine are needed. (2) Imaging and procedure room capacity: space and radiology equipment suitable for transcervical procedures in baboons must be developed and installed. (3) Dedicated histology processing capability: since projects will be histology intensive, additional resources are needed so that results can be provided to investigators without delay.

Dr. Jensen noted that space currently exists at ONPRC that is suitable to house baboons in the colony annex building, with adjacent space available that could be modified into a procedure room. Although the caging and quarantine facilities will need to be altered, no new construction would be required. Modifications to the procedure room would be needed to allow for the use of x-ray equipment that will support the anticipated experiments. Although shared radiologic facilities currently exist at ONPRC, these are not convenient to the proposed baboon housing area. The transportation of baboons would increase the stress on the animals and put human handlers at risk for injury. In addition to structural modifications, equipment needed in the proposed procedure room would include a radiology table, digital x-ray machine, fluoroscopy, and ultrasound. An anesthesia machine would also be required.

The existing ONPRC histology core is currently operating under a backlog of several weeks. To allow timely completion of projects, the proposed center would need a dedicated histology core. Space is available for a Gates-funded histology core in the Neurological Sciences Institute (NSI) building, adjacent to Dr. Jensen’s current laboratory, but a tissue processer, paraffin embedder, and digital microscope will be needed.

In addition to the major facility needs, laboratory and work space for scientific support staff and extramural investigators will be needed. The committee reviewed existing space at ONPRC to determine what could be modified and made available to outside investigators. The goal is to avoid duplicating existing services that can be shared, so that the majority of funds can be used for research projects. Molecular biology, microscopy and advanced imaging, endocrine, and pathology spaces and services can be shared. There are also common spaces that can be shared by outside investigators. Office space for the scientific support staff will be available in the Slayden and Jensen labs.
SESSION 12.2: Discussion and Brainstorming Activity: Setting a Research Agenda for the Center Grant Application

Dr. Jensen introduced the goals of the discussion and brainstorming session. First, to review the research ideas presented during the conference; and second, to prioritize projects and identify up to three initial pilot projects, two foundational projects, and one proof-of-concept study to be included in the proposed center application.

Once approved, the center will request proposals for hypothesis-driven experimental designs. At this point, none of the projects presented during the two-day experts’ meeting on permanent contraception are ready to proceed to the clinical-trial phase. Although the goal of the center is to develop one or more products that will progress into clinical trial, the purpose of the proposed ONPCC will be to conduct preclinical investigations of novel strategies for nonsurgical permanent contraception that will be applicable to women. A variety of funding mechanisms will encourage both very early-phase and more mature projects. Successful early-phase (pilot) projects will be eligible to receive additional support as Phase 2 and Phase 3 projects.

In the general discussion, participants agreed that the proposed scientific and animal resources would be attractive to outside investigators. The proposal for a dedicated histology core was endorsed, although the addition of a dedicated pathologist was discussed by some participants. Since the cost of a pathologist would be substantial, and as this expertise does exist at ONPRC, this was not recommended for funding. Funds for a histology technician and a digital scanning microscope would allow for timely review by project principal investigators. Research funds could also be used for salary support for outside expertise when needed. The availability of a dedicated staff scientist, research assistant, and administrative coordinator for the proposed center was also viewed as a strength.

Questions regarding the center proposal included:

When we talk about permanent contraception, does this exclude methods that are highly effective and very long-acting but potentially reversible? The advent of highly effective long acting reversible methods such as the levonorgestrel intrauterine system demonstrates the acceptability of a potentially reversible approach. Five or 10 years would not be considered a permanent method, but it was discussed that a method such as the microchip technology with a 20 year period of use could be considered a “reversible” permanent method. The advisory board was encouraged to consider specific criteria to evaluate products that might fit into this category. However, Dr. Jensen reminded the group that with the rapid changes in infertility treatment, even tubal occlusion is “potentially” reversible with IVF.

