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Trans Atlantic Reproductive Technologies Network

[TARTEN2011]

ABSTRACTS

Challenges in Fertility Preservation & Assisted Reproduction

14 – 17 April 2011
Swissotel The Bosphorus

ISTANBUL- TÜRKEZY

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PRESIDENTIAL WELCOME

Dear Colleagues,

It gives me joy to welcome you to the first international meeting of TARTEN, Trans-Atlantic Reproductive Technologies Network. TARTEN was established to foster collaboration between the two sides of the pond. Fields of fertility preservation and assisted reproduction are developing at a much faster pace than the specialists or the society can keep up with. Only through a collaborative approach that we can responsibly shape the future of these fields.

The first meeting has shaped up enormously well, the scientific program is extremely exciting and unique; many of the topics you will find not covered anywhere else. With that the organizing committee wanted to look into the future, the topics of the next decade. Time will prove that our presentations and and discussions in the upcoming three days will be the forbearer of pending breakthroughs in our fields of assisted reproduction and fertility preservation.

I would like to thank the organizing committee made up of Doctors Volkan Baltaci, Murat Sonmezer, and Nick Macklon for the effort they have put into making this meeting a reality. I also would like thank Turk Ureme Dernegi (Turkish Reproduction Society) executives Professors Recai Pabuccu and Timur Gurgan as well as all session chairs and speakers sparing time to support this initiative. We would also like to thank Turkish Medical Oncology Society (Tibbi Onkoloji Dernegi) and all cancer specialists for supporting our meeting-they too are visionaries in seeing the multidisciplinary nature of our fields. Finally, we thank Serenas for the great organization, and all pharmaceutical and biomedical companies for their support in the educational mission of TARTEN.

TARTEN is aiming to become a society carrying on the mission outlined above. At this inaugural meeting, all of us have the opportunity to become part of this exciting initiative. I once again welcome you and let the scientific feast and festivities begin!

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ABSTRACTS

IS THERE AN ASSOCIATION BETWEEN ART AND EPIGENETIC CHANGE?

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Assisted reproduction technologies (ARTs) have been a recent focus for research on imprinting and epigenetics as assisted conception involves the in vitro manipulation of gametes and preimplantation embryos at exactly the time when epigenetic information is being highly regulated. The epigenetic information that controls imprinted gene expression is established during mammalian gametogenesis. During oogenesis, for example, maternal methylation is established during the oocyte growth phase. In zygotes, the epigenetic inheritance from the sperm and the oocyte intersect and must be recognised, moderated and propagated during preimplantation development so as to regulate gene expression in an appropriate manner. Imprinted genes play pivotal roles in fetal growth and the interaction of the conceptus with the mother. Many imprinted genes are expressed and function within the placenta and regulate the mother-offspring relationship in utero. The incorrect regulation of genomic imprinting may lead to imprinted diseases as all epigenetic information must be correctly regulated both in vivo and in vitro during the critical window of preimplantation embryo development in order to ensure normal onward development.

A growing body of evidence suggests that ART and/or infertility per se may lead to epigenetic diseases that include disorders of genomic imprinting. This issue is important since subtle changes in the behavior of imprinted genes may not be manifest until late in development, perhaps years after birth. The need to directly quantify the epigenetic consequences of ART and infertility is becoming increasingly important as over a million babies have been born world-wide using technologies which manipulate the gametes and embryos at exactly the time when epigenetic reprogramming would normally occur in vivo. There is also widespread use of “invasive” techniques such as ICSI, extended culture, and the in vitro maturation of oocytes. A number of concerns have been raised which are founded on epidemiological studies which have documented disorders of genomic imprinting such as Angelman syndrome and Beckwith-Wiedemann syndrome in children conceived by assisted conception. Evidence is also emerging which suggests that derivation and culture methodologies may induce epigenetic modification in embryonic stem cells. Additionally, genetic analysis has revealed that specific epimutations at
imprinting control regions are causative, leading to disruption of imprinted genes, and that these epimutations are likely to have arisen in the oocyte, sperm or preimplantation embryo. These aberrations have sparked considerable debate and require close scrutiny. Unfortunately, our knowledge of imprinting and epigenetic regulation during this critical period of early human development is very limited and the great majority of the available evidence has been extrapolated from basic research in other species where the data shows that various ARTs are capable of inducing epigenetic defects in \textit{in vitro} derived embryos. However, there is a high degree of discordance of imprinting status between humans and other species and there is even variation in the regulation of epigenetic information (DNA methylation) during preimplantation development between species. It may therefore be erroneous to extrapolate data directly from animal studies to humans where very little primary research has been conducted to establish the exact sequent of events and/or the genes involved in imprint establishment, maintenance and epigenetic regulation in gametes and embryos. The debate surrounding the potential for epigenetic disturbances in human ART can only be fully resolved once a solid understanding of the epigenetic mechanisms relevant to gametogenesis and preimplantation development have been established in humans. In the absence of this fundamental information the epigenetic health of human preimplantation embryos derived by ART remains of paramount importance.

\textbf{IN VITRO MATURATION AND GENOMIC IMPRINTING}

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Several studies have linked assisted reproductive technologies such as ovarian stimulation and in vitro embryo culture to aberrant imprinting in different species. Furthermore, several studies have suggested an increased incidence in rare human imprinting disorders in children conceived after ART.

In-vitro maturation of oocytes (IVM) is a new technique suitable for oocyte banking in cancer patients. There is no evidence that IVM is associated with an increased risk for congenital malformations or abnormal fetal and neonatal growth. Two studies in human reported aberrant imprinting establishment in human oocytes after superovulation followed by IVM. However, there were a number of confounding factors in these studies: the underlying
infertility, the advanced maternal age, the fact that immature oocytes (of reduced quality) that failed to respond to superovulation were used; and the fact that the IVM oocytes had no or few cumulus cells (which play a major role in oocyte maturation) attached. Therefore, the effect of an optimal IVM procedure should be assessed in oocytes from young, fertile healthy females (not exposed to superovulation) to exclude possible confounding factors cited above.

Animal models do provide reassuring data on imprinting establishment: using an in vitro follicle culture system in the mouse model, extreme influences of treatment and culture conditions were found to have no or only minor effects and correct imprinting establishment was found after IVM in bovine oocytes.

Future studies will investigate whether the expression of imprinted genes in embryos derived from oocytes obtained after IVM is unaltered to prove that 1) other epigenetic modifications (such as histone modifications) possibly influencing genomic imprinting are not altered by the in vitro culture conditions, and 2) in vitro culture does not cause a disruption of maternal-effect gene products subsequently required for genomic imprint maintenance during pre-implantation development. Finally, epigenetic studies on cord blood and placenta from children born after IVM will be performed.

GENETIC BASIS FOR PREMATURE OVARIAN AGING

MOLECULAR MECHANISMS BEHIND PREMATURE OVARIAN AGING

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Folliculogenesis is a specialized and regulated process essential for ovarian development, embryogenesis and homeostasis. Pathologic changes in both regulatory and structural components of this pathway affect ovarian differentiation, maintenance, and early embryogenesis leading to premature ovarian failure and early embryo losses. A basic understanding of the biologic modifiers important in folliculogenesis, especially those, which act on the transcriptional level, would further our understanding of ovarian biology as well as provide insight into premature ovarian failure, reproductive life span, menopause, ovarian tumors and early embryonic losses. Identification and characterization of genes preferentially expressed in ovaries is useful in unraveling ovarian-specific pathways and their contribution to ovarian pathology. Moreover, such pathways offer novel opportunities
for ovarian specific therapeutics. For example, others, and we have identified several germ cell specific transcriptional regulators, such as Figla, Sohlh1, Lhx8, and Nobox, as well as secreted signaling molecules Gdf9 and Bmp15. Germ cell transcriptional regulators act as pro-survival factors and regulate genes essential for folliculogenesis and early post-fertilization embryo development. Transcriptional regulators upstream of Sohlh1, Lhx8 and Nobox, will give us more insight into the elusive molecular mechanisms that guide female gonadal differentiation. Loss of function mutations in Figla, Sohlh1, Lhx8 and Nobox genes lead to ovarian failure in mice. Rapid loss of oocytes observed in mouse model systems parallels some cases of non-syndromic ovarian failure in humans. A subset of ovarian-specific genes is mutated in a small percent of women with ovarian failure. Novel genomic technologies promise further insights into the genetics of human ovarian failure and will hopefully help improve our ability to identify women who are at risk for ovarian failure, and women who can benefit from advanced fertility preservation technologies. Better molecular understanding of what makes ovary such a unique organ, will much improve our ability in the future to create healthy eggs in vitro.

PRIMARY OVARIAN INSUFFICIENCY: CLINICAL TRANSLATION FROM BASIC RESEARCH

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Ovarian failure is currently referred to as primary ovarian insufficiency (POI), suggesting that the primary defect of dysfunction resides within the ovary itself. This condition is also classified as WHO, group 3. The cause of this devastating condition remains unknown in the great majority of cases. Depending on the clinic and referral patterns in up to 30% of cases a clear underlying cause can be identified: Genetic (approximate 10-20% of POI is familial) sometimes associated with rare syndromes involving many phenotypic features, previous chemotherapy or radiotherapy, pelvic surgery, inflammation or auto-immune disease.

Causes of POI may be classified as mechanisms interfering with ovarian follicle depletion (either low initial numbers or accelerated follicle loss), or follicle dysfunction (i.e. signal defects, enzyme deficiency or auto-immunity). Many mutations on the X chromosome (including BMP 15) have been associated with POI. Special attention should be paid to the associated between POI and mental retardation, in relation to fragile X (number of CGG
repeats). In addition, a series or rare mutations on autosomes, including FOXL2, FSHR, GDF9, GPR3 and LH beta have been described in these patients.

Gene knock-out studies in rats have provided additional and novel information regarding various factors involved in follicle pool depletion. Much attention is focussed towards assessing the extent of ovarian damage following the use of various chemotherapeutic agents for early age Hodgkin, leukemia and breast cancer. Anti-Mullerian hormone (AMH, produced by pre-antral an early antral follicles) seems to represent the best endocrine marker for the detection of early stages of POI.

The most intriguing questions remains whether from a genetic point of view POI should be considered the extreme of the spectrum of the normal distribution of menopausal age. Contemporary genetic technologies (including genome wide association studies) provide new insight in the possible overlap of factors involved in normal menopause and POI.

**TELOMERES IN REPRODUCTIVE AGING**

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As women increasingly delay attempts at childbearing reproductive senescence in women has become an urgent problem. Reproductive aging primarily affects the oocyte by increasing rates of non-disjunction, aneuploidy, cell cycle arrest, apoptosis, and miscarriage.

Theories to explain oocytes senescence include deficient chiasmata, mtDNA mutations; late exist from a production line during fetal oogenesis, defects in cohesion and spindle abnormalities. Telomeres, repetitive sequences of DNA, which cap chromosome ends and protect them from fusing, shorten with each replication. Oocytes do not divide, but their developmental precursors, oogonia, do. Moreover, the last eggs to ovulate in older females traversed more cell cycles during fetal development than eggs ovulated in younger females.

Telomeres also shorten in non-dividing cells from exposure to reactive oxygen through excision repair. As telomeres shorten the cell arrests and genomic instability ensues. Thus, oocytes ovulated from older women likely started with short telomeres, arising from late exist from the fetal production line, then shortened further from decades of exposure to
reactive oxygen, during the prolonged interval before ovulation. In support of this **Telomere Theory of Reproductive Senescence**, oocytes from mice with short telomeres resemble those from older women, with reduced chiasmata, spindle abnormalities, cell cycle arrest, apoptosis and genomic instability. Furthermore, telomeres in human eggs retrieved for IVF are shorter in failed compared to successful cycles and women with recurrent pregnancy loss or who gave birth to babies with trisomy 21 have shorter telomeres than normal controls. Elucidation of the role of telomeres during reproductive senescence may lead to a reliable biomarker of reproductive aging and lead to new therapies.

**REPRODUCTION AND FERTILITY PRESERVATION IN TURNER WOMEN**

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Ovarian failure and early loss of fertility is one of the features in Turner’s syndrome. Of them about 15% have signs of spontaneous puberty, but only some 5% of Turner women undergo menarche and have a possibility for pregnancies. Also those young Turner women who have spontaneous puberty, tend to undergo early ovarian failure. Therefore, fertility preservation might be important for such girls.

We have studied how many Turner girls have follicles in their ovaries, and which relationship the presence of follicles has to the karyotype, age, signs of spontaneous puberty and serum concentration of FSH, LH or AMH, in order to define indication for fertility preservation (Borgström et al. JCEM 2009). Ovarian cortical tissue was biopsied form 57 Turner girls for fertility preservation and histological follicle count. Follicles were found from 15 girls (20%). Signs of spontaneous puberty, mosaicism, and normal hormone concentrations were positive and statistically significant but not exclusive prognostic factors as regards finding follicles. Oocyte donation is an effective way to help Turner women to get children, but the high risk of aortic dissection has to be excluded by MRI and echography before the planned pregnancy.

**MINIMAL STIMULATION IVF: REALITY OR HYPE?**

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After the initial years of IVF, profound ovarian stimulation became the rule. The stimulation of growth of large numbers of follicles and the retrieval of many oocytes was viewed as an
acceptable marker of successful IVF treatment. Medication regimens to achieve profound ovarian stimulation are extremely complex and expensive, take many weeks of frequent injections, and require intense monitoring. Moreover, patient discomfort and chances for serious side effects and complications are considerable. In addition, this profound stimulation gives rise to greatly abnormal luteal phase endocrinology, and is likely to impact on endometrial receptivity and therefore IVF success.

Attitudes toward profound ovarian stimulation are changing, particularly given the growing tendency to transfer a reduced number of embryos, and there is a clear trend toward the use of lower doses of gonadotropins in conventional stimulation protocols. A recent meta-analysis of studies comparing starting doses in IVF confirmed the benefit of this approach, showing a starting dose of 150 IU FSH to provide the optimal balance between providing sufficient number of high quality oocytes while minimising burden and risk of treatment.

The combination of milder protocols with single embryo transfer has been shown to lead to an equal chance of live birth one year after starting treatment as high burden long protocols combined with the transfer of two embryos, while reducing patients’ discomfort, multiple pregnancies, and costs. Apart from clinical efficacy and costs, emotional stress should be considered an important negative side effect associated with IVF treatment. Following milder stimulation protocols, patients report fewer side effects and stress related to hormone treatment and cycle cancellation compared with conventional stimulation. Treatment-related stress has been found to be the most important reason why patients drop out of IVF treatment. The early drop-out of treatment deprives the couple of an optimal cumulative chance of achieving pregnancy, and therefore also impacts on the success of the respective IVF program.

