Key Lectures

K-1
Quality control and quality assurance in ART lab

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Introduction: The most important issues of activities in ART lab is to assure the highest level of success and outcome , with considering of prevention of errors, satisfaction of patients, following of guidelines, rules and policies. Quality control is to ensure all elements of ART lab are functioning as required with the inspection of all ART Lab activities: Data record, protocols, procedures, guidelines, safety, personnel training and skills, responsibilities and job description, equipment, supplies, corrective actions, policies, laboratory design, and proficiency testing. Quality Assurance is to evaluate, to validate and to verify all ART lab activities and performances with the assessment of elements which have been inspected and measured in Quality control program.
Conclusion: This review has been explained Quality control and Quality Assurance and elements, methods and procedures of those in details and the outcomes derived from following Quality control and quality assurance are useful to determine whether the ART lab is functioning at its optimum rate.

K-2
Influence of human oocyte morphology on embryo development, implantation and its correlation with genotype

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The appearance of the oocyte changes with the multifactor and complex mechanisms which makes the evaluation of oocyte morphology difficult. Developmental capability and implantation potential of derived embryo may be influenced by significant morphological variations of oocyte. These variations may result from intrinsic factors such as age and genetic defects or extrinsic factors such as stimulation protocols, culture conditions, and nutrition.
The aims this study was to detect if certain morphological markers are predictive for oocyte quality in assisted reproductive technology (IVF/ICSI) and also to find out if there is a correlation between oocyte morphology and its genetic status which could be predictive for the genetic health of the oocyte. The controlled ovarian-hyper stimulated IVF/ICSI patients had different qualities and morphologies of maturated oocytes (MII) after follicular puncture. In these oocytes both nuclear and cytoplasmic maturation have to be completed to ensure an optimal oocyte. Criteria's of an optimal and maturated oocyte are; clear cytoplasm with only moderate granulation, intact first polar body and morphologically normal zona pellucida.
Disorders or asynchrony of these processes may result in different morphological abnormalities depending on whether nuclear or cytoplasmic maturation has been affected. The rate of fertilisation or clinical pregnancy may be diminished by some disturbances. To sum up, non-invasive selection criteria's help to identify embryos showing a high implantation potential and reduce the high multiple pregnancies rate after IVF-treatment. In the moment, day 1 embryos turned out to be the time of choice for morphological evaluation. The object of this investigation was also to find out if there is a correlation between oocyte morphology and its genetic status which could be predictive for the genetic health of the oocyte.

K-3
Serum anti-müllerian hormone as a predictive marker in assisted reproduction technology

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Introduction: Anti-Mullerian hormone (AMH) is a dimeric glycoprotein, a member of the transforming growth factor (TGF) superfamily. It is produced exclusively in the gonads and is involved in the regulation of follicular growth and development. In the ovary AMH is produced by the granulosa cells of early developing follicles and seems to be able to inhibit the initiation of primordial follicle growth and FSH-induced follicle growth.
Materials and Methods: From an original sample of 210 patients (age from 20 to 42) serum were collected on the day 3-5 of menstrual cycle and studied for the basal level of AMH, FSH and the number of antral follicles (AFN). In further studies serum and follicular fluid (FF) were collected from 95 IVF/ICSI patients on the day of follicular puncture (FP). The aetiology of patients was tubal or male factor infertility. These patients were divided into two groups as follows: In Group 1: a) correlation between serum and FF with

respect to AMH and correlation between AMH and estradiol (E_2) in serum; and b) comparison of AMH level in serum in response to ovarian stimulation and comparison of AMH level in serum between pregnant and non-pregnant patients. In Group 2: Patients (n=25) were monitored throughout the menstrual cycle until 4 weeks after embryo transfer. In this group, AMH levels in serum were analysed throughout the different ovarian cycle phases and gestation.

Results: There was a significant positive correlation between basal AMH in serum and total number of antral follicles on the day 3-5 of menstrual cycle (r=0.7, p<0.001). We found inverse relations between serum AMH concentrations, FSH and advents patients age. In group 1 on the day of oocytes retrieval, the mean AMH level in FF (2.23±1.2 ng/ml) was significantly higher than that in serum (1.23±0.79 ng/ml (p<0.001). On the basis of normal distributed values of AMH levels in serum and FF, we found a significant positive correlation (r=0.86, p<0.001). There was also a significant and positive correlation between the AMH levels in serum and FF and number of follicles. In response to ovarian stimulation AMH levels in serum increased from low, through moderate, to high response patients (p=0.001), pregnancy rates were 17%, 25% and 48%, respectively. In group 2 the levels of AMH in serum of pregnant (n=12) and non pregnant (n=13) patients decreased throughout stimulation phase and reached a minimum on the day embryo transfer. In the post-retrieval days, from the day of ET, through implantation, to the day of confirmation of pregnancy, the AMH levels of those patients who did not became pregnant (n=13) increased and reached their highest level on the day of ET+2w. In pregnant patients the AMH levels increase slowly up to the early pregnancy time ET+4w.

