Individualized controlled ovarian stimulation and objective gamete and embryo selection

Yokohama, Japan - December 7, 2011
General information

Venue
PACIFICO Yokohama
1-1-1, Minato Mirai,
Nishi-ku, Yokohama, 220 - 00121, Japan

Language
The official language of this course will be English
Simultaneous translation Japanese from/to English will be provided

Location
Around the time of the Black Ships, Yokohama was home to barely 600 people. A century-and-a-half later, it’s Japan’s second metropolis, with a breezy atmosphere, fine food, parks and historic districts, and loads of shopping. Unlike most Japanese cities, it’s also a city of distinct neighbourhoods, including Chinatown, the historic Motomachi and Yamate districts, and the new seaside development of Minato Mirai 21. Yokohama is barely 20 minutes from central Tokyo, meaning that it’s an easy day trip or nighttime excursion. Among Japanese it’s a popular date spot.
Individualized controlled ovarian stimulation and objective gamete and embryo selection

Serono Symposia International Foundation conference on:

Individualized controlled ovarian stimulation and objective gamete and embryo selection
Yokohama, Japan - December 7, 2011

Aim of the conference
Japan is in one of the countries where research on infertility treatment is more advanced. For this reason Serono Symposia International Foundation designed an educational event that will present some relevant outcomes of basic research, will introduce advances in procedure aimed to define individual infertility profile and will discuss, in a very interactive way, treatment protocols. Renowned Japanese and international expert will contribute in this meeting providing participants with a unique opportunity to improve their knowledge and exchange experience.

Learning objectives
Participants of this meeting will:
• be updated on some of the most relevant outcomes of basic research in infertility
• acquire advanced methodologies to screen individual infertility profile
• discuss infertility treatment optimization

Target audience
This program is targeted to clinicians and scientists working on Assisted Reproduction Techniques

Accreditation
Serono Symposia International Foundation (www.seronosymposia.org) has submitted this program “Individualized controlled ovarian stimulation and objective gametes and embryo selection” (Yokohama, Japan- 7 December, 2011) for accreditation by the European Accreditation Council for Continuing Medical Education (EACCME) and the Japan Society of Obstetrics and Gynecology (JSOG).
List of faculty members

Scientific organizer
Yasunori Yoshimura
Department of Obstetrics and Gynecology
Keio University School of Medicine
Tokyo, Japan

Scientific secretariat
Serono Symposia International Foundation
Salita di San Nicola da Tolentino 1/b
00187 Rome, Italy
Associate Project Manager: Chloé Xilinas
Tel.: +39 06 420413 505 - Fax: +39 06 420413 677
E-mail: info@seronosymposia.org
Serono Symposia International Foundation is a Swiss Foundation with headquarters in
14, rue du Rhône, 1204 Geneva, Switzerland

Organizing secretariat
c/o MA Convention Consulting Inc.
Dai 2 Izumi-shoji Bldg., 4-2-6 Kojimachi, Chiyoda-ku
Tokyo 102-0083 JAPAN
Phone: +81 3 5275 1259
Fax: +81 3 5275 1192
E-mail: info@macc.jp

List of faculty members
Hiroyuki Abe
Yamagata University
Yonezawa City, Japan

David Albertini
Department of Molecular and Integrative Physiology
University of Kansas Medical Center
Kansas City, Kansas, USA

Yoshimasa Asada
Asada Ladies Nagoya Clinic
Nagoya, Japan

Robert Fischer
Fertility Centre Hamburg
Hamburg, Germany

Aisaku Fukuda
IVF Osaka Clinic
Osaka, Japan

Juan Antonio García-Velasco
IVI Madrid
Rey Juan Carlos University
Madrid, Spain

Minoru Irahara
Department of Obstetrics and Gynecology
University of Tokushima Graduate School of Medicine
Tokushima, Japan

Osamu Ishihara
Department of Obstetrics and Gynecology Saitama Medical University
Saitama, Japan

Minoru S.H. Ko
Developmental Genomics & Aging Section
Laboratory of Genetics, National Institute on Aging, IRP
NIH Biomedical Research Center
Baltimore, Maryland, USA
Henry Leese
The Hull York Medical School
University of Hull
Hull, UK

Nicholas Macklon
Department of Obstetrics and Gynecology
University of Southampton
Southampton, UK

