University of Szeged,
Albert Szentgyörgyi Medical and
Pharmaceutical Centre

Program director: Prof. László Kovács, MD, PhD, DSc
Tutor: Prof. Dr. János Szöllősi, MD,PhD

Non hormonal options in the evaluation of male infertility and their therapeutic consequences

Dr. Zsolt Kopa

National Medical Center
Department of Andrology and Urology, Budapest
Head: Prof. György Papp MD,PhD

Ph.D. thesis
2005

University of Szeged,
Albert Szentgyörgyi Medical and
Pharmaceutical Centre

Program director: Prof. László Kovács, MD, PhD, DSc
Tutor: Prof. Dr. János Szöllősi, MD,PhD

Non hormonal options in the evaluation of male infertility and their therapeutic consequences

Dr. Zsolt Kopa

National Medical Center
Department of Andrology and Urology, Budapest
Head: Prof. György Papp MD,PhD

Ph.D. thesis
2005

Publications related to the thesis

I., G. K. Papp A. Hoznek, Z. Kopa
Alloplastic spermatocele in the treatment of male infertility.
Mol. Androl. 7: 125-34. 1994

II., Papp Gy., Hoznek A. **Kopa Zs.**
Spermiumtárolók alkalmazása az andrológiában.
M. Androl. I. (1): 30-34. 1996

III., G.K. Papp, A. Hoznek, **Z. Kopa**
Do sperm reservoirs still have a place in male infertility management?
Curr. Op. in Urology 6: 340-43. 1996

IV., Papp Gy., **Kopa Zs.**, Hoznek A.
Az andrológus szerepe az asszisztált reprodukcióban.
M. Androl. I.(2): 13-16. 1997

V., Papp Gy., **Kopa Zs.**
Vizsgálataink automata spermium analyzátorral.
M. Androl. II (2): 112-14. 1997

VI., Papp Gy., **Kopa Zs.**
Climacterium virilrie - Hungarian concept.
Int. J. Androl. 20(1) 333-34 1997

VII., Papp Gy., Rajczy K., Bernárd A., **Kopa Zs.**
A direkt sebészi spermiumnyerés (SMART) első hazai andrológiai eredményei.
M. Androl. III. (3): 89-91. 1998

VIII., G.K. Papp, K. Rajczy, A. Bernard, **Z.Kopa**
Testicular sperm aspiration (First hungarian results).
Acta Chir. Hung. 37. (3-4): 195-99. 1998

IX., Balogh I. Mátyás Sz. Papp Gy. **Kopa Zs.**
Egy korszerű spermabank működése.
M. Androl. V. (3): 94-98. 2000

X., Balogh I. Papp Gy Erdei E., **Kopa Zs.**, Albert I. Mátyás Sz.
A donorprogram tapasztalatai a KRIO Intézetben.
M. Androl. VI. (3): 94-97. 2001

XI., **Kopa Zs.**, Papp Gy.
A férfi meddőség.
Háziorvosi Továbbképző Szemle VII (8): 556-61. 2002

XII., **Kopa Zs.**, Papp Gy.
Az idösödő férfiak megtermékenyítő képessége.
M. Androl VII. (2): 63-66. 2002

XIII., **Kopa Zs.,** Papp Gy.
Az urogenitalis infectiók hatása a férfifertilitásra I.
M. Androl. VII.(3) 91-93. 2002

XIV., G.K. Papp, **Z. Kopa**, F. Szabó, E. Erdei
Aetiology of haemospermia.
Adrologia 35: 317-20. 2003

XV., Papp Gy, **Kopa Zs.,** Szabó F., Erdei E.
A haematospermia okai.
M. Androl. VIII. (2): 38-40. 2003

XVI., Balogh I., Papp Gy., Erdei E., **Kopa Zs.,** Albert I., Mátyás S.
Daganatos betegek krioprezervációjának tapasztalatai a KRIO Intézetben 1999-2002.között.
M. Androl. VIII. (2): 59-61. 2003

XVII., Papp Gy., **Kopa Zs.**
Andrológiai gyógyszeres kezelések.
Pharmindex kompendium
Budapest 39-66. 2003

XVIII., Mátyás Sz., Papp Gy., Balogh I., Rajczy K., Bernard A., Kováts T., Kovács P., Krizsa F., Szmatona G., Bekes A., Palásti M., Sprőber P., Szabó Cs., **Kopa Zs.,** Erdei E., Egyed J., Gáti I., Kaáli S.G.
Terhességi eredményeink hereszövetből származó mélyfagyasztott spermiumok felhasználásával.
M. Androl. IX (1): 7-10. 2004

XIX., **Kopa Zs.,** Kálmán J., Papp Gy.
A BPH okai, kezelése és andrológiai vonatkozásai.
Medicus Anonymus XII. (2004/5): 33-37. 2004

XX., Mátyás Sz., Révész Cs., Balogh I., Papp Gy., Forgács Zs., Rajczy K., Sprőber P., Szabó Cs., Bernárd A., **Kopa Zs.,** Gáti I., Kaáli S.G.
Humán spermiumok mitokondriális aktivitásának mérése MTT-teszt segítségével fagyasztás előtt és felengedés után.
M. Androl. IX. (3): 79-82. 2004

XXI., Sz. Mátyás , G.K. Papp, P. Kovács, I. Balogh, K. Rajczy, **Z. Kopa** , A. Bernard, T. Kovács, F. Krizsa, G. Szmatona, I. Gáti I., S.G. Kaáli
Intracytoplasmatic sperm injection with motile and immotile frozen-thawed testicular spermatozoa (the Hungarian experience).
Andrologia 37 (1): 25. 2005

XXII., Papp Gy., Hoznek A., **Kopa Zs.**
Operative andrology.
Andrologia operacyjna
Andrologia (tankönyv). PZWL Poland Warszawa 1998

XXIII., **Kopa Zs.,** Papp Gy.
A férfi meddőség biokémiai alapjai és nem hormonális kezelése.
Az Andrológia tankönyve, Medicina Budapest 2000.

XXIV., Papp Gy., **Kopa Zs.**
Andrológia
Férfi meddőség.
Urológia tankönyv, Medicina Budapest 2005.

XXV., **Kopa Zs.,** Papp Gy.
A nemi érintkezéssel terjedő betegségek és a meddőség.
Budapest. STD Tankönyv in Press

XXVI., **Kopa Zs.,** Papp Gy.
A férfi húgyivarszervi gyulladások összefüggése a nemi érintkezéssel terjedő betegségekkel.
Budapest. STD Tankönyv in Press

XXVII., **Z. Kopa**, J. Wenzel, G.K. Papp, G. Haidl
The role of granulocyte-elastase and Il6 in the diagnosis of male genital tract inflammation.
Andrologia 2005 Accepted for publication

Contents

List of abbreviations

In order of their apperance

KSH	Hungarian Central Statistical Office
IvF	In vitro Fertilization
ICSI	Intracytoplasmatic Sperm Injection
DNA	Dezoxyribo Nucleic Acid
ART	Assisted Reproductive Techniques
CASA	Computer Assisted Sperm Analysis
WHO	World Health Organization
VSL	Straight Line Velocity – mm/s
VCL	Curivilinear Velocity – mm/s
VAP	Average Path Velocity – um/s
LIN	Linearity: VSL/VCL
WOB	Wobbling: VAP/VCL
IUI	Intrauterine Insemination
hMG	human Menopausal Gonadotrophin
hCG	human Chorionic Gonadotrophin
NM	Normal Sperm Morphology
CPR	Clinical Pregnancy Rate
FR	Fertilization Rate
SA	Severe Sperm Morphology Abnormalities
ROS	Reactive Oxygen Species
TAC	Total Antioxidant Capacity
RDA	Recommended Dietary Allowance
SDS	Sodium Dodecylsulfate
cGMP	cyclic Guanosine Monophosphate
cAMP	cyclic Adenosine Monophosphate
NO	Nitrogen Oxid
ATP	Adenosine Triphosphate
CPK	Chreatine-phosphokinase
NAG	Neutral Alpha Glucosidase
HSV	Herpes Simplex Virus
PCR	Polymerase Chain Reaction

ISH	"In situ" Hibridization
HCMV	Human Cytomegalovirus
AAV	Adeno-Associated Virus
HPV	Human Papilloma Virus
IL – 6 and 8	Interleukin 6 and 8
TNFα	Tumour Necrosis Factor- alpha
PMN	Polymorphonuclear
CFTR gene	Cystic Fibrosis Transmembrane Regulator gene
NCA	Numeral Chromosomal Abnormalities
AZF	Azoospermia Factor
CBVDA	Congenital Bilateral Agenesia of Vas Deferens
CUVDA	Congenital Unilateral Agenesia of Vas Deferens
EMGQN	European Molecular Genetic Quality Network

1. Introduction

The Hungarian population is continuously shrinking. There was an ascending tendency in 1980 according to the data of the Hungarian Central Statistical Office (KSH 2005), but by 1990 the natural reproduction had lessened and the number of inhabitants had decreased by 19.981 persons. In 2001 the decreasing of the population further intensified, we became 35.136 persons less and in 2003 the annual shrinking surpassed the number of 41.000 persons. The annual shrinking of the population in 2004 was 4 persons per 1000 inhabitants and it further increased in the first half of 2005. The number of vital births has also progressively decreased in the last few years. While in 1990 125.679 babies were born, in 1995 only 112.054 and in 2002 this number was 96.804 and in 2004 only 95.137.

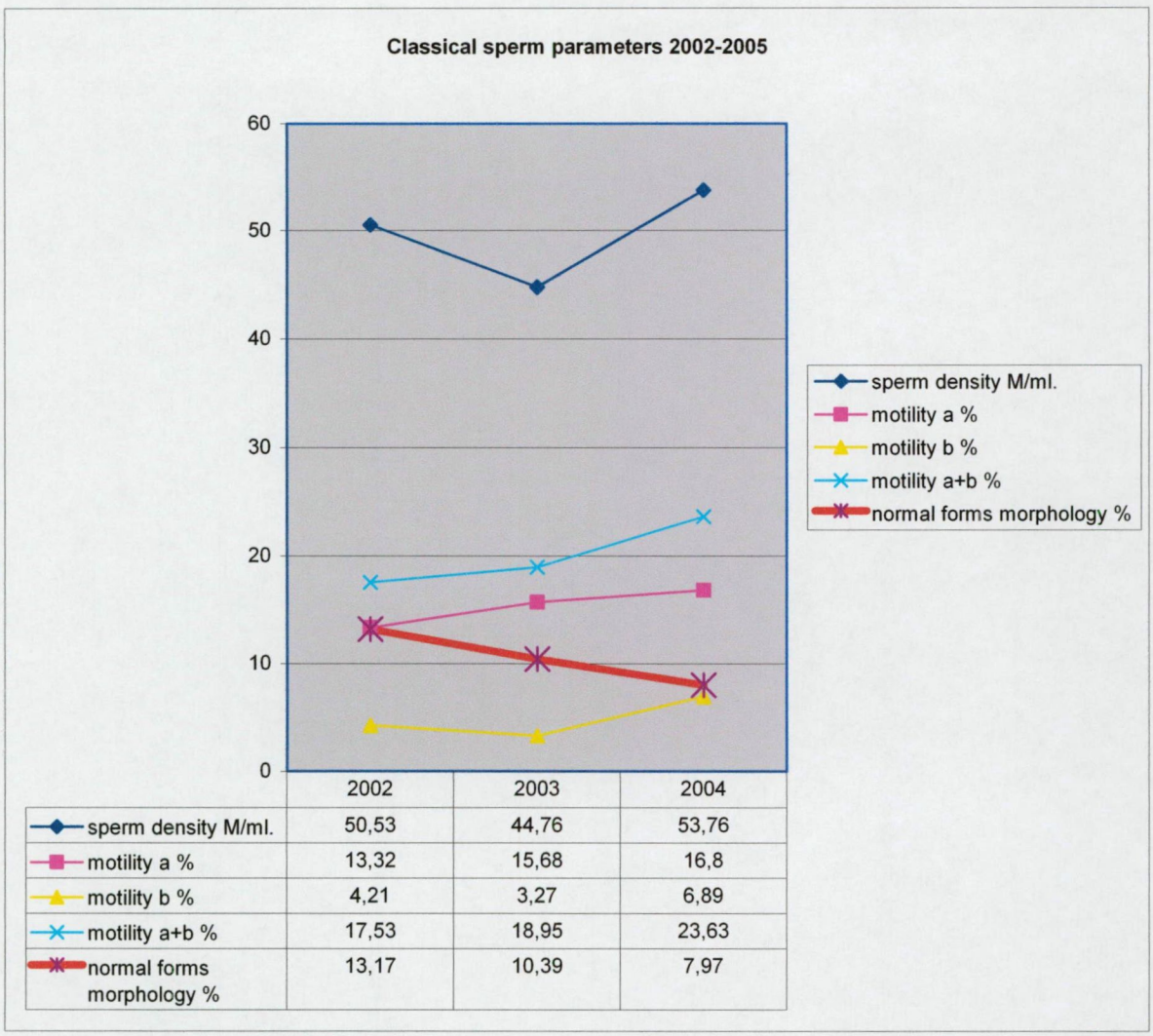
The number of families in Hungary, following a continuous rise until 1980, decreased by more than 131.000 in ten years and another 27.500 after 1990. Recently 8.360.000 people live in 2.869.000 families thus every 1.000 of families have on average of 291 persons as opposed to 339 in the year of 1949. In 1949 every hundred families had 152 and in 1960 126 children on average respectively. In 2001 this number was 108. In the last ten years the number of fertile females remarkably decreased. Nowadays the female balance (the number of females per 1000 males) exceeds the number after the Second World War.

According to a comprehensive and working out international data study every sixth relationship in the world is infertile (40). On the basis of the previous years' assisted reproduction database approximately 6.000 IVF/ICSI (In vitro Fertilization/ Intracytoplasmatic Sperm Injection) cycles take place per year currently and this results about 1500 new-born babies. In 2002 1,5% of the Hungarian new-borns were test-tube babies. Moreover we see the growing number of people who shoulder fatherhood after the age of 50 (492 in 1996 and 687 in 2001 respectively).

The procreative capacity of human males has also continuously decreased in the last decades (7, 10, 36, 78, 58, 28, 18). Unlike the volume of the ejaculate a significant worsening of all the classic parameters of spermatogram (concentration of sperm, or even more the motility and morphology) can be observed. Environmental factors and their hormonal effects as well as lifestyle elements and often occurring urogenital infections have to be taken into consideration as the

reasons of these changes. Minor genetic impairments emerging because of damaging factors of spermatozoa and it's DNA (Dezoxy-ribo nucleic acid) become apparent at the time of reproductive age. Some chemicals e.g. certain organic chlorides, alkyl phenols etc. have prenatal testicle damaging effects. There is an increased occurrence of anatomical and genetic malformations resulting in postnatal testicular function failure and a defect of the spermatogenesis (10).

During the thesis study period we summarized the classical spermatogram parameters in the last 3 years observed in our male infertility out-patient unit. (Fig.1)



	2002	2003	2004
sperm density M/ml.	50,53	44,76	53,76
motility a %	13,32	15,68	16,8
motility b %	4,21	3,27	6,89
motility a+b %	17,53	18,95	23,63
normal forms morphology %	13,17	10,39	7,97

Fig.1. Classical sperm parameters between 2002-2005. Sperm density M/ml. (blue), motility a % (rosa), motility b % (yellow), motility a+b % (cyane) and normal forms morphology % data in the examined 3 years.

In this short period of time, analyzing the data of 3427 spermatograms performed in our andrology unit (1028, 1166 and 1233 in 2002, 2003 and 2004 respectively), there is no evidence of a worsening in the sperm count and progressive motility but an unambiguous decrease in the normal forms strict criteria morphology which is a high impact factor of male fertilizing ability can be observed. To state an opinion of a tendency one need to correlate 5 or 10 years periods.

