



ANDROLOGY MINI-SYMPOSIUM

7 April 2017

University of Zagreb School of Medicine, Zagreb, Croatia



100th Anniversary
University of Zagreb School of Medicine

Organizers: **University of Zagreb School of Medicine**
EAA Training Centre Zagreb
University Hospital Centre Zagreb (KBC Zagreb)
Croatian Society for Andrology
Croatian Medical Association
Centre for Excellence in Reproductive and Regenerative Medicine (CERRM) of
the University of Zagreb School of Medicine – Research unit: Biomedical
Research of Reproduction and Development

Croatian Medical Chamber members will be awarded 4 (participants) or 6 (speakers) credits.

PROGRAMME

Friday, 7 April 2017

*Location: University Hospital Centre Zagreb – Rebro, Kišpatićeva 12,
Education Centre East, Lecture Hall 4*

- 14.30 – 14.40 Marijan Klarica, Ante Ćorušić, Željko Kaštelan, Davor Ježek: **Opening remarks**
- 14.40 – 15.00 Zsolt Kopa: **Testis sparing surgery**
- 15.00 – 15.05 *Discussion*
- 15.05 – 15.25 Dimitrios Goulis: **New aspects of Sertoli cells function**
- 15.25 – 15.30 *Discussion*
- 15.30 – 15.50 Gert Dohle: **Current approach to varicocele**
- 15.50 – 15.55 *Discussion*
- 15.55 – 16.10 *Coffee break*
- 16.10 – 16.30 *Exchange lecture with the Croatian Society for Gynaecologic Endocrinology and Reproductive Medicine, Croatian Medical Association*
Dinka Pavičić Baldani: **And now something completely different: PCOS**
- 16.30 – 16.35 *Discussion*
- 16.35 – 16.55 Davor Ježek: **Reinke's crystals and cryptorchidism: is there a link?**
- 16.55 – 17.00 *Discussion*
- 17.00 – 17.10 Dinko Hauptman: **Case report: 32-year old male with azoospermia and partial AZFb Y microdeletion with positive spermatozoa findings**
- 17.10 – 17.15 *Discussion*
- 17.15 - 17.20 Željko Kaštelan, Davor Ježek: **Concluding remarks**



Zsolt Kopa, MD, PhD (Pecs, Hungary, 1965) graduated medicine at the University of Pecs in 1990. He received his PhD degree in 2006 at the University of Szeged with the thesis titled *Non-hormonal option in the evaluation of male infertility and their therapeutic consequences*. He is currently Associate Professor and the Head of the Andrology Centre, Department of Urology at the Semmelweis University in Budapest which is a certified training centre of the European Academy of Andrology. His research interests and experience are as follows: male infertility, microsurgery, sexual medicine and aging male, late onset hypogonadism. He is also a member of the European Association of Urology (Male Infertility Guideline Committee), the European Academy of Andrology, board member of the European Society of Andrological Urology (ESAU), as well as the president of the Hungarian Scientific Society of Andrology.

Testis sparing surgery

Zsolt Kopa

Andrology Centre, Department of Urology, Semmelweis University Budapest, Hungary

The oncological aspect should be primary when treating testicular cancer. Our recent guidelines indicate organ-sparing testicular surgery only in special circumstances: in the case of synchronous bilateral or metachronous contralateral tumours and masses in solitary testicles. Testis tumour resection can have imperative, relative and elective indications. Recently, a more flexible approach can be observed in the case of non-palpable testicular tumours, which can be detected earlier and more easily more easily by means of modern ultrasound devices and examination methods. The majority of non-palpable testicular lesions are benign and in such cases radical orchiectomy would mean an overtreatment. Thus, testis-sparing surgery can be indicated in most cases according to the latest treatment guidelines. A detailed and precise preoperative patient selection is needed, taking into account the fertility aspects. The gold standard of testis-sparing surgery is the microsurgical approach with a high efficacy and low complication rate. Another important question is the treatment of the pathologically confirmed in situ carcinomas (ITGCN) from the tumour bed biopsies. Our presentation also reports the results of the microsurgical testis-sparing approach to testicular tumours in our andrology centre. The organ-sparing approach results in functional benefits, fewer fertility disturbances and avoiding the development of late onset hypogonadism, as well as being favourable in respect of aesthetic and psychogenic aspects. Recent EAU guidelines recommend testis-sparing surgery only in specialised centres.