Hideki Mizunuma
Department of Obstetrics and Gynecology
Hirosaki University School of Medicine
Aomori, Japan

Emre Seli
Department of Obstetrics, Gynecology and Reproductive Sciences
Yale University School of Medicine
New Haven, Connecticut, USA

Nao Suzuki
Department of Obstetrics and Gynecology
St. Marianna University School of Medicine
Kanagawa, Japan

Yuji Taketani
Department of Obstetrics and Gynecology
University of Tokyo
Tokyo, Japan

Takumi Takeuchi
Reproduction Center
Kiba Park Clinic
Tokyo, Japan

Atsushi Tanaka
Department of Obstetrics and Gynecology
Saint Mother Hospital
Fukuoka, Japan

Yasunori Yoshimura
Department of Obstetrics and Gynecology
Keio University School of Medicine
Tokyo, Japan
Scientific program
Wednesday - December 7, 2011

07.50 Registration
08.20 SSIF welcome
R. Fischer, Germany

Session I Biomarkers of gametes and embryo quality: from basic research to practice

Chairpersons: H. Mizunuma, Japan; E. Seli, USA
08.30 L1 - Mitochondria and ooplasmic donation
T. Takeuchi, Japan
08.50 L2 - The future is now: oocyte plasmic exchange for aged oocytes
A. Tanaka, Japan
09.10 L3 - Metabolic biomarkers of preimplantation embryo
H. Leese, UK
09.30 L4 - Preimplantation-specific genes
M. Ko, USA
09.50 Discussion
10.10 Coffee break

Session II Screening methodologies in infertility treatment

Chairpersons: O. Ishihara, Japan; Y. Taketani, Japan
10.30 L5 - Oocytes quality: reliable markers in clinical practice
D. Albertini, USA
11.00 L6 - Oocyte and embryo selection based on oxygen consumption
H. Abe, Japan
11.30 L7 - Embryo selection: from genetics to the omics
E. Seli, USA
12.00 L8 - Ovarian response prediction: from patient’s history to genetics
N. Macklon, UK
12.30 Discussion
13.00 Lunch

Session III Panel discussion on: tailoring the treatment approach to improve outcomes

Chairpersons: R. Fischer, Germany; M. Irahara, Japan
14.00 L9 - Poor response: strategies for management
A. Fukuda, Japan
14.20 L10 - Individualized ovarian stimulation
J.A. Garcia-Velasco, Spain
14.40 L11 - Personalizing ART procedure based on AMH in Japan
Y. Asada, Japan
15.00 L12 - Fertility preservation in cancer survivors
N. Suzuki, Japan
15.20 Discussion
15.45 Closing remarks
Y. Yoshimura, Japan
15.50 End of meeting and coffee
Serono Symposia International Foundation adheres to guidelines of the European Accreditation Council for Continuing Medical Education (EACCME) and all other professional organizations, as applicable, which state that programs awarding continuing education credits must be balanced, independent, objective, and scientifically rigorous. Investigative and other uses for pharmaceutical agents, medical devices, and other products (other than those uses indicated in approved product labeling/package insert for the product) may be presented in the program (which may reflect clinical experience, the professional literature or other clinical sources known to the presenter). We ask all presenters to provide participants with information about relationships with pharmaceutical or medical equipment companies that may have relevance to their lectures. This policy is not intended to exclude faculty who have relationships with such companies; it is only intended to inform participants of any potential conflicts so participants may form their own judgments, based on full disclosure of the facts. Further, all opinions and recommendations presented during the program and all program-related materials neither imply an endorsement, nor a recommendation, on the part of Serono Symposia International Foundation. All presentations solely represent the independent views of the presenters/authors.

The following faculty provided information regarding significant commercial relationships and/or discussions of investigational or non-EMEA/FDA approved (off-label) uses of drugs:

- **H. Abe**
  - Declared no potential conflict of interest

- **D. Albertini**
  - Declared no potential conflict of interest

- **Y. Asada**
  - Declared no potential conflict of interest

- **R. Fischer**
  - Declared receipt of honoraria or consultations fees from Serono Symposia International Foundation and to be member of the Scientific Committee of Serono Symposia International Foundation

- **A. Fukuda**
  - Declared no potential conflict of interest

- **J.A. Garcia-Velasco**
  - Declared receipt of grants from Merck Serono, MSD, Ferring, Angelini