Would all research funds need to be used at ONPRC? The proposed center will be a coordinating center for research activity and for distributing grant money. Outside investigators would be able to have grant funds used directly at their home center, at ONPRC, or at SNPRC. Since core resources and scientific support will be available at ONPRC, it may make sense for many investigators to perform experiments at ONPRC. As the cost of animals and scientific support will be subsidized by the center, research money may go further if projects are done here. The primary reason investigators will consider performing experiments at ONPRC will be the availability of the nonhuman primate resource. It is also important to understand that the limitations of the Bill & Melinda Gates Foundation on indirect cost recovery will apply to all awards. Support for direct research costs, supplies, and salaries are allowable.
Open discussion of projects presented during the conference

**Quinacrine sterilization (QS).** There was some concern that there might be too much controversy surrounding the previous clinical trials with QS to allow for the development of a viable product. Although toxicity remained a concern for many participants, others focused on efficacy and the need for making QS a single treatment. However, any proposed clinical projects with the existing strategy for QS would not be appropriate for the proposed ONPCC. The current QS project is ready for human phase 3 trials if the current US FDA clinical hold is lifted. But the question remains, is there anything about quinacrine that should be reexamined in an animal model? For example, could it be paired with the biomaterials proposed by Dr. Woodrow to reduce the dose and improve efficacy? Could it be coupled with an adjuvant inflammatory component such as discussed by Dr. Hennebold? We are still not 100% certain what quinacrine is doing at the molecular and cellular level. Should we investigate potential acetylcholine-receptor binding? Can we determine whether it triggers apoptosis and then investigate a mimic—this would be a pilot project. Would the use of DMPA allow for a single-treatment approach? Should these concepts be studied in baboons? Several participants commented that further research with quinacrine is not advisable as the controversies would likely limit the prospects of regulatory approval. There was discussion that alternative molecules should be used for further study of mechanism of action.

Mechanisms of cell death seem to be critical to ongoing research into permanent contraception. Much of the discussion during this conference has been around denuding the epithelium to allow stromal fusion and binding. Can the technique of QS administration be altered to lower exposure? Currently, all of the medication except for the small portion that enters the intramural tube is wasted. This would apply to many drugs, not just quinacrine.

**Polidocanol.** Additional work is needed on polidocanol. We don’t know why multiple dosing is required for tubal occlusion, even though tubal epithelial damage occurs with a single dose. We have to sort out the mechanism of repair after single dosing to learn how this can be inhibited to favor permanent occlusion. There are several possible experimental designs. All of these will require the ability to carefully time treatment and to obtain tissue samples at a variety of time points. Since the goal is a single treatment, the first priority should be to work on treatment conditions and co-treatments that will encourage initial epithelial damage, prevent re-epithelialization, and encourage collagen deposition. The initial results with PF and DMPA are very encouraging. We need to perform more detailed experiments to better understand the mechanism of this combination. For example, could another progestin work as well? Is inhibition of ovulation or the modest glucocorticoid-receptor action of DMPA a necessary part of the effect?

Although 5% PF results in tubal occlusion, this concentration is higher than the 1% PF currently FDA-approved for vein treatment. Can the concentration or dose be reduced to improve safety? A balloon-catheter system that would restrict delivery of PF to the cornual region, so as to limit exposure to the rest of the uterine cavity and thereby limit vascular uptake, would be a valuable advancement. A participant asked whether a biomaterial could be used to deliver polidocanol in a time-release fashion, but since experiments with 1% polidocanol solution did not result in tubal occlusion, this research
avenue does not seem promising. Although higher concentrations of polidocanol solution have not been studied, the mechanism of action requires that the compound enter the fallopian tube, and is not clear whether this would occur with slow release in the uterine cavity.

**Acceptability Studies.** The initial perception studies done in India and Portland done in conjunction with the PF research, and the issues surrounding QS, point to the need for additional acceptability studies of the concept of nonsurgical permanent contraception. For example, with the use of DMPA and PF, there may be religious or cultural aspects that will influence acceptance of this method. India has low acceptance of DMPA but high acceptance of sterilization, while subSaharan Africa there is high uptake of DMPA but low acceptance of permanent contraception. Further work on acceptability, perceptions and the target product profile are needed, but it was discussed that the proposed research center will not have expertise in all of these areas. It might be better to fund groups (like the Population Council) that already have experience with these types of studies. The funding might come from the proposed center, however.

**Evaluations of tubal patency.** A low-cost system is needed for verification of tubal occlusion, at least in the product development and early introduction phases to provide reassurance. Ideally, a final method would be reliable enough to not require this verification, but this will require Phase IV evidence. If uterine filling pressure measurement could be used for screening, only a subset of patients would need to have either hysterosalpingography (HSG) or more advanced imaging. This has the potential for significant resource savings. Further research is warranted in this area.

