Ovarian Reserve: Regulation and Implications for Women's Health. Proceedings of the 2012 NICHD-ASRM Conference

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Abbreviations

ASRM	American Society for Reproductive Medicine
AMH	Anti-mullerian hormone
AFC	Antral follicle count
FSH	Follicle stimulating hormone
NICHD	National Institutes of Child Health and Human Development
NGF	Non-growing follicles
OR	Ovarian reserve
PCOS	Polycystic ovary syndrome
POI	Primary ovarian insufficiency

Capsule Proceedings from workshop on the ovarian reserve summarizing latest advances and remaining obstacles in the detection management, and treatment of health disorders tied to loss of the follicle reserve.

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Purpose

Only a tiny fraction of the tens of thousands of ovarian primordial follicles initially present at birth will survive to mature, produce vital hormones and ovulate. In theory, snapshots of this remaining pool of ovarian follicles, the ovarian reserve (OR), could provide important insights for the assessment of ovarian function, and indicate a woman's maximum reproductive potential and the time remaining until menopause, with critical implications for her fertility and overall health. However, the biomarkers of ovarian reserve that are currently available in the clinic cannot be used to make such predictions.

To help advance the state of this science from theory to practice, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) and the American Society for Reproductive Medicine (ASRM) joined forces for the first time to present a satellite conference to the 2012 ASRM annual meeting. We hope that this conference, "Ovarian Reserve: Regulation and Implications for Women's Health", held October 25, 2012 in San Diego, California, will set the stage for future ASRM-NICHD co-sponsored workshops on emerging reproductive health issues.

Overview

At the opening of the conference, remarks from Dr. Robert Rebar, ASRM, and Dr. Charisee Lamar, NICHD, challenged

experts in reproductive science and reproductive medicine to outline the state of the science, identify gaps in the field, and propose future research opportunities. Two keynote speakers set the stage. Dr. Roger Gosden reviewed the history of our understanding of ovarian reserve, and forecasted the remaining challenges in terms of overarching paradigms. Dr. Marcelle Cedars then provided a clinical update on the practice and challenges in measuring and using ovarian reserve to treat women. Four scientific sessions followed: Establishment and Regulation of the Primordial Follicle Pool, Measurement of Ovarian Reserve, Clinical Considerations for Ovarian Reserve, and Advances on the Horizon. The day culminated with all 120 participants discussing the most pressing pre-clinical and clinical issues in ovarian reserve, and contributing ideas on the most fruitful and important areas to pursue with further research.

This paper summarizes the proceedings of the day, with the speaker summaries followed by synopses of breakout discussion sessions. We conclude with a list of recommendations that we hope will guide future policy making and funding for research into this significant public health problem.

Speaker Abstracts

Keynote 1: On Ovarian Reserve

Roger G. Gosden, PhD

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Since von Baer discovered the mammalian oocyte, research has revealed great subtlety in the molecular and physiological regulation of oogenesis, folliculogenesis, follicular activation and growth. For over 60 years, it has been generally held that the ovarian follicle reserve is finite, non-renewable after birth, and progressively depleted with age. Although new evidence of post-natal germ line stem cells has emerged, primordial follicle recruitment for growth, or atresia as their alternate fate, determines the functional lifespan of the ovary and, hence, the timing of menopause. Follicle recruitment into the growing pool is evidently restrained by the PI3K signaling pathway to avoid premature depletion of the reserve, but the factors responsible for activation are poorly known and require more research. Unlike the secondary reserve of FSH-dependent antral follicles, no reliable biomarkers exist for the primordial stage, which is a non-growing population, highly variable in number even for a given age, as well as endocrinologically ‘occult’. Estimating the size of the primary reserve has depended on extrapolation from other biomarkers, yet a direct method that is robust and non-invasive would likely have great clinical utility for monitoring fecundity during the reproductive lifespan. **CONCLUSION: Since the functional ovarian lifespan is determined by follicular survivorship**

and the rate of recruitment from the primary reserve, clinical benefit will emerge from a better understanding of primordial follicle biology and the discovery of reliable biomarkers.