Beyond the hype, milder stimulation regimens are being introduced increasingly into routine practice. However, the so called minimal regimens, in which no gonadotropins or very few, low doses are used, are still finding their place in clinical practice. Fewer eggs after minimal stimulation IVF may have advantages, but as long as the success in IVF is defined in terms of pregnancy rates per cycle, it will be difficult for these regimens to get beyond the hype and enter orthodox clinical practice.

Further reading

LUTEAL PHASE SUPPORT IN IVF: IS THERE AN OPTIMAL PROTOCOL?

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IVF outcomes have been demonstrated definitively to be improved when luteal phase is hormonally supported with either hCG, or Progesterone, particularly in cycles conducted with GnRH agonists or antagonists. HCG supplementation has been associated with increased incidence of ovarian hyperstimulation. Therefore Progesterone has become the main product used for luteal phase supplementation in IVF cycles. Oral progesterone products have been shown to be inferior to the parenteral formulations, and intramuscular progesterone (IMP) has been most widely used throughout the world for many years. However, IMP has also been associated with multiple adverse side effects such as infections, local reactions, abscesses, allergic, and even pulmonary complications. Patients have consistently complained of inconvenience and painful nature of intramuscular progesterone injections. An optimal protocol for luteal phase support in IVF should involve an effective medication, which is also convenient to administer, has minimal side effects, and is affordable to patients.

In this presentation we will review the data from prospective randomized studies and meta-analyses that demonstrate equal efficacy and superior patients’ acceptance of vaginal progesterone preparations. The data will lead us to conclude that vaginal progesterone preparations should be the standard of care for luteal phase support in IVF. Data on the optimal timing and duration of progesterone administration will also be presented and reviewed.

Data regarding estrogen supplementation in the luteal phase will be presented and discussed. None of the published meta-analyses show benefits of luteal estrogen supplementation in IVF cycles, and therefore routine estrogen supplementation in the luteal phase cannot be recommended.

DOES GnRHa TRIGGER IMPROVE CYCLE OUTCOMES?
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Introduction: Human chorionic gonadotrophin (hCG) has been used as a surrogate for the mid-cycle luteinizing hormone (LH) surge for several decades. Due to structural and biological similarities with LH, hCG binds to and activates the same receptor as LH, the LH/hCG receptor. This receptor is among others constitutively located on theca cells, but also on granulosa cells from a follicle size of 8-12 mm. Despite the fact that hCG effectively secures final oocyte maturation and ovulation, its use as a surrogate for LH has got several drawbacks - first and foremost a sustained luteotropic effect, facilitating ovarian hyperstimulation syndrome (OHSS).

Methods: Recently GnRH antagonist protocols for the prevention of a premature LH surge were introduced, allowing final oocyte maturation to be triggered with a single bolus of a GnRH agonist (GnRHa). GnRHa is as effective as hCG for the induction of final oocyte maturation and ovulation, and similar to the natural mid-cycle surge an FSH surge is also induced. However, significant differences exist between the natural mid-cycle surge of gonadotropins and the surge of gonadotropins elicited after GnRHa trigger, resulting in a luteal phase deficiency. Until recently, prospective randomized studies reported a poor clinical outcome when GnRHa was used to trigger final oocyte maturation in IVF/ICSI antagonist protocols, despite a standard luteal phase supplementation with progesterone and estradiol.

As GnRHa trigger possesses advantages over hCG triggering in terms of a reduced - if not eliminated risk of OHSS, the retrieval of more mature oocytes, and a higher patient convenience, the challenge has been to rescue the luteal phase. Several studies now report a reproductive outcome with GnRHa trigger comparable to that seen with hCG trigger after a modification of the luteal phase support generally used in IVF treatment. The modified luteal phase support used after GnRHa trigger focuses on individualization, according to ovarian response to stimulation; the paramount aim being to secure the reproductive outcome after GnRHa trigger without increasing the risk of OHSS.

Conclusions: GnRHa trigger is now a valid alternative to hCG trigger with potential benefits.

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**NEW CONCEPTS IN ENDOMETRIAL RECEPTIVITY: "THE EMBRYO SELECTION WINDOW"**

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Recurrent pregnancy loss (RPL) is a common and distressing disorder. Chromosomal errors in the embryo are the single most common cause, whereas uterine factors are invariably invoked to explain non-chromosomal miscarriages. These uterine factors are, however, poorly defined. The ability of a conceptus to implant in the endometrium is normally restricted to a few days in the menstrual cycle.

A limited ‘window of implantation’ ensures coordinated embryonic and endometrial development, thereby minimizing the risk of late implantation of compromised embryos. In this lecture, I will review emerging evidence, indicating that RPL is associated with impaired differentiation of endometrial stromal cells into specialized decidual cells. From a functional perspective, this differentiation process, termed decidualization, is not only critical for placentation but also signals the end of the implantation window and bestows on the endometrium the ability to recognize, respond to and eliminate implanting compromised embryos. Thus, it is proposed that spontaneous decidualization of the
human endometrium, which inevitably causes menstrual shedding in the absence of a viable conceptus, serves as functional ‘window for natural embryo selection’.

Conversely, impaired decidualization predisposes to late implantation, negates embryo quality control and causes early placental failure, regardless of the embryonic karyotype. This pathological pathway also explains the common observation that many RPL patients seem exceptionally fertile, often conceiving within one or two cycles. Thus, as the clinical correlate of inappropriate uterine receptivity, ‘superfertility’ should be considered as a genuine reproductive disorder that requires targeted intervention.

DO RECEPTOR POLYMORPHISMS HAVE ANY CORRELATION WITH GONADAL FUNCTION AND ART OUTCOME?

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FSH is fundamental for gonadal function and reproduction in both men and women. FSH act via binding to its specific receptors, the FSHR. The receptor gene is characterised by a large number of SNPs (more than 2100 listed in the NCBI SNP database), most of them located in intronic regions and of unknown heterozygosity rate. Some SNPs, especially those which are nonsynonymous and located in exons have been studied in association with gonadal function.

The FSHR SNPs at nucleotide position 919 and 2039 in exon 10 are very common (heterozygosity: 0.469) and result in the aminoacid transition Thr/Ala at codon 307 and Asn/Ser at codon 680, respectively. In the Caucasian population the two SNPs are mostly in linkage disequilibrium with the Thr307-Asn680 variant covering 55% and the Ala307-Ser680 variant 45% of the alleles. The other two possible combinations represent < 1% of all alleles in Caucasians, while they are more frequent in the far East (1, 2). In addition we described a G/A SNP in the promoter region at position –29, with the G allele covering 75% and the A allele 25% of the alleles in Caucasians, while the distribution is equal (50%) in Indonesians (3, 4).

Overall, the current literature is rather consistent in showing the association of the FSHR Asn680Ser polymorphism with menstrual cycle features.
In controlled ovarian hyperstimulation for assisted reproduction, the concept of a discrete FSH threshold implies that it is necessary to reach a distinct serum FSH concentration allowing follicular growth, for the time necessary to avoid that most follicles become atretic (5-7). Since the biological activity of FSH depend on the FSHR expressed on granulosa cells (8, 9), several studies have focused on the influence of this receptor on menstrual cycle dynamics (8, 10, 11).

To date, the unique FSHR polymorphism considerable as a clear, absolute genetic marker of reproductive features or disfunctions is the Asn680Ser polymorphism, since it modulates ovarian response to FSH (8). Indeed, women with homozygous FSHR Ser680 genotype requires a higher number of FSH ampoules in ovarian hyperstimulation, compared to the homozygous Asn680 carriers (8). These data suggests that the FSHR Ser680 genotype is less sensitive to the FSH action in vivo, compared to the FSHR Asn680 genotype, and subsequent studies in ovulatory and anovulatory women confirmed this observation (12). Basal serum FSH levels were observed to be higher in FSHR Ser680 than Asn680 carriers during the luteo-follicular transition in normo-ovulatory women, reflecting the tendency of Ser680 genotypes to be a factor of major "resistance" to FSH stimulation and explaining its association with a higher ovarian thresold of the gonadotropin (10). Moreover, we could show that the Ala307-Ser680 variant is associated with higher basal serum FSH levels and lower sensitivity to FSH stimulation in women with normal ovarian function undergoing ovarian hyperstimulation for assisted reproduction (8, 10) and during normal menstrual cycle (10), strengthening the evidence that some of FSHR SNPs might affect the ovarian response. These data were corroborated by a study in women undergoing controlled ovarian hyperstimulation which demonstrated that an equal dose of FSH results in a significantly lower level of serum estradiol in homozygous Ser680 women, compared to Asn680 homozygous carriers (13). No difference in number of follicles, retrieved oocytes, fertilization rate or cumulative embryo score were observed between the two groups. Whether the Ser680 polymorphism has any effect on pregnancy rate is currently not known, since different studies have obtained contradictory results, with pregnancy rates in homozygous Asn680 women undergoing to IVF reported to be higher (14) or lower (15) than homozygous Ser680 carriers. However, none of these studies had the statistical power necessary to arrive to conclusive results.

It is plausible that Ser680 homozygous genotype can also influence the duration of fertile age, since the timing of puberty and probably also of menopause can be due to genetic features (16, 17). In fact, the homozygosis for FSHR Asn680 allelic variant has been associated to a slightly delayed age of menarche in Italian women, but the same study did not find any correlation with menopausal age, excluding that FSHR gene may be a predictive factor for this parameter (18).
Finally, a recent meta-analysis study which included several data available from different ethnic group have confirmed that the marker Asn680Ser is associated with a poor response during controlled ovarian hyperstimulation, concluding that the FSHR genotyping could provide important information to customize the dose of FSH during controlled ovarian hyperstimulation and reinforcing the critical role of FSHR SNPs in assisted reproduction treatment (19).

The FSHR polymorphism G/A at position -29 could modulate ovarian response to FSH as well (20). Indeed, a study found that women homozygous for the A genotype undergoing controlled ovarian hyperstimulation and IVF required the highest dose of exogenous FSH, has lower levels of estradiol concentration measured before hCG administration and lower number of preovulatory follicles and of retrieved oocytes, compared to the other genotypes, suggesting an association between the SNP and a poor ovarian response (20). However, this study was performed in only 50 patients and replication in a wider population group is needed to establish the response related to the polymorphism at position -29.

To date, only the studies about Asn680Ser FSHR gene polymorphism provided a consistent association with ovarian function, suggesting that some SNPs in FSHR gene, in particular in exon 10, could be used as marker to predict differences of the response to FSH stimulation in women with normal ovarian function (21) and leading to the conclusion that FSHR is a major gene involved in controlled ovarian hyperstimulation outcome (19).

Bibliography


PROMISES AND PITFALLS OF "OMICS" IN ASSISTED REPRODUCTION

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The high success rates seen following in vitro fertilization (IVF) are attained in many cases through the simultaneous transfer of multiple embryos at the expense of multiple pregnancies. Multiple pregnancies, in turn, are associated with significant morbidity and mortality, primarily due to their propensity to result in preterm birth.

Consequently, decreasing multiple gestations while maintaining or improving overall pregnancy rates remains the most significant contemporary goal in the treatment of infertility. To achieve this goal, an improvement over our current embryo assessment strategies largely based on embryo morphology and cleavage rates by developing an objective, accurate, fast, and affordable test would be necessary. 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.

ASSOCIATION OF IVF AND BREAST AND OVARIAN CANCER

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Background: Currently, 1.2 to 2.3% of children born in the Western world is conceived by assisted reproductive technologies. The long-term effects of ovarian stimulation are unknown. In view of the assumed role of “incessant ovulation and increased gonadotrophin
levels” in ovarian cancer pathogenesis, concerns have been raised that ovarian stimulation may increase the risk of ovarian malignancies. Multiple ovarian punctures as used in IVF might also increase this risk. Furthermore, ovulation induction exposes women to higher endogenous estrogen concentrations than occur in natural menstrual cycles, sometimes for prolonged times. It is possible that these high endogenous estrogen levels, or other hormonal changes (e.g. elevations in gonadotrophin levels), provide a carcinogenic stimulus to the breast. The results of several cohort studies and case-control studies are inconsistent and based on short follow-up. Long-term effects (> 10 yrs) of ovarian stimulation for in vitro fertilization (IVF) on the risk of breast and ovarian malignancies are unknown.

Methods: We identified a nationwide historic cohort of 19,146 women who received IVF treatment in the Netherlands between 1983 and 1995, and a comparison group of 6917 subfertile women not treated with IVF. In 1997-1999 data on reproductive risk factors were obtained from 65% of women and data on subfertility (treatment) were obtained from the medical records. Detailed information on cause of subfertility and subfertility treatment (agents and doses) was collected from the medical records. Data on reproductive and lifestyle factors were obtained from the women through a questionnaire. Data on breast and ovarian cancer incidence were obtained through linkage with the Netherlands Cancer Registry (1989-2003) and the nationwide pathology network PALGA (2004-2007). We calculated population age-, sex- and calendar period specific standardized incidence ratios (SIRs) of breast cancer for comparison with the general population. Multivariable Cox regression analysis was used to quantify effects of treatment and cause of subfertility on breast cancer risk.

The risk of ovarian malignancies in the IVF group was compared with risks in the general population and the subfertile comparison group.

Results: After a median follow-up duration of 14.7 years, 378 invasive breast cancers were observed. The SIR of breast cancer overall was not increased, the risk of breast cancer was not increased after IVF and not increased in the non-IVF group compared to the general population. The SIR of breast cancer increased with follow-up to 1.1 in the 10-14 yrs of follow-up for the IVF group as compared to 0.7 in the non-IVF group. After more than 15 yrs of follow-up the risk was increased compared to the general population. After a median follow-up of 14.7 years, the risk of borderline ovarian tumors was increased in the IVF group compared with the general population with a SIR. The overall SIR for invasive ovarian cancer was not significantly elevated, but increased with longer follow-up after first IVF course; the SIR significantly increased after 15 years. The risks of borderline ovarian tumors and of all ovarian malignancies combined in the IVF group were
significantly increased compared with risks in the subfertile comparison group, adjusted for age, parity and subfertility cause). Exact SIR’s and other data will be presented during the congress.

Conclusions: From 10 years after IVF the risk of breast cancer was moderately increased compared with the general population, but from 15 years of follow-up the risk was moderately increased in the non-IVF group too. This may be explained by a lower number of children compared to the general population. Further evaluation directly comparing IVF and non-IVF treated women while adjusting for confounders will clarify the roles of fertility treatment, subfertility cause and nulliparity.

Ovarian stimulation for IVF significantly increases the risk of borderline tumors of the ovary. After 15 years or more of follow-up IVF also may increase the risk of ovarian cancer. Clearly, the outcome of weighing childwish against the potential risks associated with IVF may differ among couples considering fertility treatment. In the Netherlands the cumulative risk of ovarian malignancy (including BOT) is small, i.e. 0.45% at the age of 55 years; on the basis of our data we estimated a 0.71% risk for women who underwent IVF. This research is supported by the Dutch Cancer Society, grant nr NKI 2006-3631.