**Biomaterials.** The concept of biomaterials that could provide timed release of one or more drugs was viewed as very promising. How to deliver these materials becomes a problem. Can they be placed in the uterine cavity safely? Would a hysteroscope be needed? What types of projects and pairings could be developed, with the goal of inducing inflammation and inhibiting repair? The nitinol intrauterine system has polymer tips that could potentially deliver a drug to the tubal ostia, but currently there is no way to release a bolus of fluid or foam. Could the polymer tips release another product that would scar the tubal ostia? Perhaps the copper wire on the nitinol intrauterine device (IUD) could be replaced with drug-delivery fibers. There is a lot of good research potential here. Several early-phase projects could later be merged.

**Magnetic nanoparticles.** The initial results presented were discouraging as tissue changes were not seen with heating. Further work on this model is needed to determine whether this concept is applicable to permanent contraception. There is potential to further investigate antibody-linked magnetic nanoparticles, although the early results do not support the possibility of a systemic delivery approach. One major concern is that no thermal method alone has ever shown the ability to reliably block the tubes. Therefore, can hyperthermia from magnetic nanoparticles be combined with other strategies to make those products work better or penetrate further? A large quantity of reagent would be needed for systemic delivery; less would be needed for intrauterine infusion. We need to know which
direction to go so we know how to synthesize the particles, and whether it would be possible to scale this up at an industrial level. There was concern raised that regulatory approval may be difficult to obtain for the use of magnetic nanoparticles for ablation, but it was countered that magnetic nanoparticles are currently approved as imaging reagents. Other concepts discussed included the use of particles to release an active agent upon heating for targeted drug delivery. Further research is needed to optimize particle size and to develop a practical approach for generating a radiofrequency field to heat particles in the uterus and tubes.

There are limitations to our understanding of the biology of tubal damage with heating. For instance, does a burn need to reach a depth of 2–3 mm to be effective for permanent tubal occlusion? In addition, we do not currently understand how to effectively prevent tubal reproliferation in favor of collagen deposition.

**Inflammation and healing.** What is the state of knowledge regarding inflammatory components in the oviduct? Which toll-like receptors (TLRs) and inflammasomes are present? Basic research to determine the best targets might be a wise use of pilot funds. We should determine the function of any unique proteins and figure out how they might be manipulated to occlude the fallopian tube. This approach was enthusiastically endorsed as a promising area for research.

It is almost impossible to create a hydrosalpinx in nonhuman primates, but the phenomenon is very common in human patients undergoing in vitro fertilization. Removing the hydrosalpinx has a positive effect on fertility success. Is there an inflammatory component or something in the hydrosalpinx fluid that inhibits tubal function or is toxic to gametes? Perhaps a factor could be used to kill epithelial cells and create scarring. What is the role of inflammation in epithelial damage and repair?

A suggestion was made that lipid-mediated responses for chronic wound healing should be investigated. This could lead to better scarring methods. It was pointed out that bleomycin-induced fibrosis in the lung creates massive collagen and extracellular matrix (ECM) deposition. Can we induce that response? What is the optimal way to achieve this response? We already have a good model in Essure; nothing has yet been as successful. We could look at different time periods after Essure placement and measure the receptors present. We could then look at PF and the other agents of interest to see if they induce those levels of receptors. Will any occlusion be successful long-term without something sitting in the tube to continue the tissue response and prevent recanalization? The question was raised as to whether this research would better be conducted in humans (with hysterectomy tissue) rather than nonhuman primates. The concern, however, is that hysterectomy patients usually have other pathology that could affect the tube and compromise the purity of the tubal environment. Additionally, nonhuman primates can be used to obtain samples from multiple timepoints in the same animal.

As we want to impact the field quickly, we need to know whether we have enough information on TLRs and cytokines to begin research into how to stimulate them. Should we investigate adjuvants, such as alum, paired with a biomaterial and the nitinol wire for delivery? Transforming growth factor beta (TGFβ) is a strong fibrotic factor; perhaps in vitro culture systems could be used to investigate this fibrotic model, but we don’t know if there is any good in vitro system for investigating fallopian-tube function. Fallopian epithelial cultures grown on a flat dish do not replicate normal tubal behavior.
(oriented toward a lumen); other culture systems need to be investigated. There are three-dimensional skin and vaginal models on the market; we could let companies know that there is a potential market for tubal/uterine models.

**Other Research.** There was a suggestion that additional research investigating the electrophoretic pattern of oviductal fluid (monkeys vs. humans) is needed to identify unique proteins in human oviductal fluid that are not present in serum. This led to a discussion on whether the proposed center should support investigations into basic tubal physiology or focus on more developed leads. Some participants felt that this center should not be for basic science, while others felt that basic science will lead to the development of contraceptive applications. The decision of what projects to fund will ultimately be made by the advisory board after consulting with Gates Foundation program staff.