The above facts convincingly show infertility to be a significant issue nowadays. Several studies, completed mainly on isolated and small population, can inform us on the sexual division of the reasons of infertility with different ratios. Infertility is mostly the fault of both sides (male and female) and according to the previously mentioned comprehensive study if only one side were involved it is rather the male (40). The latest surveys show about 15% infertility among couples wanting children in Europe. Unlike the earlier renewed studies, they unanimously prove that 50% of infertility cases are caused by divergences of males. A significant proportion of infertile males have serious spermatogram discrepancies, severe oligozoospermia (sperm concentration under 5 M/ml) or azoospermia. According to our own experience 20% of spermatograms performed in our andrological laboratory belong to this class. In everyday medical practice gynecological examinations in infertility cases are performed only after the andrological examination and possible treatment of male.

In the twentyfirst century the knowledge available about the causes and treatment of infertility is limited in any case. Despite the serious ambition and continuous development of recent independent andrology dealing with male infertility, the diagnostic- and therapeutic palettes are not wide enough. The goal of professional ambition is to reduce idiopathic infertility cases. That is the reason why thorough diagnosis and the use of new methods are necessary. The role of the development of assisted reproductive techniques (ART) is indisputable in the treatment with the result that more and more children can come into the world and those people can become parents who hadn't had the possibility before. However it's important to establish that our knowledge about the causes and treatment of male infertility doesn't enlarge with the development of ART methods.

The need to get ever nearer to the causes and to make treatment more efficient determined the choice of subject of this paper. Our effort is to focus on the

introduction of new diagnostic procedures summarizing our experiences in showing their therapeutic consequences and to perform newer therapies surveying their effectiveness.

The evaluation of classical sperm parameters have reasonably been changed worldwide in the last few years. The determination of sperm concentration has not changed but the examinations of motility and morphology have undergone dramatical changes. Sperm motility is studied with the help of computer assisted sperm analysis (CASA) nowadays. The motility of the spermatozoa is classified on the basis of its progressivity and speed as a, b, c, and d categories according to WHO (World Health Organization) criteria. The device also examines different directions of movement making available a full analysis. The three most important parameters in the course of the study are VSL (straight line velocity – mm/s) referring the move between the starting- and end points of a spermatozoa, VCL (curvilinear velocity – mm/s) measuring the actual moving speed and VAP (average path velocity – um/s) showing the average spermatozoa speed. A meaningful parameter is in addition the LIN (linearity: VSL/VCL) showing the linearity of moving path and the WOB (wobbling: VAP/VCL) representing the oscillations of the path. The motion of sperms can be derived and classified into the above mentioned 4 categories with the help of that parameters. (Fig. 2. a and b)

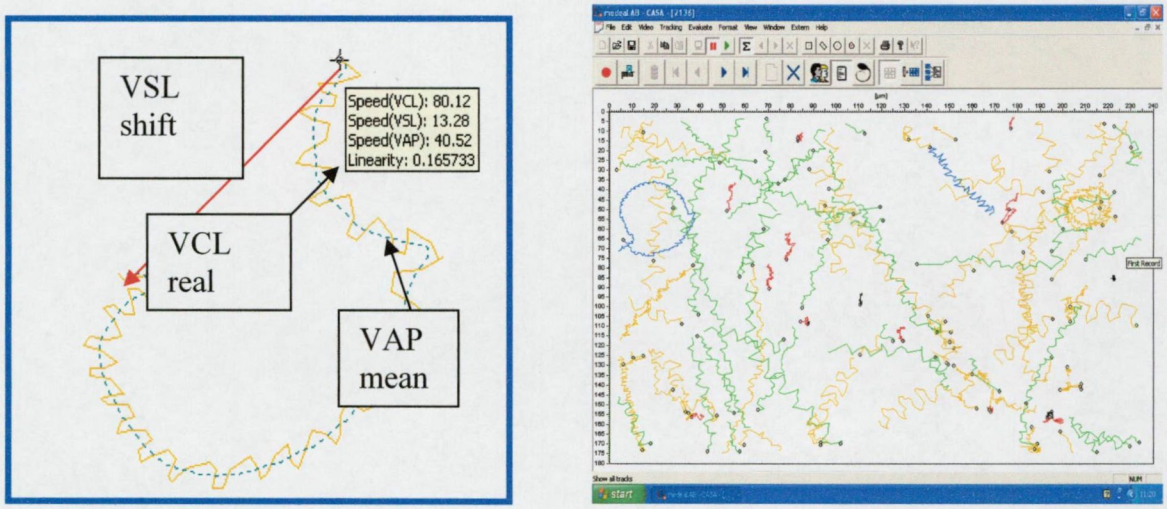


Fig.2 a. and b.: Motility assessment with the CASA system. A.: VSL, VCL, VAP velocities, B.: CASA screen; green: motility a, yellow: motility b, blue: motility c, red: motility d.

Strict criteria morphological analyses were introduced. Various techniques exist because of some minor differences of judgment but our institute uses the WHO criteria. 100 spermatozoa analyzed on stained smear marking individually abnormal sperm fragments. Head divergences are judged by the ratio of the length and width of sperm, the thickness of its midpiece and the alterations of the tail are studied too. The end values were established on the basis of different literary studies: more than 14% of normal morphologic sperm value means a good pattern, between 4-14% medium deviation is found and under 4% the prognosis is poor.

These methods were introduced in our institute making it significantly easier to classify the spermatograms and to indicate properly the assisted reproduction treatment.

The relationship between morphological disturbances and the success of ART were studied too.

2. Objectives

2.1. Impact of sperm morphology assessed by strict criteria in the classification of male infertility. Analysis of the normal morphology thresholds and their predictive value regarding the outcome of assisted reproductive techniques.

2.1.1. The first goal of our study was to confirm the impact of the established strict morphology assessment in the classification of male infertility patients.

2.1.2. The second part of our study was conducted to investigate the thresholds of the normal morphology forms correlating their pattern regarding the fertilizing ability.

2.1.3. The predictive value of the strictly assessed normal forms in assisted reproductive techniques was analyzed with special attention to the severity of the morphological disturbances of spermatozoa.

2.2. Role of biochemical markers in diagnosing male infertility, the impact of neutral alpha glucosidase activity.

2.2.1. In establishing the use of the biochemical markers in our daily andrological practice we decided to demonstrate their importance in the diagnostic procedure of male infertility reducing the occurrence of idiopathic pathozoospermia cases.

2.2.2. Our study was targeted to investigate neutral alpha glucosidase activity not only regarding the differentiation of the causes of azoospermia than as a marker of epididymal function.

2.2.3. The third goal of these efforts was intended to clarify the effects with possibly of restoring the impaired epididymal function.

2.3. The functional effect of uro-genital infections on male fertilizing capacity, new biochemical diagnostic evaluation.

2.3.1. Study of infection causing functional disturbances in the sperm function.

2.3.2. Analyzing the impact and advantages of the use of new biochemical markers in male infertility evaluation.

2.3.3. Reconsideration of the correlation of our findings with the white blood cell count in the ejaculate, differentiation in the thresholds of existing infection regarding to its localization.

2.4 Genetic analysis to reduce the number of idiopathic pathozoospermias.

2.4.1. Investigation of the occurrence of chromosomal abnormalities and CFTR gene mutation in azoospermic and severe oligozoospermic patients with more simple genetic techniques.

2.4.2. Using these methods to analyze their impact in the reduction of the idiopathic origin male infertility cases.

3. The impact of sperm morphology assessed by strict criteria in the classification of male infertility. Analysis of the normal morphology thresholds and their predictive value in relation to the outcome of assisted reproductive techniques.

3. 3.1. Introduction

For characterization of semen quality the classical sperm parameters; sperm density, motility and morphology are used. However, recently there has been general agreement that the significance of the so-called routine spermiogram is limited and that no distinction between fertile and infertile men is possible by assessing these parameters alone (except for cases of azoospermia). Among

these parameters, sperm morphology turned out to be the best indicator of male fertility in many studies based on data of assisted reproduction techniques. Introducing the morphology assessment by the WHO and using strict criteria in our department our efforts were targeted to confirm its advantages in the classification of male infertility and in the indication of assisted reproductive techniques. In trying to find a reasonable threshold in normal sperm morphology our study investigated whether the strict criteria morphology assessment can predict the outcome of the assisted reproductive procedures.

The predictive value of evaluation of sperm morphology for the outcome of IUI (intrauterine insemination) and IVF has frequently been addressed. Whereas a significant role of sperm morphology for IVF is widely accepted, it is more controversially discussed with regard to IUI, fertilization and pregnancy rates following ICSI are considered as independent from sperm morphology (59). In a recent meta-analysis on the significance of sperm morphology for the results of IUI only 8 studies during the time period from 1984 to 1998 provided sufficient data for statistical analysis (86). Most studies conclude that the percentage of normally shaped spermatozoa evaluated according to strict criteria is a good predictor of the outcome of IUI, using the 4 and 14% normal morphology as thresholds, whereas assessment of sperm morphology according to WHO criteria did not yield significant associations between sperm morphology and IUI results (86). The usefulness of sperm morphology analysis according to strict criteria as a prognostic factor for IUI has been confirmed by several recent studies as well (34). Meta-analyses of suitable studies on the correlation between fertilization- and pregnancy rates following IVF and sperm morphology have shown that in 82% and 79% of the studies positive predictive values were achieved respectively, using the 5% and 14% normal forms threshold, respectively. In general, no correlations were found between normal morphology and the results of ICSI (55). While nearly all studies only focus on the percentage of normal forms according to strict criteria, in most cases, a prognostic significance of additional consideration of the pathological forms is not discussed.

3.2. Objective

In our study we decided to evaluate the influence of sperm morphology on the clinical pregnancy rates and ongoing pregnancy rates in IUI, analyzing sperm

morphology before and after sperm preparation to evaluate whether or not consideration of type, severity and frequency of pathological forms provides additional information and prognostic significance. In addition, this evaluation should be carried out to study this question with regard to fertilization and pregnancy rates in conventional IVF. As no major impact of sperm morphology has been reported to be of significance for the ICSI-outcome – when only assessing the percentage of normal forms – we were also interested in studying a potential influence of the pattern of pathological sperm morphology on the ICSI-results.

Apart from evaluating the percentage of normal spermatozoa, Düsseldorf classification takes also in consideration pathological forms to provide more information on causes, kind and severity of the disturbed male fertility. This classification is based on comparison of testicular and epididymal histology with morphological semen analysis. Roughly, it is distinguished between acrosome defect of varying degrees that are considered as more severely disturbed spermatozoa which have been confirmed by sperm function tests, and tapering and hyperelongated forms reflecting a more favourable fertility prognosis. Moreover, combined disturbances and defects of the sperm midpiece and tail are distinguished as well (see below).

3.3. Material and method

Twenty-eight couples with so-called idiopathic infertility underwent IUI, 35 couples were referred to IVF – treatment (91 cycles) and 31 couples to ICSI (80 cycles). Apart from further semen parameters sperm morphology was evaluated before and after semen preparation (swim-up) in the patients of the IUI group whereas for the IVF- and ICSI-groups only sperm morphology after semen preparation was considered. Smears were stained according to Shorr's technique and evaluated without irrespective of the results of the artificial reproduction methods. Normal sperm morphology was evaluated according to strict criteria, whereas the pathological forms were assessed according to the characteristics of the Duesseldorf classification (37, table 1). On each smear 200 spermatozoa were evaluated whenever possible, in cases with lower sperm counts after semen preparation 100 spermatozoa were assessed. In order to get more information on the significance of sperm patho-morphology for the outcome of the artificial methods, correlations were made with morphological subgroups as follows: a) percentage of normal forms (cut-off value: > 4%), b) percentage of slightly

elongated forms (cut-off value: > 30%), and c) severe abnormalities (acrosome defects + combined disturbances + multiple and amorphous forms, cut-off-value: > 70%).

Table 1 Duesseldorf classification

Normal forms
Acrosome defect forms (I-II°)
Hyperelongated forms (I-III°)
Combined hyper elongations and acrosome defect forms (I - II°)
Multiple forms
Amorphous forms
Midpiece and flagellum disturbances (I -III°)

3.4. Statistical analysis

Computer statistical analysis was performed by SPSS™ software (version 12). Correlation and regression analysis was done. Probabilities <0.05 were considered to be significant.

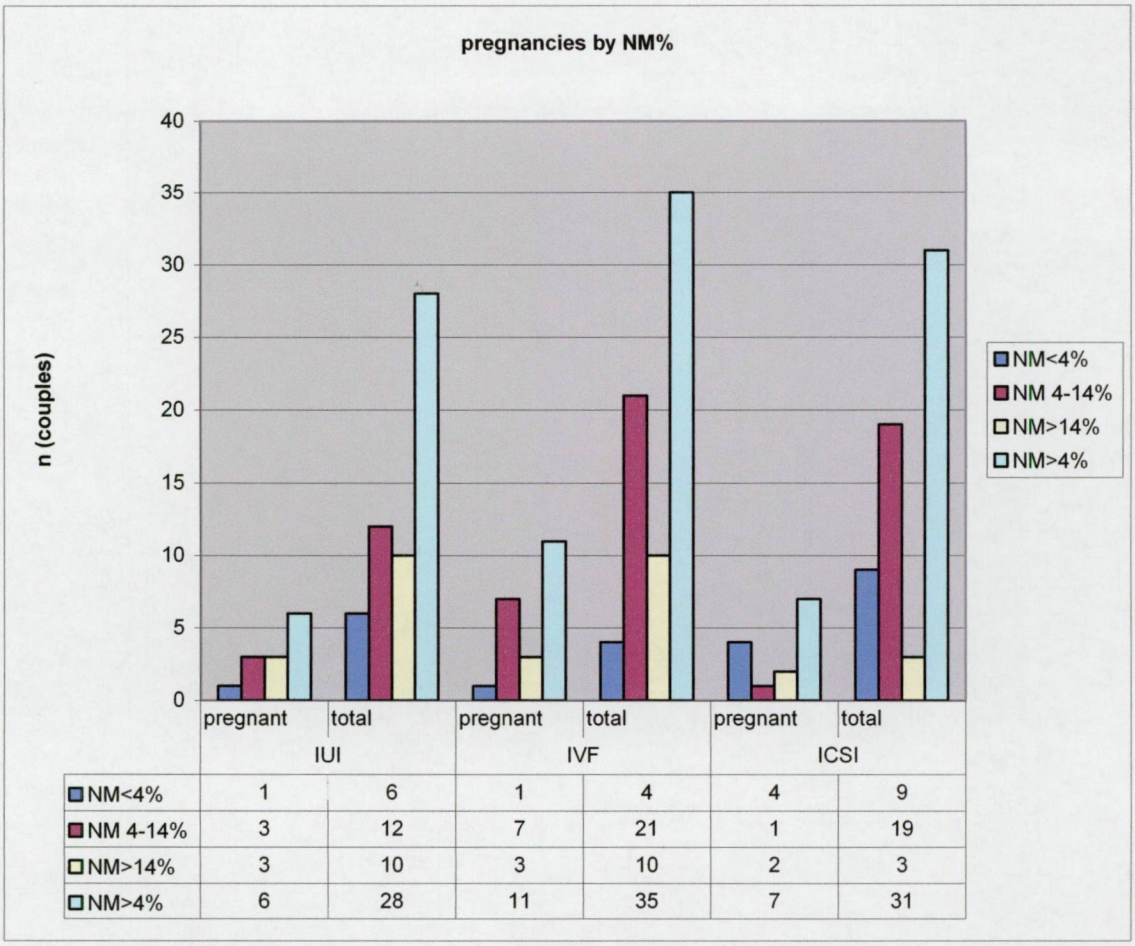
3.5. Results

Twenty-eight women underwent IUI after stimulation with Clomifene citrate+ hMG (human Menopausal Gonadotrophin) + hCG (human Chorionic Gonadotrophin) regimen. For those couples who became pregnant no significant difference could be established in the age of the male partners and sperm count and total count of motile spermatozoa correlating with the non pregnant couples. The clinical pregnancy rate was 25%/cycle, the ongoing pregnancy rate 21%. According to the 4% threshold of NM (normal morphology) a significant difference was observed with regard to the pregnancy rates.