Conclusion: Our results demonstrated a strong association between AMH and ovarian response to gonadotrophins. Serum AMH seems to result from the follicular pool and its production is independent of the gonadotrophin-dependent indicators of ovarian reserve. This makes AMH unique in providing a perspective, which is not possible with current serum markers. Moreover, for the first time, clinicians may have a reliable serum marker of ovarian response that can be measured independently of the day of the menstrual cycle.

Key words: *Serum anti-müllerian hormone, Predictive marker, Assisted reproduction technology.*

K-4
Blastulation and pregnancy rates after vitrified human zygote culture for 4 days: Preliminary results

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Introduction: Blastocyst culture has been introduced with the aim of increasing the efficacy of embryo selection. In this preliminary study, blastocyst culture was offered to patients, who had shown excessive ovarian response and thus had all 2 PN stage oocyte frozen by vitrification in order to prevent ovarian hyperstimulation syndrome (OHSS).

Materials and Methods: 29 patients were included in this study till now. These patients were stimulated with either corifollitropin alfa or recombinant FSH in a GnRH-antagonist protocol. Final oocyte maturation was triggered with GnRH agonist to avoid OHSS. The fresh embryo transfer was cancelled and all 2PN stage oocytes were vitrified by the Cryotop method (Al-Hasani *et al* (2007). Four to six zygotes were warmed per attempt to transfer and cultured in "Sage sequential media" for further 4 days under oil. A maxiumum of two blasotcysts were transferred in a programmed cycle and if more blastocysts were available they were re-vitrified.

Results: A total of 160 zygotes were warmed from 29 patients till now and 42 embryos reached the early and expanding blastocyst stage, while 9 reached the morula stage (32%). The scoring system used was according to Gardner et al. (1999). The implantation rate achieved in this study was 31.4% and the pregnancy rate was 34.5%.

Conclusion: These results show that blastulation rate after vitrification is high and thus can be offered to patients with a sufficiently high number of 2 PN stage oocytes. In combination with agonist triggering, OHSS can be avoided while efficacy is high.

Key words: *Blastulation, Pregnancy rates, Vitrified human zygote culture.*

K-5
Challenges of infertility surgery 2012

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Many technical developments and a better understanding of a combination of surgical and imaging techniques revolutionized the 20th century. The first laparoscopic surgery in the world was performed by Georg Kelling from Dresden (Germany) in 1901, when he performed an endoscopy on a dog. In the 21st century robotic endoscopic surgery with for example the Da Vinci Surgical System or the Telelap ALF-X and an increasing number of new instruments with multiple degrees of liberty, articulation (Terumo) and new hemostatic effects have enriched surgical endoscopic possibilities. Cameras with a range of settings from 0 to 120 (Endocameleon TM, Karl Storz, Tuttlingen, Germany) and improved optical systems (high-definition television; HDTV) give brilliant pictures. Today we differentiate the following endoscopic surgical techniques in the female reproductive tract.

Laparoscopic surgery: Fimbiolysis, fimbrioplasty,

salpingostomy tubal end to end anastomosis- all together with chromotubation.
Salpingolyis: Ovarian benign tumours- enucleation, ovarian cystectomies.
Ectopic pregnancy: salpingotomy and salpingectomy, Endoscopic surgery in pregnancy, Extragenital gynaecological surgery, Endometriosis surgery including bowel- shaving and- resection, Adhesiolysis and prophylaxis of adhesions, Pelvic infection surgery, Enucleation of subserous, intramural and partly subserous-partly intramural fibroids at singe and multiple locations.
Hysteroscopic surgery: Diagnostic and office hysteroscopy: adhaesiolysis, tubal canalization, enucleation of small fibroids.
Operative hysteroscopy: adhaesiolysis, myoma enucleation, septum division, polyp-excision. In many cases a combined laparoscopic and hysteroscopic approach is suggested. Transvaginal hydrolaparoscopy or fertiloscopy remains still the most simple, but in its diagnostic and therapeutic value limited, surgical procedure to primarily clarify the situation of an infertile female.
Key words: *Infertility, Surgical techniques.*

