The role of the pathologist in management of male infertility

PhD thesis

Dr. Lellei Ilona

National Medical Center

Department of Pathology

Budapest

2005

Table of contents

List of publications

Publications connected to the thesis

I. Erdei E, Magyar É, Lellei I, Rózsahegyi J, Laki A, Rusz A, Karsza A: Herebiopszia jelentősége az asszisztált reprodukció szempontjából. Magyar Andrológia, 3, 99-103, 1999

II. Erdei E, Magyar É, Klauber J, Rózsahegyi J, Rusz A, Lellei I, Drávucz S, Seager: Az elektro-ejakuláció szerepe a gerincvelő sérültek fertilitási zavarainak kezelésében. Rehabilitáció, 9, 72-74, 1999

III. Erdei E, Magyar É, Lellei I, Rózsahegyi J, Laki A, Rusz A, Papp Gy: Testicular biopsy helping assisted reproduction. Acta Chirurgica Hungarica, 38, 279-287, 2000

IV. Lellei I, Erdei E, Magyar É: A herebiopszia jelentősége férfiak meddőségében. LAM, 10, 56-61, 2000

V. Lellei I, Magyar É, Erdei E: Histological evaluation of multiple testicular biopsies. Pathology, Research and Practice 197, 727-733, 2001

VI. Erdei E, Lellei I, Laki A, Papp G, Tóth, Rózsahegyi J, Magyar É, North MO, Tritto G: Andrology in the 21th century. Proceedings of the VIIth International Congress of Andrology. Eds: B Robaire, H Chemes, C Morales. Medimond Publications

VII. Erdei E, Laki A, Lellei I, Tóth A, Rózsahegyi J, Magyar É: Mikrosebészeti módszerrel operált kétoldali varicocelék hatása a férfimeddőségre. Magyar Andrológia, 6, 69-73, 2001

VIII. North MO, Lellei I, Rives N, Erdei E, Dittmar A, Barbet JP, Tritto G: Reversible meiotic abnormalities in azoospermic men with bilateral varicocele after microsurgical correction. Cell Mol Biol noisy – le - grand, 50, 281-289, 2004

IX. North MO, Lellei I, Erdei E, Barbet JP, Tritto G: Meiotic studies of infertile men in case of nonobstructive azoospermia with normal karyotype and no microdeleted Y chromosome precize the clinical couple management. Ann Genet, 47, 113-123, 2004

X. Lellei I, Magyar É, Erdei E: A patológus szerepe a férfi terméketlenség diagnosztikájában. Magyar Andrológia, 8, 95-97, 2003

XI. Lellei I, Magyar É, Erdei E: Varicocele hatása a here csírasejtképzésére. Magyar Andrológia, 9, 13-16, 2004

Abstracts connected to the thesis

Magyar É, Erdei E, Lellei I: The importance of testicular biopsy in view of assisted reproduction. European Congress of Pathology, Barcelona, 1999

Lellei I, Erdei E, Magyar É: Testicular biopsies helping assisted reproduction European Congress of Andrology, L'Aquila, 2000

Magyar É, Erdei E, Lellei I: Importance of multiple testicular biopsy in azoospermia in view of assisted reproduction XXIII. International Congress of IAP, Nagoya, 2000

Erdei E, Lellei I, Laki A, Papp Gy, Rózsahegyi J, Magyar É, Tritto G: Value of testicular biopsy due to the microsurgical correction of bilateral varicocele

associated with azoospermia. VIIth International Congress of Andrology, Montreal, Canada, 2001

Magyar É, Erdei E, Lellei I: Importance of multiple testicular biopsies in severe oligozoospermia in view of assisted reproduction. European Congress of Pathology, Berlin, 2001

Magyar É, Lellei I, Erdei E: Correlation between varicocele and homogenity of spermatogenesis. International Congress of IAP, Amsterdam, 2002

Magyar É, Erdei E, Lellei I: Spermatogenetic function of the testis in patients of different varicocele grades. European Congress of Pathology, Ljubljana, 2003

Magyar É, Tóth A, Hegedűs Z, Erdei E, Lellei I: Histologically verified spermatogenesis in an XX man despite deletion of AZFa, b and c. International Congress of IAP, Brisbane, 2004