Thirty-five couples (91 cycles) were referred to IVF treatment indicated by both male and female factor infertility. Male age, sperm density and total motile sperm count was not found to be statistically significant in the pregnant and non pregnant patient groups. The fertilization rate (FR) was 78% and the clinical pregnancy rate (CPR) 36%. Like in the IUI treatment group, fertilization and pregnancy rates were significantly higher in the >4% group, no differences could be observed when applying a <70% threshold of severely malformed spermatozoa.

Thirty-one couples (80 cycles) underwent ICSI with a 77% fertilization rate and 28,5% clinical pregnancy rate. According to the 4% threshold, there were

even better results in the group with <4% normal forms. In the group with >70% heavily disturbed spermatozoa, a lower fertilization rate was achieved, but no significant differences in the pregnancy rates were observed. Regarding the NM our data clearly demonstrates a significant improvement of the number of pregnant couples over 4% NM in IUI and IVF, moreover a statistically significant difference exists between the pregnancy rate in IUI and IVF over the 4% NM. Controversially, we found higher pregnancy rate below 4% NM byICSI. (Fig.4 and Fig.5)



	IUI		IVF		ICSI	
	pregnant	total	pregnant	total	pregnant	total
NM<4%	1	6	1	4	4	9
NM 4-14%	3	12	7	21	1	19
NM>14%	3	10	3	10	2	3
NM>4%	6	28	11	35	7	31

Fig.4. Pregnancies by the percentage of normal forms. A significant improvement of the number of pregnant couples over 4% NM and a very small

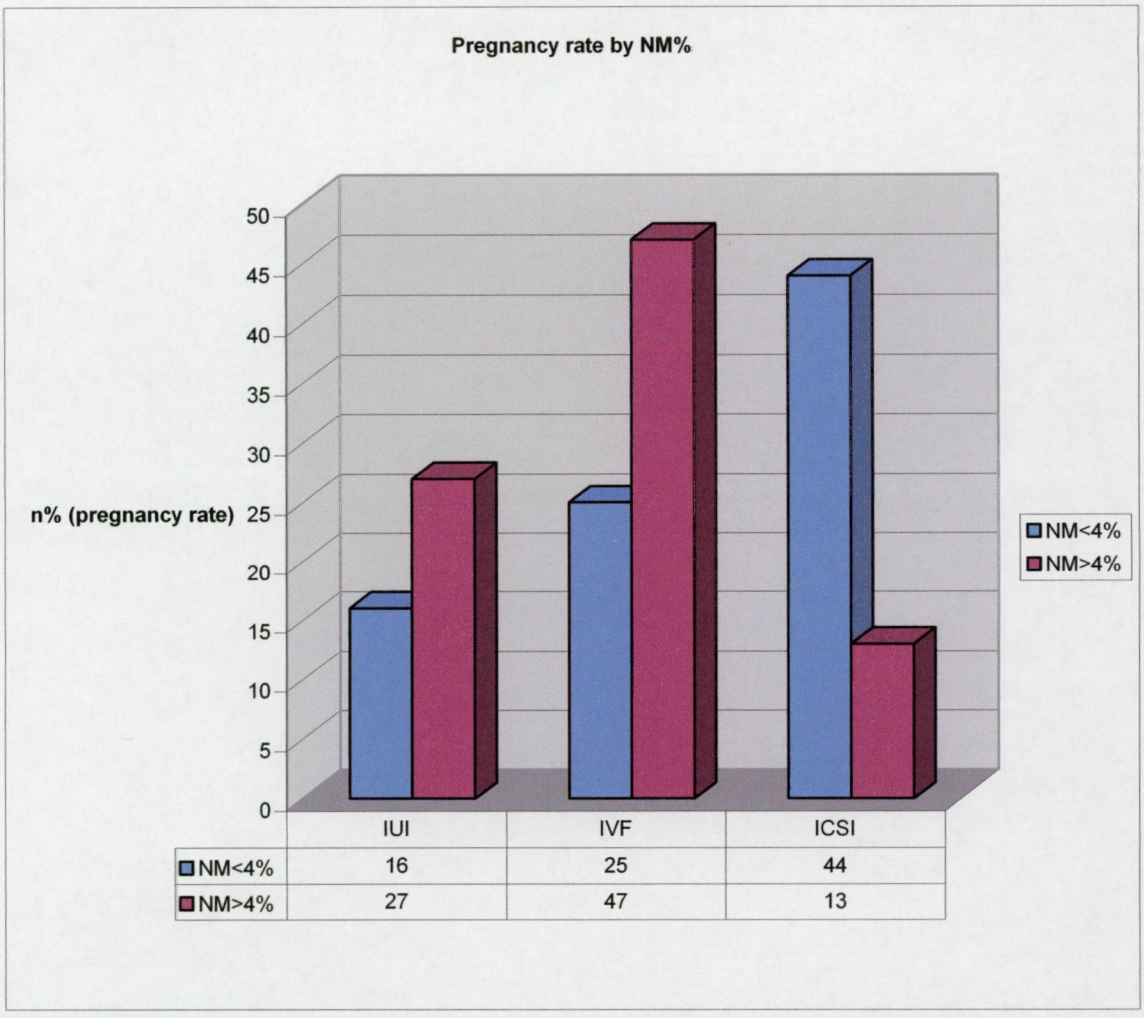


Fig.5 Statistically significant difference between the pregnancy rate in IUI and IVF over the 4% NM and controversially higher pregnancy rate below 4% NM by ICSI.

Our study was conducted to define not only the suggested thresholds of the strict criteria but to investigate whether a correlation exists between the outcome of assisted reproductive techniques and severe morphological abnormalities. Using the 70% threshold in severe abnormalities (SA: acrosome defects + combined disturbances + multiple and amorphous forms) a slight, but demonstrable difference occurred in the pregnancy rate in ICSI. Although the fertilization rate was 79,5% and 73% in the <70% SA and in the >70% SA patient group respectively, we found a 25% pregnancy rate in the group with more severe morphological abnormalities compared with the 22% in the <70% SA patient group.

(Fig.6)

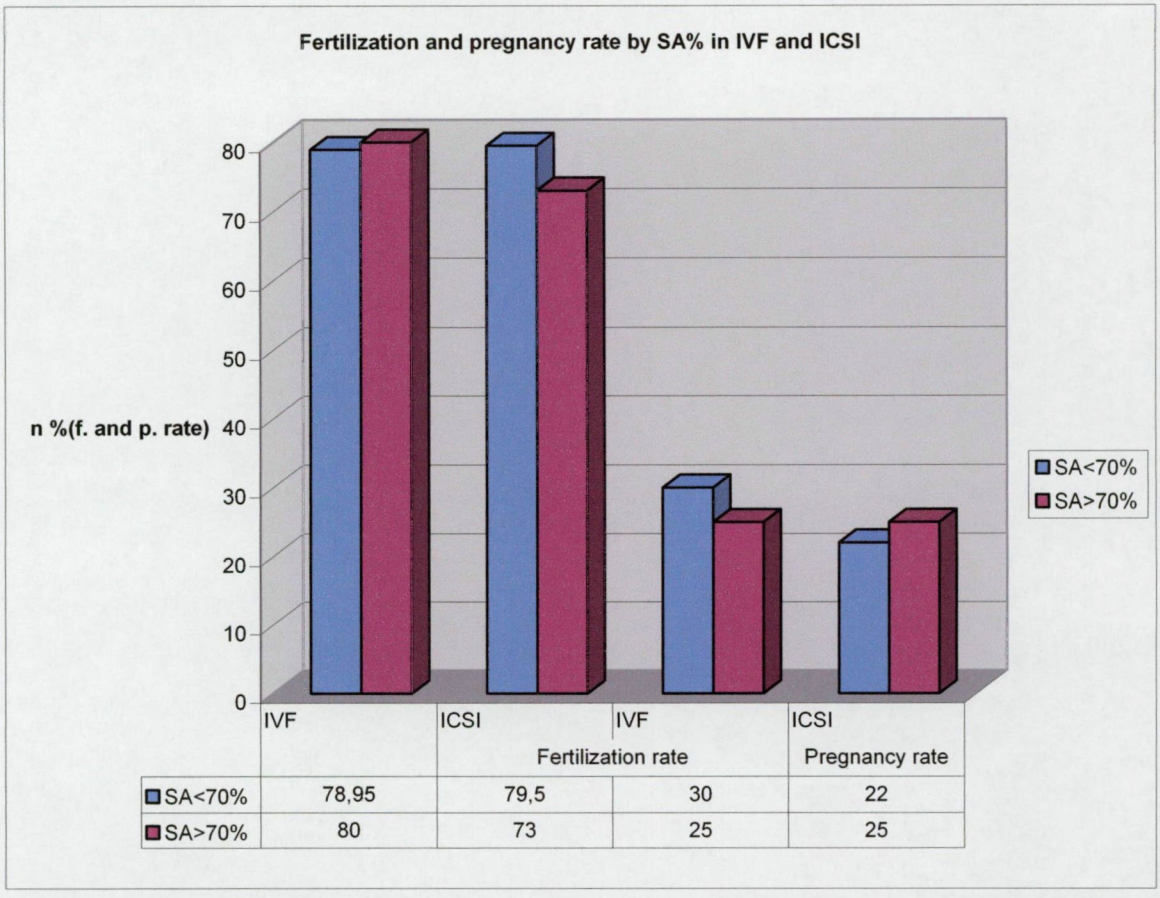


Fig.6 Fertilization rate and pregnancy rate by the pattern severe morphological abnormalities in IVF and ICSI. Conspicouos is the lower fertilization rate over 70% SA in ICSI.

Taking into consideration the percentage of severely disturbed spermatozoa by ICSI there was a significant difference at a 70% cut-off value.

Modification of the „good pattern"-group into NM + slightly disturbed forms > 30% did not yield different results compared to the group >4% normal forms.

4. Role of biochemical markers in diagnosing male infertility

4.1. Biochemical bases of male infertility

The understanding of biochemical properties and processes means the basis of non hormonal diagnostics and treatment. The investigation of biochemical aspects in conjunction with male infertility has had a different emphasis in the individual stages of developing andrology.

In the diagnosis of male infertility the known classic parameters (spermatozoa quantity, -motility, -morphology) characterize the spermatogram at

first which the fertilizing ability was tried to verify with. This so called descriptive attitude was characteristic of the '50-s and the founder of which was McLeod. Biochemical factors were still not focused on at that time. Biochemistry has appeared later in the so called functional attitude, cervical penetration, chromosomic reaction, membrane fusion were studied and a new procedure has been developed using standardized parameters and computerized measurements. Nowadays the so called molecular biochemical attitude is popular. We are closer to the molecular bases of spermatozoa function and the biochemical backgrounds of defects via bioassay. Biochemical protocols have been evolved by now which are easily performed and standardized. In Hungary Corradi et al. published pioneer data in the field of andrological biochemistry. (17)

4.1.1. Sperm membrane injury, oxidative stress.

The integrity of the cell membrane guaranties the proper function of sperms. The functional loss primarily depends on the injury of the cell membrane. To establish biochemical diagnosis and subsequent treatment consequences one has to clear up the biochemical properties and processes which have special importance in maintaining or damaging of fertilizing ability.

The plasmatic membrane of spermatozoa contains unsaturated fatty acids in large quantities thus it is very sensitive to lipid peroxidation. Every cell existing in an aerobe milieu produces certain amount of reactive oxygen species (ROS). Simultaneously, a protective mechanism develops in every cell against the oxidative damages caused by ROS. It was first published in 1987 that human spermatozoa were able to produce reactive oxygen species too. Numerous studies have provided evidence that the sperms of infertile males produce significantly (2-3 times) more ROS than the fertile ones and the ROS producing ability of sperms is proportional to the degree of the lesion (62). Leukocytes also produce significant amounts of ROS (8) which is important because the extracellular generated ROS also damages the neighborhood spermatozoa (53). In the course of lipid peroxidation from superoxide anion with the action of the dysmutase enzyme hydrogen peroxide forms, and an oxidative stress develops in the plasmatic membrane. The membrane loses integrity and fluidity thus sperms are not able to perform their function. The achromosomic reaction suffers damage and the fusion is not able to exist. These fatty acids make the spermatoplasma suitable to

oxidative processes which decrease the degree of plasm fluidity. DNA fragmentation occurs in addition. Protective mechanisms called scavenger are also available. These enzymes are described as total antioxidant capacity (TAC)(49). The ROS production, measurable via biochemical tests, and TAC or their ratio refer to the functional integrity of sperms. The abnormality of this ratio in any reason consequently indicates the loss of spermatozoa function. The damage does not only exists on testicular level, but can be present in any part of the seminal tract and such changes in ratio occur in conjunction with infections, toxins and the course of aging. The infections and the intactness of testicular tubuli and epidydimis have great importance.

4.1.2. Magnesium and zinc

Detailed examination of the metallic content of the ejaculate yielded major results. Previously published data suggested especially zinc and magnesium having a role in spermatozoa motility.

After previously favorable experiences with oral magnesium therapy a placebo controlled, randomized prospective clinical pilot study did not confirm the previous optimal data, magnesium did not improve the conventional semen parameters compared to either placebo or the baseline values, although some increases were detectable in the cumulative data of all patients. Although Mg considered as bioavailable magnesium in seminal plasma was elevated due to magnesium therapy, any direct biological effect of magnesium on spermiogenesis was not detectable. (96)

Numerous biochemical mechanisms are zinc dependent, including more than 200 enzymes in the body. Zinc deficiency is usually associated with decreased testosterone levels and sperm count. Previous literature demonstrates that an adequate amount of zinc ensures proper sperm motility and production. Zinc levels are generally lower in infertile men with diminished sperm count, and several studies have found supplemental zinc may prove helpful in treating male infertility. (46, 57, 51, 92) Zinc's role in the production of testosterone is known. In one trial, the effect of zinc supplementation on testosterone, dihydrotestosterone, and sperm count was studied. Thirty-seven patients with idiopathic infertility of more than five-year duration and diminished sperm count received 24 mg elemental zinc from zinc sulfate for 45-50 days. The results were dramatic in the

22 subjects with initially low testosterone levels; a significant increase in testosterone levels and sperm count (from 8 to 20 million/ml) was noted, along with nine resulting pregnancies. Fourteen infertile males with idiopathic oligospermia were supplemented with 89 mg zinc from oral zinc sulfate for four months. Serum zinc levels were unaffected, but seminal zinc levels significantly increased. There were also improvements in sperm count and in the number of progressively motile sperm. Three pregnancies occurred during the study. (63) A study from Wayne State University School of Medicine found that men who consumed a diet lacking in zinc had decreased levels of testosterone and lower sperm counts than the control group. Upon restoring a daily dose of 15 milligrams, testosterone and sperm count levels rebounded to acceptable levels within 12 months. The recommended dietary allowance (RDA) for an adult male is 15 milligrams per day. Zinc supplementation appears warranted in the treatment of male infertility, especially in cases of low sperm count and decreased testosterone levels. (67) In the study of Kvist et al. (46) a positive relation was found between zinc in sperm nuclei (X-ray microanalysis) and the resistance of the chromatin to decondense in sodium dodecylsulfate (SDS). The infertile men had lower degree of sperm chromatin stability and lower sperm zinc content than the fertile donors. A subgroup of the infertile men, which all had minor clinical signs of prostatic inflammatory reaction, had the lowest content of zinc in the chromatin and the lowest degree of chromatin stability. A low content of nuclear zinc would impair the structural stability of the chromatin and thereby increase the vulnerability of the male genome. This mechanism may be one explanation for the reduced fertility of the men with minor inflammation of the prostate.

In our practice the zinc level analysis of the seminal plasma was established in the time period of this work. Our first experiences regarding 118 male infertility patients 13,6% (16 patients) were found with lower seminal plasma zinc level. The detailed analyzis with the correlation of inflammatory markers and hormone levels are ongoing. The preliminary results seem to underline a beneficial effect of zinc treatment amended with antiphlogisitcs.