K-6
Endometriosis, secrets of symptoms and possible treatments with success

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Introduction: According to a global collaboration report through the Endometriosis research foundation in 2010 about 176 million reproductive-age women worldwide are affected and the cost of annual expenses is estimated to be for example in the U.S.A. more than 22 billion Dollars per year. While infertility associated endometriosis receives considerably clinical attention the symptom of pain is often chronic and even debilitating and receives less attention. From the surgical point of view excision of endometriotic lesions by laparoscopy stands still in the center of diagnosis and treatment. For symptomatic adenomyosis hysterectomy is currently considered the most and only effective treatment besides selective excision.
Objectives: Let me today concentrate on the surgical excision of endometriosis, focal adenomyosis and adenomatoid tumours, which often are connected to severe pain.
Materials and Methods: 1) Endometriotic lesions were biopsied and the pathohistological outcome was compared to the suspected diagnosis in 216 patients. 2) We performed histological diagnosis either by ultrasound guided needle biopsy or by endometrial resection or by needle biopsy during laparoscopy (n=15). 3) Two women of reproductive age with uterine adenomatoid tumours.
Results: 1) In black and red lesions, including endometriomas, the suspected diagnosis was confirmed in >90% of cases. In white lesions, however, the diagnosis could only be verified in 53% of cases. 2) In all patients we performed a laparoscopic resection partly combined with a resectoscopic resection in cases of menorrhagia. 3) Tumour excision is difficult because of the missing capsule. Adenomatoid tumours needs to be cut out of the myometrium, there is no clear plane of cleavage.
Conclusion: 1) Purely morphological criteria are not sufficient for the diagnosis of endometriosis but these laparoscopic findings are still our most reliable points of reference. 2) Vaginal ultrasound combined with transabdominal or transvaginal myometrial biopsy established the diagnosis of adenomyosis in 15 infertility patients. 3) The proper laparoscopic handling of these tumours is crucial, because malignancy exclusion is only possible by histologic work-up.
Key words: *Endometriosis, Symptoms, Treatments.*

K-7
Endometriosis and infertility, sclerotherapy in recurrent endometrioma

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Endometriosis is a common hormone-dependent gynecologic disease with a high recurrence. Pathogenic mechanisms in endometriosis-associated infertility are:

- Inflammatory changes in peritoneal fluid (proliferation of macrophages and phagocytic dysfunction, release of proinflammatory and angiogenic factors, changes in peritoneal fluid can affect sperm-oocyte interaction, increased E2 levels in the peritoneal fluid).
- Changes in steroidogenic factor such as production of estrogen in situ and resistance to progesterone that affect endometrium.
- Reducing ovarian reserve by endometriomas or surgery.
- Changes in response to ovarian stimulation.
- Increased peritoneal fluid concentrations of prostaglandins, interleukin-1, tumor necrosis factor and proteases.
- Increased PAPP-A and VEGF in the peritoneal microenvironment.
- Above changes may induce these situations:
- Impaired folliculogenesis and oocyte quality.
- pelvic anatomy distortion.
- immunologic dysfunction.
- Sperm dysfunction.
- Impaired fertilization.
- Impaired implantation.

One condition that put the patients in severe endometriosis is endometrioma. In endometrioma all of

above mechanisms are involved but the most important factors are impaired folliculogenesis and oocyte quality and decreased ovarian reserve.
Laparotomy or laparoscopy is the standard surgery for the large endometrioma, but in recurrent endometrioma repeated surgery is not recommended by many researches because of decreased ovarian reserve. Also, sclerotherapy is basically used to treat different diseases, one of which is endometrioma. In a clinical trial study, we compared 20 patients underwent transvaginal ethanol sclerotherapy for ovarian endometrioma with 20 patients with endometrioma who had no treatment by ethanol sclerotherapy. The result showed the recurrence rate of 20% after 6 months. Most patients undervent IVF after Sclerotherapy and pregnancy rate was increased in this group compared to control group (33.3% vs. 15%, p>0.05). According to these findings, ethanol sclerotherapy is an effective strategy for the treatment of recurrent endometrioma especially before IVF.
Key words: *Endometriosis, Alcohol, Sclerotherapy, Recurrent endometrioma.*