Keynote 2: State of the Science in Clinical Ovarian Reserve Research

Marcelle Cedars, MD

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Clinical studies on the ovarian reserve have for the most part focused on the aging ovary in the context how assisted reproduction—how many eggs are anticipated and how best to treat the ovary for optimum clinical care. In principle, the relationship between ovarian age and follicle loss is easy to conceptualize but the accuracy and reliability of currently available methods (AFC, AMH) may not be adequate to assess the gradual decline in follicle quantity due to the challenge of identifying biomarkers for the non-growing follicle (NGF) pool size. This presentation draws attention to deficiencies in the predictive value of some tests for OR and to the fact that some measures (e.g. FSH) are indirect and together fail to distinguish populations of women ranging from fertile to infertile and/or the slow losers or fast losers. Even with the available measures for OR, the cause of age-related loss in oocyte quality, which likely contributes to decreasing fertility and increasing pregnancy loss, has not been determined and no direct correlation has been shown between lower AFCs/AMH and increased rates of aneuploidy. Given this background, there is a pressing need to develop reliable biomarkers and to understand the implications for any *individual* woman with respect to natural fecundity and long-term health. **CONCLUSION: Currently available biomarkers for the OR are insufficient as predictors of fertility potential or advancement to menopause.**

Session I: Establishment and Regulation of the Primordial Follicle Pool

Facilitator: Lawrence Nelson, MD, NICHD

Mouse Models for the Study of Primordial Follicle Maintenance, Diego H. Castrillon, MD, PhD, University of Texas Southwestern Medical Center, Dallas TX

The mechanisms regulating early ovarian follicle growth and the utilization of the primordial follicle pool—particularly primordial follicle activation (i.e. the primordial-to-primary follicle transition)—were until recently, largely unknown. In the past 10 years, mouse models have provided substantial mechanistic insights into the nature of these molecular pathways. The Forkhead transcription factor(Foxo3) is a key factor

whose activation state and subcellular localization regulates primordial follicle activation; when Foxo3 is active and nuclear, primordial follicle activation is restrained. In mice, inactivation of Foxo3 either by direct genetic ablation, or indirectly by inactivation of Pten (which leads to PI3K/AKT activation, Foxo3 hyperphosphorylation, and relocalization of Foxo3 protein from the nucleus to the cytoplasm) triggers activation of all primordial follicles, leading to a dramatic syndrome of premature reproductive senescence. Other studies have shown that genetic inactivation of TSC1/2, critical regulators of the MTOR pathway that interacts with the PI3K/AKT pathway at multiple levels, also results in global primordial follicle activation. **CONCLUSION: The mechanisms that maintain the primordial follicle pool are essential to understanding both normal ovarian aging and female infertility syndromes due to the accelerated depletion of primordial follicles (i.e. primary ovarian insufficiency and primary amenorrhea). Genetic mouse models have revealed that the PI3K/FOXO and TSC/MTOR constitute the central pathway regulating primordial follicle activation, and hence, the utilization of the primordial follicles during reproductive life. For a variety of reasons, including the impracticability of obtaining human ovarian tissue for research, mouse models will continue to provide unique opportunities to elucidate the molecular mechanisms that regulate the utilization (and longevity) of the primordial follicle pool.**

In Vitro Activation of Ovarian Follicles

Aaron Hsueh, PhD

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Although multiple follicles are present in mammalian ovaries, most of them remain dormant for years or decades. During reproductive life, only a small number of primordial follicles are activated for development. Earlier studies demonstrated that oocyte-specific deletion of the PTEN (Phosphatase with TENsin homology deleted in chromosome 10) gene led to the activation of dormant primordial follicles, resulting in a premature ovarian failure phenotype in mice. Using an inhibitor of PTEN and a PI3K (phosphatidylinositol-3-kinase) stimulating peptide mimicking the phosphorylated intracellular region of the activated PDGF receptor, we found that short-term treatment of neonatal mouse ovaries in vitro increased nuclear exclusion of Foxo3 in oocytes of primordial follicles. After treatment with these in vitro activation drugs, ovaries were transplanted under kidney capsules of ovariectomized hosts and primordial follicles in ovarian grafts were observed to develop to the preovulatory stage. After in vitro fertilization and embryo transfer, healthy progeny with proven fertility were delivered. In addition to the activation of dormant

follicles from neonatal mice, a similar in vitro activation and allo-transplantation protocol also allowed the activation of residual follicles from aging mice at >1 year of age to yield mature oocytes, suggesting this procedure could be useful for senescent ovaries. Using human cortical fragments containing mainly primordial follicles obtained from patients with ovarian tumors, we further demonstrated the utility of this in vitro activation procedure to generate mature human oocytes in xeno-transplants of immune-deficient SCID mice. **CONCLUSION: This in vitro activation (IVA) protocol could be useful for the activation of dormant primordial follicles for infertile patients that lack responsiveness to traditional gonadotropin treatment.**