Dr. Dimitrios G. Goulis was born in Thessaloniki, Greece. He completed his basic medical training (MD) at the Aristotle University of Thessaloniki and received his PhD degree from the same University. Having a scholarship from the European Union, he worked for four years as a clinical research fellow at St. Mary and Hammersmith Hospitals, London, UK, acquiring the specialty of Endocrinology, Diabetes and Metabolism. He is currently an Associate Professor of Reproductive Endocrinology in the First Department of Obstetrics and Gynaecology, Aristotle University of Thessaloniki, Greece. Dr. Goulis has published more than 180 full papers in peer-review international journals and 150 in Greek journals. He was an invited speaker in more than 300 national and international congresses and seminars. He has been a reviewer

in 30 international medical journals and associate editor of "Human Reproduction", "Andrology", "Journal of Endocrinological Investigation", "Hormones", "Anir" and "Human Reproduction Open". He is currently the president of the Hellenic Society of Andrology (2013-2017) and general secretary of the executive council of the European Academy of Andrology (2014-2018). Dr. Goulis' main research interests include male infertility (anti-Müllerian hormone and inhibin-B, sperm DNA fragmentation), endocrine complications of pregnancy (gestational diabetes, thyroid disease, vitamin D deficiency in pregnancy), polycystic ovary syndrome (metabolic syndrome in women with PCOS) and medical research methodology. He was a member of the writing group of the "Thessaloniki European Society of Human Reproduction & Embryology (ESHRE)/American Society of Reproductive Medicine (ASRM) – Sponsored PCOS Consensus Workshop", which produced the "Consensus on infertility treatment related to polycystic ovary syndrome" in 2008.

New aspects of Sertoli cells function

Dimitrios G. Goulis

Unit of Reproductive Endocrinology, First Department of Obstetrics and Gynaecology, Medical School, Aristotle University of Thessaloniki, Greece

Originally described by Enrico Sertoli in 1865, the somatic Sertoli cell performs a crucial nursing function. The Sertoli cell is important for endocrine and paracrine control of spermatogenesis. Functions attributed to Sertoli cells are: (1) supportive and trophic functions for the cells of the seminiferous epithelium, (2) transport of mature spermatids towards the lumen of seminiferous tubules, (3) secretion of androgen binding protein, (4) production of substances with endocrine or paracrine action for spermatogenesis control, and (5) interaction with intertubular endocrine Leydig cells. Sertoli cells are connected to each other by specialized zones of tight junctions of cellular membranes separating the germinal epithelium into a basal and an adluminal compartment. These tight junctions form the blood-testis barrier of the testis. During spermatogenesis, the germ cells pass this barrier entering the adluminal compartment, where they find protection from diffusion of extraneous substances. Thus, the blood-testis barrier does not separate the blood from testicular tissue, but rather constitutes a delicate boundary between diploid and haploid germ cells. A well-functioning Sertoli cell provides the developing germ cells with appropriate mitogens, differentiation factors and sources of energy, as well as protects them from harmful agents and from the host's own immune system. Sertoli cells produce: (1) transport proteins and enzymes, e.g. ABP, transferrin, ceruloplasmin, plasminogen activator (PA), (2) growth factors, e.g. transforming growth factors α and β (TGF- α , TGF- β), insulin-like growth factor (IGF-1), and (3) hormones, such as inhibin B and anti-Müllerian hormone (AMH). Inhibin B and anti-Müllerian hormones (AMH) are glycoproteins belonging to the transforming growth factor β (TGF- β) superfamily; they are produced almost exclusively by the Sertoli cells and have been proposed as direct markers of their function and indirect markers of spermatogenesis. Serum Inhibin B and AMH concentrations seem to constitute additional diagnostic parameters in male subfertility, as they reflect Sertoli cell function. Stimulated concentrations of serum Inhibin B and AMH do not add clinically relevant information in subfertile men compared to basal concentrations of these hormones. Serum Inhibin B and AMH concentrations correlate with testicular histology/cytology but are not superior to FSH as predictors of the presence of sperm in testicular sperm extraction (TESE)/fine-needle aspiration (FNA) biopsy in men with azoospermia.