IMPACT OF CHEMOTHERAPY ON OVARIAN FUNCTION

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According to 2006 SEER statistics, approximately 120,000 women under age 50 develop cancer each year in the United States. Several studies have shown that loss of reproductive potential after cancer treatment can negatively impact quality of life in young survivors. Unfortunately, many young women unknowingly face reproductive compromise. While about 7% of women across the United States report 12-month infertility, the rates of infertility in young cancer patients are unknown. This lecture will focus on the overall decreased reproductive potential in women of reproductive age that have been diagnosed with cancer and have undergone systemic chemotherapy. We will provide estimates of age-specific rates of infertility and early menopause for use in counseling reproductive age women receiving chemotherapy about their additional risks of reproductive impairment beyond acute ovarian failure (AOF). In order to give patients appropriate, age specific counseling, it is critical that they understand the increased risk of infertility and early menopause beyond that of acute ovarian failure. These findings can provide improved counseling regarding reproductive impairment for young women facing a cancer diagnosis.
OVARIAN STIMULATION IN CANCER PATIENTS

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Ovarian stimulation for oocyte or embryo cryopreservation can be a challenge in cancer patients due to time restrictions, medical complications as well as estrogen sensitivity. Over the last decade we developed specialized approaches in overcoming these barriers. These include use of aromatase inhibitors, random start controlled ovarian stimulation, in vitro maturation of immature oocytes as well as utility of tamoxifen as an ovarian stimulant. Surrogacy has also been used as a complementary approach. At the conference we will share our recent findings on these approaches including the follow up on pregnancies from letrozole stimulated cycles. Use of GnRH analogs for trigger and to minimize estrogen exposure, while potentially improving maturation rates will also be discussed. The lecture will be a valuable review of the topic for both cancer specialists and those who work in the field of reproductive medicine.

BRCA MUTATIONS AND FERTILITY

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Mutations in the BRCA genes, which are associated with breast and ovarian cancer susceptibility, are transmitted through the germ line, raising the question of what impact these mutations have on germ line survival, and ultimately on ovarian reserve and fertility.

The proteins encoded by BRCA genes play important roles in DNA repair and localize to telomeres, which have been implicated in reproductive aging. Mutations in the BRCA genes, when present in the homozygous state are embryo lethal. The impact of heterozygous BRCA mutations on reproductive function is less well understood. Understanding the effects of BRCA mutations on reproduction is an important priority because survival rates following cancer have increased markedly allowing more survivors to worry about the effects of cancer and its treatment on their fertility. Moreover, the biology underlying aging and cancer are intertwined, so as women increasingly delay attempts at childbearing the effects of aging on reproduction and cancer risk become
increasingly impactful. Because BRCA mutations affect DNA repair and telomere integrity we sought to investigate whether fertility is reduced in women carrying mutations in the BRCA genes (BRCA1 and BRCA2), compared with non carrier family members. Using a matched case-control design we studied 2,254 BRCA carriers and 764 non carrier controls from the same families. We obtained histories of fertility problems (yes/no) and prior use of fertility medications (yes/no) by questionnaires in cases and controls. We found no difference in mean parity between carriers (1.9) and non carriers (1.9) and no effect of the BRCA gene mutation on fertility.

Identification of effects of BRCA mutations on fertility at the end of reproductive life, however, would require a larger study with age-stratified analysis. The high frequency of BRCA mutations among the Ashkenazi Jewish population led Moslehi et al to hypothesize the opposite effect of BRCA mutations on reproduction - positive selection for BRCA mutations. 260 Ashkenazi Jewish women with ovarian cancer and 331 controls, unselected for age or family history of the disease, had similar pregnancy success as 96 mutation carrier (0.84) and 164 non carrier cases (0.87) and controls (0.83). After adjusting for covariates, no significant differences between BRCA carrier and non carrier cases and controls emerged with regards to fertility, despite lower pregnancy rates among all cases compared to controls (P = 0.0049). Comparisons among the three groups yielded statistically significant distortion against males among the offspring of known and obligate BRCA carriers compared to non carriers (OR = 0.74, 95% CI:0.55- 0.99) and controls (OR = 0.71, 95% CI:0.54-0.94). Both of the above investigations were limited by their study of parity as the reproductive outcome of interest, since parity is a crude assay of ovarian reserve. Indeed, women with defective BRCA function would be expected to initially exhibit normal fertility, but eventually develop precocious oocyte dysfunction.

Moreover, concerns over cancer risk may discourage women with BRCA mutations from attempting pregnancy later in life. Ovarian response to stimulation provides a more sensitive assay of ovarian reserve. Oktay et al performed ovarian stimulation in 126 women with breast cancer using letrozole and gonadotropins for fertility preservation. Oocyte yield and incidence of low response were compared following ovarian stimulation according to BRCA mutation status. 47 of 82 women meeting criteria (57%) had undergone BRCA testing, and 14 carried a BRCA mutation. Low ovarian response was significantly higher in BRCA mutation-positive patients compared with BRCA mutation-negative patients (33.3 v 3.3%; P = .014) and with BRCA-untested women (2.9%; P = .012).

All BRCA mutation-positive low responders carried BRCA1 mutations. Low response was not encountered in women who bore only BRCA2 mutations. Compared with controls,
BRCA1 mutation- but not BRCA2 mutation-positive women produced lower numbers of eggs (7.4 [95% CI, 3.1 to 17.7] v 12.4 [95% CI, 10.8 to 14.2]; P = .025) and had 38.3 times the odds ratio of low response (95% CI, 4.1 to 353.4; P = .001). BRCA1 mutations, therefore, are associated with occult primary ovarian insufficiency. This finding should be shared with women harboring BRCA1 mutations in counseling them for fertility preservation. We do not know whether oocytes from women harboring BRCA1 mutations also exhibit reduced developmental competence. Mutations in genes such as BRCA1 also may contribute to the link between infertility and breast and ovarian cancer risks.

CGH AND OTHER TECHNOLOGIES IN PGD OF CANCER GENES AND OTHER INHERITABLE DISORDERS

Joris Robert Vermeesch

We developed several FISH approaches to enable preimplantation genetic diagnosis of cancer predisposition syndromes. In addition we developed several novel tools to genome wide screen for CNVs and SNPs in single cells. Those technologies are now being applied for polar body, blastomere and blastocyst screening for chromosomal imbalances and an overview of the current status of the technology will be provided. We applied the technologies to cleavage stage embryos from young fertile couples and discovered, unexpectedly, an extremely high incidence of chromosomal instability, a hallmark of tumorigenesis (Vanneste et al., Nature Medicine, 2009; Vanneste et al., Hum.Reprod., 2011) Based on the copy number changes that were observed in the blastomeres it was hypothesised that chromosome breakages and fusions occur frequently in cleavage stage human embryos and instigate subsequent breakage-fusion-bridge cycles. In addition, it was hypothesized that the DNA breaks present in spermatozoa could trigger this CIN. To test these hypotheses, we genotyped both parents as well as 93 blastomeres from 24 IVF embryos and developed a novel SNP-array based algorithm to determine the parental origin of (aberrant) loci in single cells. Paternal as well as maternal alleles were commonly rearranged in the blastomeres indicating that sperm-specific DNA-breaks do not explain the majority of these structural variants. The parent-of-origin analyses together with microarray-guided FISH analyses demonstrate the presence of inv dup del chromosomes as well as more complex rearrangements. These data provide unequivocal evidence for breakage-fusion-bridge cycles in those embryos and suggest that the human cleavage stage embryo is a major source of chromosomal disorders.
Significant progress has been made in techniques and technologies for fertility preservation in women who face battles with cancer, chemotherapy and radiation treatments. However success rates with respect to achieving a pregnancy are variable. Oocyte donation from a third party is a powerful treatment option for those women who cannot use their own oocytes because of severely diminished ovarian reserve or ovarian failure secondary to chemotherapy, radiation, or both. Women who receive pelvic radiation experience difficulties in conceiving and carrying pregnancies because of impaired blood supply to the uterus after radiation exposure, even if the ovaries are pexed outside of the radiation field. Gestational surrogacy is another powerful treatment modality that has been successfully offered to women who wish to have their genetic child but either lack uteri because of prior hysterectomy, congenital absence, or severe impairment due to previous radiation exposure. Medical protocols for ovulation induction for donors and endometrial preparations for recipients have been well established and are similar to those employed for conventional IVF treatments and frozen embryo transfer cycles. Synchronization protocols between oocyte donors/genetic mothers and oocyte recipients/gestational surrogates are easily accomplished with the use of oral contraceptives. In conducting donor oocyte and gestational surrogacy cycles, great care and attention should be paid to the social, psychological and legal aspects of all procedures. Selection of appropriate candidates to serve as oocyte donors and/or gestational surrogates requires meticulous medical and psychological screening.

GERMLINE STEM CELLS AND POSTNATAL OOGENESIS IN MAMMALS

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Although several factors contribute to loss of female fertile potential with age, the progressive depletion of follicle numbers to near-exhaustion is arguably the most critical event. For many years, scientists and clinicians have viewed the ovarian reserve, once
established at birth, as a non-renewable resource. In other words, dogma has taught us that female mammals permanently lose their capacity for oogenesis prior to birth. However, this concept was challenged by a study in 2004, which provided evidence for not just the existence of mitotically-active germ cells, but also ongoing oogenesis, in postnatal mouse ovaries (Johnson et al., 2004). This publication thus raised the possibility of using female germline stem cells (GSCs) as both agents and therapeutic targets for increasing the ovarian reserve.

Although these initial studies identifying the existence of GSC in juvenile and adult mouse ovaries were met with skepticism by some (Tilly et al., 2009; Tilly and Telfer, 2009), female GSCs have recently been isolated from postnatal mouse ovaries by two different research groups using different purification strategies (Zou et al., 2009; Pacchiarotti et al., 2010). These cells, which closely resemble male spermatogonial stem cells responsible for maintenance of spermatogenesis in the adult testes, can be stably propagated in vitro for months and spontaneously form immature oocytes in culture. Moreover, following intraovarian transplantation into chemotherapy-conditioned hosts, cell-tracing experiments have shown that GSCs generate developmentally-competent eggs that fertilize and yield normal viable offspring (Zou et al., 2009).

Although it remains unknown if GSC transplantation can replenish the oocyte pool in aging females, subsequent studies have shown that aged mouse ovaries devoid of oocytes retain a small pool of premeiotic germ cells that remain capable of producing oocytes when exposed to a young adult ovarian environment following transplantation (Niikura et al., 2009). Accordingly, failure of GSCs to sustain oogenesis, and thus support the ovarian reserve, with age may reflect both germ cell-intrinsic changes (e.g., replicative senescence) as well as deterioration of somatic microenvironments that support GSC function. Parallel studies from our lab have also focused on identification of agents that enhance oogenesis in adult ovaries, an objective that will be facilitated by our recent validation of an in-vitro screening assay that predicts the ability of a given compound to stimulate oocyte formation when administered to adult female mice in vivo (Wang and Tilly, 2010).

Despite these advances, several challenges lay ahead for this new, and still controversial, field. First, while the purification of GSCs from adult mouse ovaries provides definitive proof of their existence, additional studies are needed to elucidate the physiological role of these cells, if any, in contributing to normal adult ovarian function. Second, efforts are needed to delineate if and why GSC-supported oogenesis declines with advancing age, and how this event plays into exhaustion of the ovarian reserve. Finally, and perhaps most
importantly, priority must be placed on identification of GSCs in, and their purification from, adult ovaries of healthy reproductive-age women. It is on progress in these latter three areas of investigation that this lecture will focus. (Supported by NIH MERIT Award R37-AG012279).

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**OOCYTE FREEZING: IS VITRIFICATION REALLY BETTER THAN SLOW FREEZING?**

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Studies conducted in the last few years suggest that oocyte cryopreservation can be applied in a systematic and reproducible fashion, in some cases with success rates which appear to compete with those routinely achieved with embryo freezing. Vitrification techniques, recently introduced in the field of human IVF, have further enhanced the hope of developing oocyte storage as a viable assisted reproduction strategy. The overall ability of oocyte cryopreservation to generate a viable pregnancy depends on the inclusion in the calculation of all the events of attrition at pre- and post-storage stages. Only under such conditions do certain differences between alternative methods become apparent. For example, it is well known that a major improvement (from 35–40% to 70–75%) in the
survival rate of oocytes frozen and then thawed via slow cooling may be obtained by increasing the sucrose concentration in the freezing solution from 0.1 to 0.3 mol/l (Fabbri et al., 2001). This change also improves the rate of fertilization (Borini et al., 2006; De Santis et al., 2007; Levi Setti et al., 2006). However, the different degree of attrition at the steps of survival after thawing and on fertilization in the 0.3 mol/l sucrose protocol is counterbalanced by a higher implantation rate of embryos generated by the protocol involving the lower sucrose concentration (Borini et al., 2004; De Santis et al., 2007). The overall outcome, considered as the proportion of implantations per thawed oocyte, ultimately makes the efficacy of the two methods very similar (2.4 versus 2.6%) and in any case insufficient for competing with embryo freezing whose efficiency per used oocyte is approximately 5-6%. Our group reported an implantation rate per thawed oocyte used of 5.9%, following the application of a protocol based on differential concentration of sucrose in the freezing (0.2 mol/l) and thawing (0.3 mol/l) solutions (Bianchi et al., 2007). A similar implantation rate per thawed oocyte has been more recently reported by another Italian group (Parmegiani et al., 2008). In recent years, the alternative cryopreservation approach of vitrification has been adopted by several groups as well. By adopting the cryotop vitrification method in cycles involving young donors (mean age 26.7 years), Cobo et al. (2008) achieved an implantation rate per embryo transferred of more than 40%. Nevertheless, when this study is analyzed considering the original number of oocytes used the implantation rate corresponds to a value (8.6%). Such a rate is not very dissimilar from the one (7.3%) resulting from a study conducted by a slow cooling method and including patients with a mean age of 33.7 years (Bianchi et al., 2007). In the literature just a couple of studies comparing the two techniques are available: Grifo and Noyes in 2010 showed no difference between vitrified and slow frozen eggs in terms of clinical outcome while instead Smith et al. (2010) showed a better outcome using vitrification. It is important to point out though the these authors used a slow freezing protocol that has been previously proved to give poor clinical outcome by several groups (Borini et al. 2006; Levi Setti et al. 2006). Evidence gained from approximately one thousand babies born from cryopreserved oocytes has not suggested so far that the process of low temperature storage is associated with an increase in birth abnormalities. This result is very encouraging and brought several IVF clinic to deem oocyte cryopreservation a standard ART procedure.

ETHICAL ISSUES IN FERTILITY PRESERVATION FOR SOCIAL INDICATORS

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Introduction: The breakthrough of the vitrification of oocytes opened the debate on the use of this technology for healthy women. Very soon, clinics all over the world offered fertility preservation to anyone who wanted it. All kinds of concerns were raised against these applications: it would encourage women to further postpone their pregnancies, it would be inappropriate use of medical technology, etc. We will look at these objections in more detail to see whether they stand scrutiny.