Primate Follicular Development: Anti-Müllerian Hormone (AMH) Production and Action

Richard L. Stouffer, PhD

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Very little is known about the processes and regulation of early follicle development in primate species. We recently described advances in three-dimensional (3-D) culture that permit the growth of individual primary and secondary follicles from rhesus monkeys to the small (1 mm diameter) antral stage. However, follicle survival, growth and maturation were dependent on a number of factors. Notably, “fast grow” follicles (which at least tripled their diameter) produced significantly more steroid hormones and were more likely to provide a healthy oocyte that could reinitiate meiosis and fertilize in vitro. This model offers novel opportunities for investigating early folliculogenesis in primates, with the information relevant to maintaining or improving fertility in women. Since data from nonprimate species suggest that AMH is an important regulator of early follicular development, and clinicians use circulating AMH levels to estimate “ovarian reserve” in patients, studies were designed to quantitate AMH production by macaque follicles during 3-D culture and to consider whether AMH acted directly on preantral-to-antral follicles. Media levels of AMH were near the limit of detection and remained low throughout culture of “nongrowing follicles”. In contrast, AMH levels were detectable and higher in growing as compared to nongrowing, follicles as early as week 1 of culture, and then peaked at 3–4 weeks at the onset of antrum formation. Pilot studies suggest that AMH added to secondary follicles at the onset of culture reduces the percentage of nongrowing follicles, increases the cohort of growing follicles, but reduces steroidogenesis by antral follicles. **CONCLUSION: Further studies are warranted to examine the apparent stage-specific actions of AMH in primate follicles and its potential value as a marker of early follicle growth/quality.**

Session II: Measurement of Ovarian Reserve

Facilitator: Esther Eisenberg, MD, MPH, NICHD
The Emerging Role of AMH as an Index of the Ovarian Reserve Across the Female Reproductive Lifespan.

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The basis for the value of AMH as a biomarker is that granulosa cells start producing AMH as soon as follicle growth is initiated, and that expression rapidly declines in antral follicles at approximately 8 mm diameter. This means that serum AMH concentrations are distinct from periovulatory events, although it is the smaller antral follicles rather than the preantral follicles that contribute the bulk of circulating concentrations. The field is hampered by the absence of an international standard against which assays can be calibrated but it has become clear that AMH predicts the ovarian response in IVF (the ‘functional’ ovarian reserve) and also reflects the size of the histologically determined primordial follicle count in humans as well as rodents (the ‘true’ ovarian reserve). AMH declines during the later reproductive years, with emerging data investigating the potential for prediction of the menopause. It also appears to reflect increasing ovarian activity in childhood. A small decline at pubertal onset has been confirmed in longitudinal studies, before a secondary rise to a peak in the mid 20s. This changing pattern in AMH concentration can be mapped against the rate at which follicles are lost from the histologically-determined non-growing pool, showing closely associated rises during childhood. This finding is consistent with the idea that most follicles leaving the non-growing pool start growing rather than becoming atretic directly. **CONCLUSION. Recent years have seen rapid emergence of the value of AMH in a number of different clinical contexts. It promises to be of further value in our understanding of ovarian development during childhood and puberty, and of the relationship between the rate of follicle loss and time to the menopause. In addition to being a valuable biomarker in observational studies this will lead to new experimental approaches advancing our understanding of follicle dynamics across the lifespan.**

Biomarkers of Ovarian Aging as Predictors of Fertility

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As the ovary ages, the granulosa cell products antimüllerian hormone (AMH) and inhibin, decline leading to a rise in