Other publications

Kiss F, Csaba A, Lellei I, Csellár M, Szerdahelyi A: Gyors képi dokumentáció műtéti preparátumok metszéslapjáról. LAM 4, 462-463, 1994

Magyar É, Lellei I: Histological appearences of granulosa cells in different stages of ovarian follicles. CME Journal of Gynecologic Oncology 1. 121-122, 1996

Tóth J, Cserni G, Kálmán E, Kulka J, Lellei I, Mágori A, Orosz Zs, Péter I, Tóth V, Vass L: Az emlőrák patológiai feldolgozása és kórszövettani leletezése. Magyar Onkológia, 44, 14-16, 2000

Radácsi A, Lévay Gy, Lellei I, Truszka F, Nyakas Cs, Góth M, Szabolcs I: Histochemical detection of 8-oxodeoxyguanosine (DNA adduct) in thyroid cancer tissue. Cytometry, 46, 211, 2001

Kovács G, Lellei I., Gonda G., Szabolcs I: Pajzsmirigy microcarcinoma. Orvosképzés 3, 184-188, 2001

Bauer Z, Simon, Görömbey Z, Vörös A, Lellei I: Hypertoniás krízis hátterében álló phaeochromocytoma. Gyermekgyógyászat 53, 475-8, 2002

Abbreviations

OATS	oligo-astheno-teratozoospermia
ATS	astheno-teratozoospermia
TESE	testicular sperm extraction
MESA	microepididymal sperm aspiration
PESA	percutan epididymal sperm aspiration
IVF	in vitro fertilization
ICSI	intracytoplasmic sperm injection
ART	assisted reproduction technique
HE	haematoxylin eosin

Introduction

General aspects and history

According to demographic data the population of Europe is decreasing. The international data of the Hungarian Central Statistical Office show that this tendency can be observed in well-developed Western European countries as Luxembourg, the Netherlands or France and in the countries of the Eastern European region as Russia, Bulgaria, Romania and Hungary either. The "greying" of the population leads to economic and social problems, which are striking in Hungary as well.

One of the reasons of the population reduction is that the number of spontaneous births has fallen back. There are more and more childless marriages due to fertility problems. In the early time of investigations of infertility primarily the female partner was examined and treated. Recently it is well known that in 30% of the infertile marriages the male partner is alone responsible for the infertility, in 30% exclusively the female and in 40% both of them. [4]. The amount of viable spermatozoa in the ejaculate of the normal male population shows a tendency to decrease.

The two main etiologic problems that lead to male infertility are obstruction of the seminal vasa and spermatogenetic failure. Both categories can be

divided in more subgroups. By obstruction the spermatogenesis of the testes is normal but seminal vasa are blocked and the ejaculate contains no spermia at all. The reason of obstruction can be:

- developmental anomaly
- cystic fibrosis
- previous inflammation.

By spermatogenetic failure ejaculate contains no spermia because they are not produced by the testis. The cause of that may be:

- genetical (Klinefelter's syndrome, deletion of chromosome Y) [2,11]
- endocrinological (diseases of the hypothalamo-hypophyseal system or the adrenals)
- vascular (oxygen supply of testis is insufficient, for example torsion of the testis or varicocele) [17,37]
- previous damage of germ cells (irradiation or chemotherapy in clinical history)
- occupational (vibration damage or toxic agents at work)

Any of the problems listed above may result azoospermia but the treatment is completely different. There are therefore important reasons to investigate the causes of male infertility.

Varicocele is one of the most common reasons of fertility problems in men [17]. The dilatation of the veins of the plexus pampiniformis was described first by Ambroise Pare in 1541. For the pathogenesis of the disease there are several theories (insufficiency of the valves in the internal spermatic veins, different inflow angle of the right and left testicular vein, anastomoses between the left and right testicular veins). The first surgical correction methods of varicocele originate from the beginning of the last century. More recently (in the 1970's) microsurgical methods were developed [18, 19]. Another method of interventive treatment is angiographic embolisation or sclerotisation of the spermatic vein. According to the literature varicocele repair may improve semen quality [7].

Development of the advanced methods of assisted reproduction helps more and more previously infertile couple to achieve pregnancy. According to 2002 data 1,5% of the babies born in Hungary was conceived by using of ART (assisted reproduction technique). The methods available for that are those of homologous or heterologous insemination, in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) [1, 25, 27, 33].