Results: The problem of the application of medical techniques for non-medical applications has a long history. The distinction between medical and non-medical applications has never been clear-cut. Moreover, analysis of the use of the label reveals that the concept ‘medical’ is used as a normative concept; it sanctions the use of a technique for certain problems and it justifies reimbursement from public funds. Simultaneously, the ‘medical’-‘non-medical’ tandem frequently overlaps with the need-desire tandem. Desires, contrary to needs, are a matter of personal choice and autonomy.

A key question is whether medical technology (fertility preservation) should be used to cure a health problem (infertility) that is caused by individual decisions (postponed parenthood) strongly determined by the social and cultural context (career, education). An analogy can be made with medical interventions (e.g., gastric bypasses) for morbidly obese persons. Some argue that one should alter the social and cultural context so that women can and want to have children earlier. This is easier said than done. Moreover, even if we believe that this is the way forward, this does not tell us what we should do in the meantime. Autonomy is a very strong argument to allow women to decide for themselves whether the burden of the intervention is worthwhile in order to preserve the chance of having genetically related children in the future.

An interesting exercise of prospective ethics is to work out the (unlikely?) scenario of a large scale uptake of this option. What will be done with the oocytes if they are not requested by the progenitors for their own reproduction? Should they be donated? How should this be organized?

Conclusion: Fertility preservation for non-medical reasons is a highly complex issue that touches upon general views on the appropriate use of medicine, beliefs about motherhood and women’s role in society, long-term consequences for society etc. The blunt rejection of these applications that dominate the present discussion in Europe is too simplistic. We need stronger arguments than those offered at the moment to made a convincing case for a prohibition of egg freezing for social reasons.
OVARIAN CRYOPRESERVATION AND TRANSPLANTATION: WHERE ARE WE NOW?

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Since the first report of ovarian transplantation with previously cryopreserved ovarian tissue there have been less than 50 attempts at ovarian transplantation, and some with fresh tissue. While the origin of some pregnancies are debated as the remaining ovary might have continued to function, the number of live births stand at <15. There are a number of factors affecting the low utility of ovarian cryopreservation and transplantation, as well as limiting success. One of the major limiting factors is the massive ischemic follicle loss during the revascularization phase of ovarian transplants. We have recently developed pharmacological approaches to improving ovarian revascularization immediately after transplantation and the detailed results will be discussed at this meeting. Moreover, improvements in surgical techniques such as the use of robotic surgery or extracellular matrix scaffolds may further enhance success, our preliminary findings will also be discussed at this meeting. Finally, an exciting data on a connection between ovarian transplantation and germ stem cell renewal in the remaining ovary will be presented.

FERTILITY PRESERVATION IN CHILDREN

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Young girls suffering from a malignant disease or other diseases requiring treatment with radiation therapy or chemotherapy are at risk of loss of ovarian function due to the gonadotoxic nature of the treatment. Depletion of the follicular pool in the ovaries can cause absent menarche in the pre-pubertal girls and premature ovarian failure in the post-pubertal girls with subsequent loss of ovarian steroid production and infertility. This risk is age- and dose-dependent and it seems that the younger the girl the less the risk of loss of ovarian function. Certain treatment regimes, such as bone marrow transplantation and protocols containing alkylating agents as well as abdominal radiation, are more gonadotoxic than other treatment protocols. Different options for fertility preservation in cancer patients exist, but presently, for the young cancer patient, the most appropriate technology is cryopreservation of ovarian tissue with the aim to thaw and autotransplant pieces of the cryopreserved tissue at a later stage.
Data from the Danish fertility preservation programme will be presented on a cohort of girls < 18 years of age who have had an entire ovary cryopreserved with regards to treatment received, pubertal status and risk of ovarian failure.


TESTICULAR TISSUE FREEZING AND TRANSPLANTATION

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Male fertility preservation usually focuses on spermbanking. But patients may need other options to preserve or restore their fertility. In some patients, fertility may be suppressed by factors related to the cancer. These patients can be offered banking after surgical sperm recovery by testicular sperm extraction (TESE).

Since spermatogenesis only starts at puberty, prepubertal boys also do not have the possibility of cryobanking spermatozoa before starting cancer treatment. The only spermatogenic cells that are present before puberty are the spermatogonia. Two options for fertility preservation through the application of spermatogonial stem cell banking are currently under research: the spermatogonial stem cell transplantation (SSCT) and the grafting of testicular tissue pieces, both aiming at for regenerating spermatogenesis in-vivo.

Spermatogonial stem cell transplantation: SSCT was developed in mice were it has proven succesfull with both fresh and frozen/thawed cells. SSCT was also successfully applied in a number of other animal species, including primates.

SSCT is a potential solution for fertility preservation in male prepubertal cancer patients. Harvesting and cryostoring spermatogonial stem cells before the start of cancer treatment, and retransplanting them into the testis of the patient after cure, could theoretically result in initiation of autologous spermatogenesis.

One of the major concerns regarding a clinical translation of this technique is the possible malignant contamination of the testis tissue, e.g. in case of leukaemia. Retransplantation of these cancer cells to the patient could cause malignant relapse. It is therefore utterly important to deplete the germ cell fraction from any malignant cell before transplantation.

Apart from this safety hazard, also the effect on the genetic content of the germ cells being generated, cultured or transplanted has to be studied. In a mouse model we evaluated the pre-implantation development of foetuses obtained after SSCT and. After controlling for
strain-related phenomena, we observed no major developmental disorders in offspring obtained after in-vivo conception. These findings were corroborated by a subsequent study in which we studied the imprinting of spermatozoa and offspring obtained after SSCT.

**Grafting of testicular tissue:** An alternative technique for reintroducing the cryopreserved SSCs into the testis is the intratesticular grafting of pieces of tissue. Both fresh and frozen/thawed grafts can induce efficient colonisation of the endogenous seminiferous tubules of the recipient mouse and initiation of donor-derived spermatogenesis. Intratesticular grafting of testis tissue has as a main advantage that the SSCs are transplanted within their original microenvironment and can be supported by donor-derived Sertoli cells. In a clinical set-up this might be important, since it has been hypothesised that cancer treatment might also affect the somatic niche environment of the testis and therefore lead to an inefficient transplantation.

**Clinical application:** Different strategies for testicular stem cell regeneration have been successfully applied in animal models, making them of great promise for application in the human. Although, the translation of any of these techniques from research to clinic will still require a considerable amount of further research, at present, no possibility for fertility preservation for prepubertal patients exists. Therefore, cryobanking testicular tissue should be offered to boys having a high risk for sterility. Both the parents and the boy must be informed about the experimental character. According the guidelines of the British Fertility Society, we propose prepubertal testicular tissue banking to boys having a risk of 80% or more to become sterile. To date 25 samples were stored in patients facing this risk.

**IS IN VITRO GROWTH OF PRIMORDIAL FOLLICLES A REALITY?**

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At present our ability to cryopreserve ovarian tissue is far ahead of the development of methods to realise the fertile potential of this tissue by the complete in vitro growth (IVG) and maturation of follicles. The development of technologies to grow and mature oocytes from the most abundant primordial follicles holds many attractions for research and fertility preservation in humans and animals. Indeed, early staged mammalian follicles lend themselves to culture, however the complete IVG of oocytes from primordial follicles can
only be achieved on the basis of an in-depth understanding of the biology of follicle and oocyte growth in vivo. Any culture strategy designed to support the complete IVG of oocytes and follicles from cryopreserved tissues must mimic the developmental sequence of events and cellular checkpoints seen in vivo as oocyte development is reciprocally linked to follicle development. Despite the demanding biology, several different follicle culture strategies have been tested and significant advances are being made. It is possible to isolate primordial follicles from the ovarian cortex of many species however these follicles tend to have only a short life span in vitro as they undergo abnormal activation or apoptosis as soon as they are put into the culture environment. In contrast, the culture of primordial follicles in situ either in whole ovaries (in rodent species) or in fragments of cortical tissue (ruminants, primates and humans) has proven to be a far more effective method of inducing and maintaining long-term primordial follicle growth in vitro. To date the in vitro development of oocytes from primordial follicles to maturity with the production of fertile oocytes and offspring has still only been achieved in murine models where the normal follicular growth span from the primordial to Graafian follicle stages is relatively short, the follicles are small and the trophic requirements and the interactions between the follicular cells and oocytes are well characterised. However, in large animals and humans it is now possible to produce multi-layer preantral follicles in vitro from primordial and primary follicles. Thereafter, isolated in vitro-derived secondary-staged follicles can be induced to form antral cavities while the somatic cell compartment undergoes differentiation for steroid biosynthesis. In some, but by no means all, culture systems these changes are supported by follicle encapsulation within biological scaffolds such as alginate gels. Our own extensive studies have revealed that the in vitro growth of sheep and human preantral follicles can be achieved with equal efficiency in serum-free cultures, without biological scaffolds or extracellular matrix and using both fresh and cryopreserved tissue. Electron microscopy has shown that the in vitro grown cells have a similar morphology to oocytes and follicles grown in vivo, and that the full sized oocytes grown from sheep preantral follicles over 35-40 days can be induced to undergo nuclear maturation and progression to MII in response to the appropriate physiological stimuli. We consider that this major advance has two implications for the restoration of fertility in girls and adolescents. Firstly, it is possible for the first time to test the fertility and health of in vitro grown oocytes derived from cryopreserved ovarian tissues in large animals. Secondly, the stage is set to incorporate the methodological advances derived from our sequential sheep follicle culture system into a multistage IVG strategy for human follicles. Confirmation of the safety and efficacy of follicle culture strategies remains a priority before they can be used for the restoration of fertility for young patients.
Pharmacological methods to prevent ovarian toxicity of aggressive cancer therapy have been investigated.

**In vitro studies and animal studies:** Gonadal protection by ovarian suppression via gonadotropin-releasing hormone analogues (GnRHa) agonists or antagonists during chemo- and radiotherapy has been studied in vitro and in animal models including mice, rats and monkeys. Although some studies seem to indicate a protective effect of ovarian suppression, data are still conflicting.

The administration of tamoxifen concomitantly with gonadotoxics has recently shown an association with lesser ovarian follicular depletion after exposure to ovarian toxicants and chemotherapeutic agents in rodents, which deserves further investigation.

**Human studies:** Most of available human studies of ovarian protection with GnRHa during cancer therapy are uncontrolled. Although some of those studies seem to indicate a protective effect and authors had argued a protective effect provided by the pre-pubertal hormone milieu, it is currently known that pre-pubertal cancer patients still develop ovarian failure after chemotherapy suggesting a limited benefit of this treatment. A few clinical randomized trials are available and up-to-date most of them do not support a preservation of fertility potential when current ovarian markers were investigated as surrogates of ovarian reserve. Most studies indicating ovarian protection by GnRHa have included clinical menstrual data such as resumption of menses or amenorrhea as surrogates of fertility potential. Studies reporting fertility outcomes after ovarian suppression with GnRHa are scarce and they do not support a beneficial effect of this therapy. The bulk of data from human studies is thus inconclusive.

**Potential agents in development:** Current reports of a reduction of chemo- and radiotherapy-induced follicle loss in vivo by administration of sphingosine-1-phosphate (S1P), an apoptosis inhibitor, raise the possibility of developing therapeutic agents for fertility preservation.

**POLYCYSTIC OVARIAN DISEASE: CONSIDERING PATHOPHYSIOLOGY WHEN TREATING WITH IVF**
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PCOS is the commonest endocrine abnormality in women of reproductive age, estimated to affect 6-10% of women in most populations (1-3). It represents the major cause of anovulatory infertility, involving up to 20% of infertile couples, and is also associated with distressing cutaneous manifestations of androgen excess such as hirsutism and acne (1, 3). The typical biochemical feature is an elevated serum concentration of testosterone but PCOS is also associated with a characteristic metabolic disturbance that includes insulin resistance, hyperinsulinaemia and abnormalities of energy expenditure (4, 5). Crucially, PCOS is now recognised as a major risk factor for the development of type 2 diabetes (T2D) and cardiovascular disease in later life. Women with PCOS have a 3-7 fold increase in risk of T2D (6-8). At least in part, this reflects the strong associations between PCOS and obesity (9, 10), with the latter a frequent concomitant, and likely amplifier, of the PCOS state.

Before any intervention is initiated, preconceptional counseling should be provided emphasizing the importance of lifestyle, especially weight reduction and exercise in overweight women, smoking, and alcohol consumption (11). The recommended first-line treatment for ovulation induction remains the anti-estrogen clomiphene citrate (CC). There are several large prospective studies of predictors of success in terms of ovulation, conception, pregnancy, and live birth after ovulation induction using CC in women with PCOS. Women with PCOS can be counseled on their likelihood for live birth with front-line infertility therapy by the use of the following clinical parameters: BMI, age, degree of menstrual cycle irregularity, duration of attempting conception, the free androgen index (FAI) as well as a hirsutism score. For women with projected poor pregnancy success, the poor utility of this intended treatment should be discussed with the patient, and serious consideration should be given to the pursuit of a more aggressive initial therapy, such as ovarian diathermy, injectable gonadotropins, or in vitro fertilization. Adjuvant metformin therapy seems not to be of any value as far as the outcome live birth is concerned (12). Recommended second-line intervention, should CC fail to result in pregnancy, is either exogenous gonadotropins or laparoscopic ovarian surgery (LOD). The use of exogenous gonadotropins is associated with increased chances for multiple pregnancies and, therefore, intense monitoring of ovarian response is required. Recommended third-line treatment is in vitro fertilization (IVF). During the second line treatment using gonadotrophins initial serum concentrations of LH, testosterone and androstenedione constitute significant predictors for the probability of multi-follicular development. FSH
treatment results in about 40% in an ongoing pregnancy. Predictors for pregnancy are serum insulin-like growth factor-I (IGF-I), testosterone and a women's age. (13). Classical ovulation induction produces very good results in normogonadotrophic anovulatory infertility. Alternative treatment options may not be indicated as first-line therapy in these patients, except for subgroups with poor prognosis (14). The use of individual patient characteristics may lead to a more patient-tailored and more efficient treatment of WHO 2 anovulatory patients. The classical sequence of treatment is efficient for young patients with normal serum androgen levels, but the second-line FSH treatment step could be skipped in older patients with elevated serum androgen levels. Depending on assumptions concerning the costs of FSH, the indication to skip FSH could be widened to younger patients with elevated serum androgen levels (15).