K-8
Ovarian slice freezing, *in vivo* and *in vitro* growth of follicles from ovarian slices and *in vitro* maturation of oocytes

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In-vitro maturation (IVM) of oocytes offers an alternative strategy to patients requiring clinical ART, particularly PCO patients and poor responders. The procedure avoids ovarian stimulation and reduces time and cost by avoiding the requirements for gonadotrophins, and greatly reduces risks such as ovarian hyperstimulation syndrome. Collection of immature oocytes in an unstimulated cycle, combined with vitrification of the resultant mature oocytes, also offers an effective method of fertility preservation for recently diagnosed cancer suffers. This technique can replace or can be combined with ovarian slice freezing, depending on resources and expertise available at the time of treatment. IVM protocols, ovarian slice cryopreservation protocols and the production of developmentally competent oocytes from thawed ovarian slices and in both human and animal species will be reviewed in this talk.
Key words: *Ovarian slice freezing, In-vitro maturation, Oocytes.*

K-9
Vitrification of oocytes and embryos; what's important?

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With the plethora of media and devices available on the market these days it is important to understand the basic principles and concepts of vitrification of oocytes, cleavage stage embryos and blastocysts. A particular media and device in one embryologist's or trainer's hands can work perfectly with '100% success, while in many others this is not the reality. Here we will discuss the key components of the various commercial vitrification media and the principles behind the various devices on the market, and discuss whether the same media can be used for the different stages of embryos and what temperature and equilibration times are acceptable. The concept of a universal thaw solution will also be discussed.
Key words: *Vitrification, Oocytes, Embryos.*

K-10
Embryo assessment and selection

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Over the years it has been an important goal to optimize the treatment of subfertility in order to obtain a better pregnancy rate and baby-take-home rate, usually combined with the goal of decreasing the rate of multiplet pregnancies. This implies optimizing the development of embryos through improved in vitro culture methods. And it also implies optimizing the methods of selection of embryos for fresh and frozen transfers, as well as the timing and location of the deposition of the embryos during transfer. This lecture will deal with embryo morphology and pre-implantation development. Automated time-lapse micro-cinematography is being used to make exact determinations of fertilization, cleavage pattern, and number of nuclei in the blastomeres, degree of fragmentation etc. As opposed to standard microscopy this method provides a continuous microscopy and can be done without interfering with temperature and pH – and without personal being present at critical times.
Another aspect of the present study is the investigation of metabolomics in connection with embryonic development. Our aim is to study the possibility of correlations between metabolomic profiles and morphological development. This study has just started.
Key words: *Embryo assessment, Embryo selection.*

K-11
Assessing the Sperm

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Assessment of sperm has over the years by many been regarded as something secondary to assessment of ova, oocytes and embryos. Often the most inexperienced of the laboratory staff have been asked to evaluate the semen sample. It is our belief that the outcome of

subfertility treatment could be improved considerably, if semen and sperm assessment would be taken more seriously. This may not be as simple as it may sound. This lecture will deal with subjective and objective semen analysis. We will discuss the mistakes, which can inadvertently be made during a subjective analysis, and the advantages-and disadvantages-of an automated CASA (Computer Aided Sperm Assay) system.
The total concentration of sperm is fairly easy to determine, but makes only sense, if we know the motility and morphology, both of which can be very hard to determine. If the motility is very poor, we still may be able to use the sperm for ICSI, if we can show that an immotile spermatozoon is actually alive. The presence of antisperm antibodies may prevent fertilization by IUI or IVF, but ICSI may still work. Severe sperm DNA fragmentation can make IUI and even IVF impossible, but again ICSI can maybe lead to success and a healthy baby. DNA fragmentation assays are relatively expensive, but in cases where the sperm sample has a good concentration, good motility, good morphology and good vitality, but does not bring about fertilization, it may be worthwhile to do a DNA fragmentation assay.
Key words: *Sperm, Assessment, Semen, CASA (Computer Aided Sperm Assay) system.*