- **O. Ishihara**
  - Declared receipt of honoraria or consultation fees from Merck Serono, MSD

- **M. Ko**
  - Declared no potential conflict of interest

- **H. Leese**
  - Declared to be scientific advisor and shareholder of Novocellus LTD

- **N. Macklon**
  - Declared receipt of grants from Merck Serono, Ferring, MSD, Anelova and receipt of honorarium from Merck Serono, MSD, Anelova. Declared to being stakeholder of Complete Fertility Services LTD

- **E. Seli**
  - Declared no potential conflict of interest

- **N. Suzuki**
  - Declared no potential conflict of interest

- **Y. Takeuchi**
  - Declared no potential conflict of interest

- **T. Tanaka**
  - Declared no potential conflict of interest

- **Y. Yoshimura**
  - Declared no potential conflict of interest

The following faculty has provided no information regarding significant relationship with commercial supporters and/or discussion of investigational or non-EMEA/FDA approved (off-label) uses of drugs as of November 2, 2011:

- **M. Irahara**
- **H. Mizunuma**
The fertility declines with maternal age, this is particularly evident by age 40 and it appears to be primarily related to an increased incidence of oocyte aneuploidy. Such age-related oocyte aneuploidy occurs at a specific maturational step predominantly during the first meiosis and is likely due to ooplasmic dysfunction. It has been postulated that insufficient ATP production due to mitochondrial dysfunction is responsible for structural abnormalities of the meiotic spindle leading to malsegregation of chromosomes/chromatids in oocytes of aging women. 

In order to assess the effect of mitochondrial damage on meiotic maturation in germinal vesicle (GV) stage oocytes, a mitochondrial insult was imposed by specifically inhibiting the ATP production pathway, or by damaging the mitochondria. Briefly, some GV oocytes retrieved from PMSG-primed B6D2F1 mice were exposed to oligomycin, a specific inhibitor of the mitochondrial F1FO ATPase; another group being exposed to 250 µM CMXRos, a mitochondrial photosensitizer, with photoirradiation. These mitochondria damaged oocytes displayed clustering and swelling of their mitochondria, and a reduced membrane potential. These mitochondrial features were confirmed in ultrastructural analysis of photosensitized oocytes arrested at the GV stage, with more aggregated or dilated/swollen mitochondria in the photosensitized oocytes. The meiotic spindles of those treated oocytes were then examined by immunofluorescence. In another set of experiments, GV karyoplasts isolated from such mitochondria-impaired oocytes were transferred into control healthy ooplasts, following which the reconstituted oocytes were allowed to mature in vitro and their spindles were imaged.

GVT was accomplished with a high efficiency. Maturation rate following reconstitution was comparable to that in control oocytes, as was the incidence of aneuploidy among the reconstituted oocytes. In the control oocyte group, 82.1% matured to MII stage while only 53.1% and 50.8% did so after oligomycin exposure or photosensitization, respectively (P < 0.05). Among the arrested oocytes, the meiotic spindle configuration was abnormal in 37.5% after oligomycin treatment and 47.0% with photosensitization, compared to only 6.9% in the controls. The transfer of the GV's recovered from the mitochondria-damaged oocytes into control ooplasts resulted in 66.7% being reconstituted and 77.2% underwent maturation. The majority (84.6%) had a normal MII spindle with 60% of these mature oocytes being successfully fertilized by ICSI. Ultimately full term offspring were obtained from the rescued oocytes.

In conclusion, healthy mitochondrial replenishment by nuclear transfer can reverse maturational block brought by damaging mitochondria ensuring full developmental competence. Thus, the GVT technique contributes and stimulates research aimed to prevent chromosomal defects associated with oocyte aging.
The main causes of repeated failure in assisted reproduction such as IVF-embryo transfer are believed to be ooplasmic deficiencies, abnormalities and ageing rather than nuclear deficiencies. It is a common phenomenon that pregnancy rates decrease, but miscarriages increase, as women grow older. Also, the percentage of fetal chromosomal abnormalities in miscarriages increases according to female age, reaching >90% when women are over 40 years old; surprisingly, about 90% of them are cases of autosomal trisomy. Such aneuploidy is mainly induced by the chromosomal pre-division, in which homologous chromosomes fail to pair during meiosis I and segregate before it is complete, resulting in disomic gametes. Nuclear transfer into the metaphase-II (M-II) oocytes shows promise as a means of repairing female infertility due to ooplasmic deficiency and abnormalities. We therefore conducted nuclear transfer of in vitro matured metaphase-II oocytes (recipient oocytes) into enucleated freshly ovulated metaphase-II oocytes (donor oocyte).