4.1.3. Pentoxyfillin

Research concentrates of course on measuring and finding the opportunities to decrease ROS production. A number of materials were proved to

decrease ROS production or inhibit lipid peroxidation in vitro. One of them is a metilxantine derivate called pentoxifylline (52). It increases spermatozoa motility in vitro and even in vivo sperm parameters improve (62). ROS production decreases and pentoxifylline plays an important role in the patomechanism as a phosphodiestherase inhibitor. It inhibits decomposing cGMP (cyclic Guanosine Monophosphate) and cAMP (cyclic Adenosine Monophosphate) following cAMP and cGMP level increase which results intracellular Ca level increase enhancing the cell's kinematics, though some study disagrees (56). The in vitro effect of it is more pronounced maybe because of the in vivo antioxidant mechanisms (62).

4.1.4. Basic amino acids

From 1941, when tyrosine was isolated from the ejaculate the investigation of basic amino acids functions in fertility have been developed. L-Arginine as a NO (Nitrogen oxide) donor plays a crucial role in the above process. The action of NO being torn off from L-Arginine is to increase the levels of cAMP and cGMP. In the 1970'-ies Papp et al. (65) underlined the role of L-Arginine in the male reproductive system.

Beside this main guidelines the importance of other material was discovered e.g. phosphatase and prostaglandin which also increase the levels of cAMP and cGMP.

4.1.5. Callicrein

Callicrein as part of the callicrein-kinin system is a kinin releasing enzyme. The major components of the system are the substrate kininogen, the activator kininogenase enzyme, the effector kinin and the inactivator kininogenase. All of the components are both present in male genitals and the seminal fluid. The kinins are polypeptides having great biological potency. They have hormonal-like effects on cellular level, existing only for a short time and derivate fast. Callicrein activates callidin and bradykinin (2 types of kinins) from seminal kininogen and the increase of motility in vitro plus the improvement of fusion capacity in animal tests were described as a result (87). The physiological importance of the callicrein-kinin system is not entirely clear. The system enhances blood vessel permeability, helps the contraction of flat muscles, improves glucose transportation through cell membrane and plays a role in the proliferation of different cells. In the processes of

reproduction they cause flat muscle contraction of the uterus, increase the motility of sperms and stimulate the spermiogenesis. Their inhibitors are presented in the spermatoplasma in a very low concentration only (ά-1-antitripsine and ά-antichimotripsine). The homoeostasis of the system in physiological circumstances exists and any changes seem to be pathological.

4.1.6. Antioxidants

The efficiency of the antioxidant "scavenger" side was also studied of course. Literary data varies and the in vivo efficiency has not been proved (73, 89). In animal tests following the use of drinking water supplemented with ascorbic acid decrease of free derivatives' production and improvement in sperm parameters were described (94).

4.1.7. Metabolic energy

The releasing of metabolic energy in the course of the glucolysis and the increased ATP level indicated the investigation of glucose. Not significant but positive changes of motility and fusion have been demonstrated following glucose nutrition though this effect lasts for a very short time. Fructose was first isolated from the seminal vesicle in 1946 which assures 60% of the whole energy according to later studies. It is also a significant diagnostic factor in these days and gives reliable information on the function of vesicula seminalis. The scientific importance of the remaining energy-providing materials (citric acid, isonit) has been vigorously reduced by now. Lipids and ATP (Adenosine Triphosphate) have been more and more focused on.

4.1.8. Other biochemical approaches

There is another measurable biochemical parameter of damaged sperm function: the most important enzyme of energy balance inside the sperm cell namely chreatine-phosphokinase (CPK). This enzyme controls the synthesis, transportation and dephosphorilation of chreatine-phosphate. There is significant negative correlation between spermatozoa concentration and the CPK production of sperms.

A simple test is also available to investigate the acrosine enzyme. It gives information on the fertile ability. Acrosine together with hyaluronidase play an important role in liquidification and chorion dissolving in the course of fusion.

4.2. Impact of Neutral Alpha Glucosidase (NAG) activity

4.2.1. Introduction

The epididymis plays a crucial role in the maturation of spermatozoa and their progressive motility and fertilizing capacity (14). The final sperm function is dependent on epididymal transit where spermatozoa maturation occurs. The most important epididymal marker in the seminal plasma is the alpha glucosidase produced mostly in the corpus and cauda of epididymis (93) but a proportion of this enzyme is secreted by the seminal vesicles, whereas the contribution of the prostate is probably negligible (15).

The determination of the neutral isoenzyme of alpha glucosidase has been claimed a rapid, sensitive and non-invasive method to differentiate azoospermia type and to localize the site of obstruction in the male genital tract, and to identify partial obstruction at epididymal level (50). Low levels of alpha glucosidase in semen can be related to epididymitis (90) and have been associated with defective sperm maturation in the epididymis (31). Its low levels in the seminal plasma of patients with oligozoospermia may reflect either varying degrees of epididymal obstruction, or epididymal hypofunction due to the influence of the testis on the epididymis.

A high level of the isoenzyme correlates with a strong binding capacity of the spermatozoa to the human zona pellucida (3) and with a high probability of success following assisted reproduction (54). It appears to be a sensitive indicator of epididymal function (15) its activity correlates positively with conventional sperm characteristics (16) and with seminal ATP, whose factors are indicative of the fertilizing capacity of spermatozoa (12).

Also, it is negatively correlated with both ROS and the concentration of peroxidase-positive white blood cells, which are known to affect fertilization in vivo (54) and in vitro (1).

4.2.2. Objective

The role of NAG in the differential diagnosis of azoospermia is well known. Since we know that low levels of NAG in seminal plasma of patients with oligozoospermia may reflect either epididymal obstruction, or epididymal hypofunction due to the influence of the testis on the epididymis efforts have been targeted the correction of the epididymal function. Experiences demonstrated the testosterone therapy as the most effective treatment modality. (31) Our study was conducted to define the impact of the testosterone therapy at the lower NAG level diagnosed epididymal dysfunction, and whether it results in an improvement in sperm density and motility. This study has needed an especially careful selection determained by the other circumstances causing deterioration in the level of the seminal neutral alpha glucosidase. Urogenital infections and varicocele can reduce the level of NAG. Nevertheless, patients with in the evaluation of the therapeutic results disturbing abnormal hormonal conditions had to be excluded from the trial.

4.2.2. Material and method

Baseline characteristics

Finally oligozoospermic and severe oligozoospermic or severe asthenozoospermic patients attending our andrology department with infertility with normal hormonal status and neither having varicocele or history of varicocele operation or uro-genital infections were investigated. At the baseline characteristics of the recruited 54 infertile patients 18,47 M/ml mean sperm density was observed with the range of 0,1 M/ml. to 77 M/ml. The mean progressive, rapid motility assessed by the WHO criteria as motility grade „a" was 11,62% with the range between 0 and 50% and the mean value of the progressive, slow motility classified as category „b" by the WHO was 4,16% with the range of 0-20%. The alpha glucosidase assessment showed a mean level of 12,35 U/ejaculate with the range of 1-19,7 U/ejaculate where the normal cut off value is 20 U/ejaculate.

Our patients underwent a testosterone therapy with Andriol tablets (testosterone undecanoate 40 mg, Organon). This version of testosterone is based in oil and is sealed in a capsule to be taken orally. A daily dosage of 120 mg through 3 month was given orally. Controll spermatograms were analyzed by the same criteria as in the baseline examination.

4.2.4. Statistical analysis

Statistical analysis was performed by computer based by SPSS™ software (version 12). The nonparametric Mann-Whitney-U test was used. Correlation analysis was done by Spearmans rho (ρ). Probabilities <0.05 were considered to be significant.

4.2.5. Results

By the control sperm analysises we observed an improvement regarding the sperm density, where the baseline mean density 18,49 M/ml. elevated to 27,56 M/ml (p= 0,40586821) with the range of 0,1 M/ml. to 82 M/ml. (Fig.7)

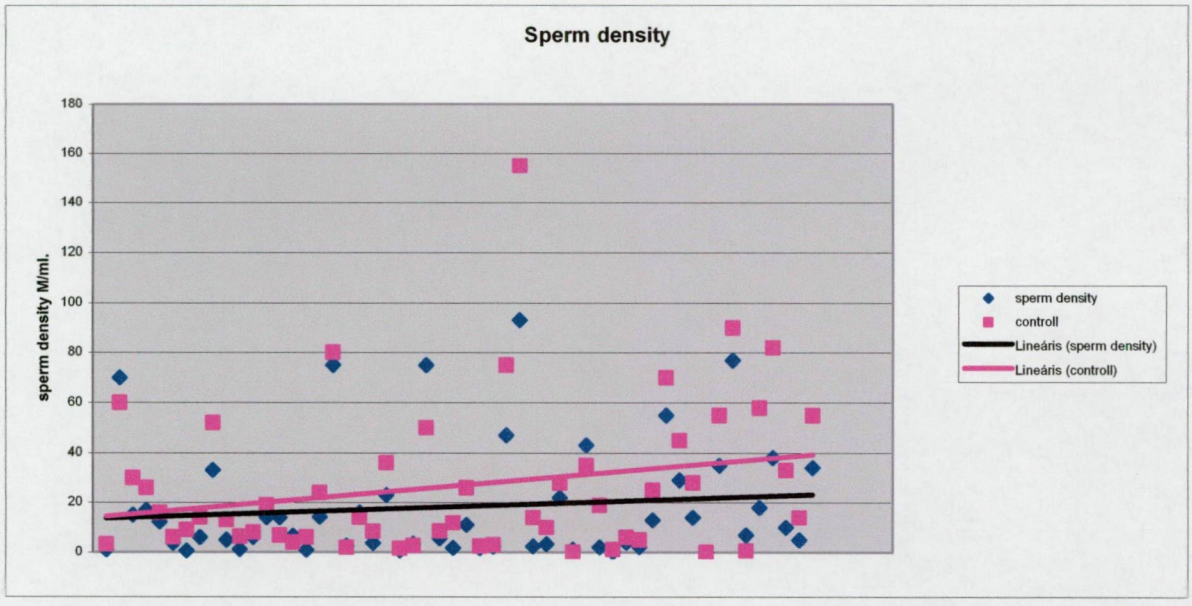


Fig.7 Sperm density M/ml. at the baseline (black) and after treatment (rosa) with linear drawing of the mean values. Unambigous raising in the controll levels.

The mean motility grade „a" was 17,56% with the range of 0-60% compared to the baseline 11,62%. The mean motility grade „b" improved to 5,41% (range: 0-25%) from the beginning assessed 4,16%. The by the beginning 15, 71% total progressive motile sperm percentage improved to 22, 97% (p=0, 049616). (Fig.8)

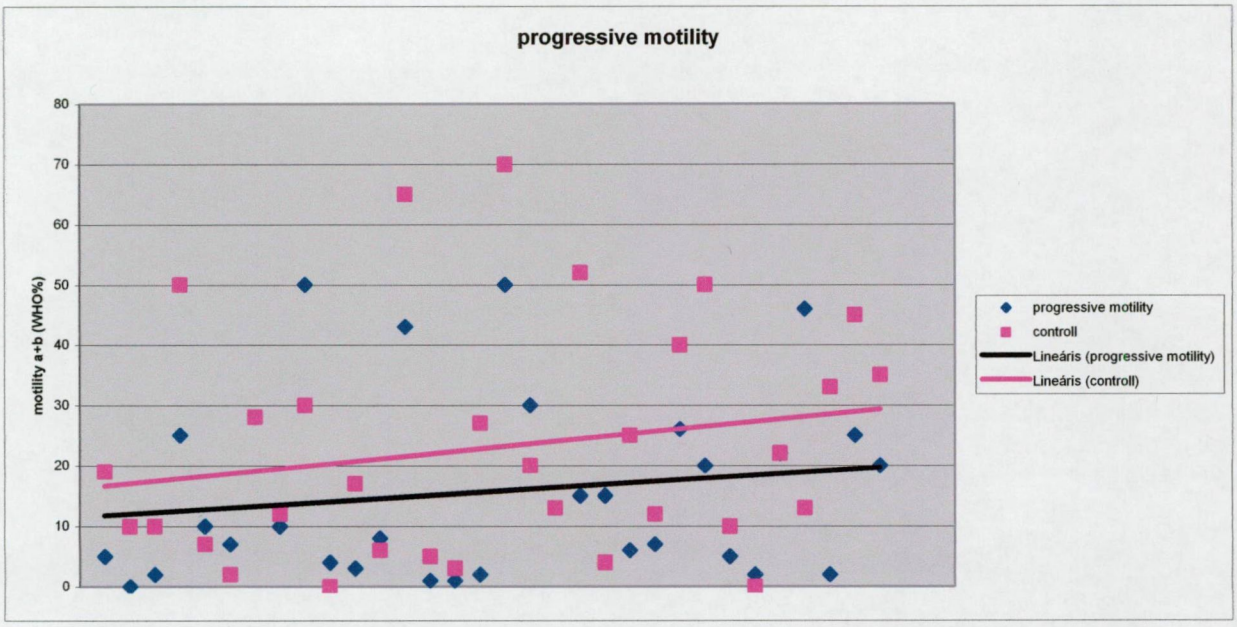


Fig.8 Percentage of progressive motile sperms at the baseline (black) and after treatment (rosa) with linear drawing of the mean values. Univocal rising in the control levels.

5. The functional effect of uro-genital infections on male fertilizing capacity, new biochemical diagnostic evaluation.

5.1. Functional effect of uro-genital infections on male fertilizing capacity

The majority of urogenital infections are proved not to influence spermatogenesis in reality and the induction of autoimmune processes is thought to be their most important impact resulting the appearance of auto-antibodies and the following immune-infertility.

The peroxidase positive white blood cell count above 1 million/ml in the ejaculate is considered pathological according to the currently valid WHO criteria established in 1998. The contamination of seminal sample is indicated by the presence of bacteria, viruses and fungi. Damage can be observed under 1 million/ml white blood cell count but often no functional loss experienced even with high white blood cell count concentration (e.g. patients with spinal cord lesions) (77). Analyzing asymptomatic infertile patient-groups, no correlation was found between white blood cell count and the degree of lesion (6), likewise with bacteria concentration and the seriousness of deviations (43).

According to a comprehensive study spermatogram suffers in 73,8% vitality, in 70,2% motility-, in 34,5% morphological losses in the course of infection and only 13% of cases produced a spermatozoa concentration reduction (27).

Classic sperm parameters don't consequently worsen and most of all divergences of motility can come out. The acrosomal reaction, the sperm-ovum fusion thus the fertile capacity suffer a clearly damage. Post-inflammation obstruction is also a harmful factor having understandably greater importance in the cases of narrow seminal tracts (22).

Bacterial and viral infections

Gram negative anaerobic bacteria cause major damage in the spermatogram but Gram positive anaerobic ones seem not to have these kinds of effects (39, 83). Gram negative aerobic ones (E.Coli) are surely harmful unlike Gram positives (21). In the course of a laboratory test a type of intracellular pathogens appearing in a great number, namely Ureoplasma urealyticum, was analyzed and unambiguous, significant damage of sperm motility was found on the expiration of long time incubation but sperm parameters were improved within short time incubation. A theoretical interpretation of this can be the higher pH supposing short time incubation and glycolysis providing energy for motility and its degree is increased by the Ureaplasma urealyticum resulting in improvement. In the case of low pH the source of energy is due to the oxidative phosphorization which is inhibited by this pathogen and the impairing of parameters can be explained in this manner (70).

Currently the investigation of the function of viral infections in fertility is a new and popular scientific subject. The HSV (Herpes simplex virus) 2 type has placed itself in the focus of interest in the last few years. The role of HSV in male infertility was not established until 1998. El Borai et al. (24) have detected HSV DNA in 24% of male infertility patients with nested PCR (Polymerase chain reaction) technique. Kotronias and Kapranos (42) published HSV2 infections as an important causative factor of male infertility. They found 46% of the examined semen samples to harbor HSV DNA. (ISH – "In situ" hibridization technique) In oligo-asthenospermic patient group this rate was 70%. Sperm count and motility were significantly affected and correlated inversely with the number of infected spermatozoa.

Other studies have also shown that the presence of viral particles in the semen is correlated with a lower percentage of motile sperm (47).