**Key Ideas.** We need to establish a model in which we can compare new products or agents. For example, could we develop a tissue culture system suitable for screening agents for the ability to damage tubal epithelium and promote collagen deposition? This would improve the pace of research and reduce the need to perform experiments in whole animals.

We need to know more about the effects of thermal injury. A preliminary study could compare thermal with chemical burns in an animal model and evaluate the tissue responses, determining how deep the burn needs to penetrate. This might help us learn more about responses that favor normal repair versus scar formation. Establishing a benchmark might lead to a systematic method to evaluate agents such as PF and learn if the outcomes are comparable or different.

The main mandate of this conference is to identify new research ideas and collaborations to develop nonsurgical permanent contraception for women. There still seem to be gaps concerning the mechanism of action of molecules that have been shown to be useful so far, and what new agents could be promising. We need to know more about the best mode of administration and drug delivery system? The availability of an appropriate research center with dedicated facilities and scientific expertise to advance research in the field would be useful. Developing collaborations between basic and clinical investigators will be needed.

Looking at inflammation we realize that we need to know more about the common pathways and how these can be exploited to cause localized tissue damage followed by collagen deposition. The use of adjuvants and immune stimulating factors such as chlamydia heat-shock protein are novel approaches. The new biomaterials offer promise of an effective delivery system. There are opportunities to test the time-release biospun materials and possibly use the nitinol wires for focal delivery of these agents. These are potential high-priority projects.

**Devices.** The concern was raised that any product developed should be pain-free and should have a safety and side effect profile no worse than current permanent methods. Reversible inhibition of sperm under guidance (RISUG), thermal ablation, and nitinyl expansion wires with an occlusive polymer that does not damage the tube are all potentially viable in low-resource settings and should be studied further. A technique that only requires a single intervention would obviously be best.
limitation of female RISUG is the need to deliver the polymer through a hysteroscope. Most meeting participants agreed that a nonsurgical approach should not involve the need for a hysteroscope.

Concerns were expressed about the low-cost endoscope. First, do techniques using this endoscope have an advantage over Essure? Operative intervention can be a problem in low-resource settings. Instrumentation, even using a “simple” model, is always more of a problem than giving someone an injection. It might be better to focus on methods that do not require hysteroscopy.

There was not a lot of optimism expressed for the silicone tubal plug proposed by Dr. Wang, as the risk of spontaneous expulsion was high. Since the method was not designed to cause permanent occlusion, there is no contraceptive effect if expulsion occurs. The need for hysteroscopy is another limitation.

The prospects for development of a very long implant were greeted with enthusiasm, but concerns were voiced over the acceptability of the device given the relatively large size. However, it was agreed that a method that could last 20 plus years could be considered “permanent”, and that the ability of a method to have easy reversibility would not rule out funding as a permanent method at the proposed center.

**Implementation science.** Karen Katz presented the Family Health International 360 (FHI 360) experience with an advisory board, composed of members of the nonscientist community and healthcare advocates, during studies with QS. Participants felt that it would be premature to invite nonscientists to the advisory board of the ONPCC. However, additional research evaluating acceptability and behavioral studies should accompany the ongoing product development to ensure that money is not wasted on products with low potential for impact (low acceptability or deliverability).

Participants felt that although lay advisory board members could be useful, it is more important to find advisors who understand the work that we are doing. We do not want to limit innovation because someone is worried about the implication in a particular country or culture. But it is important to be sure we have an audience for the product and that it meets women’s’ needs. Some focused behavioral research would be helpful in guiding our projects. We might need to do more work on preliminary perceptions and cultural acceptability, or that work might be better for a different funding source. We could consider collaborating with organizations that get funding to do that work, such as the Population Council.