In both in-vitro matured oocytes and freshly ovulated oocytes, the M-II chromosomes were easily recognized as a round transparent substance in which the chromosome body was centrally located, and they were usually beneath or adjacent to the 1PB with the aid of an inverted microscope equipped with a Nomarski differential interference contrast system. The aspirated M-II karyoplast of recipient oocytes was transferred into the perivitelline space of an enucleated donor oocyte. The grafted oocyte was transferred in Zimmerman cell fusion medium. Membrane fusion was facilitated by electrical stimulation (10V for 1 second AC + 10V for 45 microsecond DC) with an electro cell fusion generator (LF 201). After fusion, the constructed oocytes were cultured in HTF medium for 2 hours and ICSI was performed.

The percentage of identification of M-II chromosome was 91.1% (41 out of 45) in freshly ovulated oocytes and 96.0% (48 out of 50) in vitro-matured oocytes.

The M-II karyoplast was removed successfully in 35 of 41 (85.4%) of the donor oocytes and 40 of 48 (83.3%) of the recipient oocytes. All of 35 karyoplasts of recipient oocytes were replaced in the perivitelline space of enucleated donor oocytes and 28 of these 80.0% were fused to form a reconstituted oocyte. The fertilization rate, cleavage rate and blastocyst formation rate following ICSI for constructed oocytes and recipients oocytes were \( \frac{77.1}{27/35}, \frac{65.7}{23/35}, \frac{25.7}{9/35} \) and \( \frac{59.0}{58/98}, \frac{26.1}{25/98}, \frac{3.4}{3/98} \) respectively. Chromosomal analysis of 4 embryos following nuclear transfer indicated they were all diploid sets of 46 chromosome.

In conclusion, it has been demonstrated that oocytes constructed following the karyoplast transfer of in-vitro matured M-II oocytes into enucleated freshly ovulated M-II oocytes clearly had more efficient and chromosomally normal embryonic development than did in-vitro matured oocytes after ICSI. These results demonstrate that this technique can be applied to the treatment of female infertility due to ooplasmic deficiency and abnormalities in aged oocytes.
The major challenge facing human In Vitro Fertilisation (IVF) and related techniques is to devise a rigorous, non-invasive test to select single embryos for transfer and overcome the problem of multiple births, which pose serious risks for mother and child [http://www.oneatatime.org.uk/].

After briefly reviewing the history of this topic, the focus will be on metabolic markers, especially, amino acid profiling (AAP). In AAP, individual human embryos are cultured with a close to physiological mixture of amino acids. When the subsequent development of the embryos is recorded, those in which the depletion and appearance (i.e. ‘turnover’) of amino acids lies within a lower range have a higher viability in terms of their capacity to develop to the blastocyst stage in culture [Houghton et al Hum Reprod 2002;17:999-1005] or give rise to a pregnancy following embryo transfer in clinical IVF [Brison et al Hum Reprod 2004; 19:2319-24]. Sturmey et al [Mol Reprod Dev 2010; 77:285-96] have now shown that AAP offers the possibility of predicting prospectively the ability of single bovine zygotes to develop to the blastocyst stage. This same study also highlighted differences in profiles between male and female embryos, those derived in vivo and in vitro, and the influence of maternal nutrition. One explanation for these data is in terms of the ‘Quiet Embryo Hypothesis’ (Leese Bioessays. 2002; 24:845-849; Leese et al Hum Reprod 2007;22:3047-50) which proposed that viable embryos have a ‘quieter’ metabolism than those which fail to develop. The hypothesis was developed by Baumann et al [Mol Reprod Dev 2007;74:1345-53] who speculated that those embryos with a quieter metabolism were subject to less damage to the genome, transcriptome and proteome, or were better equipped to deal with damage when it occurred, and thus devoted fewer resources (such as amino acids) to repair processes; colloquially termed ‘running repairs’. As a test of the hypothesis, Sturmey et al [Hum. Reprod 2009;24:81-91] compared the amino acid turnover of cattle, pig and human blastocysts with levels of DNA damage in each individual cell of the embryo. For each species, there was a strong, positive correlation between amino acid turnover and DNA damage, in line with the Quiet Embryo Hypothesis. In order to translate these findings into the IVF clinic, there needs to be a large-scale clinical trial, first to define retrospectively which biomarker correlates most closely with a successful pregnancy outcome and then to test this finding prospectively. In reality it is likely such biomarker profiles will be combined with conventional morphological embryo scores to generate an algorithm predictive of embryo viability.
Mouse preimplantation embryogenesis is marked by three waves of gene expression: massive degradation of maternal RNAs, zygotic genome activation (ZGA) at the 2-cell stage, and mid-preimplantation gene activation (MGA) [1]. Recently, it has been shown that human preimplantation embryos also undergo waves of gene activation starting from the 2-cell stage [2].