early follicular phase follicle stimulating hormone (FSH) and subsequent shortening of the follicular phase and menstrual cycle length. These biomarkers of ovarian aging—chronologic age, menstrual cycle length, serum or urinary early follicular FSH, and AMH—have been proposed as potential measures of female fertility. Chronologic age has been the most commonly used biomarker to predict fertility following assisted reproductive technology (ART) and natural fertility. Menstrual cycle length also appears to be associated with fecundability in assisted and unassisted attempts to conceive. However, the value of menstrual cycle length as a predictor is limited by the low prevalence of short menstrual cycles, oral contraceptive use, and menstrual cycle irregularity. While FSH has been shown to be a predictor of assisted fertility, it appears to have low sensitivity for non-pregnancy and has unproven value as a predictor of unassisted fertility. AMH appears to have the most promise. Serum levels of AMH have been shown to be directly associated with the probability of conceiving following ART and naturally; however, there are no studies to date that examine AMH test characteristics, e.g. sensitivity, specificity, in the prediction of natural fertility and infertility. **CONCLUSION: While historical and laboratory biomarkers of ovarian aging are associated with assisted and unassisted fertility, their value as predictors are unproven. These biomarkers require further study as “fertility tests” in the general population.**

Genetic Factors Influencing the Timing of Natural Menopause

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Understanding the genetic determinants influencing the timing of natural menopause may provide insights into normal reproductive function and clarify the biologic mechanisms underlying the association between menopause and key health conditions (e.g. cardiovascular disease, cancer, and osteoporosis). Heritability studies demonstrate that about 50 % of the variation in age at natural menopause is due to genetic factors. Candidate gene studies of menopause age have largely yielded inconsistent results in part due to small samples and lack of replication. Large genome-wide association studies conducted in women of European ancestry have successfully identified 17 genetic loci associated with age at menopause including genes implicated in hormone regulation, DNA repair, and immune function. At least two genes, *POLG* and *TDRD3*, may be associated with primary ovarian insufficiency. Many of the identified variants for normal menopause are also associated with early menopause suggesting some shared genetic factors. Initial

work from one study provides evidence for shared genetic variants for multiple markers of ovarian reserve including menopausal age, antral follicle count, FSH, and anti-mullerian hormone. Much of the genetic etiology of age at menopause remains to be discovered. New advances including whole exome and whole genome sequencing and ExomeChip genotyping will permit investigation of low frequency and rare genetic variants. **CONCLUSION: The timing of menopause is under genetic control. Genes influencing normal age at natural menopause also influence early menopause and possibly several markers of ovarian reserve. Additional work needs to be done to understand the biologic function of the genes to further our understanding ovarian aging.**

Session III: Clinical Considerations for Ovarian Reserve

**Facilitator: Alan DeCherney, MD, NICHD
BRCA Gene Function and DNA Repair in Oocyte Aging
Kutluk Oktay, MD, New York Medical College, Valhalla
NY**

Current understanding dictates that ovarian reserve is established in utero. Either some primordial follicles are formed faulty and/or they are passively subjected to environmental exposure which eventually results in oocyte aging. As a result, oocyte aneuploidy rates increase and fecundity rates decline. This phenomenon is collectively referred as the decline in oocyte quality. Furthermore, 99.9 % of primordial follicle reserve is wasted and this wastage appears to accelerate with age. The mechanism behind this accelerated wastage or the decline in oocyte quality has not been explained. If the mechanism can be understood, targeted treatments can be developed to slow down or prevent reproductive aging. BRCA genes are critical in double-strand DNA break (DSB) repair and their mutations are associated with predisposition to numerous cancer types. While studying an innovative protocol of aromatase inhibitor use during ovarian stimulation for women undergoing oocyte or embryo freezing before breast cancer chemotherapy, we observed that, compared to BRCA mutation-negative women, those with BRCA1 mutations yielded a significantly lower mean number of oocytes (7.4 vs. 11.3, $p=0.025$). The same women were 38 times more likely (OR, 95 % CI 4–353, $p=0.001$) to have low response to ovarian stimulation. Furthermore, women with BRCA mutations experience early menopause. Recent studies in the rodent also suggest BRCA1 plays a role in meiotic spindle function. **CONCLUSION: These novel and recently published findings (see Commentary in this issue of JARG) suggest that ATM-mediated DNA DSB repair has a central role in oocyte aging and may be linked to the early onset of menopause in women carrying BRCA 1 mutations.**

Anti-Mullerian Hormone (AMH) and Measurement of Ovarian Reserve in Women with PCOS