Regarding the assisted reproductive techniques high concentrations of HSV2 or HCMV (human cytomegalovirus) do not have a strong effect on the percentage of motile sperm regardless of the viral concentration. It would therefore appear that the in-vivo correlation between the reported reduction of percentage of motile sperm and the presence of viral particles (47, 42) is due to indirect interactions between the virus and the sperm rather than to a direct effect on motility. In the absence of seminal fluid, sperm is able to adhere to HSV2. It cannot be excluded that this is a ligand/receptor relationship that only occurs when sperm have been separated from its seminal fluid. In conclusion, sperm motility is not a good enough criterion on which to prove the presence of viral elements in the medium or on the sperm.

ICSI creates a new risk of contaminating sperm with viruses in cases of ICSI for azoospermia. Sperm inside the seminiferous tubules are protected from viruses by the haematotesticular barrier. When this barrier is ruptured, sperm can come into contact with viral particles. Indeed, this barrier is always destroyed when spermatozoa are collected from the testes. This increases the risk of sperm coming into contact with high local concentrations of HSV2 due to contamination from the blood or interstitial tissues. The washing procedure cannot eliminate viral particles, meaning that they are directly injected into the ooplasm.

HSV could be responsible for the high rate of fertilization failure observed using low quality semen for assisted reproduction.

Relatively limited data is available concerning other viral infections but relying on the strength of these findings the most frequent pathogen is the adeno-associated virus (AAV) and its DNA can be traceable in 30% of infections. It surely damages sperm function. The fertility damaging effect of the human papilloma virus (HPV), being also often traceable with the help of the polymerase chain reaction (PCR) technique nowadays, is not proved and this virus seems to participate as a "helper" in the processes. It was also detected in 11% of the analyzed healthy men population. The human cytomegalo virus may have a similar role. HCMV was demonstrated not to be transmitted if fertilization occurs in its active phase and to damage spermatogenesis (82).

The level of ROS significantly increases in the course of urogenital infections, functional loss is evident and the gravidity rate in this group of patients reaches only one seventh comparing normal population.

In the course of the patomechanism detailed in the chapter called Biochemical Bases of Male Infertility actually oxidative stress process happens by means of the forming of free derivatives, thus oxygen induced membrane lesion exists. The degree of lesion depends on white blood cell activity, the amount of ROS production and the available antioxidative systems (61, 74, 79).

The cytokines are other leading factors of the pathomechanism of membrane lesion. They are immune regulating polypeptide growing factors produced by macrophages and their major representatives are the interleukins and TNF- alpha. These mechanisms control immune response and are balanced with opposing cytokin inhibitors and their soluble receptors. Unbalance occurs in the course of infection with subsequent membrane lesion.

The basal membrane of spermatozoa together with the extensions of Sertoli cells form the so called blood-seminal barrier. The lesion of the membrane leads to the overturn of the blood-seminal barrier thus the filtering mechanism damages, anti-sperm antibodies are formed and immune-infertility develops.

Consequently up to date spermatograms cannot be limited to classic sperm parameters and biochemical analysis of the ejaculate composes the fundamental part of it. As the limitation of the financial background is well-known in our country it's hard to spread these methods but we should follow this direction. The activity of the alpha-glycosidase indicating possible seminal tract blocks and being the most reliable epididymis-marker; the metallic ion content especially Zn and Mg; and the level of fructose as a marker of energy supply are eventually tried to measure. A modern approach would require the measurement of ROS and TAC together with their ratios. It would be practical to determine interleukin 1, 2, 8 and 4 subtypes, to analyze the soluble TNF receptor and to check membrane integrity. These methods are still not available in everyday andrology in Hungary. The presence of pathogens can certainly be examined in traditional bacteria cultures, by means of ELISA- and PCR methods from urine or ejaculate, postmassage urin, prostatic exprimate and urethral secretion.

The adequate and longused antibiotic treatment produces the best results. The use of antiphlogistic materials is also indicated and Zn and Mg treatment subsequent to infection produce positive effects too. Antioxidant therapy seems to be a good choice but the efficiency has not been proved clearly by studies until now. Under antibiotic therapy levels of interleukin 4 (defending mechanism)

increases and type 2 and 8 decrease. The antimicrobial effect of the treatment is unequivocal but has a proven partial antioxidant action too (89). The efficiency of the independent antioxidant therapy is controversial, examination experiences show inefficiency (73) but reasonable results were reported using carnitine (89). The positive effects of vitamins are well known and proved for a long time. Albumin has been protected under laboratory environment against the lipid peroxidation of spermatozoa membrane but the therapeutic effect is not such clear. The intake of essential fatty acids produced ROS level reduction thus giving these materials good therapeutic effects on patients with oligozoospermia (11).

5.2. New biochemical diagnostic evaluation in uro-genital infections

5.2.1. Introduction

The diagnosis of andrological infection is based on the occurrence of leukocytospermia, i.e. more than 1 million leukocytes per ml of seminal fluid, besides the culture of pathogenic bacteria, increased viscosity of seminal plasma and/or abnormal biochemistry (WHO, 1999). The mainstay, however, of the diagnosis of seminal tract infection/inflammation is leukocytospermia. Many studies have excluded a correlation between leukocytospermia and semen quality (23), antibiotic treatment has been shown to be effective against bacteria but did not significantly influence semen quality and fertility (75). The threshold of 1 mill leukocytes for diagnosis of male genital tract infection/inflammation has recently been questioned with regard to potentially detrimental effects on spermatozoa exerted by ROS produced by leukocytes in a lower concentration (68). As leukocytospermia only detects granulocytes which are intact, degranulated granulocytes could be missed. Therefore, determination of additional markers of inflammation in the seminal plasma may be helpful.

Inflammation is accompanied by a variety of chemical reactions, such as the release of various enzymes from polymorphonuclear leukocytes. One of the main changes during the inflammatory process is the discharge of large amounts of proteases. The most important proteinases produced by granulocytes are elastase, cathepsin G and collagenase. Elastase is released from the azurophilic granules of neutrophil leukocytes during phagocytosis, degranulation, or cell death and may also lead to proteolytic damage of spermatozoa; on the other hand, the protease may serve as an objective indicator for granulocyte activity in semen. Cytokines

play an important role in intercellular communication. They are involved in numerous physiological and pathological processes, particularly in the mediation of inflammatory responses. Inflammatory cytokines are produced by white blood cells in response to foreign antigens and pathogens and are mainly acting in the initiation of the immuno-inflammatory cascade.

Previous studies have found elevated levels of cytokines like IL- 6 or 8 (Interleukin 6 and 8) and TNFα (tumour necrosis factor- alpha) or of granulocyte elastase in infertile patients compared to healthy controls, however, lacking any significant correlations between elevated cytokines and semen parameters (Jochum et al., 1986; Comhaire et al., 1994). On the other hand, there is a growth in the number of contributions which report on a beneficial effect of an antiphlogistic treatment – at least in terms of semen quality (30, 33).

5.2.2. Objective

In our study we compared the concentrations of both diagnostic markers, IL-6 and granulocyte elastase, and correlated them with the number of peroxidase-positive cells and conventional sperm parameters to detect potential influences on semen quality. In particular, we were interested in the relationship between silent genital tract inflammation and sperm DNA-integrity, which has been associated with fertilization, blastocyst development and ongoing pregnancy rates in IVF and ICSI-cycles. The site of DNA-damage has been attributed to a posttesticular origin, probably caused by oxidative stress (29).

5.2.3. Material and methods

Semen was obtained by masturbation after 3 – 5 days of abstinence from 340 consecutive men aged 18 – 59 (mean 37,5) who attended the infertility clinic for fertility assessment. In addition to the 340 patients, semen samples of 50 healthy men with normozoospermia (mean age 36,5, range 24 - 47), however, not with proven fertility, served as controls. Semen samples were processed 30 to 60 minutes after liquefaction according to WHO recommendations (WHO, 1999). Smears stained according to Shorr (Merck No. 9275, Darmstadt, Germany) were used for morphological analysis as previously described (31). In addition to conventional semen parameters the number of peroxidase-positive cells, PMN elastase and Interleukin 6 in the seminal plasma were determined. The peroxidase

test is based on cytochemical visualization of peroxidase activity in polymorphonuclear granulocytes. The method is recommended by WHO and several authors (10). Our protocol was adapted from Entz (1974). A stock solution was prepared by mixing 50 ml distilled water with 50 ml 96% ethanol and adding 125 ml benzidine. The working solution was obtained by addition of 5 µl 30% H_2O_2 to 4 ml stock solution. Twenty µl of working solution were mixed with 20 µl of ejaculate in a small test tube. After incubation for 5 min at room temperature, peroxidase-positive, intensively brown round cells were counted. PMN (Polymorphonuclear) elastase and Interleukin-6 were determined in frozen-thawed seminal plasma by means of enzyme linked immunosorbent assays strictly according to the manufacturer's instructions (PMN elastase Enzyme immuno assay, No. MKEL, Milenia, Bad Nauheim, Germany; Il-6- ELISA-Kit, No. KAC 1262, Biosource, Solingen, Germany). Elastase levels were subdivided into the following groups: up to 250 ng ml^{-1} (normal), 250 to 1000 ng ml^{-1} (moderate inflammation), and >1000 ng ml^{-1}(acute inflammation)(41). An IL-6 threshold at $\geq$30 pg ml^{-1} was defined „high" , concentrations of $\geq$ 50 ng ml^{-1} were considered as „very high"(23) In a subgroup of patients (n = 40) DNA-integrity was assessed by means of the sperm chromatin structure assay using Acridine-orange staining as described elsewhere (25). More than 70 % of spermatozoa with native double-stranded DNA were considered as compatible with good fertility (25). Moreover microbiological investigations of semen samples were carried out. For screening of chlamydia trachomatis infection a commercially available Elisa-test was used (Chlamydia trachomatis Iga-p-Elisa, No. 498 TMB, Medac, Hamburg, Germany). For detection of Chlamydia antigen in semen samples an immunoflourescent test was performed (Chlamydia trachomatis - AG-IFT, No. 466, Medac, Hamburg, Germany). For further microbial screening for aerobic and anaerobic bacteria, ureaplasma and mcyoplasma, standard methods of colonization were used.

5.2.4. Statistical analysis

Statistical analysis was performed by computer based SPSS™ software (version 12). The nonparametrical Mann-Whitney-U test was used to compare the expression of specific markers in different subsets and controls. Correlation analysis was done by Spearmans rho (ρ). Probabilities <0.05 were considered to

be significant (*), p-values <0.01 as highly significant (**), p<0.1 is indicated as "tendency".

5.2.5. Results

An increased number of peroxidase-positive cells (> 1mill ml^{-1}) was detected in 22 out of 340 semen samples (6.5%).

The seminal plasma concentration of IL-6 ranged between 0 pg ml^{-1} and 2350 pg ml^{-1}. „High"concentrations of ≥30 pg ml^{-1} were found in 200 out of 340 samples (58.8%), „very high"concentrations of ≥ 50pg ml^{-1} were measured in 159/340 (46.7%), and of ≥ 100pg ml^{-1} in 93/340 (27%).

Of the 340 samples examined, 218 showed elastase levels up to 250 ng ml^{-1} (64.1%), 101 had levels up to 1000 ng ml^{-1} (29.7%), and 21 patients had elastase levels >1000 ng ml^{-1}(6.2%). Thus, elastase concentrations were increased (> 250 ng ml^{-1}) in a total of 122 men (35.9%) indicating silent genital inflammation.

The correlation between the seminal plasma concentrations of IL-6 and elastase was highly significant (r = 0.539, p< 0.01) (Fig. 9).

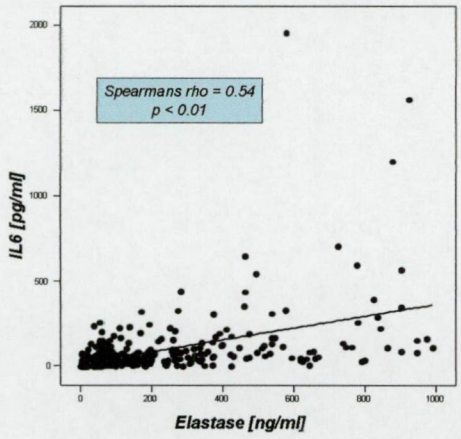


Fig.9 Correlation of the seminal plasma concentration of IL-6 and elastase.

Determination of IL-6 concentrations in the seminal plasma from 50 men with normozoospermia showed the following results: mean: 18.0 ng ml^{-1} ± 2.6.SEM, (95% interval of confidence 12.2 - 23.7). Mean IL-6 concentrations of men with genital tract inflammation as defined by elevated elastase levels (> 250 ng ml^{-1}) were 32 pg ml^{-1} (Fig.10).

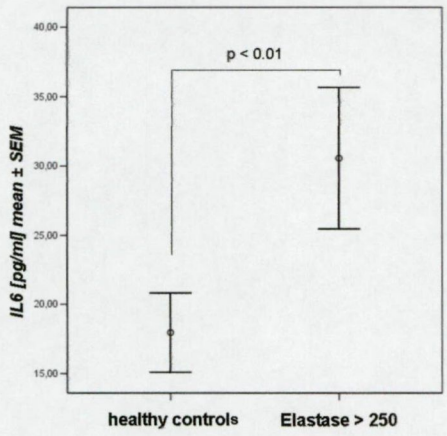


Fig.10 Mean IL-6 concentrations in genital tract inflammation as defined by elevated elastase levels.

A significant correlation was observed between IL-6 levels and the number of peroxidase positive cells ($r = 0.459$, $p<0.01$); moreover, IL-6 concentrations correlated negatively with the vitality of spermatozoa as determined by staining with eosin ($r = - 0.208$, $p<0.01$); a weaker, however, still significant association was observed between IL-6 levels and the total motility of spermatozoa ($r= -0.103$, $p < 0.05$) .There were no correlations with further semen parameters like sperm count and progressive motility, and no correlation with the extent of DNA-integrity.

Elastase-concentrations were highly significantly correlated with the number of peroxidase-positive cells ($r = 0.577$, $p< 0.01$); in addition, there were inverse correlations with the vitality of spermatozoa ($r = -0.166$, $p < 0.01$), total motility ($r= -0.112$) and progressive motility according to WHO a quality ($r = - 0.112$), and sperm morphology ($r = -0.117$;$p<0.05$). No association was found with the sperm count. Interestingly, there was a significantly negative correlation with the percentage of spermatozoa revealing decreased DNA-integrity ($r= -0.643$, $p < 0.05$). Subdivision of patients into groups with normal (> 70%) and disturbed DNA-integrity (< 70%) (Evenson et al., 1999) shows the relationship with elevated elastase levels and the missing correlation with IL-6 even more clearly. (Fig.11)

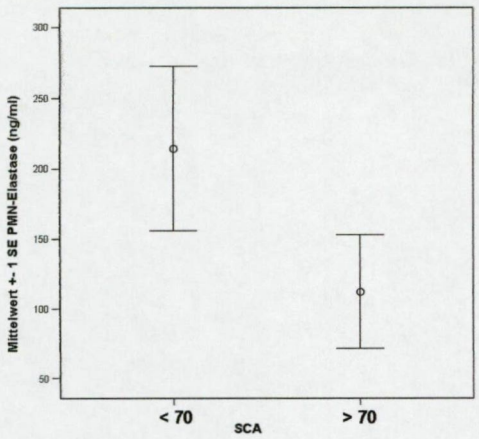


Fig.11. the relationship with elevated elastase levels and the missing correlation with IL-6

There were no correlations either between microorganisms and elastase-, and IL-6 levels, or with the number of peroxidase positive cells (data not shown).