ICSI was developed in 1992 by Palermo and since 1994 it is successfully performed in Hungary as well [25]. Viable spermatozoa or spermatides can be used for these techniques not only from the ejaculate but also from the epididymis (microepididymal sperm aspiration-MESA, percutan sperm aspiration-PESA) or testis (testicular sperm extraction-TESE) as well [1, 28, 29, 33]. These time and money consuming techniques might only be used by cases when they are really needed. By azoospermic individuals first evidence of spermatogenesis should be proven in the testis [3]. The only way for this is histological evaluation of testicular biopsies [30, 32]. Testicular biopsy is also essential to elucidate the etiology of infertility (obstructive or testicular failure) and to detect the amount of normal spermatozoa produced in the testis [12, 15, 28, 33, 34, 40]. Evaluating testicular biopsies in the treatment of male infertility began in the late 1930's. Charny [6] and Hotchkiss [16] carried out the first testicular biopsies. They published the first papers on this topic between 1940-42. The technique of the operation to gain biopsy specimen and its histological evaluation has made a great evolution since then. Double bilateral testicular biopsy was introduced using microsurgical method [10, 14]. For histological report the Johnsen score has been developed.

Management of infertile male patients

Management of infertile couples requires multidisciplinary teamwork [4, 22]. The team includes andrologist, radiologist, genetist, gynaecologist and pathologist. The first step is a complex examination of both male and female

partner to evaluate the cause of infertility and in order to choose further diagnostic and therapeutic procedures.

At first a complex andrological examination is carried out by childless men. Physical examination detects testicular volume and turgor, evidence of any palpable alteration of testis, epididymis or vasa (for example varicocele) [13]. Presence of focal changes and obstructions are diagnosed by testicular ultrasound. Color Doppler is used to grade varicocele [9], which is one of the main reasons of male infertility [26].

Semen is taken for morphological examinations. Quantity and quality (viscosity, concentration, and pH) is checked. Number of viable spermia is counted. Morphological changes of sperm cells (head, body, tail) are detected light- and electron microscopically [20]. Rate of moving spermia is recorded at start, after one and two hours. Movement abnormalities are also detected by the light microscope.

Blood is taken for clinical pathological, molecular biological and genetical examinations. Hormone levels (FSH, LH, PRL, testosterone) should be checked, too. Molecular biological examinations are used to detect microdeletions of chromosome Y (AZF a, AZF b, AZF c, SRY, ZFY) [31]. Presence or absence of Barr body and karyotype is examined by genetist.

Having evaluated the results of first step examinations, further diagnostic and therapeutic plan is to be made.

The treatment can be surgical, conservative-including hormonal, or using of an ART method, which also might need surgery for sperm retrieval. Among of operational possibilities reconstruction of seminal vasa, varicocelectomy and orchidopexy can be listed. Conservative treatment includes cure of possible infections (antibiotics, nonsteroid antiinflammatory drugs), using of callicrein, pentoxyphillin, mast cell blockers or vitamins. Applying of hormonal treatment (androgens or gonadotropins) is helpful in azoospermia caused by endocrine disorders. Assisted reproduction techniques and sperm retrieval techniques are listed above.

Aim of the study

The aim of this study is to describe how histopathologist can participate in this multidisciplinary teamwork.

Concerning the etiology

- What kind of additional information can histology give about the etiology of infertility, especially of azoospermic cases?
- Are these changes irreversible?

Concerning the homogeneity

- Are there any focal differences of spermatogenesis?
- Which location shows best-preserved spermatogenesis?

Concerning the treatment

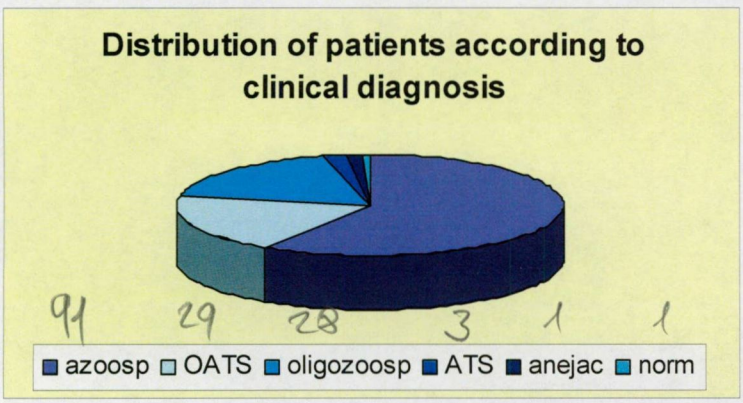
- What could be the next therapeutic step to choose?
- Can hormonal treatment or varicocelectomy improve spermatogenesis at this patient?
- Is there any chance of successful sperm retrieval?
- Which method of sperm retrieval and assisted reproduction is to be used?
- Which is the optimal location of later therapeutic biopsy?