**How do we request research proposals from the broader scientific community?** How do we get the best research in the world and have a broad number of projects to choose from? If the proposed center is funded, the mechanism to request research proposals should target societies related to reproduction, physiology, and toxicology (e.g., Society for the Study of Reproduction, Society for Gynecologic Investigation). These associations have newsletters in which we could advertise. Another possibility is using the Gates Foundation as a conduit, sending out an announcement to their stakeholders (similar to their grand challenge announcements). A web site for the Center will need to be developed and maintained. It was suggested that FHI 360 would be a useful model to emulate, as they have a similar Gates-funded center devoted to long-acting contraceptive methods. We should also try
to reach non-traditional sectors: Materials scientists, biomedical engineers, etc., not just reproductive biologists. We need to attract smart people who can problem solve or we run the risk of not getting any new thinking/ideas

A web-based system for submission of applications for funding was discussed. One concern is that we run the risk of inundation. It will be important to be very clear about what is in scope and what is out of scope for the research proposals. A suggestion was made that the advisory board determine two or three high-priority areas for requests for applications; we can then solicit preliminary abstracts or single-page, focused concept papers in these areas. A web-based format would allow us to limit the length and/or word count of submissions and would allow for an organized, standardized submission format. We would ask for estimations on cost, length of development, and ease of use. The advisory board will ultimately make the decision on projects, guided by input from the Gates Foundation. It was pointed out that the Bill & Melinda Gates Foundation will likely want to invest mainly in applied projects and highly focused basic science directly related to product development.

At the conclusion of the discussion, Dr. Jensen mentioned that the advisory group will put together ideas for first-year projects to move forward. He invited meeting participants to submit brief research proposals to be considered for first-year funding.

Thank you to everyone for attending and participating.
# OHSU Permanent Contraception Conference Summary

## Index of Abbreviations

Compiled by Julie Quinn, MD, ELS

Definitions redefined in each session

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANDA</td>
<td>Abbreviated new drug administration (application)</td>
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<tr>
<td>BrdU</td>
<td>Bromodeoxyctydine</td>
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<tr>
<td>CDER</td>
<td>Center for Drug Evaluation and Research</td>
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<td>CDRH</td>
<td>Center for Device and Radiological Health</td>
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<tr>
<td>cHSP</td>
<td>Chlamydial heat shock protein</td>
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<td>CMC</td>
<td>Critical micelle concentration</td>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<td>CREST</td>
<td>Collaborative review of sterilization</td>
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<td>DMPA</td>
<td>Depo medroxyprogesterone acetate</td>
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<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
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<td>EB</td>
<td>Elementary body</td>
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<td>ECM</td>
<td>Extracellular matrix</td>
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<td>EU</td>
<td>European Union</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<td>FHI 360</td>
<td>Family Health International 360</td>
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<td>FSH</td>
<td>Follicle stimulating hormone</td>
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<td>GnRH</td>
<td>Gonadotropin releasing hormone</td>
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HCG  Human chorionic gonadotropin
HIV  Human immunodeficiency virus
HSG  Hysterosalpingogram/hysterosalpingography
IDE  Investigational drug (application)
IL  Interleukin
IND  Investigational new drug (application)
ISAF  International Services Assistance Fund
ITD  Intratubal device
IUD  Intrauterine device
LH  Luteinizing hormone
MMP  Matrix metalloproteinase
MNP  Magnetic nanoparticle
MTD  Maximum tolerated dose
NDA  New drug application
NICHD  National Institute of Child Health and Human Development
NIH  National Institutes of Health
NLR  Nod-like receptor
OHSU  Oregon Health and Science University
ONPCC  Oregon Nonsurgical Permanent Contraception Center
ONPRC  Oregon National Primate Research Center
OVGP  Oviductal glycoprotein
PAMP  Pathogen-associated molecular pattern
<table>
<thead>
<tr>
<th>PID</th>
<th>Pelvic inflammatory disease</th>
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<tr>
<td>PF</td>
<td>Polidocanol foam</td>
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<td>PMA</td>
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<td>PRR</td>
<td>Pattern recognition receptor</td>
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<td>PTH</td>
<td>Parathyroid hormone</td>
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<tr>
<td>QS</td>
<td>Quinacrine sterilization</td>
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<tr>
<td>RB</td>
<td>Reticulate body</td>
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<tr>
<td>RISUG</td>
<td>Reversible inhibition of sperm under guidance</td>
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<tr>
<td>SCID</td>
<td>Severe combined immunodeficiency</td>
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<tr>
<td>SMA</td>
<td>Shape memory metal alloy</td>
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<tr>
<td>SMA</td>
<td>Styrene maleic anhydride</td>
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<td>SNPRC</td>
<td>Southwest National Primate Research Center</td>
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<td>STD</td>
<td>Sexually transmitted disease</td>
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<td>TGFβ</td>
<td>Transforming growth factor beta</td>
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<td>Toll-like receptor</td>
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<td>Tumor necrosis factor alpha</td>
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