6. Genetic analysis to reduce the number of idiopathic pathozoospermias.

6.1. Introduction

Recent biochemical studies concentrate primarily on the investigation of DNA content of sperm's head. These genetic tests represent the largest step forward in the diagnostics of male infertility nowadays. Firstly determination of the karyotype and the investigation of the microdeletion of the Y chromosome in as much locus as possible and of the mutation of the Cystic Fibrosis Transmembrane Regulator (CFTR) gene have to be mentioned here. (84)

Apart from that fundamental idea to decrease of the occurrence of idiopathic pathozoospermias, the expressive development of assisted reproduction techniques especially the ICSI have also contributed to the promotion of andrological genetics. Our accurate skill makes it possible to decrease genetic abnormalities which may have not been noticed earlier in the absence of ICSI screening. (64, 5)

Among the numeral chromosomal abnormalities (NCA) the Klinefelter syndrome is the most frequent one and exists in 7-13% of azoospermic men according to literary data. There is an extra X chromosome in men thus the classic karyotype is 47 XXY. It occurs in 90% and the remaining 10% represents the mosaic cases. The incidence of the Klinefelter syndrome in male infants counts 1/500, and is recorded as a cause of male infertility. The greater the abnormality of

the chromosome the more severe is the manifestation of the disease. The classic form is the meiotic nondysjunction of gamete's X chromosome and the mosaic form is the mitotic nondysjunction of X chromosome. The man having classical Klinefelter syndrome has small atrophied testicles with associated azoospermia. The histological examination of testicle biopsy reveals sclerosis, hyalinization of tubuli, absence of spermatogenesis and increased number of Leydig cells. The other form reveals tall, fatty, varicose male patient with associated slight mental retardation, thyroid functional disorder, diabetes mellitus and chronic bronchitis. Serum testosterone level is significantly low and the level of oestradiol is high. In the case of mosaicism spermatogenesis takes place. Several scientists proposed that in many cases of severe oligozoospermia such divergence persists in the background having been often undiscovered until now. (20, 48)

Structural chromosomal abnormalities follow the Klinefelter syndrome considering frequency. The greatest interest tends towards to the azoospermia factor gene (AZF) and the 46 XX karyotype. Tiepolo and Zuffardi described in 1976 in the course of the genetic examination of 6 azoospermic patients the complete absence of distal fluorescent heterochromatin in the Yq section. Research has focused on this region afterwards and the AZF test being part of the daily routine has recently been developed.

AZF discrepancies occur in 10-13% of non obstructive azoospermic, 46 XY karyotype men according to literary data. The rate of occurrence is 1/10.000 among male infants. Wide spectrum of the disorder of spermatogenesis is detectable in the course of testicular biopsies. They are routine examinations in the cases of azoospermia and severe oligozoospermia in modern andrology. (26, 72)

In the last few years mutations of the cystic fibrosis conductance regulator (CFTR) gene being responsible for the cystic fibrosis have been demonstrated without the appearance of classic cystic fibrosis in the cases of Wolff tube anomalies. Its andrological significance is concealed in congenital bilateral and unilateral agenesia of vas deferens (CBVDA, CUVDA) whose deviations are slight or incomplete forms of cystic fibrosis. These divergences have also been shown in conjunction with epididymal obstructions. CBVDA occurs in 1-2% of infertile men and 50-82% of patients having CBVDA show at least one detectable CFTR mutation and two mutations are present in 10%. The occurrence of one mutation is 43% in the case of CBVDA and CFTR mutation was found in 47% with

examinations of epididymal obstructions. Not every Wolff tube abnormality implies CFTR mutation: the simultaneous occurrence of CBVDA or CUVDA with renal agenesia or ectopy is not related to CFTR mutation. In recent times correlations have been found between the abnormal viscosity of ejaculate and the mutations of CFTR gene. In vas deferens abnormalities the examination of it is part of the everyday routine. (81, 85)

6.2. Objective

Our study was conducted to define the occurrence of numeral and structural chromosomal abnormalities and CFTR gene mutation in azoospermic and severe oligozoospermic patients to ascertain whether it reduces the number of idiopathic pathozoospermias.

6.3. Material and method

In our study numeral chromosomal abnormalities (NCA), structural chromosomal abnormalities including the microdeletions of Y chromosome and finally the mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR) were analyzed in 159 azoospermic and severe oligozoospermic patients who attended the andrology department.

XY aneuploidity: assessing the numeral chromosomal abnormalities a multiple fluorescent PCR method was developed as a rapid alternative of the relatively long lasting cytogenetic analysis or rather for pre-screening available test which was with capillary gelelectrophoresis on ABI310 genetic analyzer detected. On one X and Y chromosome existing but different length showing (amelogenin) one polymorph and on the common pseudoautosomal part of the X and Y chromosome localized (X22) two the X chromosomes specified polymorph (DXS6803, DXS6809) and one Y chromosome specific marker (SY134) were investigated. The automatised marker detection and the automatic evaluation of the peaks were performed by an own developed Genotyper template. The plus X chromosome was detected either by a plus peak or/and by the increased area under the plus peak. All Klinefelter cases were confirmed by cytogenetic tests.

Detection of numeral chromosomal abnormalities (Fig.12)

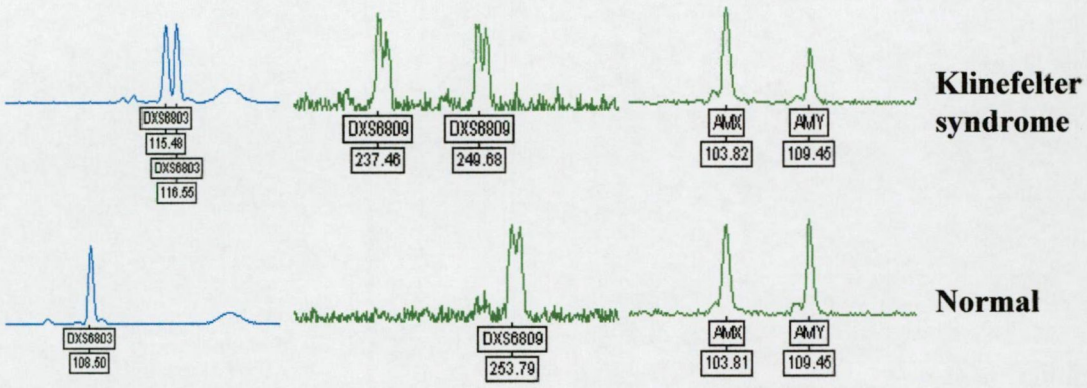
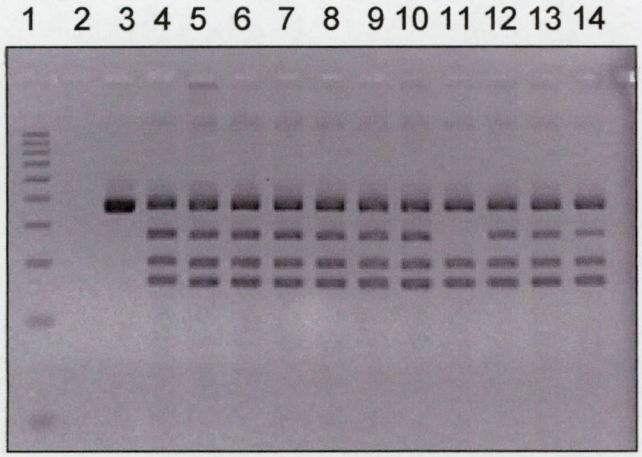


Fig.12. Detection of numerical chromosomal abnormalities. In Klinefelter-syndrome 2 peaks are presented by the 2 X chromosomes. The presence of the 2 X chromosomes can also cause a dose difference as seen in the case of amilogenin. The area under the curve is 2 times greater in the Klinefelter patient than the Y chromosome specific markers.

DXS6803 and DXS6809 markers localized on the X chromosomes. In a normal case exists only one X peak, in the case of Klinefelter-syndrome 2 peaks are presented by the 2 X chromosomes. The presence of the 2 X chromosomes can also cause a dose difference as seen in the case of amelogenin. The area under the peak is 2 times greater in the Klinefelter patient than the Y chromosome specific marker's peak.

Y chromosome microdeletions: the genetic examinations were performed using the recommendations of the "European Molecular Genetic Quality Network" (80) with multiple PCR amplification and detection system. PCR primers and analyzed DNA markers were selected the same way. In the first part of the study the existence or absence of 2-2 markers in each AZF region were investigated; in the AZF a region sY84 and sY86 markers, in the AZF b region the sY127 and sY134 markers and in the AZF c region the localized sY254 and sY255 using SRY and ZFY markers as inner controls. In the positive cases additional markers recommended by the EMQN were used in the second part of the trial. To visualize the PCR products traditional agarose gels and Agilent Lab-on-Chip instrument

were used. More than 90% of the AZF deletions can be confirmed with these methods. (Fig.13)



Investigated markers:

- ZFY
- SY254 (AZFc)
- SY84 (AZFa)
- SY127 (AZFb)

Fig.13 Y chromosome microdeletion detection with agarose gel electrophoresis detection after multiplex PCR amplification of genetic markers (sY254, sY84, sY127).
In the sample No. 11 the sY254 marker is deleted in the AZF c region, other samples without microdeletions.

CFTR gene mutations: the most common classical mutations and in the male infertility confirmed variants were investigated by real time PCR technique: the intronic 5T allele, deltaF508, R117H, N1303K and the G542Xmutations were isolated by an own constructed system using real time PCR and melting-point analysis. By the difference in the melting-point of the mutant and wild types the homozygote normal, the heterozygote and homozygote mutant samples can be well separated in each mutation.

6.4. Results

Examining the numeral chromosomal abnormalities (NCA) Klinefelter syndrome was found in 5 cases from 148 (3,37%) with 47 XXY karyotype in every ones. Concerning the spermatograms of the 5 patient's azoospermia was verified in 4 cases and 1-2 motile spermatozoa were seen in 1 case. 154 patients were examined in the course of structural chromosomal abnormalities analyzis and divergence was found in two cases (1,29%): partial deficiency and azoospermia in one and the complete absence of the AZFc region in other. The latter patient had 14 cm3 testicles on both sides and 0,1 M/ml sperm concentration, cryptozoospermia was found. Examining 140 patients via CFTR 15 positive cases were found (10,7%), 3 compound heterozygote (2,1%), in which DF508 and 5T

occurred in 2 cases, and DF508 and R117H occurred in 1 case. However in 9 cases only IVS85T mutation was shown. Regarding the classical CFTR gene mutations the IVS85T mutations must be separated. Thus, the occurrence of CFTR gene mutations is 4,3% (6 patients) which number is equivalent in correlation with the international data.

All of the investigated genetic abnormalities occur in 14,57% according to our study which corresponds to international clinical data dealing with large groups of patients.

7. Discussion

7.1. The impact of sperm morphology assessed by strict criteria in the classification of male infertility. Analysis of the normal morphology thresholds and their predictive value regarding the outcome of assisted reproductive techniques.

In our study previous results with regard to sperm morphology and ART-outcome could be confirmed, i.e., there were positive correlations between the percentage of normal spermatozoa and pregnancy rates following IUI (34, 88), positive correlations between sperm morphology and fertilization and pregnancy rates after IVF, and no correlation between normal morphology and fertilization and pregnancy rates after ICSI (5, 9). Using the morphology-index – as described by Kruger et al. earlier (44, 45), i.e. the percentage of strictly normal forms plus slightly disturbed, borderline spermatozoa with a threshold of 30%, did not provide a further advantage over determination of the normal forms alone. There was no additional value of consideration of the pathological forms for choosing the appropriate treatment in the groups with IUI- and IVF-treatment, when using the 4% threshold of normal morphology. The significant negative correlations between the percentage of severely malformed spermatozoa and fertilization and pregnancy rates during these procedures were more or less associated with a percentage of normal forms below 4 %. Therefore, determination of normal forms alone seems to be sufficient for the decision of IUI or IVF as opposed to ICSI. However, the negative correlation between pathomorphology of spermatozoa with the fertilization rate in ICSI may be of more importance. This result is not helpful for selecting the most suitable ART-method, because these couples have no other option than ICSI, but it should make aware physicians and biologists of the potential significance of

severely disturbed spermatozoa for the ICSI-outcome. Most recently, it has been reported on a higher rate of chromosomal abnormalities in abnormally shaped spermatozoa which can influence fertilization and implantation (89). Although it is widely accepted that the average percentage of normal spermatozoa is not predictive of the ICSI-outcome, it has been pointed out, that individual sperm morphology, i.e. morphology of the sperm used for ICSI, correlated well with fertilization outcome. A lower implantation rate was achieved when using an abnormal sperm for injection (19). In addition, a significant sperm effect on blastomere cleavage has been demonstrated, which could be referred to sperm morphology as determined according to strict criteria rather than sperm count or progressive motility (76). Moreover, unexplained recurrent pregnancy loss has been positively associated with sperm chromosome aneuploidity and abnormal sperm morphology (8). In addition to sperm chromosome aneuploidy which has been described in severely disturbed spermatozoa, for example globozoospermia, acrosome defect forms according to the Dusseldorf classification have been shown to exhibit disturbed chromatin condensation (38). Sperm chromatin anomalies have been found to exert profound effect on fertilization failure in ICSI (69). Therefore, it is useful not only to determine normal morphology but also to look at the pathological forms. In cases where no normal spermatozoa are available, it is suggested that we select the less disturbed for injection into the oocyte and spermatozoa with elongation of the postacrosomal region, rather than those with acrosomal maldevelopment or enlarged heads, because of the worse chromatin condensation and increased aneuploidy rates the in the latter ones (89).

7.2. The role of biochemical markers in diagnosing male infertility, the impact of neutral alpha glucosidase activity.

The assessment of the neutral isoenzyme of alpha glucosidase is a sensitive and straight forward method to differentiate azoospermia type. Moreover the method helps to localize the site of obstruction in the male genital tract and to identify partial obstruction especially at epididymal level. Low levels of alpha glucosidase in semen can be related to disturbed epididymal function and have been associated with defective sperm maturation in the epididymis. In oligozoospermic patients NAG deterioration means either epididymal obstruction or epididymal dysfunction. NAG became the marker of epididymal function, marker of

sperm maturation. Interestingly, our result showed a more clear and statistically significant correlation between the corrected NAG levels hereby corrected epididymal function and sperm density and percentage of progressive motile spermatozoa than previously it was hypothesized flaring the field of indication int he evaluation of oligozoospermic or asthenozoospermic male infertility patients.

7.3. The functional effect of uro-genital infections on male fertilizing capacity, new biochemical diagnostic evaluation.

Due to imprecise definitions of male genital tract inflammations/infections there is still much confusion on how to diagnose these conditions and how to estimate their influence on male fertility. With regard to chronic prostatitis studies show contradictory results: while some authors have found an alteration of basic ejaculate parameters like sperm density, motility or morphology, most have not (91). Compared to inflammations of the prostate and seminal vesicles where normally higher numbers of leukocytes are detected in the seminal fluid, because 90% of the ejaculate volume derive from these organs, this is not the case in chronic torpid inflammations of the epididymis, in many cases occurring as epididymo-orchitis, which is difficult to diagnose, but is much more harmful for fertility (33). The patients hardly feel any discomfort, due to the low percentage of epididymal secretions in the seminal plasma -about 5 to 10%- there will only be a low number of leukocytes. Therefore, the additional determination of proinflammatory cytokines or granulocyte elastase has been suggested for diagnosis of silent genital inflammations (71).