Materials and methods

Patients

Multiple testicular biopsies of 154 young men were evaluated histologically [10]. By 143 of them bilateral double biopsy was carried out according to our protocol seen below. In four cases one of the four biopsy samples was insufficient, only three could be evaluated. Seven patients had only 2 biopsies from the same testis. By four of them the other side was not operated because of severe atrophy. By two other cases one testis was removed (seminoma or embryonal carcinoma in clinical history). The remaining one individual had congenital aplasia on the left side. Four of the 154 patients had more than 4 biopsies (one 6, three 5). By one person the andrologist decided to take 5 biopsies by the operation. By the other three one or two biopsies was proved insufficient histologically so rebiopsy was carried out from the same location. The distribution according to the clinical categories is summarized in table 1.

Table 1.



The majority of the patients examined were azoospermic (91). The mean age of this group was 31,8+/-5,86 years. In these cases the ejaculate contained no spermia at all. Severe oligozoospermia coexisted with morphological and movement abnormalities of the spermia: oligo-astheno-teratozoospermia (OATS). The spermium concentration of the ejaculate ranged 0,1-5 million/ml. In the material in question 29 men presented with OATS. The mean age of them was 32,7+/-6,7 years. In oligozoospermic patients the amount of spermatozoa was 5,1-20 million/ml. There were 28

oligozoospermic cases detected. The mean age of them was 29,71+/-6,48 years. Normozoospermia indicated by more than 20-million spermatozoa/ml ejaculate and only one normozoospermic patient was recorded. He was 19 years old. In three other cases the spermium concentrate of the ejaculate was normal, but morphological and movement abnormalities of the spermia were detected so they belonged to the astheno-teratozoospermic (ATS) group. Their age was 33,33+/-5 years. Two patients suffered of anejaculation, one of them had spine cord injury and the other had semicastration and chemotherapy in clinical history. Both of them were 34 years old.

Among the 154 individuals examined 61 presented coexisting varicocele. In 17 cases it was unilateral and in 44 bilateral. For grading of varicocele a four grade complex clinico-radiological scale is used which is summarized in table 2.

Table 2.

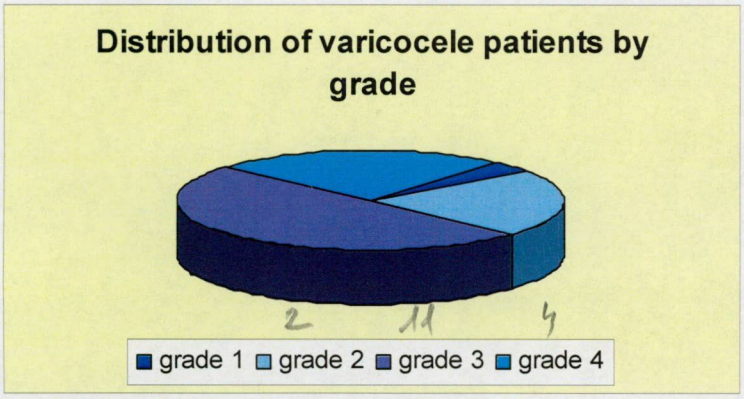
Grading of varicocele

Grade	Palpability	Valsalva	Doppler
1	Not palpable	+/-	+
2	Palpable only in standing position	+	+
3	Palpable in standing and lying position	+	+
4	Palpable in standing and lying position	-	+

All unilateral varicoceles were detected on the left side. The grade distribution was as follows (table 3.): two grade 2, 11 grade 3 and four grade 4. By all but one bilateral varicoceles right-sided symptoms were same or lower grade than left sided. The grade distribution of left side was as follows:

one grade 1, three grade 2, 33 grade 3 and 24 grade 4. On the right side three grade 1, 20 grade 2, 19 grade 3 and only two grade 4 cases were recorded.