Laparoscopic ovarian surgery alone is usually effective in less than 50% of women, and additional ovulation induction medication is required under those circumstances. There is no evidence of a difference in the live birth rate and miscarriage rate in women with clomiphene-resistant PCOS undergoing LOD compared to gonadotrophin treatment. The reduction in multiple pregnancy rates in women undergoing LOD makes this option attractive. However, there are ongoing concerns about long-term effects of LOD on ovarian function (16). Metformin use in PCOS should be restricted to women with glucose intolerance. Based on recent data available in the literature, the routine use of this drug in ovulation induction is not recommended. Insufficient evidence is currently available to recommend the clinical use of aromatase inhibitors for routine ovulation induction. Even singleton pregnancies in PCOS are associated with increased health risk for both the mother and the fetus (11,17). Miscarriage rates among women with PCOS are believed to be increased compared with normal fertile women, although supporting evidence is limited. Pregnant women with PCOS experience a higher incidence of perinatal morbidity from gestational diabetes, pregnancy-induced hypertension, and preeclampsia. Their babies are at an increased risk of neonatal complications, such as preterm birth and admission at a neonatal intensive care unit. (18).

Treatment of normogonadotrophic anovulatory infertility using the classical treatment algorithm is cost effective. However, not all patients will become ovulatory or will conceive with this treatment. Others, exhibiting multifollicular instead of monofollicular development, may encounter complications such as ovarian hyperstimulation and multiple pregnancy. Based on initial patient characteristics, it may be possible to identify specific patient subgroups with altered chances of success or complications while using one of these interventions. This approach may enable us to improve safety, cost-effectiveness, and patient convenience in future ovulation induction.
References:

4. Robinson S, Chan SP, Spacey S, Anyaoku V, Johnston DG, Franks S 1992 Postprandial thermogenesis is reduced in polycystic ovary syndrome and is associated with increased insulin resistance. Clin Endocrinol (Oxf) 36:537-543


THE PROMISE OF IN VITRO MATURATION IN ASSISTED REPRODUCTION AND FERTILITY PRESERVATION

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Oocyte in vitro maturation (IVM) is a technique suitable to banking of female gametes for Cancer patients. IVM has been limited in particular by reduced embryo development and approximately 10% implantation rate and high levels of early miscarriage (compared to IVF). There is no evidence that IVM is associated with perturbations in fetal and neonatal growth or congenital malformations. Oocyte pick-up can be performed in a natural cycle. In these conditions, oocyte maturation occurs in vitro over a 30-36 h period and is referred to as 'spontaneous IVM'. In most cases the pre-treatment protocol involves administration of hCG (10,000 I.U.), which appears to initiate a cascade of molecular events which possibly mimics an ovulation, perhaps inducing a blunted ovulatory EGF-like cascade in the small follicles. However, even without hCG several follicles <6mm diameter can be aspirated at any moment of the cycle. Progress in laboratory culture conditions appropriate for IVM can significantly enhance not only blastocyst production yields, but also fetal survival following transfer: recent advances include the use of FF- cAMP modulating agents such as phosphodiesterase inhibitors. Addition of oocyte-secreted factors such as GDF-9 and BMP-15 into the culture medium is adding another level of improvement, as is being investigated in several animal models. Appropriate paracrine communication within the cumulus-oocyte complex during IVM is essential for the attainment of an increase in oocyte developmental competence. The latest achievements with IVM from follicles < 12 mm will be presented.
PROTEIN-FREE EMBRYO CULTURE – THE WAY AHEAD?

Jaffar Ali

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and
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Present-day embryo culture medium (ECM) contains donor serum proteins that have the potential to transmit harmful pathogenic protein-bound agents such as viruses and prions to patients/babies/healthcare workers. There is a need to develop culture conditions and systems that are safe and non-hazardous for utilization in therapeutic assisted reproduction, regeneration medicine and food production. The European Union recommends where possible avoidance of the use of non-uniform biological preparations (EU Tissue Directive No.2004/23/EU) by April 2007. IVF workers have not been able to comply with this directive due to non-availability of efficacious alternatives to biological supplements in ECM. This presentation aims to discuss the relevance of protein-free embryo culture in human and animal ART (food production), the research efforts spanning nearly two decades of work to develop/formulate an efficacious protein-free medium and the outcome of a clinical trial using embryos generated in the protein-free medium in the human.

Fifty (50) experiments were performed using mouse zygote assay to determine the individual tolerance levels/optimal concentrations of 50 chemical components that could serve as alternate energy substrates, inorganic salts, antioxidants, chelators, osmolytes, amino acids, vitamins, antioxidants and macromolecules in an effort to replace the multifunctional serum proteins in the ECM. These findings were utilized to formulate different ECM. The best ECM’s were evaluated and modified to PF medium during the course of another 23 experiments, then tested for suitability and safety, specific for human embryos.

In the mouse 100% of 2- and 4-cell embryos developed in PF medium to blastocyst stage (95.5 and 100% respectively in control medium containing proteins; p>0.05). In sibling human oocytes the fertilization rate in PF medium was similar to or better than in control commercially prepared embryo culture medium containing proteins (CECM) for both conventional IVF (85.3%, 116/136 vs 79.2%, 118/149 respectively; p=0.2352) and ICSI (77.8%, 196/252 vs 69.4%, 175/252; respectively, p=0.0432). Quality of day 2 embryos in PF medium was superior to CECM. The average blastomere number was significantly
higher in embryos generated in PF medium than CECM (3.7 vs 3.4 respectively; p=0.0011). Embryo grade was also significantly better in PF medium compared to CECM (3.0 vs 2.8 respectively; p=0.0007; Embryo grading range: 4=excellent; 1=poor). Human day 2 embryos generated in PF medium resulted in viable pregnancies (48%, n=114; all age groups; 55% n=95 in women <39yrs; as opposed to 33%, n=1515 in control group. This long-term systematic research effort concluded in the formulation of, for the first time, an efficacious PF medium specific for human embryos. The PF medium eliminates the risk of disease transmission, has less regulatory profile for users, and provides a safe chemically non-variable option in ART. The formulation of the PF medium has created interest in other areas as well. The protein-free culture technology has ramifications in the meat and dairy industries, stem cell technology, cell and tissue transplantation technology, cryopreservation, etc, which as in human ART could benefit from the application of protein-free culture technology.

FREE COMMUNICATIONS (ORAL)

[OP1]

BARRIERS TO FERTILITY PRESERVATION VIA ASSISTED REPRODUCTION TECHNOLOGIES: LOOKING BEYOND COST

Molly Moravek, Matt Will, Xiao Xu, Senait Fisseha
University of Michigan, Ann Arbor, Michigan

Objective: To examine barriers preventing patients with fertility-threatening conditions from pursuing assisted reproductive technologies (ART).
DESIGN: Retrospective chart review.

Materials-Methods: The medical records of women who presented to the University of Michigan Fertility Clinic for fertility preservation counseling from 2004-2010 were reviewed. Demographic data, age, diagnosis, treatment plan, reproductive history, method of fertility preservation, and barriers to pursuing ART were examined.

Results: 72 charts were reviewed, with a mean age of 28.7 years (range 14-43). There was no age difference between the group that chose fertility preservation and the group that did not (mean age 28.8 vs. 28.3). Of 47 women that chose fertility preservation, 17 used embryo cryopreservation, 12 used oocyte cryopreservation, 1 used combined
embryo and oocyte cryopreservation, and 17 used GnRH agonist. Women scheduled to receive high or intermediate risk chemotherapy for amenorrhea were more likely to use ART than women who received low risk chemotherapy (85% vs. 35%, p<0.01). The group that used GnRH agonist was younger than the group that used ART (mean age 24.4 vs. 29.8). The most common barrier to pursuing ART was administration of a gonadotoxic treatment prior to fertility counseling (24%, 17/72). Ten patients (14%) cited cost as a factor; however, only 8 cited it as the primary barrier. Other barriers included medical instability (7), concern about delaying chemotherapy (3) or preexisting infertility (3).

Conclusions: The patient’s health status must always be the primary concern; however, many patients encountered other, potentially modifiable, barriers to pursuing ART. Despite increasing awareness and ASCO guidelines regarding fertility preservation with cancer treatment, almost 25% of patients presented after their fertility potentially had already been compromised. Better efforts should be made to increase coordination between care providers to maximize fertility preservation options for these patients. Financial support: University of Michigan

Keywords: Fertility preservation, Barriers to fertility preservation, oocyte cryopreservation

[OP2]

DEVELOPMENT AND FIELD TEST OF A WEB BASED PATIENT DECISION AID ABOUT FERTILITY PRESERVATION FOR BREAST CANCER PATIENTS

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Objectives: According to the recommendations of the American Society of Clinical Oncology, options of fertility preservation should be considered early after the diagnosis of breast cancer. To inform patients about such options and the consequences of their choice, a web based patient decision aid (pDA) was developed by the authors. A values
clarification exercise with online summary is part of the pDA. The pDA was next revised by medical oncologists, gynaecologists, nurse practitioners, and a textwriter. Before the start of an evaluation study, a field test was conducted to investigate acceptability and to get suggestions for improvement.

**Methods:** The field test of the web based pDA consisted of semi-structured interviews with 10 patients, selected from the database of patients with breast cancer, who had been informed about fertility preservation options in the past. Every paragraph of the website was evaluated for understanding, length of information, relevance, and use of pictures, graphics and tables, using questions with open answers or answers on a five-point scale. Every comment was registered. The interviews were audio-recorded, transcribed, and coded by two independent researchers.

**Results:** All patients were enthusiastic about this type of information. They found the website to be informative, useful, worth reading and easy to understand. They suggested to shorten some text parts. Patients stated that the website was a source of information that they had missed at the time of diagnosis, when they had to decide about options of fertility preservation. The website will be presented.

**Conclusions:** The positive evaluation of this web based pDA has led to improvements. According to this small group of patients web based information can be of great help for newly diagnosed breast cancer patients in decision making about fertility preservation options. Our research group is preparing a nation wide study to compare the web based pDA and usual care.

**Keywords:** fertility preservation, breast cancer, webbased patient decision aid

[OP3]

**AGE BASED EFFICIENCY OF OOCYTE CRYOPRESERVATION: A META ANALYSIS OF RAW DATA FROM 3185 CYCLES**

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We aimed to determine the efficiency of oocyte cryopreservation based on age, cryopreservation method, and oocyte type.

MEDLINE was searched for oocyte cryopreservation reports with pregnancy outcome from 1986 to May 2010. Only IVF cycles with ICSI and mature oocytes were included. The search provided 88 reports of which 36 were case reports and 52 were prospective or retrospective clinical studies. Of the 88 reports 59 used the slow freezing technique (SF) whereas in 29 vitrification (VF) was used. We attempted to contact 33 investigators of the clinical studies in order to get the raw data of each thaw cycle. Seventeen investigators sent their raw data and 16 investigators either did not reply or were unwilling to send their raw data. Therefore, we analyzed raw data from 3185 thaw cycles of which 2299 were SF and 886 were VF cycles.

The details of the results will be discussed at the meeting.

**Keywords:** oocyte cryopreservation, vitrification, slow freezing, age

**[OP4]**

**GENETICS AND "OMICS" (INCLUDES PREIMPLANTATION GENETIC DIAGNOSIS AND NON INVASIVE EMBRYO MONITORING)]**

**LESSON FROM 10 YEARS EXPERIENCE IN MALE INFERTILITY MANAGEMENT AN APPROACH FOR CYTOGENETICS INVESTIGATION OF INFERTILE MEN**

Seyed Mehdi Kalantar, Hossien Fazli, Hossien Khodai, Azam Rasti, S. Mohamad Seyed Hassani, Mohamad Hassan Sheikhha
Research & Clinical Centre for Infertility, Reproductive & Genetic Unit, Shahid Sadoughi, Medical Sciences University, Yazd, Iran

**Objective:** Fifteen percent of couples do not achieved pregnancy after one year of sexual intercourse & at the end of their reproductive life, 2-7% of couples remain childless. Male
factor infertility accounts for about half the cases of couple infertility. Up to 40% of MF the etiology remains unknown. These cases of idiopathic MF the question arises whether these could be explained by, at least in part, genetic factors. Chromosomal abnormalities is one of Genetic cause of infertility.

**Methods:** It was retrospectively reviewed the genetic abnormalities detected clinically in 625 infertile men. Metaphase spreads were made from phytohaemaglutinin-stimulated peripheral lymphocytes using standard cytogenetic techniques. The chromosomal status was analyzed using CytoVision Ultra ver.4.0 from Applied Imaging.

**Results:** In this study, out of 800 infertile males, 96 men had chromosomal aberration as 38 numerical; 47,XXY, 46,XY/47,XXY, 46,XY/47,XY+mar, 48XXYY and 18 cases with structural aberration; 46,XY,dup(4)(q31.1q32), and 46XY,qh+(ch, 1,9,Y), 46,XX,qh+, 46,XY,del 13p, 46,XY/47,XY,+8, del (8)(pter), 46,XY,t7;14(10p;10q).

**Conclusion:** Concerning the potential of various sperm retrieval techniques to transmit genetic defects causing male infertility raises the need for a systematic genetic screening of these patients. An algorithm was designed to be used by andrology evaluation for referring to Genetic counseling during infertility program.

**Keywords:** Male infertility, chromosomal abnormality, cytogenetic.

**POSTER PRESENTATIONS**

**[PP01]**

**BREAST SELF EXAMINATION PRACTICE AND ITS EFFICIENT FACTORS AMONG WOMEN IN ARDABIL**

Pouran Akhavanakbari
Ardabil Universiy of Medical Science

**Introduction:** Breast cancer is the most common cancer and the second major cause of cancer deaths in women. Monthly Breast self examination (BSE) is the strategy to early detection of breast cancer. With performance of Breast self examination, clinical breast examination and mammography can prevent progression of breast cancer in 95%. Studies have demonstrated that practice of Breast self examination is influenced with many
different sociocultural factors. The Purpose of this study was to examine the performance conditions of BSE practice among women in Ardabil.

**Methods:** Using descriptive- analytic study method, 149 women aged over 20 year were selected through random simple sampling. This study was conducted in five health centers located in Ardabil, Iran. The data were collected through a self administered questionnaire. Descriptive and inferential statistics (Chi square and fisher’s exact test) were used to analysis the data by SPSS software (11/5 version).

**Results:** In all 42.9% of the women performed BSE. Only 17.4% of them performed BSE on a regular basis (monthly) and 57.1% never performed BSE. The main reasons for women who not having BSE were being: unaware of breast self examination, unaware of benefits of BSE, unaware of time of BSE, forgetfulness and not having family history of breast cancer. Performing of BSE was significantly correlated with levels of education (p=0.002), income (p=0.007), employment (p=0.000), gravid (p=0.023), marriage age (p=0.01) and mother age in first delivery (p=0.02).

**Conclusion:** The finding revealed that the performance of Breast self examination in women is low and many women had never performed BSE. Providing essential education regarding Breast self examination to promote monthly BSE compliance among women through health care personal, seems to be necessary.