In our series of 340 patients 36% revealed elevated levels of granulocyte elastase, and 58% showed increased concentrations of IL-6 (23), indicating inflammation of the genital tract. This frequency is higher as proposed in the literature, where the frequency of infections as cause of male infertility is quoted to account for approximately 15% of cases of male infertility (22). On the other hand, most of the samples in our study were in the group of moderate inflammation (elastase concentrations between 250 and 1000 ng ml^{-1}; IL-6 concentrations between 30 and 50 pg ml^{-1}). The comparison of IL-6 levels in normozoospermic men with those with genital tract inflammation as defined by elevated elastase concentrations confirm the definition of „high"IL-6 values (> 30 pg ml^{-1}) reported by Eggert-Kruse

et al (23); the unexpectedly high number of patients with elevated concentrations of IL-6 may indicate that this marker is highly sensitive but possesses lower specificity compared to elastase. Moreover, the different origin of IL-6 and granulocyte elastase may have contributed to this finding. While granulocyte elastase is released from polymorphonuclear leukocytes, inflammatory cytokines like IL-6 are mainly produced by macrophages. We found a good correlation between levels of Il6 and granulocyte elastase within a number of peroxidase positive cells; however, in a considerable number of patients there were elevated concentrations of the biochemical markers despite peroxidase positive cells below 1 mio ml^{-1}. These results support previous reports suggesting a change of the normal value of peroxidase positive cells in the seminal fluid considering the different origin of the components of seminal plasma (35). Moreover, our data reconfirm that the determination of standard ejaculate parameters according to WHO is not sufficient for diagnosis of silent genital tract inflammation and increased levels of cytokines, elastase or increased seminal oxidative stress (60). In contrast to previous reports elevated levels of IL-6 as well as of granulocyte elastase showed significant correlations with semen quality (71, 23). This observation may have been influenced by the higher number of samples investigated in our study. Interestingly, disturbed sperm chromatin integrity as reflected by the sperm chromatin structure assay was correlated with elevated concentrations of elastase. These findings support recent contributions reporting on the role of disturbed DNA-integrity for implantation and pregnancy rates after IVF and ICSI. Moreover, a couple of months ago, increased DNA-fragmentation as assessed by the TUNEL-assay has been addressed as major cause for repeated ICSI-failure and has been attributed to a posttesticular origin, probably caused by oxidative stress (29). This observation is in agreement with our results of increased concentrations of granulocyte elastase in semen samples with disturbed sperm chromatin. In addition, a poorer blastocyst development rate was observed during an IVF-program with the male partner showing elevated seminal elastase-levels, indicating a role of silent genital inflammation (95). As a consequence of our new finding of elevated elastase concentrations in semen samples from patients with decreased DNA-integrity screening for silent genital tract inflammations should be performed on all patients undergoing methods of artificial reproduction. An antiphlogistic approach in such patients seems logical, rather than a testicular

sperm extraction which has been suggested for patients with increased DNA-damage (29). First promising results after treatment with diclofenac have been reported most recently which will have to be confirmed in future studies (2). The value of an antiphlogistic treatment not only in patients with repeated ICSI-failure and disturbed DNA-integrity but also in those with increased concentrations of IL-6 or elastase and a concomitant reduced semen quality has to be clarified in adequate studies which are urgently needed as well. Both markers of inflammation investigated are similarly useful for diagnosis of silent inflammations of the male genital tract; in our study, elastase revealed better correlations with semen quality compared to IL-6; in addition determination of elastase requires only 10 µl of seminal plasma whereas for determination of IL-6 100 µl are needed. Not least, determination of elastase is cheaper, which may - all together - represent a slight advantage for elastase compared to IL-6.

7.4. Genetic analysis to reduce the number of idiopathic pathozoospermias.

These examinations can be quickly completed; however their importance is indisputable on the strength of the above data. The controlled divergences in our study are not lethal but have remarkable relevance. Genetic mutations are found in nearly 15% of patients that means the cause of infertility was cleared in a meaning number of men thus idiopathic pathozoospermic cases decreased with this number. It had been impossible for these men to be a father before the era of assisted reproduction but lots of them have been given the chance in the past years by means of the development of IVF or mainly ICSI and the opportunity for fertilization with round spermatides. These abnormalities could have been transmitted without genetic analyzis via ICSI and genetic discrepancies exist at a greater rate among the descendants than in the normal population. Having been detected the genetic failure the couple can be informed fairly and they have the chance of making a responsible decision understanding clearly the possibilities to complete ICSI, thus reducing the number of infants with genetic abnormalities.

In the case of a serious pathozoospermia with testicular origin a check-up of the Klinefelter syndrome is necessary and if cytogenetic analysis is not available (unfortunately this often happens in Hungary) the numeric divergence of the chromosome can be checked via the above method and cytogenetic examination has to be ordered only if pathological findings are present. The Y chromosomal

microdeletions are transmitted further in male descendant resulting in infertile descendant afterwards. The screening of CFTR mutations and the examination of the wife completing with preimplantational and prenatal diagnostics make possible a fair genetic counselling and the prevention of cystic fibrosis. (66)

8. Conclusion

8.1. The impact of sperm morphology assessed by strict criteria in the classification of male infertility. Analysis of the normal morphology thresholds and their predictive value regarding the outcome of assisted reproductive techniques.

A positive correlation exists between the percentage of normal spermatozoa and pregnancy rates in IUI and between sperm morphology and fertilization and pregnancy rates by IVF. For ICSI no correlation exists between normal morphology and pregnancy rate. The percentage of strictly normal forms plus slightly disturbed spermatozoa with a threshold of 30% does not provide any advantage over determination of the normal forms. Assessing the normal forms using the strict criteria is sufficient for the indication of IUI or IVF vs. ICSI.

Special interest must be put on the negative correlation between severe morphological abnormalities of spermatozoa with the fertilization rate in ICSI. These couples have no other option of achieving pregnancy but it should draw the attention on the significance of severely disturbed spermatozoa for the ICSI-outcome. We suggest determining the severity of pathological forms and select the less disturbed for injection into the oocyte.

Thus, the application of the Dusseldorf criteria for the determination of sperm morphology provides additional information concerning patients undergoing the ICSI-treatment. Apart from the usefulness for selection of the appropriate ART-method, assessment of sperm morphology according to these criteria enables the andrologist to recognize the pattern of a disturbed male fertility and the degree of its severity, and may therefore be helpful as an additional tool to the strict criteria.

8.2. The role of biochemical markers in diagnosing male infertility, the impact of neutral alpha glucosidase activity.

Summarizing our experiences there is no doubt that the modern andrological evaluation of infertility means the use of special biochemical markers.

The previous published data about idiopathic infertility cases showed 30% occurrence. Using this methods andrologists can come closer to the origin of the pathozoospermia and there is the possibility of prefering a causative treatment modality offering much more success regarding the sperm parameters and function. These methods allow the reduction of the unknown origin male fertility cases, moreover giving suitable data for decision about the further therapy of male infertility.

Magnesium did not fulfil previously hopes in the therapy of male infertility, randomized prospective clinical pilot studies did not confirm the previous optimal data. Although Mg considered as bioavailable magnesium in seminal plasma was elevated due to magnesium therapy, any direct biological effect of magnesium on spermiogenesis was not detectable.

Zinc deficiency is usually associated with decreased testosterone levels and sperm count. Infertile men had lower degrees of sperm chromatin stability and lower sperm zinc content than the fertile. Zinc therapy has a beneficial effect amended with antiphlogisitcs especially in postinflammatory asthenozoospermia. Further studies are needed to clarify its real impact.

Pentoxyfillin increases sperm motility in vitro and even in vivo sperm parameters improve. ROS production decreases and pentoxifylline plays an important role in the patomechanism as a phosphodiestherase inhibitor. Using pentoxyfillin improvement in sperm motility could be observed.

L-Arginine is to increase the levels of cAMP and cGMP. In the 1970'-ies Papp et al. underlined the role of L-Arginine in the male reproductive system.

Callicrein enhances blood vessel permeability, helps the contraction of flat muscles, improves glucose transportation through cell membrane and plays a role in the proliferation of different cells. In the processes of reproduction they cause flat muscle contraction of the uterus, increase the motility of sperm and stimulate the spermiogenesis. Long term results did not confirm their real importance in the therapy.

The scientific importance of the remaining energy-providing materials (citric acid, isonit) has been vigorously reduced by now. Lipids and ATP have been more and more focused on.

Checking their effects and considering their cost-effectiveness mast cell blockers and nucleotides were rejected from the therapeutic regimen.

Concluding these findings the above mentioned drugs and compounds have their basic place int he treatment of male infertility but only in the case when their seminal plasma concentration has decreased. The so-called specific (according the origin of the deterioration) treatment is an important slice of the therapy. Int he case of idiopathic pathozoospermia these treatment modalities are not recommended.

Special interest must be placed on the neutral alpha glucosidase activity. Our results confirm the hypothesis based on the previously published data suggesting that neutral alpha glucosidase is a sensitive indicator of epididymal function (15). Several other studies have shown the NAG activity to correlate positively with conventional sperm characteristics (16) and with seminal ATP, which factors are indicative of the fertilizing capacity of spermatozoa (12). Our results clearly demonstrate the correctness of the diagnosis of epididymal hypofunction or dysfunction and the indication of the testosterone therapy with the statistically significant improvement in the sperm density and in total count of progressive motile spermatozoa which are the most important factors of the male fertilizing capacity. Of note, we have to underline the impact of the precise adherence of the exclusion criteria namely the normal hormonal status and the absence of varicocele and urogenital infections. The results verify the importance of restoring epididymal function with a new field of indication of testosterone therapy. Further studies are needed with greater strength of patients to demonstrate this new indication for the testosterone treatment.

8.3. Functional effect of uro-genital infections on male fertilizing capacity, new biochemical diagnostic evaluation.

Most recently the diagnosis of male uro-genital infections involves the effect of the inflammation on the sperm function. The andrological evaluation became mostly a biochemical evaluation regarding fertilizing ability. The commonly and traditionally used parameters are insufficient for the correct diagnosis and management. New and safe methods must be established for this reason.

Our results confirmed the definition of „high"IL-6 values (> 30 pg ml^{-1}), this marker is highly sensitive but possesses lower specificity compared to elastase. A high correlation exists between IL6 and granulocyte elastase with the number of peroxidase positive cells. Our experiences suggest a change of the normal value

of peroxidase positive cells in the seminal fluid considering the different origin of the components of seminal plasma. Moreover, our data reconfirm that determination of standard ejaculate parameters according to WHO is not sufficient for diagnosis of silent genital tract inflammation and increased levels of cytokines, elastase or increased seminal oxidative stress has to be considered.
It seems to be advisable the screen for silent genital tract inflammations in all patients undergoing methods of artificial reproduction. An antiphlogistic approach in such patients seems logical. Elastase revealed better correlations with semen quality compared to IL-6; in addition determination of elastase requires only 10 µl of seminal plasma whereas for determination of IL-6 100 µl are needed. Not least, the determination of elastase is cheaper, which may - all together - represent a slight advantage for elastase compared to Il-6.

8.4. Genetic analysis to reduce the number of idiopathic pathozoospermias.

Genetic mutations are found in nearly 15% of patients which means the cause of infertility was clear in so many men thus idiopathic pathozoospermic cases decreased with this number with relatively simple and not long lasting methods.

These abnormalities could have been transmitted without genetic analyzis via ICSI and genetic discrepancies are surely exist at a greater rate among the descendants of these parents than in a normal population. Having been detected the genetic failure, the couple can be informed fairly and they have the chance of making a responsible decision understanding clearly the possibilities of completing ICSI, thus reducing the number of infants with genetic abnormalities.

In the case of a severe pathozoospermia with testicular origin the check-up for the Klinefelter syndrome is necessary. Numeral divergence of the chromosome must be controlled. Y chromosome microdeletions are transmitted further in the male descendant resulting infertile descendant afterwards. The screening of CFTR mutations and the examination of the wife, with preimplantational and prenatal diagnostics, make possible a fair genetic councelling and prevention of cystic fibrosis.

Our efforts must be put towards a nationwide introduction of the genetic analyzes also in the andrological practice, especially recent and difficult to obtain cytogenetic examinations. We must come closer to the causes of unknown origin

male infertility cases and find the possible male background of spontaneous abortions.

9. Acknowledgments

I would like to thank to my PhD program co-ordinator, Prof. László Kovács and to my tutor, Dr. János Szöllősi for their help, advises and support my scientific activity during these years.

I would like to express my grateful thanks to Prof. György Papp, who entered me in the world of andrology and took my hands making the first steps with his support and orientation and is standing until today by my side not only in professional activities. Grateful thanks to Prof. Mihály Berényi having the possibility working with him in laboratory circumstances.

Here I would like to thank Prof. Dezső Frang that I became urologist and the possibility to know andrology.

Special thanks to Prof. Gerd Haidl in the Department of Dermatology Andrology Unit of the University Bonn for his instrumental, technical, scientific and personal support.

Many thanks for all my colleagues and assistants and nurses in the Department of Andrology and Urology of the National Medical Center and previously in the Urology Department of the Semmelweis University providing the circumstances of the work and making a friendly strain, special thanks to Molnárné Kedves Ildikó for her outstanding doll.

A meaning part of the work was supported by Laborigo, special thanks to Dr. Tímea Tisza.

Last, but not least warmest thanks to my family, for everything to my parents to have all these possibilities and to be forced to do this work and heart-felt thanks to my wife, Bernadette and my children, Eszter and Miki for their patience, support, kindness and love.

References

1 Aitken R.J., Irvine D.S., Wu, F.C.
Prospective analysis of sperm–oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility.
Am. J. Obstet. Gynecol. 164: 542–51. 1991

2 Alvarez J.G.
Efficient treatment of infertility due to sperm DANN damage by ICSI with testicular spermatozoa. Letter to the editor.
Hum. Reprod. 20: in press. 2005

3 Ben Ali H., Gue´rin J.F., Pinatel M.C.
Relationship between a-Glucosidase and male infertility semen characteristics, alpha-glucosidase and the capacity of spermatozoa to bind to the human zona pellucida.
Int. J. Androl. 17: 121–26. 1994

4 Bonduelle M., van Assche E., Joris H., Keymolen K., Devroey P., van Steirteghem A., Liebaers I.
Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters.
Hum. Reprod. 17: 2600 – 14. 2002

5 Bonduelle M., Wilikens A., Buysse A., Van Assche E., Devroey P., Van Steirteghem A.C., Liebaers I.
A follow-up study of children born after intracytoplasmic sperm injection(ICSI) with epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI.
Hum. Reprod. Suppl. 1, 13: 196-207. 1998

6 Cardoso E., Arregger A., Coumroglon M., Andrada E.C., Andrada J.A.
Seminal plasma modulates lymphokine-activated killer cell activity in vitro.
Min. Endocrin. 18(3):109-14. 1993

7 Carlsen M.
Evidence for Decreasing Quality of Semen during Last 50 Years.
B. M. J. 305: 609-13. 1992

8 Carrell D.T., Wilcox A.L., Lowy L., Peterson C.M., Jones K.P., Erickson L., Campbell B., Branch D.W., Hatasaka H.H.
Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss.
Obstet. Gynecol. 101: 1229 – 35. 2002

9 Check M., Check J.K., Summers-Chase D., Swenson K., Yuan W.
An evaluation of the efficacy of in vitro fertilization with intracytoplasmic sperm injection for sperm with low hypoosmotic swelling test scores and poor morphology.
J. Assist. Reprod. Gen. 20: 182 – 85. 2003

10 Comhaire F. Van Waeleghem K. De Clercq N. Vermeulen L. Schoonjans F.
Statement on the general reduction in sperm quality.
International Journal of Andrology. 18 Suppl 2:1-2.1995

11 Comhaire F., Bosmans E., Ombelet W., Punjabi U., Schoonjans F.
Cytokines in semen of normal men and of patients with andrological diseases.
Am. J. Reprod. Immun. 31: 99 – 103. 1994

12 Comhaire F., Vermeulen L., Ghedira K.
Adenosine triphosphate in human semen: a quantitative estimate of fertilizing potential.
Fertil. Steril. 40. 500–04. 1983

13 Comhaire F.H., Mahmoud A.M., Depuydt C.E., Zalata A.A., Christophe A.B.
Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist`s viewpoint.
Hum. Reprod. Update 5: 393 – 98. 1999

14 Cooper T.G.
Physiology and evaluation of epididymal function.
In Comhaire F.H. (ed.), Male Infertility. Chapman & Hall, London, pp. 97–119. 1996

15 Cooper T.G., Weidner W., Nieschlag E.
The influence of inflammation of the human genital tract on secretion of the seminal markers alpha-glucosidase, glycerophospocholine, carnitine, fructose and citric acid.
Int. J. Androl., 13: 329–36. 1990