Gert R. Dohle, MD, PhD (The Hague, Netherlands, 1956) studied medicine at the Free University in Amsterdam and graduated in 1983. He was a research fellow at Leiden University from 1983 to 1985, studying genetic instability in prostate cancer. In 1985 he started his surgical training and proceeded with urological specialization in 1987 at Leiden University Hospital. Since 1991 he is a urological surgeon and fellow of the European Board of Urology (FEBU). He developed an interest in male infertility and was trained in microsurgery in Rotterdam and in New York (with Prof. Marc Goldstein). In 1993 he was appointed a staff member-urologist at the Erasmus University Hospital (Erasmus MC) in Rotterdam and started developing the first andrology unit in The Netherlands. In 1999 he passed the examination of the European Academy of Andrology (EAA-clinical andrologist). In 2001 he finished his PhD on obstructions of the male genital tract at Erasmus University Rotterdam. In 2009 he became Associate Professor of urology at Erasmus MC. He is the head of the Andrology unit of the Reproductive Centre of Erasmus MC and specialized in reconstructive surgery of the male genitalia and urethra. From 2004 to 2010 he was the chairman of the EAU-guidelines on male infertility. In 2010 he was appointed as chairman of the EAU-guidelines on male hypogonadism. Since 2010 he is a board member of the European Academy of Andrology (EAA) and associate editor of *Andrology*, the joint journal of the EAA and ASA. Since 2008 he is board member of the European Society of Andrological Urology (ESAU).

Current approaches to varicocele treatment

Gert Dohle, MD, PhD,

Erasmus MC Rotterdam, The Netherlands.

Varicocele is a dilatation of the veins of the spermatic cord due to reflux of blood from the spermatic vein in an upright position. It is usually left-sided, but can also occur bilaterally in about 15% of cases. Varicoceles are found in 11,7% of men with normal semen, but is more common in men with fertility problems, affecting 25,4% of those with abnormal semen. Analysis of the WHO data of 3468 men attending a fertility clinic indicated that varicocele is related to semen abnormalities, decreased testicular volume and a decline in Leydig cell function. The mechanism by which a varicocele influences male fertility is still unknown, but it is suggested that the impairment of semen in men with a varicocele is due to increased scrotal temperature, impaired blood drainage from the testis and reflux of renal and adrenal metabolites. In some men the presence of clinical varicocele is associated with progressive testicular damage from adolescence onwards and consequent reduction in fertility. In several retrospective studies varicocele repair was associated with improvement of sperm quality and spontaneous pregnancy rates. In a large series of patients Dubin and Amelar in 1977 reported a pregnancy rate of 53% with a follow-up period of five years. Thirty percent of the pregnancies occurred in the first year of follow-up. A recent meta-analysis of randomised controlled trials and observational studies showed that surgical varicocelectomy significantly improves semen parameters in men with abnormal semen, but only in men with clinical varicoceles. A 2013 Cochrane Database system review of randomised controlled trials, comparing treatment of a varicocele to no treatment concluded that there is evidence that treatment of a varicocele in men from couples with otherwise unexplained subfertility improves a couple's chance of pregnancy. Five randomised controlled studies, only including men with abnormal semen analyses and a clinical varicocele, showed that there was a benefit in favour of treatment with a combined OR 2.39 (95% CI 1.56 to 3.66). The best treatment of a varicocele is a microsurgical subinguinal correction: it has the lowest chance for recurrence and shows significant improvements in semen quality in 50-60% of men. Other approaches to varicocele repair show a higher recurrence rate and hydroceles in about 5% of cases.