This lecture provides a broad overview of gene expression regulations in mouse and human preimplantation embryos. We describe the identification and characterization of Zscan4, an example of a ZGA gene, which shows a sharp spike-like expression level at the late 2-cell stage [3]. We also describe the identification and characterization of Trim43a, Trim43b, and Trim43c genes, which are examples of MGA genes [4].

This lecture also covers recent findings about the molecular processes operating commonly in preimplantation embryos, germ cells, embryonic stem (ES) cells, and induced pluripotent (iPS) cells.

References
Introduction: There continues to be a need for objective measures of oocyte and embryo quality in order to improve ART outcomes. Current methodologies still rely heavily on morphologic criteria that accurately gauge oocyte nuclear, but not, cytoplasmic status. Our work has emphasized identification of parameters of cytoplasmic maturation necessary for embryonic development that might at some point be consistent with non-invasive biomarker testing at the level of the cumulus-corona cells.

Approach: We have monitored alterations in protein phosphorylation, ATP, and glutathione content in mouse or bovine oocytes subjected to varying conditions of IVF. For mouse oocyte studies, cleavage and blastocyst cell counts were used as output measures for oocytes matured under controlled conditions that allowed for the generation of different levels of cytoplasmic maturation.

Results: Biomarkers of receptor tyrosinase and M-phase kinase activities reveal a distinct concentration of substrate phosphorylation in the oocyte cortex under conditions of high cytoplasmic quality. These were paralleled by elevated levels of ATP and developmental competencies that included higher cleavage rates and increased numbers of cells in blastocysts. Reduced glutathione levels were not appreciably different between high and low quality oocytes.

Conclusions: Indirect measures of oocyte quality will gain application once those qualities of cytoplasmic maturation are better defined and the dependence for such alterations on metabolic profiles in the cumulus-corona is more completely understood. Current approaches fall short of providing reliable markers of oocyte quality.
The mitochondrial respiration is an important indicator in assessment of several cellular activities. Our previous studies demonstrated that mitochondrial activity is a ubiquitous parameter also used to gain valuable information on embryo quality. To establish an accurate method for embryo quality evaluation, we have employed a scanning electrochemical microscopy (SECM) technique. SECM is a technique in which the tip of a microelectrode is used to scan and monitor the local distribution of electro-active species near the sample surface. Recently, we succeeded in development of a modified SECM measuring system. This modified SECM system includes a measuring instrument mounted on the stage of an inverted optical microscope, a potentiostat, and a notebook computer for component control and analysis. Pt-microdisk electrodes sealed in tapered soft-glass capillaries can monitor the oxygen consumption rate of single cells. Our studies focus on the high sensitive and non-invasive nature of SECM system, to examine the correlation between the oxygen consumption activity of single embryos and its mitochondrial development and its quality. This system can measure respiration activity by single embryos and oocytes of several species including human. Oxygen consumption of individual embryos at different developmental stages was monitored. Development of mitochondria corresponds to the increase of oxygen consumption rates during the development of embryos. Furthermore, the embryos with higher oxygen consumption are better candidates to further development into good quality embryos and yielded higher pregnancy rates after embryo transfer. These studies suggest that the respiration activity correlates with the embryo quality. SECM technique may be a valuable tool for accurately assessing the quality of embryos and thereby contribute to improving outcomes associated with assisted reproduction, including human in vitro fertilization clinics.
Soon after the report of the first successful pregnancy after in vitro fertilization (1), and development of controlled ovarian stimulation in order to obtain more than one egg in a given cycle (2), it became apparent that morphology and cleavage rate of embryos correlate with their implantation potential (3). Thereafter, grading systems based on embryo cleavage rate and morphology were developed [reviewed in 4] leading to significant improvements in implantation and pregnancy rates and reductions in multiple gestation rates. Unfortunately, their precision is still insufficient to compel most patients and clinicians to reduce the number of embryos transferred to a point where twins are uncommon and high order multiple gestations are rare or eliminated [reviewed in 5].