16 Cooper T.G., Yeung C.H., Nashan D., Nieschlag E.
Epididymal markers in male infertility.
J. Androl. 9: 91–101. 1988

17 Corradi, Gy., Jaszovszky,S.-né,Hegedüs, M .
A prostatovesiculitis diagnosztikája biokémiai módszerrel.
Magy.Urol. 6(2): 121-25. 1994

18 de Mouzon J. Thonneau P. Spira A. Multigner L.
Declining sperm count. Semen quality has declined among men born in France since 1950.
B.M.J. 313(7048):43; 44-5. 1996

19 De Vos A., van de Velde H., Joris H., Verheyen G., Devroey P., van Steirteghem A.
Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection.
Fertil. Steril. 79: 42 – 48. 2003

20 Denschlag D., Tempfer C., Kunze M., Wolff G., Keck C.
Assisted reproductive techniques in patients with Klinefelter syndrome: a critical review.
Fertil.Steril. 82(4):775-9. 2004

21 Diemer T., Huwe P., Michelmann H.W., Mayer F., Schiefer H.G., Weidner W.
Escherichia coli induced alterations of human spermatozoa. An electronmicroscopy analysis.
Int.J.Androl. 23(3): 178. 2000

22 Diemer T., Ludwig M., Huwe P., Hales D.B., Weidner W.
Influence of urogenital infections on sperm function.
Curr.Opin.Urol. 10(1): 39. 2000

23 Eggert-Kruse W., Boit R., Rohr G., Aufenanger J., Hund M., Strowitzki T.
Relationship of seminal plasma interleukin (IL)-8 and IL-6 with semen quality.
Hum. Reprod. 16: 517 – 28. 2001

24 el Borai N., Inoue M., Lefevre C., Naumova E.N., Sato B., Yamamura M.
Detection of herpes simplex DNA in semen and menstrual blood of individuals attending an infertility clinic.
J. Obst. Gynaec. Res. 23(1):17-24. 1997

25 Evenson D.P., Jost L.K., Marshall D., Zinaman M.J., Clegg E., Purvis K., De Angelis P., Clausen O.P.
Utility of the sperm chromatin structure assay (SCSA) as a diagnostic and prognostic tool in the human fertility clinic.
Hum. Reprod. 14: 1039 – 49. 1999

26 Foresta C., Moro E., Ferlin A.
Y Chromosome Microdeletions and Alterations of Spermatogenesis
Endocrine Reviews 22 (2): 226-39. 2001

27 Gdoura R., Keskes-Ammar L., Bouzid F., Eb F. Hammami A., Orfila J.
Chlamydia trachomatis and male infertility in Tunisia.
Eur. J. Contracept. & Reprod. Health Care 6(2):102-7. 2001

28 Glockner D. Gaevert K. Kleinstein J.
Declining sperm quality in men of childless couples.
Andrologia. 30(1):55. 1998

29 Greco E., Scarselli F., Iacobelli M., Rienzi L., Ubaldi F., Ferrero S., Franco G., Anniballo N., Mendoza C., Tesarik J.
Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa.
Hum. Reprod. 20: 226 – 30. 2005

30 Haidl G.
Macrophages in semen are indicative of chronic epididymal infection.
Arch. Androl. 25: 5 – 11. 1990

31 Disturbances of sperm flagellae due to failure of epididymal maturation and their possible relationship to phospholipids.
Hum. Reprod. 7: 1070–73. 1993

32 Haidl G., Schill W.-B.
Is research on epididymal functions still of clinical relevance?
Andrologia 29: 117–19. 1997

33 Haidl G., Weidner W.
Epididymitis and orchitis – andrological implications.
Egypt. J. Androl. Reprod. 17: 3 – 16. 2003

34 Hauser R., Yogev L., Botchan A., Lessing J.B., Paz G., Yavetz H.
Intrauterine insemination in male factor subfertility: significance of sperm motility and morphology assessed by strict criteria.
Andrologia 33: 13 – 17. 2001

35 Schill W.B.
Urogenital inflammation: changes of leucocytes and ROS.
Andrologia 35: 309 – 13. 2003

36 Hirosima K., Mita F.
Prefectural differentials in recent fertility
Jinko Mondai Kenkyu. 50(4):1-30 1995

37 Hofmann N., Haider S.G.
New results in the morphologic diagnosis of spermatogenesis disorders
Gynäkologe 18: 70 – 80. 1985

38 Hofmann N., Hilscher B.
Use of aniline blue to assess chromatin condensation in morphologically normal spermatozoa in normal and infertile men.
Hum. Reprod. 6: 979 – 82. 1991

39 Huwe P.,Diemer T., Ludwig M., Liu J., Schiefer H.G., Weidner W.
Influence of different uropathogenic microroganisms on human sperm motility parameters in an in vitro experiment.
Andrologia. 30 Suppl. I. 55. 1998

40 Irvine D.S.
Falling sperm quality.
B.M.J. 309(6952):476, 1994

41 Jochum M., Pabst W., Schill W.B.
Granulocyte elastase as a sensitive diagnostic parameter of silent genital tract inflammation.
Andrologia 18: 413 – 19. 1986

42 Kapranos N., Petrakou E., Anastasiadou C., Kotronias D.
Detection of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in the semen of men attending an infertility clinic.
Fertil. Steril. 79 Suppl 3:1566-70. 2003

43 Köhn F.M., Erdmann I., Oeda T., el Mulla K.F., Schiefer H.G., Schill W.B.
Influence of urogenital infections on sperm functions.
Andrologia. 30 Suppl 1:73-80. 1998

44 Kruger T.F., Acosta A.A., Simmons K.F., Swanson R.J., Matta J.F., Oehninger S.
Predictive value of abnormal sperm morphology in in vitro fertilization.
Fertil. Steril. 49: 112 – 117. 1988

45 Kruger T.F., Menkveld R., Stander F.S., Lombard C.J., van der Merwe J.P., van Zyl J.A, Smith K.
Sperm morphologic features as a prognostic factor in in vitro fertilization.
Fertil. Steril. 46: 1118 – 23. 1986

46 Kvist U, Bjorndahl L.
Zinc preserves an inherent capacity for human sperm chromatin decondensation.
Acta Physiol Scand. 124:195-200. 1985

47 Lai Y.M., Lee J.F., Huang H.Y., Soong Y.K., Yang F.P., Pao C.C.
The effect of human papillomavirus infection on sperm cell motility.
Fertil. Steril. 67(6):1152-5. 1997

48 Lanfranco F., Kamischke A., Zitzmann M., Nieschlag E.
Klinefelter's syndrome.
Lancet. 364(9430):273-83. 2004

49 Lewis S.E., Boyle P.M., McKinney K.A., Young I.S., Thompson W.
Total antioxidant capacity of seminal plasma is different in fertile and infertile men.
Fertil. Steril. 64(4):868-70, 1995

50 Mahmoud AM, Geslevich J, Kint J, Depuydt C, Huysse L, Zolata A, et al.
Seminal plasma alpha glucosidase activity and male infertility.
Hum. Reprod. 13(3):591–5. 1998

51 Marmar J.L..
Semen zinc levels in infertile and postvasectomy patients and patients with prostatitis.
Fertil Steril 26:1057–63. 1975

52 McKinney K.A., Lewis S.E., Thompson W.
Persistent effects of pentoxifylline on human sperm motility, after drug removal, in normozoospermic and asthenozoospermic individuals.
Andrologia. 26(4):235-40, 1994

53 McKinney K.A., Lewis S.E., Thompson W.
Reactive oxygen species generation in human sperm: luminol and lucigenin chemiluminescence probes.
Arch. Androl. 36(2):119-25, 1996

54 Milingos S., Comhaire F., Liapi A., Aravantinos D.
The value of semen characteristics and tests of sperm function in selecting couples for intra-uterine insemination.
Eur. J. Obstet. Gyn. Reprod. Biol. 64: 115–18. 1996

55 Nagy Z.P., Liu J., Joris H., Verheyen G., Tournaye H., Camus M., Derde M.C., Devroey P., van Steirteghem A.C.
The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters.
Hum. Reprod. 10: 1123 – 29. 1995

56 Nassar A., Morshedi M., Mahony M., Srisombut C., Lin MH., Oehninger S.
Pentoxifylline stimulates various sperm motion parameters and cervical mucus penetrability in patients with asthenozoospermia. Andrologia. 31(1):9-15, 1999

57 Netter A, Hartoma R, Nahoul K.
Effect of zinc administration on plasma testosterone, dihydrotestosterone and sperm count. Arch. Androl. 1981;7:69–73.

58 Nieschlag E. Lerchl A.
Declining sperm counts in European men--fact or fiction?
Andrologia. 28(6):305-06. 1996

59 Novero V., Camus M., Tournaye H., Smitz J., Verheye G., Joris H., Derde M.P., van Steirteghem A.,
Relationship between serum follicle stimulating hormone in the male and standard sperm parameters, and the results of intracytoplasmic sperm injection.
Hum Reprod 12: 59 – 63. 1997

60 Ochsendorf F.R.
Infections of the male genital tract and reactive oxygen species.
Hum. Reprod. Update 5: 399 – 420. 2000

61 Oehninger S., Blackmore P., Mahony M.,Hodgen G.
Effects of hydrogen peroxide on human spermatozoa
J. Assist. Reprod. Genet. 12(1):41.1995

62

Okada H., Tatsumi N., Kanzaki M., Fujisawa M., Arakawa S., Kamidono S. Formation of reactive oxygen species by spermatozoa from asthenospermic patients: response to treatment with pentoxifylline.
J. Urol. 157(6):2140-6. 1997

63 Painter F.M.
Male infertility: Nutritional and environmental considerations
Alt. Med. Rev. 5(1): 23-38. 2000

64 Palermo GD, Colombero LT, Schattman GL, Davis OK, Rozenwaks Z.
Evolution of pregnancies and initial follow-up of newborn delivered after intracytoplasmic sperm injection.
JAMA 276:1893-97. 1996

65 Papp Gy. szerk. : Az andrológia tankönyve
Medicina – Budapest 2001.

66 Pauer H.U., Hinney B., Michelman H.W., Krasemann E.W., Zoll B., Engel W.
Relevance of genetic counselling in couples prior to intracytoplasmic sperm injection.
Hum. Reprod. 12(9):1909-12. 1997

67 Prasad A.S.
Zinc status and serum testosterone levels of healthy adults..
Nutrition, 12(5):344-48. 1996

68 Punab M., Loivukene K., Kermes K., Mändar R.
The limit of leucocytospermia from the microbiological viewpoint.
Andrologia 35: 271 – 78. 2003

69 Razavi S., Nasr-Esfahani M.H., Mardani M., Mafi A., Moghdam A.
Effect of human sperm chromatin anomalies on fertilization outcome post-ICSI.
Andrologia 35: 238 – 43. 2003

70 Reichart M., Levi H., Kahane I., Bartoov B.
Dual energy metabolism-dependent effect of Ureaplasma urealyticum infection on sperm activity.
J. Androl. 22(3):404.2001

71 Reinhardt A., Haidl G., Schill W.B.
Granulocyte elastase indicates silent male genital tract inflammation and appropriate anti-inflammatory treatment.
Andrologia 29: 187 – 92. 1997

72 Roberts K.P.
Y-chromosome deletions and male infertility: State of the Art and Clinical Implications.
J. Androl. 19:255-59. 1998

73 Rolf C., Cooper T.G., Yeung C.H., Nieschlag E.
Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study.
Hum. Reprod. 14(4):1028-33, 1999

74 Roth E.
Oxygen free radicals and their clinical implication.
Acta Chir. Hung. 36(1-4):302.1997

75 Rowe P.J., Comhaire F.H., Hargreave T.B., Mahmoud A.M.
WHO manual for the standardized investigation, diagnosis and management of the infertile male.
Cambridge University Press, Cambridge, UK 2000

76
Salumets A., Suikkari A.M., Möls T., Söderström-Anttila V., Tuuri T.
Influence of oocytes ans spermatozoa on early embryonic development.
Fertil. Steril. 78: 1082 – 87. 2002

77 Sharma R.K., Pasqualotto A.E., Nelson D.R., Thomas A.J., Jr. Agarwal A.
Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic.
J. Androl. 22(4):575-83. 2001

78 Sharpe R.M.
On the importance of being earnest.
Decline in semen quality among fertile men in Paris during the past 20 years.
Hum. Exp. Toxicol. 14(5):463-64. 1995

79 Sikka S.C.
Relative impact of oxidative stress on male reproductive function.
Curr.Med.Chem. 8(7):851.2001

80 Simoni M., Bakker E., Krausz C.
EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004.
Int. J. Androl. 27(4):240-9. 2004

81 Stuppia L., Antonucci I., Binni F., Brandi A., Grifone N., Colosimo A.
Screening of mutations in the CFTR gene in 1195 couples entering assisted reproduction technique programs
Eur. J. Hum. Gen. 13: 959–64. 2005

82 Tebourbi L.. Testart J.. Cerutti I.. Moussu J.P., Loeuillet A., Courtot A.M.
Failure to infect embryos after virus injection in mouse zygotes.
Hum. Reprod. 17(3):760-4. 2002

83 Török L., Szőke I., Sinkó I., Nagy E., Pajor L.
Anaerob baktériumok hatása a spermiumok motilitására.
M.Androl. 6 (3): 89. 2001

84 Tuerlings J.H., Kremer J., Meuleman E.
The practical Application of Genetics in the male infertility Clinic.
J. Androl. 18(6): 576-81. 1997

85
Tzetis M, Efthymiadou A, Strofalis S, Psychou P, Dimakou A, Pouliou E, Doudounakis S, Kanavakis E.
CFTR gene mutations--including three novel nucleotide substitutions--and haplotype background in patients with asthma, disseminated bronchiectasis and chronic obstructive pulmonary disease.
Hum. Genet. 108(3):216-21. 2001

86 Van Waart J., Kruger T.F., Lombard C.J., Ombelet W.
Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review.
Hum. Reprod. Update 7: 495 – 500. 2001

87 Vandekerckhove P., Lilford R., Vail A., Hughes E.
Kinin-enhancing drugs for unexplained subfertility in men.
Cochrane Database of Systematic Reviews. (2):CD000153, 2000.

88 Vicari E., de Palma A., Burrello N., Longo G., Grazioso C., Barone N., Zahi M., D'Agata R., Calogero A.E.
Absolute polymorphic teratozoospermia in patients with oligo-asthenozoospermia is associated with an elevated sperm aneuploidy rate.
J. Androl. 24: 598 – 603. 2003

89 Vicari E., La Vignera S., Calogero A.E.
Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds.
Fertil. Steril. 78(6):1203-8, 2002

90 von der Kammer H., Scheit K.H., Weidner W., Cooper T.G.
The evaluation of markers of prostatic function.
Urol. Res. 19: 343–47. 1991

91 Weidner W., Krause W., Ludwig M.
Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis.
Hum Reprod Update 5: 421 – 32. 1999

92 Wong W.Y., Merkus H.M., Thomas C.M., Menkveld R., Zielhuis G.A., Steegers-Theunissen R.P.
Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial.
Fertil. Steril. 77(3):491-8, 2002

93 Yeung C.H., Cooper T.G., Senge T.
Histochemical localization and quantification of a-glucosidase in the epididymis of men and laboratory animals.
Biol. Reprod. 42: 669–76. 1990

94 Yousef M.I., Abdallah G.A., Kamel K.I.
Effect of ascorbic acid and Vitamin E supplementation on semen quality and biochemical parameters of male rabbits.
Anim. Reprod. Sc. 76(1-2):99-111, 2003

95 Závaczki Z., Szöllösi J., Kiss A.S., Koloszár S., Fejes I., Kovács L., Pál A. Magnesium-orothate supplementation for idiopathis infertile male patients: A randomized, placebo controled clinical pilot study Magn. Res. 16(2): 131-36. 2003

96 Zorn B., Virant-Klun I., Vidmar G., Sesek-Briski A., Kolbezen M., Meden-Vrtovec H. Seminal elastase-inhibitor complex, a marker of genital tract inflammation, and negative IVF outcome measures: role for silent inflammation?
Int. J. Androl. 27: 368 – 74. 2204

COPIES OF THE PUBLICATIONS RELATED TO THE THESIS