Dinka Pavičić Baldani is a Professor at the University of Zagreb School of Medicine, and the gynaecologist at the Clinical Hospital Centre Zagreb. She is a subspecialist in Gynaecological Endocrinology, Reproductive Medicine and Menopausal Medicine. Her main areas of research/interest are: female infertility and methods of assisted reproduction, anovulation especially PCOS, and menopausal medicine. She was a research fellow at many scientific projects of Croatian Ministry of Science, and principal investigator on the project of the Ministry of Science of Republic of Croatia *The aetiology and pathogenesis of PCOS – selection of therapy and metabolic consequences*. Currently, Dr. Pavičić Baldani is a member of the Scientific Centre of

Excellence for Reproductive and Regenerative Medicine at the University of Zagreb School of Medicine, a clinical consultant and project partner in the FP-7 THYMISTEM, and investigator in three other international projects. She is the co-author or editor of four books and 25 chapters in books and scripts, and the author of about 40 peer-reviewed papers. She is the president of the Croatian Society of Human Reproduction and Gynaecological Endocrinology, vice president of the Croatian Society for Menopause and a member of the executive committee of the Croatian Society of Gynaecology and Obstetrics Medical Association. She is the member of the National Committee for Medically-assisted Conception of the Ministry of Health Republic of the Republic of Croatia.

And now something completely different: PCOS

Dinka Pavičić Baldani,

Professor at the University of Zagreb School of Medicine, Gynaecologist at the Clinical Hospital Centre Zagreb.

The PCOS was first recognized by Stein and Leventhal in 1935 as a triad of amenorrhea obesity and hirsutism. Since then, the PCOS has gained clinical and public health importance as it is very common, affecting up to one in five women of reproductive age. The importance of the PCOS is not only in its numerosity. This syndrome is associated with a range of cosmetic, reproductive, obstetric, metabolic, cardiovascular, psychological and oncogenic risks which make this syndrome a major health risk in the reproductive age of women. It is estimated that the economic burden of the PCOS in the USA is about 4 billion dollars per year, making the PCOS not only a health, but also a significant economic burden. The etiology of the PCOS is complex and remains largely unclear. There is emerging evidence that the PCOS is a complex genetic disorder in which a variety of predisposing genes, especially those responsible for metabolism of androgens and insulin could be activated by some intrauterine and environmental factors (particularly nutritive) or by influence of endocrine disruptors. In the absence of the clear etiology, this syndrome is defined by consensus criteria and the therapy is symptom orientated. As PCOS accounts for approximately 80% of anovulation, the majority of PCOS will need fertility treatment for achieving pregnancy. The principles of anovulation therapy in PCOS patients are induction of mono-follicular development, whilst, at the same time, minimizing the risks of OHSS and multiple pregnancies. Recently, there has been a shift in some clinics from mono-follicular ovulation induction to IVF, based on the false premise of greater cumulative pregnancy rates. Ovarian stimulation for IVF in PCOS patients presents substantial risk for developing life-threatening OHSS, and exemplifies significantly increased cost, therefore IVF in PCOS patient is indicated only for those not responding to ovulation induction therapies or in those who require IVF due to other indications.



Davor Ježek has a double appointment: he is a full professor University of Zagreb School of Medicine; he is also acting as a Head of Testicular Biobank at Dept. of Transfusion Medicine and Transplantation Biology, University Hospital Centre "Zagreb". His main areas of research/interest are: male infertility, andrology, testicular sperm extraction (TESE), biobanking of testicular biopsies as well as genital ridge development. Currently, Dr. Ježek is a co-chair of Scientific Centre of Excellence for Reproductive and Regenerative Medicine at the University of Zagreb School of Medicine. He is a project partner in the FP7 BIOCOMET project and was a principal

investigator of many international and national projects. Dr. Ježek is also a project partner in the newly approved BIOCHIP Horizon 2020 project. He published more than 60 publications in internationally renowned journals. Dr. Ježek is a member of many international societies in the area of reproductive medicine, including European Academy of Andrology.