The limitations of morphologic evaluation of embryos have led many investigators to pursue adjunctive technologies for the assessment of the reproductive potential of a given embryo, with the aim to develop an objective, accurate, fast, and affordable test. Recently, global assessment strategies involving genomic, transcriptomic, proteomic, or metabolomic profiling of oocytes, granulosa or cumulus cells, embryos, or culture media have been applied to assisted reproduction. These technologies are at different stages of development and present unique advantages as well as limitations [reviewed in 6 and 7].

References
The most prominent determining factor for outcomes after ART is the individual variability in ovarian response to stimulation. There is therefore considerable interest in identifying factors which enable the prediction of response and thus appropriate selection of patients and of the individual dose likely to optimise outcomes of treatment.

The prediction of ovarian response starts with assessing the patients history. In addition to age, duration and cause of subfertility and response to previous ovarian stimulation, lifestyle factors such as smoking and BMI are known to impact on response. In recent years attention has been given to the identification of sensitive and specific markers of ovarian aging which may enable prediction of poor or good response to ovarian stimulation. The most widely used endocrine marker for ovarian reserve remains the early follicular phase FSH level. While baseline FSH levels predict poor response to ovarian stimulation, age appears to be more closely related to the chance of implantation and ongoing pregnancy.

The age-related decrease in number of antral follicles present in the ovary at the start of the cycle is considered to correlate with the number of primordial follicles remaining in the ovary. It should be emphasized, however, that direct evidence to support this contention is lacking. The antral follicle number assessed by ultrasound during the early follicular phase has been shown to correlate with ovarian response to stimulation, and to predict the number of immature oocytes retrieved from unstimulated ovaries prior to in-vitro maturation.

Recently, there has been increased interest in the use of AMH to help predict dosing regimes. The advantage of AMH over any menstrual cycle dependent predictor marker is its low inter and intra-cycle variability. Cohort studies have shown that serum AMH levels can predict response to exogenous gonadotropins, and that it may provide the basis for a simple means of individualising doses regimes in IVF. However, while serum AMH measurements may be effective in predicting response to treatment, as with other markers, they have not been shown to predict the likelihood of achieving pregnancy after ART.

In recent years there has been increased interest in pharmacogenetics as applied to ovarian response. A number of specific polymorphisms of the FSH receptor have been identified which affect the response to exogenous gonadotropins. However, in a recent genome wide study of SNPs, no specific SNPs were shown to be significantly predictive of response.