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Anti-mullerian hormone (AMH) is produced by granulosa cells of preantral and early antral follicles. During life, serum AMH levels rise in childhood until puberty when values plateau. A second rise occurs in late adolescence and peaks at about age 25 after which there is a gradual and progressive decline until menopause. In adult women, circulating levels of AMH are significantly correlated with the number of small antral follicles (2–5 mm). As such, AMH has been suggested to serve as a surrogate indicator for antral follicle number. In addition, compared with other sized follicles, these small follicles appear to provide estimates of reproductive performance in assisted reproductive technology. The relationship of AMH to the number of small follicles is also observed in women with polycystic ovary syndrome (PCOS). However, in this disorder the number of follicles is considerably higher than that of normal women and, accordingly, serum AMH concentrations are higher. In late reproductive age, AMH levels in PCOS women remain greater than those of normal women which may preclude any accurate assessment of residual follicle number. **CONCLUSION: The clinical utility of AMH with respect to ovarian reserve is unclear in women with PCOS. Presently, there is insufficient data to determine whether serum AMH measurement provide any predictive value of reproductive outcomes or pregnancy in these women.**

Correlations Between Natural and Iatrogenic Ovarian Aging

**Clarisa Gracia, MD
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Fertility preservation strategies for females have improved dramatically over the past decade. Available methods and technologies are now being offered to a variety of women at risk for premature ovarian insufficiency (POI) or for those who wish to defer reproduction. However, the application of these technologies is not straightforward, in large part because it is difficult to predict who is at highest risk of ovarian insufficiency and infertility in the population. Clinically available measures of ovarian reserve, such as AMH, may be useful surrogate biomarkers to help predict POI in both naturally aging populations and patients exposed to gonadotoxic therapies. For example, data from the Penn Ovarian Aging study indicate that a woman's AMH level, the rate of change of AMH over time, and age are important predictors of

time to menopause in naturally aging late reproductive age women. Additionally, prospective studies in patients exposed to gonadotoxic therapies suggest that pretreatment AMH levels may be helpful in determining the degree of impairment and recovery of ovarian reserve after treatment. In young adult cancer survivors remote from therapy, measures of ovarian reserve including AMH appear to reflect underlying follicular reserve since these biomarkers are impaired in a dose dependent manner. However, while these biomarkers may reflect depleted follicular reserve in young cancer patients, it does not appear that they accurately predict pregnancy in this population. **CONCLUSION: Currently available biomarkers of ovarian reserve, including AMH, appear to be a better reflection of oocyte quantity rather than quality. More research is needed to better understand the significance of clinically available biomarkers of ovarian reserve with respect to fertility and time to menopause in naturally aging females and females exposed to gonadotoxic therapies.**

Session IV: Advances on the Horizon

Facilitators: Charisee Lamar, PhD, MPH, NICHD and Susan Taymans, PhD, NICHD

Genomic Markers of Primary Ovarian Insufficiency

**Aleksandar Rajkovic, MD, PhD
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Clinical diagnostic studies, on the genetic contribution to primary ovarian insufficiency (POI), have been limited to karyotype analysis and FMR1 pre-mutation testing. POI is a genetically heterogeneous disorder and candidate gene studies have yielded little information about genetics of human POI. Personalizing genetic care for women with POI will require novel diagnostic modalities to understand the underlying genetic contribution to their condition. In order to begin devising treatment and screening options, we need to better understand the etiology of idiopathic POI. This is especially important in light of current ability to cryopreserve ovarian tissue and oocytes, and future potential to generate oocytes through regenerative medicine. Chromosomal microarrays to identify genomic imbalances, and whole exome studies on familial cases are yielding novel information about the genetic contributions to POI. These and other technologies on the horizon will lead the way to identify novel genomic markers of POI. **CONCLUSION: Idiopathic POI has a significant genetic component, especially in young women with primary amenorrhea and early loss of ovarian function. The current practice of diagnosing and using genetic markers to guide therapy and follow up is in its infancy. Genomic approaches, rooted in the family unit and its history, will be critical to delineate the role of genes in women with POI.**

From Within or Without: the role of ovarian somatic cells in the determination of oocyte quality.