Table 3.



$= \Sigma 17$ OK ✓

Technique of testicular biopsy

Bilateral testicular biopsy was carried out under general anaesthesia using an operation microscope Leica Wild (magnification 3-15x). Scrotal exploration was made with an incision in the line of the raphe. By the exploration the turgor and status of the testis and epididymis was checked. Two biopsies of 1 mm^3 using the atraumatic microsurgical method were taken from each testis (from the upper inner and lower outer part) [18]. For closing of the wounds 4-0 Monocryl was used.

Handling of specimens

Samples were fixed in Bouin's fixative or buffered formalin for 15 hours at room temperature. After paraffin embedding four sections of 3-4 µm were cut and stained by HE.

Histological evaluation

By reporting following data were given: [21,36]

1. The number of round shaped tubules present in the slides examined

2. The percentage of the tubules showing spermatogenesis (spermatogenetic activity)
3. The number of mature spermatozoa counted in 10 round shaped tubules.
4. Thickness of tubular wall. The severity of atrophy was evaluated with a qualitative method based on the thickening of the tubular walls. A three-grade scale (mild-moderate-marked) was used. The presence of completely hyalinised tubules always indicated a marked atrophy.
5. Pathological changes in the interstitium (blood vessels, Leydig cells etc.)
6. Desquamation of the germinal epithelium
7. Evidence of maturation arrest was described. The type and stage of maturation arrest (early or late, complete or incomplete) was also recorded

Statistical evaluation

Examining for histological distribution the grouping variable was the clinical diagnosis. From the number of mature spermia counted in 10 round shaped tubules average, standard deviation and variation coefficient, level of significance (one way ANOVA) were calculated.

The 61 varicocelic cases were also evaluated according to the varicocele. In this context the grouping variable was the grade of the varicocele. Left- and right-sided cases were not separated. Spermatozoa numbers of one testis (two biopsy samples) were summarized, frequency tables and histograms were created. Correlation of histological findings and grade of varicocele was also investigated. Another examination was made to compare spermatogenetic activity of uni- and bilateral varicocelic cases with each other and nonvaricocelic control cases (five obstructive azoospermic patients). For this reason a percent difference from the spermatozoa numbers counted in 10 round shaped tubules for each testis was calculated using the following formula: (a-b)/ (a+b)x100. Values a and b are spermatozoa numbers counted in 10 round shaped tubules in the upper inner and lower outer quadrant of the same testis. From this percent difference average and standard deviation were calculated.

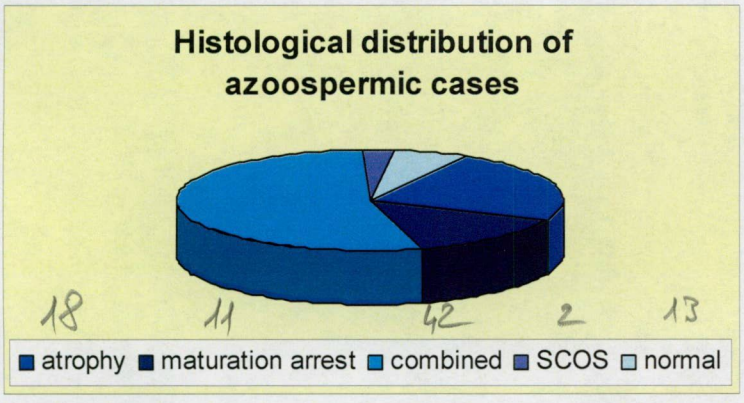
Results

Six patients were excluded from the study because of the poor quality of at least three biopsies. One of them suffered of anejaculation the other five were azoospermic. To evaluate the inhomogeneity among the clinical diagnosis groups, level of significance was calculated. P value was lower than 0,001 which showed the mean of spermatozoa numbers were significantly different among the groups.

Etiological distribution of the different patient groups

Histological distribution is summarized in table 4.

Table 4.



86

Nearly the half (42 patients) of the 86 **azoospermic** person showed a combination of maturation arrest and atrophy on histology. 18 patients presented only atrophy (figure 1) while 11 exclusively maturation arrest (figure 2, 3). A rare histological condition, Sertoli Cell Only syndrome (figure 4) was found at two cases. Normal spermatogenesis (figure 5) with no pathological changes was detected in 13 patients.