**Keywords:** Breast self examination Practice, women.

**[PP02]**

**APPLICATION OF ELI-P-COMPLEX IN REPEATED PREGNANCY LOSS**

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²Nano fanavar pishro Co. Tehran-Iran

**Objective:** Miscarriage is the common during pregnancy. The cause of repeated pregnancy loss (RPL) remained unknown. It is estimated nearly 30% of all RPL after standard evaluation. Evaluation of natural embryo tropic auto antibodies, IgG class in the
blood serum of the women suffering from habitual miscarriage is be seen necessary in order to prediction of gestation course events and pregnancy outcomes.

**Methods:** ELI-P-Complex is based on the ELISA; assay the probability of pathology in the pregnancy. With this method, was evaluated the immuno reactivity of na-Abs against antigens related to gestation course, in the 50 women (average age 25+3.7) who suffering from habitual miscarriage. The control group include 15 healthy women (average age 25+2.5), has not any miscarriage, and even has only healthy child to the study time. Then, after measurement of OD of Ags, IR of na-Abs was estimated. The results were analyzed with SPSS statically software and p value was considered lower than.05 significant.

**Results:** there was a significant change for all Abs in 43% of patient group, but none of control group. In 24 patients the amount of IR against TG reduced (M=49 cu; p<0.05) and IR in serum against Collagen IV was increased in highest level (M=42 cu;p<0.05). change in IR against S100 in 13 patients significantly reduced (M=41 cu;p<0.05).

**Discussion:** The results showed that ELI-P-Complex proposed as useful tools for screening, monitoring and prediction of pregnancies outcomes, and for choosing and to application the best treatment procedure specific to individuals. However, it is necessary to perfume most study in larg population to confirm the value of test.

**Keywords:** ELI-P-Complex, repeated pregnancy loss, immuno reactivity.

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**[PP03]**

**EVALUATION OF DIAGNOSTIC CRITERIA OF POLYCYSTIC OVARIAN SYNDROME IN ADOLESCENTS**

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¹Dept of Pediatric Endocrinology,Ufuk University,Faculty of Medicine,Ankara,Turkey
²Dept of Gynecology and Obstetrics, Ufuk University,Faculty of Medicine,Ankara,Turkey

**Aim:** Polycystic Ovary Syndrome (PCOS) is a frequently observed endocrinopathy of unknown etiology affecting about 5-10% of women at reproductive age. It is also known as the most common cause of hyperandrogenism in adolescence. But there isn't a consensus
about PCOS diagnose in adolescence, also PCOS and symptoms of exaggerated puberty can overlap. For this reason, diagnosis is difficult in this age group.

**Cases and Methods:** 23 adolescents that referred to pediatric endocrinology outpatient clinic with menstrual irregularity, acne, hair growth and weight gain complaints, between 12-18 years of age, and with menarche at least during the past two years, PCOS diagnosis was made according to having at least two of the Rotterdam criteria. The degree of hirsutism was calculated using Ferriman-Gallwey scoring system. For the diagnosis, on the 3rd day of period blood samples were collected for hormonal (ACTH stimulation test and 17OHP response to LHRH test for the etiology of hyperandrogenism) and biochemical tests. Basal FSH, LH, prolactin, total testosterone, DHEA-S, fT4, TSH, and fasting insulin were measured as hormonal parameters. IR-HOMA was used as the insulin resistance index. An ultrasound examination was made on the 2nd-5th days of the menstrual cycle.

**Results:** Hyperandrogenemia were detected in 82.6% all of the cases. Of these cases, 43.5% were with biochemical hyperandrogenism. 30.4% of the cases (n = 7) were obese. HDL levels were lower in patients with hyperandrogenemia. 73.9% of patients group were found to have the PCO morphology. DHEAS were higher in the group with PCO morphology.

**Conclusion:** Therefore, the presence of hyperandrogenism and chronic anovulation should be investigated in the patients with clinical findings, even if they do not have PCO morphology. However, ultrasound appearance of PCO in patients should be separated from cystic ovarian morphology developed during normal pubertal development. This result shows that imaging of ovaries in adolescents for the diagnosis of PCOS doesn’t help for confirming the diagnosis all the time. It is difficult to make a distinction between exaggerated puberty and PCOS in adolescent girls, but with early treatment interventions in patients that diagnosed as PCOS by clinical and hormonal assessments, we believe that it would be possible to minimize the acute and chronic results of this situation.

**Keywords:** Adolescent, PCOS, diagnosis

[PP04]

**LONG-TERM OUTCOMES OF LETROZOLE-FSH STİMULATİON İN PRE-CHEMOTHERAPY BREAST CANCER PATIENTS**
Erol Arslan, Michael Karsy, Kutluk Oktay

Objectives: Letrozole, a selective aromatase inhibitor, has been used along with FSH to reduce estrogen levels in women with breast cancer (BCa) undergoing ovarian stimulation for oocyte/embryo cryopreservation prior to chemotherapy. Our aim was to study the long-term outcomes of Letrozole-FSH (LF) protocol and compare BCa recurrence rates between patients with LF stimulation and un-stimulated controls.

Methods: This was a prospective study of women (n=141) with BCa <=Stage 3 who underwent ovarian stimulation with letrozole (5mg/day) starting on cycle day 2 (CD2) and gonadotropins 150-450 IU on CD4. Oocyte maturation was triggered either with hCG or GnRHa. Female controls (n=151) with BCa <=Stage 3 who did not undergo stimulation were compared.

Results: The mean age, BCa stage, receptor status (estrogen, progesterone and Her2/neu receptors), and the incidence of BRCA mutations did not differ between LF patients and controls. Cancellation rate for LF patients was 12% (17/141). Age at IVF was significantly higher for canceled vs. non-canceled patients (38.9±0.9 years vs. 35.0±0.4 years, p<0.001) but the starting FSH dose, total FSH, and letrozole dose were not significantly different. Among the LF group (n=124), 102 women underwent embryo cryopreservation, 13 underwent oocyte cryopreservation, and 9 underwent both. Out of 23 women who underwent 29 FETs, clinical pregnancy per ET (CP/ET) and livebirth per ET (LB/ET) rates were 69% (20/29) and 34.5% (10/29), respectively. In comparing self-transfer vs. gestational groups, the CP/ET (8/10 vs. 12/19) and LB/ET rates (5/10 vs. 5/19) were not significantly different. Of the 16 babies born (4 singletons and 6 set of twins; 7 males, 9 females) no birth defects were encountered. Recurrence rate was significantly lower for LF stimulated patients (4%) compared to un-stimulated controls (17%). The mean follow-up period for un-stimulated controls was not significantly different from LF stimulated patients (3.1±0.3 years vs. 2.5±0.2 years).

Conclusion: This long-term study showed that the pregnancy rates are comparable to standard IVF success rates and children born after this protocol are healthy. Moreover, LF
was not associated with increased recurrence rates compared to un-stimulated controls. We conclude that the LF protocol is a safe and effective method of ovarian stimulation and fertility preservation, with age appropriate pregnancy success rates.

**Keywords:** Letrozole, stimulation, breast cancer, pregnancy, live birth.

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### Characteristics of breast cancer patients undergoing ovarian stimulation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean±SE (n=141)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at IVF (years)</td>
<td>35.0±0.4</td>
<td>(34.2, 35.8)</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>2.9±0.5</td>
<td>(1.9, 3.9)</td>
</tr>
<tr>
<td>Follow up period (years)</td>
<td>2.5±0.2</td>
<td>(2.1, 3.0)</td>
</tr>
<tr>
<td>Recurrence rate</td>
<td>3.7%±0.03%</td>
<td></td>
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<tr>
<td>Starting FSH dose (IU)</td>
<td>237.1±5.9</td>
<td>(225.5, 248.7)</td>
</tr>
<tr>
<td>Total FSH dose (IU)</td>
<td>1787.6±63.4</td>
<td>(1662.1, 1913.0)</td>
</tr>
<tr>
<td>Total letrozole (mg)</td>
<td>48.1±1.0</td>
<td>(46.5, 50.0)</td>
</tr>
<tr>
<td>Total follicle number</td>
<td>9.8±0.7</td>
<td>(8.3, 11.2)</td>
</tr>
<tr>
<td>E2 at TD (pg/ml)</td>
<td>543.6±35.2</td>
<td>(473.8, 613.3)</td>
</tr>
<tr>
<td>Total oocytes retrieved</td>
<td>14.3±0.8</td>
<td>(12.7, 15.9)</td>
</tr>
<tr>
<td>Mature oocytes retrieved</td>
<td>9.4±0.5</td>
<td>(8.4, 10.4)</td>
</tr>
<tr>
<td>Maturity rate</td>
<td>71.8%±2.1%</td>
<td>(67.6, 76.0)</td>
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<tr>
<td>Inseminated oocyte number</td>
<td>8.6±0.7</td>
<td>(7.2, 10.1)</td>
</tr>
<tr>
<td>Fertilized 2PN oocyte number</td>
<td>6.9±0.5</td>
<td>(6.0, 7.8)</td>
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<tr>
<td>Fertilization rate</td>
<td>77.1%±2.6%</td>
<td>(72.0, 82.2)</td>
</tr>
</tbody>
</table>

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[PP05]

**UTILITY OF SERUM FSH MONITORING IN LETROZOLE-FSH IN VITRO FERTILIZATION CYCLES AND OOCYTE RETRIEVAL**
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**Objectives:** Letrozole (LF) is a highly potent, selective aromatase inhibitor previously used for treatment of breast cancer in post-menopausal women in conjunction with gonadotropins. Letrozole-induced estrogen suppression also results in FSH secretion by an unaffected negative feedback loop. In our previous studies, we proposed serum FSH monitoring as a method of cycle monitoring in LF level at trigger day (TD) of 15.5IU/ml was discriminatory in predicting pregnancy outcomes (ASRM 2010). The purpose of this current work was to analyze whether there is a discriminatory value of TD- FSH that can determine LF cycle outcomes.

**Methods:** One hundred and twenty-seven patients under the age of 45 were evaluated via a prospective study. Inclusion criteria was: patients with intact ovaries, diagnosis with breast cancer stage <=3, LF (5mg/day) treatment starting on cycle day 2 (CD2), and gonadotropin dose at >=150-400IU from CD4.

**Results:** Our previous study utilized logistic regression analysis and determined a discriminatory FSH at TD value of <15.5 for optimal cycle outcomes, therefore the comparison was made between FSH at TD <15.5 vs. >=15.5. When compared this way, there was no significant difference in patient age at the time of IVF, cancer diagnosis, receipt of chemotherapy prior to LF-IVF, overall clinical pregnancy rate per ETs, or total letrozole dose. However, FSH start dose (210±11.7 IU vs. 270.2±5.7 IU) and total FSH dose (1430.6±100.4 IU vs. 2137.3±76.6 IU) were significantly lower in the FSH <15.5 IU/ml group. FSH on CD2 (7.4±0.8 mIU/ml vs. 9.4±0.5 mIU/ml) and, as expected, on TD (11.8±0.6 mIU/ml vs. 28.9±1.0 mIU/ml) were also significantly lower in the FSH <15.5 IU/ml group. Furthermore, the change in FSH from CD2 to TD (ΔFSH) was significantly lower in the FSH <15.5 IU/ml group. E2 on CD2 was not significantly different but E2 on TD (849.2±136.3 pg/ml vs. 445.6±25.0 pg/ml) was significantly higher in the FSH <15.5 IU/ml group. The total number of follicles (14±2.5 vs. 8.0±0.5), number of mature oocytes retrieved (11.1±1.4 vs. 7.6±0.6), and total number of oocytes retrieved (17.7±2.2 vs. 12.2±0.8) were significantly higher in the FSH <15.5 IU/ml group. The total number of oocytes inseminated, number of fertilized oocytes 2PN, oocyte fertilization rate, antral
follcle count, and oocyte maturation rate were not different. Multivariate regression analysis indicated that age at IVF, E2 at TD, and FSH at TD predicted total oocyte retrieval (R2=0.57) while both E2 and FSH on TD predicted the number of mature oocytes retrieved (R2-squared=0.61).

**Conclusion:** Paradoxically, FSH levels <15.5 IU/ml on TD are associated with higher response to LF stimulation. Furthermore, a larger change in FSH from CD2 to TD (ΔFSH) was indicative of a poorer response to LF.

**Keywords:** letrozole, FSH at TD, oocytes retrieved, mature oocytes, outcome

### Patient comparison by FSH level on trigger day cutoff values

<table>
<thead>
<tr>
<th></th>
<th>FSH Level on Trigger Day &lt;15.5 IU/ml (mean±SEM)</th>
<th>FSH Level on Trigger Day &gt;=15.5 IU/ml (mean±SEM)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=21</td>
<td>n=106</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current age [years]</td>
<td>38.9±1.1</td>
<td>38.8±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Age at IVF [years]</td>
<td>34.7±1.0</td>
<td>34.9±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Age at diagnosis [years]</td>
<td>33.6±0.9</td>
<td>34.5±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Percentage of post-chemotherapy patients</td>
<td>10%</td>
<td>14%</td>
<td>NS</td>
</tr>
<tr>
<td>Total letrozole dose [mg]</td>
<td>48.0±2.7</td>
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<tr>
<td>FSH start dose [IU]</td>
<td>210±11.7</td>
<td>270.2±5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total FSH dose [IU]</td>
<td>1430.6±100.4</td>
<td>2137.3±76.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH on CD2/3 [mIU/ml]</td>
<td>7.4±0.8</td>
<td>9.4±0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FSH on TD [mIU/ml]</td>
<td>11.8±0.6</td>
<td>28.9±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E2 on CD2/3 [pg/ml]</td>
<td>66.7±10.9</td>
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<tr>
<td>E2 on TD [pg/ml]</td>
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<td>445.6±25.8</td>
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<tr>
<td>ΔFSH [mIU/ml]</td>
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<td>20.3±0.9</td>
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<tr>
<td></td>
<td>Value 1</td>
<td>Value 2</td>
<td>P-value</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Total follicles number</td>
<td>14±2.5</td>
<td>8.0±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total inseminated oocyte number</td>
<td>10.2±1.5</td>
<td>7.7±0.6</td>
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</tr>
<tr>
<td>Fertilized oocytes 2PN number</td>
<td>7.8±1.2</td>
<td>5.9±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Oocyte fertilization rate</td>
<td>73.7%±6.6%</td>
<td>71.7%±3.1%</td>
<td>NS</td>
</tr>
<tr>
<td>Antral follicle count</td>
<td>11.4±1.0</td>
<td>10.2±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Retrieved mature oocytes number</td>
<td>11.1±1.4</td>
<td>7.6±0.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Retrieved total oocytes number</td>
<td>17.7±2.2</td>
<td>12.2±0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oocyte maturation rate</td>
<td>67.6%±4.2%</td>
<td>63.8%±2.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