**David F. Albertini, PhD
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The relative contributions of ovarian somatic and germ cell interactions to the problem of ovarian aging is poorly understood because emphasis has been placed historically on deficiencies intrinsic to the oocyte. With increasing evidence to suggest that the oocyte is at the mercy of the metabolic status of granulosa cells, age-related alterations in somatic cells are becoming a focus in our understanding of the establishment and maintenance of the ovarian reserve. Given the now well-documented examples of oocyte-granulosa cell signaling that occurs at sequential stages of follicle development, it is likely that the loss of oocyte quality with advancing maternal age will find origins in the breakdown between the somatic germ interactions. Evidence is reviewed from animal models demonstrating the role of oocyte-specific factors such as GDF9 and BMP15 in the maintenance of metabolic cooperation via specialized structures known as Transzonal Projections (TZPs). Importantly, the ability to maintain oocyte quality and metabolic status is shown to be inversely related to FSH dosage due to the transient retraction of TZPs in response to FSH, possibly explaining the consequences of rising FSH levels on oocyte quality in older individuals. **CONCLUSION: Precocious sensitivity to FSH, or pharmacological dosing, is likely to contribute to a loss of oocyte quality with advancing maternal age due to a breakdown in metabolic communication between the somatic and germ cell compartments of the ovarian follicle.**

Isolation and Characterization of Adult Ovary-derived Oogonial Stem Cells

**Jonathan L. Tilly, PhD
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For decades it was believed that mammalian females rely on primordial germ cell (PGC)-derived oogonia to generate their entire quota of oocytes during embryogenesis, leading to the endowment of a non-renewable pool of oocyte-containing follicles at birth. However, the recent discovery of mitotically active germ cells, referred to hereafter as oogonial (oocyte-producing) stem cells (OSCs), in adult mouse and human ovaries opens the prospects that the mammalian oocyte pool is actively replenished at least during early adulthood. Such a paradigm shift would draw strong parallels to maintenance of adult spermatogenesis by spermatogonial stem cells (SSCs) in the testes as well as to the continuous production of oocytes

during adulthood in less evolved species such as flies and teleost fish. This presentation will highlight new advances in the field of mammalian OSC biology, including as-yet unpublished observations from our lab regarding the functionality of eggs generated by transplanted mouse OSCs, as tested in natural mating trials, as well as insights into the signaling pathways that guide the decisions of self-renewal (proliferation) versus differentiation (meiotic commitment) in OSCs in vitro and in vivo. Lastly, experimental evidence supporting a central role for OSC dysfunction with age to deterioration of the ovarian reserve, and eventual ovarian failure, will be overviewed. **CONCLUSION: Mounting evidence suggests that the mammalian ovary may harbor a population of OSCs capable of replenishing the oocyte pool.**

Break out Discussion: Moving Towards a Predictive Model for Ovarian Lifespan

Presentation of Pre-Clinical Research Directions and Priorities

David F. Albertini, PhD
University of Kansas Medical Center

The discussion focused on two major aspects of ovarian lifespan that clinicians and basic scientists alike felt should be targeted for future pre-clinical and clinical research. First, participants expressed a need for improved diagnostic and experimental imaging approaches. Current clinical evaluation of ovarian reserve relies on the evaluation of serum FSH, E2, and AMH, which are known to be problematic, and only allow for the evaluation of the current state of ovarian reserve, and do not predict the trajectory of ovarian aging nor the gradual loss of oocyte quality. New imaging approaches could expand the set of clinical criteria used to evaluate a given patient and, in the best of circumstances, would have predictive and/or prognostic value for lifestyle and aging parameters affecting future cardiovascular or bone health. Development of novel predictive, mathematical models would benefit substantially if new biomarkers are identified that combine with existing biomarkers and data generated from imaging modalities beyond ultrasound.

In this context, participants strongly voiced a need for new ovarian imaging protocols that would provide real time data on the dynamics of follicle development and associated changes in extrafollicular compartments of the ovary. For example, shifting, patterns of ovarian innervation and vascularity accompany the menstrual cycle and with improved technology, we may better be able to define how these or other parameters change across the ovarian lifespan. While animal models continue to provide new information on the molecular bases of ovarian aging, participants agreed that most assays in hand fail to provide a comprehensive

assessment of follicle dynamics. Opportunities exist currently given recent advances in whole animal imaging in the fields of cancer biology and neurobiology and adopting established paradigms for pre-clinical studies in these fields for the study of the ovarian reserve seems fully warranted at this time

The second focus of discussion derived from recognizing the critical roles of primordial follicle endowment, arrest and activation in determining the rate of ovarian aging and the evaluation of ovarian reserve. Although our mechanistic understanding of the processes of primordial follicle arrest and activation has improved, the practical implications and conservation of mechanisms between animal models and humans remain largely unexplored. What controls differential rates of follicle loss? How do the recently identified genetic factors, such as those regulating DNA damage repair and immune system involvement, contribute to ovarian aging in humans? Are the biomarkers in hand sufficiently indicative of states of ovarian or follicular physiology through the pre and post reproductive lifespan or in women with Turner's syndrome (XO), primary ovarian insufficiency (POI), polycystic ovary syndrome (PCOS) or cancer?