Many factors are therefore predictive of ovarian response, and prediction models which account for multiple factors can be useful in clinical practice. The performance of a prediction model can be measured by assessing its ability to discriminate between poor and good response (‘discrimination’) and the extent to which it predicts observed response (‘calibration’). However, before a prediction model can be introduced into every day clinical practice, prospective external validation is required.
The number of aged female patients has been increasing worldwide due to changes in the social environment. Women who do not respond well to ovarian stimulation or premature ovarian insufficiency (POI), previous premature ovarian failure (POF) or precocious menopause are considered to be traditional poor responder, primarily. However, majority of aged female patients, especially older than 40 year old, are realistically considered to be poor responder at present. Assisted reproductive technology [ART] in Japan has peculiar or unique hardship for treating poor responders compared to many other countries. We have to treat the poor responders by their own oocytes, because oocyte donation is not allowed officially by the guideline of Japan Society of Obstetrics and Gynecology (JSOG). The percentage of patients older than 40 year old among new patients at our clinic was 10.4% in 2005, but increased to 17.4% in 2011. Likewise, average age of new patients was 33.4 year old in 2005, but 34.8 year old in 2011. On the other hand, average number of antral follicle counts (AFC) and AMH (pmol/L) value decreased from 14.1 and 27.4 in 2005 to 9.3 and 22.5 in 2011, respectively. The number of poor responders due to female patient age increased without doubt. Average number of oocyte retrieved drops to less than 10, when AMH value is lower than 10 pmol/L or serum FSH level is higher than 10 IU/L. Overall pregnancy rate (PR) improves when the number of oocytes retrieved increases, regardless of patient age. However, PR of frozen-thawed embryo transfer cycles (FET) (18.2%) in the patients of 40 year old or older is significantly higher compared to fresh transfer cycles (10.4%). Moreover, PR of FET improves from 6.4% to 15.7% when more than 3 embryos are thawed for transfer compared to 2 embryos. This does not mean that number of embryos transferred increases, but better quality embryos could be chosen for transfer. Our strategy for older female patients is to preserve the embryos frozen more than 3 by several trials of natural or mild stimulation IVF, and transfer in subsequent FET cycles. Another strategy for poor responders such as POI/POF and sometimes repeated IVF failures is in vitro maturation (IVM). IVM is a relatively new technology in ART, but very effective when other methods do not achieve pregnancy. We supplement estrogen to POI/POF patients with or without FSH administration to expect follicular growth in the ovary. When follicle develops to more than 8-10 mm in diameter, immature oocyte is retrieved and matured in vitro for ICSI. Otherwise, full maturation of follicles is not expected in POI/POF patients in many instances. Two pronuclear stage oocytes are frozen for future FET. IVM should be significant alternative for such severe cases. IVM is another strategy for poor responders at our clinic. In conclusion, integral part of our strategy is not only high quality of IVF and IVM techniques, but also efficient freezing technique such as vitrification.
Individualized treatment must be part of the evolution of fertility treatment. Humans are not a particularly fertile species, and there is not always an obvious reason for infertility. For example, patients with no observable, abnormalities, illness, or abnormal laboratory test results may still be unable to conceive. The existence of this patient profile suggests that we do not fully understand all of the reasons for infertility. In daily practice, many patients are beyond the age range of highest fertility and require careful consideration of their individual situations and needs. Despite the availability of multiple new approaches to COS, no single treatment protocol will be able to optimally treat all patients and their individual needs. Many patients seen in routine practice are more complex than the typically younger and healthier subjects in clinical trials thus, the conclusions from clinical trials may not always be appropriately extended to all patients outside of the trial. These real-world patients must be considered on an individual basis and treated with an individualised COS tailored to meet their specific needs.
Ovarian reserve is now a important competent of evaluation for old patients. Because FSH is insufficient to evaluate their ovarian reserve, AMH has recently been seen as an alternative marker for such patients. Since 2008, we began measuring the serum AMH levels in my patients. The methods for ART can be selected by checking the AMH levels. AMH is an excellent indicator of the number of collectable eggs in ART procedures. In my opinion, the AMH levels are most important to select appropriate procedures of controlled ovarian stimulation for my patients.

Japanese people are marrying later in life. Consequently, women have their babies when they are much older recently. The mean age of patients for infertility has also increased. The targets of infertile treatment are shifted away from younger patients with well-defined causes of infertility to older patients with age-related infertility of unknown etiology. Ovarian stimulation as part of in vitro fertilization has changed along with these societal changes. Ovarian reserve is now a important competent of evaluation for such patients. Because of basal follicle-stimulating hormone (FSH) levels insufficient to evaluate their ovarian reserve, anti-Müllerian hormone (AMH) has recently been seen as an alternative marker for such patients.

Three years ago, I began measuring the serum AMH levels in my patients and have reported on the importance of this hormone as a marker of ovarian reserve in reproductive medicine.

AMH, a hormone expressed in the testes of the male embryo, promotes the formation of the male reproductive organs by the regression of the Müllerian ducts. AMH, coded for by a gene on chromosome 19, belongs to the transforming growth factor (TGF)β super-family. Its protein is consisted by a dimer of two 72-kDa units. The expression of AMH is detectable in the fetal ovaries around week 32 of gestation. AMH is secreted from granulosa cell of the preantral and small antral follicles for promoting primordial follicles to be primary follicles. AMH is characteristically not modified through the menstrual cycle. Large amounts of AMH are secreted from the granulosa cells of the preantral and antral follicles. Its secretions are decreased by the maturing procedures of follicles. By such characters, the hormone is a quite useful marker for infertile treatment. More than 5000 patients visiting in my clinics are checked serum AMH levels by 2nd step ELISA. The method for infertility treatment are decide by this results.