*T-tests or Mann-Whitney tests used

**Prediction of the total number of oocytes retrieved by multivariate regression**

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
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<td>7.2488</td>
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</tr>
<tr>
<td>Age at IVF</td>
<td>-0.5339</td>
<td>0.1658</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E2 on CD2/3 [pg/ml]</td>
<td>0.003</td>
<td>0.0175</td>
<td>NS</td>
</tr>
<tr>
<td>E2 on TD [pg/ml]</td>
<td>0.0113</td>
<td>0.0021</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔFSH [mLH/ml]</td>
<td>0.3007</td>
<td>0.1756</td>
<td>NS</td>
</tr>
<tr>
<td>FSH on TD [mIU/ml]</td>
<td>-0.373</td>
<td>0.1627</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total letrozole dose [mg]</td>
<td>0.0436</td>
<td>0.088</td>
<td>NS</td>
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<tr>
<td>FSH start dose [IU]</td>
<td>-0.0027</td>
<td>0.0202</td>
<td>NS</td>
</tr>
<tr>
<td>Total FSH dose [IU]</td>
<td>-0.0014</td>
<td>0.0018</td>
<td>NS</td>
</tr>
</tbody>
</table>

*8-term model showed an R2 value of 0.57

**Prediction of the total number of mature oocytes retrieved by multivariate regression**
<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Regression Coefficient</th>
<th>Standard Error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
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<td>Intercept</td>
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<td>5.0934</td>
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</tr>
<tr>
<td>Age at IVF</td>
<td>-0.2309</td>
<td>0.1171</td>
<td>NS</td>
</tr>
<tr>
<td>E2 on CD2/3 [pg/ml]</td>
<td>-0.0031</td>
<td>0.0114</td>
<td>NS</td>
</tr>
<tr>
<td>E2 on TD [pg/ml]</td>
<td>0.0083</td>
<td>0.0014</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ΔFSH [mIU/ml]</td>
<td>0.2016</td>
<td>0.1163</td>
<td>NS</td>
</tr>
<tr>
<td>FSH on TD [mIU/ml]</td>
<td>-0.239</td>
<td>0.1093</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total letrozole dose [mg]</td>
<td>0.1014</td>
<td>0.057</td>
<td>NS</td>
</tr>
<tr>
<td>FSH start dose [IU]</td>
<td>0.0033</td>
<td>0.0134</td>
<td>NS</td>
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<tr>
<td>Total FSH dose [IU]</td>
<td>-0.0023</td>
<td>0.0012</td>
<td>NS</td>
</tr>
</tbody>
</table>

*8-term model showed an R2 value of 0.61

[PP06]

IMPACT OF CHEMOTHERAPY ON IVF OUTCOMES

Erol Arslan1, Michael Karsy2, Kutluk Oktay1

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2Institute for Fertility Preservation, Department of Obstetrics/Gynecology, Department of Pathology, New York Medical College, Valhalla, NY, USA

Objectives: Though chemotherapy is known to damage ovarian reserve and cause premature ovarian failure, some women retain their menses and appear to have normal ovarian reserve by standard assessment methods such as baseline E2 and FSH. The fertility potential of those women who appear to maintain normal ovarian function post-chemotherapy is unknown. The purpose of this study was to determine whether post-chemotherapy-IVF (PCIVF) success is comparable to IVF performed pre-chemotherapy (IVF).

Methods: This was a secondary analysis of cancer patients undergoing IVF before or after...
chemotherapy. Patients (104 breast cancer and 7 other types of cancers) underwent controlled ovarian stimulation using the following protocols: 86 IVF vs. 16 PCIVF with letrozole + gonadotropin, and 5 IVF vs. 4 PCIVF with gonadotropins only. The great majority of patients undergoing ovarian stimulation had breast cancer (n=104, 86 IVF vs. 18 PCIVF). PCIVF patients received a variety of chemotherapy regimens including: adriamycin and cyclophosphamide (AC, n=7); adriamycin, cyclophosphamide, and taxol (ACT, n=6); adriamycin and taxol (AT, n=4); methotrexate, vinblastine, adriamycin, cisplatin (MVAC, n=1); vincristine, adriamycin and cyclophosphamide (VAC, n=1), and 1 unknown. Patients with day 2/3 serum levels of FSH>13 or E2>70 and age >45 years at the time of ovarian stimulation were excluded.

Results: The mean ages at the time of IVF, CD2/3 FSH and E2 levels, and E2 at TD were not statistically different between PCIVF and IVF patients. The mean interval from completion of chemotherapy to IVF was 5.1±1.3 years. The total number of oocytes (13.5±0.9 vs. 8.7±1.6) and the total number of mature oocytes retrieved (8.9±0.6 vs. 6.6±1.3) were significantly higher in the IVF patients. PCIVF patients received a significantly higher total FSH dose (1751±77IU vs. 2455±278IU). Cancellation rate was significantly higher in the PCIVF (14% [13/91] vs. 30% [6/20], p<0.01). Of those who were not cancelled, 67/78 (85%) in IVF and 7 of 14 (50%) in the PCIVF group underwent embryo or oocyte cryopreservation while the remainder underwent fresh embryo transfer (ET). The clinical pregnancy rate was significantly higher in the IVF group (73%; 8/11) compared to the PCIVF group (42%; 3/7). Restriction of the analysis to breast cancer patients did not alter results.

Conclusion: Ovarian reserve, as assessed by the oocyte yield after ovarian stimulation, is significantly diminished in patients who previously received chemotherapy despite the presence of normal baseline FSH and E2. These results indicate that normal baseline E2 and FSH levels do not guarantee normal ovarian function and response to ovarian stimulation post-chemotherapy. Likewise, IVF pregnancy rates are altered in these women who previously received chemotherapy. Further studies are ongoing to test the utility of more sensitive markers such as AMH in this setting.

Keywords: Chemotherapy, FSH, Estradiol, IVF outcomes
Comparison of pre-chemotherapy and post-chemotherapy patients who underwent ovarian stimulation (IVF vs. PCIVF)

<table>
<thead>
<tr>
<th></th>
<th>IVF (n=91)</th>
<th>PCIVF (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at IVF (years)</td>
<td>35.1±0.4</td>
<td>35.6±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>FSH on CD2/3 (mlU/ml)</td>
<td>7.9±2.6</td>
<td>6.7±0.6</td>
<td>NS</td>
</tr>
<tr>
<td>E2 on CD2/3 (pg/ml)</td>
<td>40.3±2</td>
<td>34.1±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total FSH dose (IU)</td>
<td>1,751±77</td>
<td>2,455±278</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E2 on TD (pg/ml)</td>
<td>566.6±48.5</td>
<td>641.1±194.5</td>
<td>NS</td>
</tr>
<tr>
<td>Retrieved total oocyte number</td>
<td>13.5±0.9</td>
<td>8.7±1.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Retrieved total mature oocytes number</td>
<td>8.9±0.6</td>
<td>6.6±1.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cancellation rate</td>
<td>14% (13/91)</td>
<td>30% (6/20)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Clinical pregnancy/ETs rate</td>
<td>73% (8/11)</td>
<td>42% (3/7)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

[PP07]

THE ROLE AND DISTRIBUTION OF WNT/B-CATENIN PATHWAY IN UNEXPLAINED INFERTILITY PATIENTS

Hafize Seda Vatansever¹, Burcu Kara¹, Yıldız Uyar², Yeşim Baytur², Mahmut Kemal Özbilgin¹

¹Celal Bayar University, Faculty of Medicine, Department of Histology-Embryology, Manisa
²Celal Bayar University, Faculty of Medicine, Department of Gynecology and Obstetrics, Manisa

The Wnt family play a role during cell polarity, proliferation, apoptosis and cell migration during both embryonic and pathologic conditions. Signaling of Wnt is control with two different pathways. The first one that the activation of Dishevelled (Dvl) via occurring of Wnt/Fzd complex which provide the connection of β-catenin and the cell transcription is trigger. The second pathway is β-catenin independent pathway and activate profilin, Rac and Rho.
Success of the implantation depends on blastocysts and receptive uterus. Secretion of growth factors from both embryo and endometriyum play a role during implantation. In unexplained infertility patients infertility could fertilization complete, but, implantation can not be occur. While Wnt/β-catenin signaling pathway was observed in proliferation and early secretion phases of normal endometriyal cycle, there was still no information their roles in unexplained infertile patients.

The role and distribution of Wnt/β-catenin signaling pathway were investigated in proliferation and early secretion phases of endometriyal samples from control and unexplained infertile patients. The samples were done routine paraffin embedding protocol after fixing in 10% formalin solution. The distribution of Wnt7a, β-catenin, Fzd6 and Dkk1 was examined using indirect immunohistochemistry and in situ hibridization tecniques.

After immunohistochemical analyses, the distribution of Wnt7a in the luminal epithelium during proliferation phase was more detectable in control groups then infertile patients, it was observed in luminal epithelium during the secretory phase of infertile patients. The immunoreactivity of β-catenin in both phases was negative in the luminal and glandular epithelium in both groups. Distribution of Fzd6 and Dkk1, in both groups and all three regions were similar during both proliferation and secretion phase and the intensity of immunoreactivity were moderate.

After in situ hybridization analysis, the expression of Wnt7a was different in the luminal and glandular epithelium of both groups during in the proliferation phase, while it was similar in stroma. In secretory phase, the expression of Wnt7a was higher in the luminal epithelium in infertile group. β-catenin expression was different in luminal and glandular epithelium from both groups in proliferative phase, however, in secretory phase, β-catenin expression in the luminal and glandular epithelium was higher in infertile group than the control group. The expression of Fzd6 and Dkk1 were similar in both proliferation and secretory phase in both groups.

Wnt7a was secreted during proliferation phase and prepared to endometrium for implantation, but, in infertile patients the increased of secretion of Wnt7a than control group, epithelial expression of Wnt7a was need for maternal receptivity for embryo. β-catenin expression is observed no difference between control and infertile groups, showed that it was not changed in infertile group. The expression of Fzd6 was similar in both groups and required for invasion of embryo. In conclusion, because of decreased
expression of both the epithelial Wnt7a and stromal Fzd6 in infertile patients, the implantation may be failed. The decreased of Wnt7a expression may be also inhibited expression of Dkk1.

**Keywords:** Wnt, β-catenin, Fzd, Dkk, implantation, infertility, immunohistochemistry, in situ hybridization

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**[PP08]**

**MAKING THE BEST OF ELECTİVE FREEZİNG, BOTH CLİNİCALLY AND ETHİCALLY**

Heidi Mertes, Guido Pennings
Bioethics institute Ghent, Ghent university, Ghent, Belgium

**Objectives:** The possibility for healthy women to cryopreserve their oocytes in order to counter future infertility has gained momentum in recent years. However, women tend to cryopreserve oocytes at a time that is suboptimal from a clinical point of view - in their late thirties - when both oocyte quantity and quality have already considerably diminished and success rates for eventually establishing a pregnancy are thus limited. This also gives rise to ethical concerns, as the procedure is seen as giving false hope to (reproductively speaking) older women.

We evaluate which measures can be taken to turn elective freezing into a procedure that is both clinically and ethically better than the current practice. The main objective of these measures is to convince those women who are most likely to (want to) reproduce at an above average age to cryopreserve their oocytes at a time when this intervention is still likely to lead to a life birth (after cryopreservation).

**Methods:** Three main categories of measures are discerned. First, we consider which measures can be taken to create public awareness, both about the decline in natural fertility, the limitations of artificial reproductive technologies and oocyte cryopreservation. Second, we consider which specific information and counselling should be offered to women who express interest in oocyte cryopreservation and whether gynaecologists should address the issue of freezing with all women of 30 to 35 who have no partner and indicate a child wish in the future. Third, we consider which specific tests can be offered to
those women who decide that they want to freeze their eggs, so that they are better informed about their chances of success.

**Results**: Private cryobanks are likely to do a good job at informing women about their services but may paint an overly optimistic picture of elective freezing. Therefore an important role in making women aware of their reproductive options should also be played by gynaecologists. Women who are interested in elective freezing should be presented with uncomplicated, age-specific live birth rates and be given an individual estimation of the number of stimulation cycles that will be needed. Tests such as antral follicle count or antimullerian hormone (AMH) measurements can help women make an informed decision.

**Conclusions**: Although women in their late thirties express most interest in elective freezing, specifically targeting them to use the procedure gives cause for ethical concern as these women are often offered a false sense of security. This does not mean that elective oocyte freezing is unethical per se, as it can be a very useful procedure if those women who are most likely to benefit from it present themselves before age 35. There are three main routes to optimise the elective freezing ‘clientele’: by creating public awareness, by offering age-specific information and individual counselling and by offering predictive tests.

**Keywords**: Oocyte cryopreservation, fertility preservation, elective freezing, social freezing

**[PP09]**

**RANDOM-START CONTROLLED OVARİAN HYPERSTİMULATİON (RS-COH) FOR EMERGENCY FERTİLİTY PRESERVATION IN LETROZOLE CYCLES**

Murat Sönmezer¹, Ilgün Türkçüoğlu², Sinan Özkavukcu³, Kutluk Oktay⁴

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²Department of Obstetrics and Gynecology, School of Medicine, Inonu University, Malatya, Turkey
³Center for Research on Human Reproduction, Ankara University, Ankara, Turkey
⁴Division of Reproductive Medicine & Infertility, Department of Obstetrics & Gynecology,
New York Medical College, Valhalla, NY, and Institute for Fertility Preservation, New York, NY, USA

**Objectives:** We report an emergency approach of random start controlled ovarian stimulation (RS-COH) in the late follicular or luteal phase of the menstrual cycle for embryo cryopreservation in cancer patients.

**Methods:** Three patients with a diagnosis of breast cancer were referred on menstrual cycle day 11-17 for fertility preservation. Following baseline pelvic ultrasound and hormonal evaluation, RS-COH was commenced immediately using letrozole 2.5 mg/day and rFSH 150-300 IU/day. GnRH antagonist was administered to prevent ovulation on the first day of stimulation in one case, the fifth day in the remaining two. For the ovulation trigger 250 µg of r-hCG or 10,000 IU of u-hCG was administered.

**Results:** Baseline characteristics and the outcome of RS-COH are given in the Table. Following the RS-COH protocol, 9-17 oocytes were harvested and 7-10 embryos were cryopreserved. The mean maturity and fertilization rates ranged between 58.8-77.7% and 69.2-87.5%; respectively.

**Conclusions:** In an emergent setting, ovarian stimulation can be started at a random cycle date for the purpose of fertility preservation without compromising fertilization rates in letrozole cycles.