In response to these questions, the group agreed that in addition to understanding primordial follicle endowment as a source of variation in ovarian reserve, understanding the causes of premature follicle activation represents a major challenge to the field. Our concept of the timing of follicle development after activation is woefully incomplete and requires further experimental dissection in animal models. Is the oocyte itself a sensor or integrator of signals being generated from somatic compartments within the ovary? And finally, with so many factors now implicated in local control of follicle activation and development, how will future research catalogue and integrate the roles of these players to make clinical management or manipulation of the ovarian reserve a realistic possibility?

Presentation of Clinical Research Directions and Priorities

Clarisa Gracia, MD, MCSE University of Pennsylvania

The participants reached a general consensus that currently, clinical data regarding measures of ovarian reserve are quite limited. Most of the clinical information stems from relatively small cross sectional studies in select populations, which have generally focused on indirect measures of ovarian reserve such as FSH in infertile populations or among older women during the menopausal transition. There is a dearth of normative population based data across the lifespan for newer clinically available measures of ovarian reserve such as AMH and AFC. Little is known about factors associated with variability in newer measures of ovarian reserve such as genetic factors, age, race, body composition, the effects of hormone and other medication use, timing with respect to the menstrual cycle, and the

diseases such as endometriosis, fibroids, endocrinopathies and the effects of cancer therapies. The participants enthusiastically agreed that developing methods to predict ovarian reserve in select populations would allow for improved counseling regarding fertility preservation strategies to maximize future reproductive potential.

Moreover, the participants recognized that we need longitudinal studies assessing changes in ovarian reserve over time in order to better understand the impact of age on these measures. At this time, it is not clear that currently available measures of ovarian reserve reflect oocyte quality, so large prospective population based studies are needed to determine if these measures correlate with clinically important reproductive outcomes such as pregnancy and menopause. Furthermore, while preliminary data suggest that there may be long term health risks related to impaired ovarian reserve, more data are needed to better understand the pathophysiology behind these findings and to identify interventions to optimize long term health in such women.

Ultimately, more work is needed to develop better surrogate measures of ovarian reserve to counsel women about future reproductive potential and long term health. Fertility preservation strategies and medical therapies may be available to optimize outcomes in women at risk for early ovarian insufficiency.

Conclusions and recommendations:

Ovarian reserve is an important driver of fertility, the timing of menopause, and a woman's overall health and quality of life. However, our current clinical measures do not provide adequate information to fully understand the true level of ovarian reserve nor predictive power in terms of future fertility and health outcomes. To optimize the measurement and predictive power of ovarian reserve, new methods must be equally applicable to all women, regardless of their age, race and health status. Future models will need to incorporate the data collected from normal, healthy women before, during and after their reproductive lifespan. Moreover, con-

founders such as environmental or lifestyle factors potentially harmful to the ovaries, and fertility compromising diseases, such as primary ovarian insufficiency (POI), polycystic ovary syndrome (PCOS), and certain cancers and rheumatic diseases will require scrutiny and evaluation to have a widespread impact on woman's health.

Going forward, it is important to:

Better understand follicle dynamics, especially the role of primordial follicle endowment, early follicle attrition and the control of follicle activation in determining later ovarian reserve

Develop and validate new measures of ovarian reserve, especially new ovarian imaging techniques, that can apply to all women

Establish the longitudinal arc of ovarian reserve and develop predictive mathematical models for remaining fertility, time to menopause, and effects on cardiovascular and bone health of post-menopausal women

Understand the connection between ovarian reserve and oocyte quality, and its influence on a woman's future fertility and the health of her offspring

Explore possible ways to manipulate the ovarian reserve through lifestyle alterations or treatments that could rescue or re-programming ovarian follicles, and/or delay or reduce follicle atresia and attrition.

Expanding on opportunities that have emerged from the field of fertility preservation, such as optimizing oocyte and ovarian tissue cryopreservation technology

Develop in vitro models for follicle and oocyte maturation, which would add to our basic understanding of follicle dynamics and afford more widespread access to women of all ages.

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