Result

Although there are standard and mean AMH levels, there is no range seems to be normal. Mean levels in women in their early 20s about 7 ng/ml, however its levels linearly go down 0 in women in their late 40s. Surprisingly, the ages of our infertile patients are not statistically correlated with AMH levels (Figure 1). AMH levels, moreover, show irregular distributions not only in infertile patients but also in pregnant women and fertile women. The AMH levels are not correlated with age in all these groups. Previously in my Clinic, the ovarian reserve are evaluated by basal FSH, the numble of antral follicle, age, the patient history and outcomes of prior treatment. By these results, we selected the appropriate methods for ART, such as the long, antagonist, short protocol or simple ovulation induction by clomiphene and HMG.

The results clearly demonstrate the methods for ART can be selected by checking the AMH levels (Figure 2). AMH levels are particularly well correlated with the number of eggs can be collected. Convincingly, AMH is an excellent indicator of the number of collectable eggs in ART procedures (Figure 3). In my opinion, the AMH levels are most important to select appropriate procedures of controlled ovarian stimulation for my patients. Although I also consider age, I have found that patients with AMH levels of below 1 ng/ml, are candidates for simple ovarian stimulation because they do not respond well to the short protocol. The patients with beyond 3 ng/ml seem to respond well to the antagonist protocol. Since November 2009, I quit using the long protocol and use the antagonist protocol as the first choice, because my patients now are getting older and having lower AMH levels. Figure 4 shows the AMH-based criteria I for selecting controlled ovarian stimulation procedures.

A high percentage of quality ova are required for a high conception rate. High AMH levels and a large number of ova increase the quality of eggs can be available in the operations. While high AMH levels facilitate conception, pregnancy quality is largely dependent on age, meaning that AMH cannot be used to predict conception rates. Moreover, AMH is a very effective predictor of ovarian hyperstimulation syndrome and should be measured to prevent this side effect.

In Japan, women delay her marriage and childbearing, fertility should treat women beyond their peak reproductive years, even in...
premenopausal or menopause period. In perimenopausal women, FSH levels are very unstable, sometimes normal, but in some cases are high or even abnormally high by a patient’s. Evaluating a patient based on FSH levels is therefore not feasible for them. AMH levels are more conducive to accurate choices for such patients.

Due to the effects of FSH, follicles vary in size and may fail to develop and mature in perimenopausal women. Moreover, there may be few ova despite a normal E2 level, or mature ova may be difficult to collect by the operation. An examination of E2 levels on the day of HCG administration classified by the number of eggs collected shows that levels vary according to age and AMH. E2 levels per egg collected are consistently higher in older women and women with lower AMH levels (Figure 5).

I emphasize that mature egg can be collected in the operation by checking AMH levels and the patient age. The conventional by-the-book decision are no longer effective because of this methods.

By measuring AMH levels in many patients, I realize that there is no mean range consistent enough to indicate ovarian age. Women cannot foresee their reproductive potential by their age. AMH is the only true indicator. It can be concluded that the range of reproductive years are different in person. I can advice women, whether married or unmarried, is to check her AMH level to know their true reproductive age and obtain data to decide when she should be married and pregnant.
Young female cancer patients face various problems, including a decrease in their quality of life (QOL) due to early menopause or loss of fertility after remission. Chemotherapy and radiotherapy can cause loss of reproductive function in young women due to adverse effects such as ovarian failure. The frequency of ovarian failure depends on the age of the patient, the anticancer agents used, and the dose of each agent. In these patients, improvement of the post-treatment QOL and fertility preservation can be achieved by measures such as protection of ovarian function against the effects of anticancer agents by administration of gonadotropin-releasing hormone analogs, transposition of the ovaries outside the radiation field, or cryopreservation of unfertilized and fertilized ova. However, ovarian stimulation to obtain ova is time consuming and sometimes considered a taboo depending on the type of cancer. The self-solution to problem to occur frequently at the same time is demanded from the patient, and the patient is forced with too many choices in a short term. It is often less than one month until the cancer treatment begins after an underlying disease is diagnosed since chemotherapy cannot be delayed. In these cases, cryopreservation of ovarian tissue is currently proposed for fertility preservation. In this lecture, I will discuss a topic about fertility preservation in the young cancer survivors including the recent knowledge of cryopreservation of ovarian tissue.