**Keywords:** breast cancer, emergency fertility preservation, embryo freezing, letrozole

<table>
<thead>
<tr>
<th>Table: Baseline characteristics and COH outcome of the patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case I</td>
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</tr>
<tr>
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<tr>
<td>Histology</td>
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<tr>
<td>COH start day</td>
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<tr>
<td>FSH (mIU/mL)</td>
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<tr>
<td></td>
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<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
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<tr>
<td>Estradiol (ng/ml)</td>
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<td>Progesterone (pg/ml)</td>
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<td>Endometrial thickness (mm)</td>
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<td>AFC (n)</td>
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<td>GnRH-ant start day</td>
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<td>Peak E2 (pg/ml)</td>
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<td>Duration of COH (days)</td>
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<td>Oocytes retrieved (#)</td>
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<tr>
<td>M II (#,% )</td>
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<tr>
<td>M I+GV (#,% )</td>
</tr>
<tr>
<td>Fertilization rate (n,% )</td>
</tr>
<tr>
<td>Cleavage rate (%)</td>
</tr>
<tr>
<td>Embryos frozen (#)</td>
</tr>
</tbody>
</table>

¹ One dominant follicle measuring 20-mm in diameter was observed ² A corpus luteum was observed

GnRH-ant start day was defined as to the day of COH start Fertilization rate was calculated as the ratio of cleaving embryos divided by the sum of number of MII plus in vitro matured MI oocytes

[PP10]

DECISION MAKING IN BREAST CANCER; PRE-IMPLEMENTATION OF A DECISION-AID FOR FERTILITY PRESERVATION (DECIDE)

Mirjam Garvelink¹, Moniek Ter Kuile¹, Leoni Louwe¹, Carina Hilders¹, Anne Stiggelbout²

¹ Leiden University Medical Center, department of gynaecology, Leiden, the Netherlands
² Leiden University Medical Center, department of medical decision making, Leiden, the Netherlands
**Objectives:** Information provision about fertility preservation is not always sufficient for informed decision making, and often late. To improve information provision we developed a web based decision-aid (DA). A pre-implementation study is carried out to reach consensus between different end-users of the DA regarding their attitude towards implementation of the DA.

**Methods:** Delphi expert panel consisting of three rounds. In round 1 and 2 respondents complete a questionnaire, in round 3 respondents take part in an online focus group. We will assess attitudes towards fertility preservation, the procedure of informing patients, and the (implementation of the) DA. Answering categories in the questionnaire range from 1 (totally disagree) to 5 (totally agree). Consensus is considered significant when at least 80% of the participants score either the lowest or the highest two categories. The online discussions will be analyzed qualitatively. Study population (n=27) exists of 10 (ex)patients who have received information on fertility preservation before their breast cancer treatment started, 4 medical oncologists, 3 breast cancer surgeons, 2 radiotherapists, 4 gynecologists, and 4 specialized nurses from different parts of the Netherlands.

**Results:** Data collection will take place from January-February 2011.

(Expected) **Conclusions:** Consensus on the way of implementing a DA to improve information provision about fertility preservation, and on the procedure of introducing it to patients. In case of no consensus we will have important information on heterogeneity of procedures. We expect increased motivation for all end-users to provide or use the DA.

**Keywords:** Decision aid, Fertility preservation, Pre-implementation

[PP11]

**PREVALENCE OF FETAL MACROZOMIA İN NEONATES BORN AT 17 SHAHRİVAR HOSPİTAL, MASHHAD, İN 2009-2010**

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**Objectives:** Macrosomia, resulting from excessive intrauterine fetal growth over 4000 grams, is associated with increased fetal-neonatal and maternal risks. The prevalence of macrosomia in the general population is 8% to 10%, with a significantly greater prevalence
among diabetic mothers. The purpose of this study is determine the prevalence of macrosomia in 3388 delivered women at 17 Shahrivar hospital in Mashhad, the years 2008 to 2009.

**Method:** In this descriptive retrospective study, 3388 women referred for delivery to hospital 17 Shahrivar Mashhad, during the years 2008 and 2009 were studied. 14 spss software and the methods of descriptive statistics were used for data processing.

**Results:** Newborn’s weight was between 200 and 5200 grams. The mean weight was 3218 grams. The incidence of macrosomia according to definition, weight over 4000 grams, was 3.9% (131 cases). Shoulder dystocia rate were reported 0.5 percent (16 cases) in total. Dystocia rate in macrosom neonates was 2.3% (3 in 131 cases of macrosomia), 0.4% in normal weight neonates (12 in 3081 cases) and 0.6% in infants less than 2500 grams (about 1 in 176 newborns). Shoulder dystocia rate was higher significantly in macrosomia (p = 0.002, df = 1). The mean of birth weight was not associated with episiotomy and types of perineal laceration. Meconium before delivery 3.3% and after delivery 6.4% in total 9.7% was detected.

**Conclusion:** From the data it can be concluded that the prevalence of macrosomia in the studied population was less than the general population (3.9% vs. 8 to 10%). Further studies are recommended to determine the cause of low prevalence of macrosomia in our studied population.

**Keywords:** macrosomia, neonates, 17 Shahrivar Hospital

[PP12]

THE EFFECT OF HYDROALCOHOLİC EXTRACT OF BLACK SEED (NİGELLA SATİVA L.) ON THE PITUITARY- OVARY AXIS IN MICE

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The black seed a member of the family of ranunculaceae, have been employed as a spice and curative remedy for numerous disorder. The aim of this study was the effect of hydroalcoholic extract of black seed on the level of pituitary- ovary hormones in Balb/C mice. Five groups of mice, each including eight adult mice were selected. First all of mice
received Cloprostenol, after three days plus Progesterone, for synchronization. The control group received no drugs. While placebo group received normal saline, and three experimental groups received intrapretoneally(IP) injection of 50,100,200mg/kg/2day extract for 20 days. After 10 injection,blood samples were taken from all groups, hormonal measurement, including LH,FSH, estradiol, progesterone were performed by RIA technique. The results is analyzed to use of analysis of unilaterally variance and Duncan test in signification level about %95 by SPSS program. These groups compared with control group. The results indicated significance decrease the levels of FSH,LH for the three experimental groups and significance increase the level of progesterone in dose of 100 mg/kg extract, while no significance increase in the level of estradiol. According to the results of this study, the hydroalcoholic extract of Nigella sativa L. stimulus on the follicle cell and increase excretion of estradiol and progesterone of these, that it is negative feed back on pituitary- ovary axis activities, effectuate decrease of FSH,LH.

Keywords: Niella sativa, Female Mice, Estradiol, Progesterone, FSH, LH

[PP13]

EFFECT OF OXCARBAZEPINE ON MALE REPRODUCTIVE PHYSIOLOGY

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Introduction: Oxcarbazepine is a widely used novel antiepileptic drug. In vitro electrophysiology studies indicate that oxcarbazepine produced blocked of voltage-sensitive sodium channels, resulting in stabilization of hyper exited neural membranes, inhibition of propagation of synaptic impulses. In this research the effect of oxcarbazepine has been studied on the amount LH, FSH, testosterone Hormones and male Reproductive physiology.

Material-Method: In this research 40 adult male was stricken to epilepsy used accidentally between the conferring to neurological clinics of shiraz in four groups which each of them was consist of 10 members, was as follow: Control group not received each material and experimental groups was received 150,300,600 mg/day amount of oxcarbazepine as oral ( tablet ) for 30 days and after the termination of this period for measuring the amount LH, FSH, testosterone Hormones of
blood shooting from vessels of hand. And obtaining result was analyzed, by using ANOVA, Duncan, Tukey and Test.

**Results:** According to the obtained result, the in plasma concentration of LH presented a significant increased at \( p < 0.05 \), whereas in plasma concentration of FSH weren’t seen any significant change. However, in plasma concentration of testosterone presented a significant decrease at \( p < 0.05 \) in using of oxcarbazepine on three experimental groups of min (150 mg/day), med (300 mg/day) and max (600 mg/day) observed that the amount of male reproductive potential decrease with the increasing of drug dose.

**Conclusion:** Obtaining results demonstrated that use up of oxcarbazepine can have negative effect on leydig cells in testis and the production of testosterone was decrease and for this reason the Hypophysis axis is decided to compensate this reduction with more decrease of LH.

**Keywords:** Oxcarbazepine - Male human - FSH - LH - Testosteron

[PP14]

**THE EFFECT OF LEAD ACETATE ON SEXUAL BEHAVIOR AND CHANGES IN THE LEVEL OF TESTOSTERONE IN ADULT MALE RATS**

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**Background:** Nowadays, much attention is paid to the environmental contaminations caused by the toxic effects of lead on the plants and vegetables, animals and humans. In the present study, the oral effect of lead acetate on the parameters related to sexual behavior and also changes in the level of testosterone hormone in adult male rats has been investigated.

**Materials-Methods:** In this experimental study, 40 adult male wistar rats, each weighing 200-220 g were used. They were divided into 5 groups of 8. The control group received nothing and the sham group was given only distilled water and the experimental groups received orally 25, 50, 100mg/kg lead acetate, respectively for 28 days. The changes in testosterone hormone level and sexual behavior parameters have been investigated including, mount latency (ML), intromission latency (IL), post ejaculatory interval (PEI),
mount frequency (MF), ejaculatory latency (EL), intromission frequency (IF), copulatory efficacy (CE) and inter copulatory interval (ICI) at the end of 28 days among the experimental and control groups. The obtained results were analyzed using statistical methods such as TUKEY and ANOVA.

**Results:** Level of testosterone hormone in the group receiving 50, 100 mg/kg lead acetate, showed significant decrease than that in control group. Also, the same doses of lead acetate caused significant increase in ML, IL, PEI and EL, compared to the control group. No significant decrease was observed in MF, but a significant decrease was detected in IF and CE in the experimental group receiving lead acetate with level of 100 mg/kg, than in the control group. ICI showed significant decrease in the experimental groups receiving 50.100 mg/kg lead acetate, compared to the control group.

**Conclusion:** According to the results, lead acetate causes decrease in sexual motivation and sexual performance via affecting the central nervous system and disorder in the regulation and secretion of different neurotransmitters such as serotonin, norepinephrine, in particular dopamine. Also, its effects on leydig cells and reduction in testosterone level causes decrease in sexual behavior and reproductive activities.

**Keywords:** lead acetate, Sexual behavior, Testosterone, Rat.

**[PP15]**

**COMPARISON OF THE RESULTS OF FRESH EMBRYO DONATION IN AZOOSPERMIC COUPLES WITH EMBRYO TRANSFER RESULTS IN TUBAL FACTOR INFERTILITY**

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**Objective:** Our objective was to compare fresh embryo donation embryo transfer results in azoospermic couples with embryo transfer results in tubal factor infertile couples.

**Material-Methods:** A retrospective study was conducted on two hundred twenty five women in a private infertility center. Fresh embryo donation and their comparison group
(female partners with tubal problem) consisted of 158 and 67 patients, respectively. Donors entered the stimulation cycle; simultaneously endometriuma of the recipients were prepared by hormonal drugs. After transvaginal, ultrasound guided oocyte retrieval and ICSI; fresh embryo was transferred transcervically to the recipient. In tubal factor infertile group the same procedures were performed as the above with the gametes of the genetic parents. Fertilization and implantation rates, clinical pregnancy, and chemical pregnancy were compared between the 2 groups.

**Result(s):** Fertilization and implantation rate, clinical pregnancy, chemical pregnancy and blighted ovum were not significantly different between two groups.

**Conclusion(s):** Our study showed that fresh embryo donation has comparable results with embryo transfer in tubal factor infertile couples, so encouraging this policy in azoospermic infertile couples is accepted, especially in our society which sperm donation is prohibited.

**Keywords:** Embryo donation, ICSI, embryo transfer, azoospermia, tubal factor.

[PP16]

**COMPARISON OF THE EFFICACY OF CLOMIPHENE CITRATE, TAMOXIFEN AND LETROZOLE IN OVULATION INDUCTION IN ISOLATED UNOVULATION**

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  of Medical Sciences, Kurdistan, Sanandaj, Iran.

**Background:** There are many drugs have been used for induction ovulation among isolated unovulation (non polycystic ovarian syndrome). The first line oral treatment is anti-estrogens. clomiphene has been the mainstay for induction ovulation since 1962. There may be resistance to clomiphene. So alternative treatments like aromatas inhibitors have been developed.
The aim of this study was to compare the effectiveness of clomiphene, tamoxifen and letrozole for ovulation induction, endometrial thickness, pregnancy, multiple pregnancy, live birth and miscarriage in isolated nonPCOS unovulatory patient.

**Design:** prospective randomized clinical trial.

**Intervention:** 150 infertile women who had isolated nonPCOS unovulation, randomized to 3 groups. Group A received clomiphene 50 mg up to 150mg for 5-7days, Group B received tamoxifen 10 mg up to 30mg for 5-7days, Group C received letrozole 2.5 mg up to 7.5mg for 5-7days. Drugs have been increased in dose and days until six months to maximum dose and 7days. If there was not response to treatment with maximum dose for 7days or achievement of pregnancy treatment was discontinued.

**Results:** Demographic characteristics between 3 groups showed main age and duration of infertility no significant differences. Non-responder patient was 11(33.3%) with clomiphene, 10(30.3%) with tamoxifen and 12(36.4%) with letrozole.

Pregnancy was 32(41.5%) in group A, 20(26%) in group B, 25(32.5%) in group C end abortion was 10(31.3%) in group A, 3(15%) in group B, 4(16%) in group C.

Twin pregnancy was one with clomiphene and tamoxifen, but all pregnancy with letrozole was singletons. Endometrial thickness was higher but not significant with tamoxifen.

**Conclusion:** Ovulation rate were same in three groups. Pregnancy rate and miscarriage were higher with clomiphene then tamoxifen and letrozole (P=0.05 X2=9.37), but no significant differences between tamoxifen and letrozole. Abortion rate was lower if patient conceived with tamoxifen or letrozole then clomiphene.

**Keywords:** Infertility, unovulation, non-polycystic ovarian syndrome, ovulation induction, clomiphene, tamoxifen, letrozole

[PP17]

**EMBRYOLOGICAL ASPECTS OF HCG IN VITRO MATURATION FOR PATIENTS WITH POLYCYSTIC OVARIES**
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**Background:** In this review, recent advances in the laboratory as well as embryological aspects of hCG in vitro maturation (IVM) are described.

**Methods:** This report is based on publications from literature searches and the authors' experience.

**Results:** In IVM cycles, priming with hCG the recovery of a certain number of oocytes with an expanding/dispersed cumulus pattern which facilitates its identification within follicular fluid as compared with non-primed IVM cycles. The immature oocytes with dispersed cumulus cells (CC) at collection have high IVM rates and embryo development potentials. Moreover, a few in vivo matured oocytes with dispersed CC can be obtained, and these have produced good quality embryos. hCG can be given to patients when a dominant follicle reaches 10–12 mm to avoid negative effects on the sibling immature oocytes. ICSI should be performed at least 1 h after the first polar body extrusion. Embryo transfer time depends on quantity and quality of the embryos produced after IVM. Compared with slow freezing, vitrification is a more efficient method for freezing the embryos produced from IVM.

**Conclusions:** The data from the meta-analyses suggests that the effect on clinical outcome of gonadotrophin priming of IVM still needs to be studied. In order to improve the IVM programs, it is essential to define not only the clinical aspects but also the laboratory and embryological aspects.

**Keywords:** hCG, immature oocyte, in vitro maturation, polycystic ovary syndrome
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