Reinke's crystals and cryptorchidism: is there a link?

Davor Ježek^{1,2}, Viviana Kozina¹

¹University of Zagreb School of Medicine, Department of Histology and Embryology

²University Hospital Centre Zagreb, Department for Transfusion Medicine
and Transplantation Biology

Within the human testis, Reinke's crystals are found in Leydig cells but their nature and function are poorly understood. The aim of our study was to investigate the properties of Reinke's crystals in men with the normal morphology of the testis (control group) and infertile patients diagnosed with cryptorchidism. 20 biopsies from infertile patients and 6 biopsies from men with regular spermatogenesis (20-30 y.) were used. Sections of the testis tissue were stained with haematoxylin and eosin and a modified Masson's method. Specimens were observed by bright field, confocal and transmission electron microscopy (TEM). The number of Reinke's crystals in investigated groups was determined applying stereological methods. In both groups, Reinke's crystals were noted within the cytoplasm and nuclei of Leydig cells. Some "free" crystals were found within the interstitial space, outside Leydig cells. Confocal microscopy proved to be very useful in the assessment of the shape and 3D reconstruction of the crystal. TEM analysis confirmed a hexagonal form of the crystal while crystallographic data on sections of 70-300 nm thickness provided a better insight into the organization of the crystal lattice. Stereological analysis revealed a significant increase in the number of crystals in cryptorchid testes when compared to controls. Increased number of crystals in cryptorchid specimens leads to the assumption that the prolonged exposure to higher (abdominal) temperature might stimulate enzymes involved in the synthesis of the proteins of the crystal. However, the exact molecular nature of the crystal lattice remains in both normal and cryptorchid testis obscure.



Dinko Hauptman obtained his MD in 2004 at the University of Zagreb School of Medicine, Croatia. In 2011 he became a urologist at the Department of Urology, University Hospital Centre Zagreb, Croatia where he still works. His fields of interest are diagnosis and treatment of male infertility, reconstructive urethral surgery and kidney transplantation.

Case report: 32-year old male with azoospermia and partial AZFb Y microdeletion with positive spermatozoa findings

Dinko Hauptman¹, Zoran Zimak¹, Davor Ježek^{2,3}, and Željko Kaštelan¹

¹Clinic for Urology, University Hospital Centre Zagreb, University of Zagreb School of Medicine,

²University of Zagreb School of Medicine, Department of Histology and Embryology,

³University Hospital Centre Zagreb, Department for Transfusion Medicine and Transplantation Biology

Infertility accounts about 10-15% of all couples. Half of them refer to men. Two major genetic infertility reasons are Klinefelter syndrome and microdeletions of the Y chromosome. Three regions are regularly checked in men with spermatozoa counting below 5 mill/ml (AZFa, AZFb and AZFc). Nowadays, even further microdeletions are investigated to help infertility treatment management. Although patients with microdeletion of the Y chromosome in AZFa and AZFb region have virtually zero possibility of having children, we showed that azoospermic men with partial AZFb deletion of Y chromosome had positive spermatozoa findings and even successful IVF/ICSI procedure. The patient was a 32-year old male with azoospermia. A detailed patient history was taken and a genital examination was done. No infertility reason was observed during the examination. The patient's testicles demonstrated normal volume and consistency. Hormone, semen and genetic testing were done. FSH level was 5.7 IU/L, LH 7.7 IU/L and testosterone 20.46 nmol/L. During genetic analysis, a normal karyotype but a partial AZFb microdeletion of Y chromosome was diagnosed. Genetic counselling was also performed. After detailed analysis and genetic counselling, TESE procedure was performed. Histology findings revealed late spermatids that could be used in IVF/ICSI procedure. ET was successfully performed but after 6 weeks miscarriage occurred. Couple decided to make second TESE procedure and the same histology of the testicular parenchyma was observed. Second IVF/ICSI procedure is ongoing. Even though patients with AZFb microdeletion of Y chromosome have small fathering chances, in some special cases and after proper genetic counselling it could be possible.