K-12
Bio-molecular aspect of Embryo Implantation

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Implantation is an event that happens early in pregnancy in which the embryo adheres to the wall of uterus. At this stage of prenatal development, the embryo is a blastocyst. The fetus receives the oxygen and the nutrients from the mother to be able to grow. Implantation is an important step in establishing a pregnancy and is of major concern in the management of infertility. Failure at this step greatly limits the success of all approach to infertility treatments as well as assisted reproductive technology (ART).
Implantation is predominantly difficult to study, for it requires a blastocyst to interact with a receptive endometrium, which is an in vivo condition. Indeed, our knowledge of what happens during the first week of human life in vivo is limited to a handful of observations. The implantation window is started by preparations in the endometrium of the uterus, both structurally and in the composition of its secretions. The phenomenon of endometrial receptivity has been broadly studied. The endometrium increases in thickness, becomes more vascularized and its glands grow to be tortuous and boosted in their secretions. These changes reach their maximum about 7 days after ovulation. Furthermore, the surface of the endometrium produces a kind of rounded cells, which cover the whole area toward the uterine cavity. This happens about 9 to 10 days after ovulation. These cells are called decidual cells, which emphasizes that the whole layer of them is shed off in every menstruation if no pregnancy occurs. The uterine glands, on the other hand, decrease in activity and degenerate already 8 to 9 days after ovulation in absence of pregnancy. The endometrial epithelium consists of two types of cells that are easily distinguishable by scanning electron microscopy: The secretory and the ciliated cells. The morphology of ciliated cells does not change much during the cycle. In contrast, the secretory cells bear microvilli and develop dependent changes. The apical membranes of the secretory cells lose their microvilli and develop large ectoplasmic protrusions. These protrusions were found to be involved in pinocytosis and were thereafter termed pinopodes. These are abundant experimental evidence that pinopodes provide a specific marker for uterine receptivity in rats. Structures resembling pinopodes present at the time of implantation have been described in all mammals studied so far, including humans. The stromal cells originate from the stromal layers that are always present in the endometrium. However, the decidual cells make up a new layer, the deciduas. The rest of the endometrium, in addition, expresses differences between the luminal and the basal sides. Decidualization succeeds pre-decidualization if pregnancy occurs.
Implantation is characterized by the interaction of two immunologically and genetically distinct tissues. During implantation, local and systemic immune factors, and growth factors may interact with adhesion molecules and other matrix-associated proteins, glycoproteins, and peptides. The embryo differs from the cells of the mother, and would be rejected as a parasite by the immune system of the mother if it didn't secrete immunosuppressive agents. Thus, immunological rejection of the fetus due to recognition of paternal antigens by the maternal immune system, resulting in abnormal immune cells and cytokine production, is postulated to be one cause of unexplained pregnancy loss. Most of the recent investigations suggest differences in the expression of some immune cells and molecules in women with recurrent miscarriage such as CD56+, CD4+.
Most important mother immunosuppressive agents are Platelet-activating factor, human chorionic Gonadotropin (hCG), Prostaglandin E2, Interleukin 1-alpha, Interleukin 6, interferon-alpha, leukemia inhibitory factor (LIF) and Colony-Stimulating Factor (CSF). In addition, new studies have investigated the role of autoimmune factors in implantation in women undergoing in- vitro fertilization. Antiphospholipid antibodies are identified more frequently in women undergoing in-vitro fertilization, but their presence does not appear to influence the outcome of pregnancy, miscarriage, or live birth rates. Antithyroid antibodies are commonly found in women of reproductive age, but implantation rates and miscarriage rates are not altered when women have normal thyroid function. Antinuclear

antibodies may be a marker for underlying autoimmune disease when coupled with certain signs and symptoms, but low-titer antibodies do not influence in-vitro fertilization outcome. Antisperm antibodies are more often associated with fertilization failure when found in high titers in seminal plasma, in sperm, or in the mucosal immune system of women. Antisperm antibodies are uncommon but most often associated with ovarian hypo function.

Embryo implantation in the uterus involves the trophoblast cells apposing and adhering to, then invading across the epithelium lining of the endometrium. However, ethical concerns regarding experimentation with primary human tissue during this period of life necessitates creation of in vitro models for understanding the basic mechanisms involved. Toll-like receptors (TLRs) play a crucial role in defense against pathogens invading the female reproductive tract. Recently, we suggest a novel mechanism by which the presence of intrauterine infection through TLR5 activation may result in implantation failure. These data may provide a new opportunity in the management of infertility cases.

Consequently, understanding the roles of local and systemic immune factors, cytokines, growth factors, adhesion molecules and other matrix- associated proteins in uterine receptivity for implantation is necessary to develop approaches to enhance reproductive health and fertility in humans.

Key words: *Implantaion, Endometrial receptivity, Interleukin, Toll like receptor.*