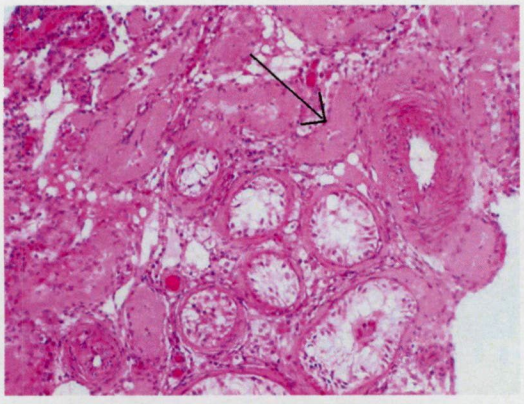


Figure 1. : Marked atrophy. The tubular walls are thickened, some tubules are completely hyalinised (arrow). The number of germ cells is decreased. HE, 50x

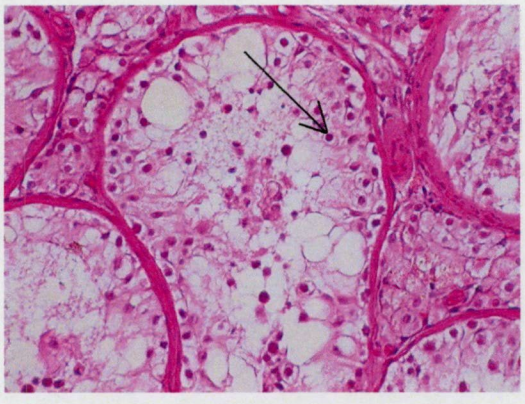


Figure 2. Early maturation arrest. The hypocellular tubules show normal wall thickness. Among the Sertoli cells primer spermatocytes (arrow) are present, there is no evidence of later stage germ cells. HE, 120x

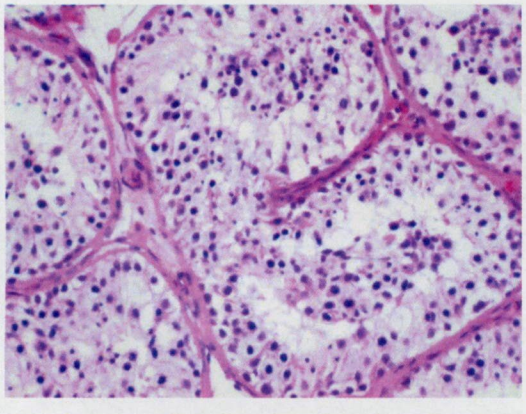


Figure 3. Late maturation arrest. The tubules are more cellular. Spermatogenesis is blocked in stage of round spermatids. No mature spermia are seen. HE, 120x

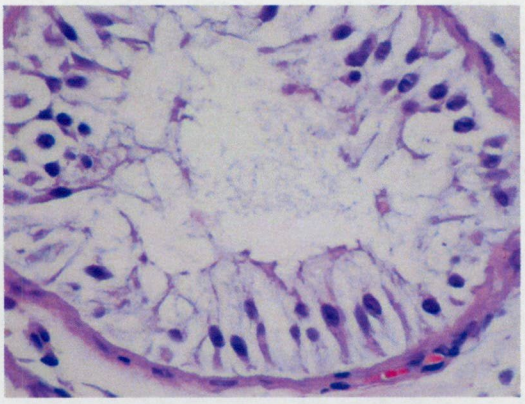


Figure 4.SCOS. The wall thickness is normal; the tubules are lined by Sertoli cells. There is no evidence of germ cells at all. HE, 300x

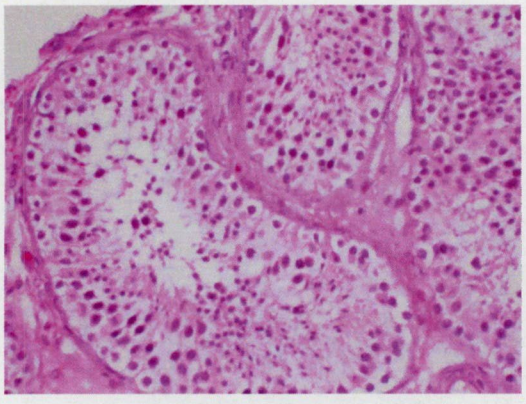
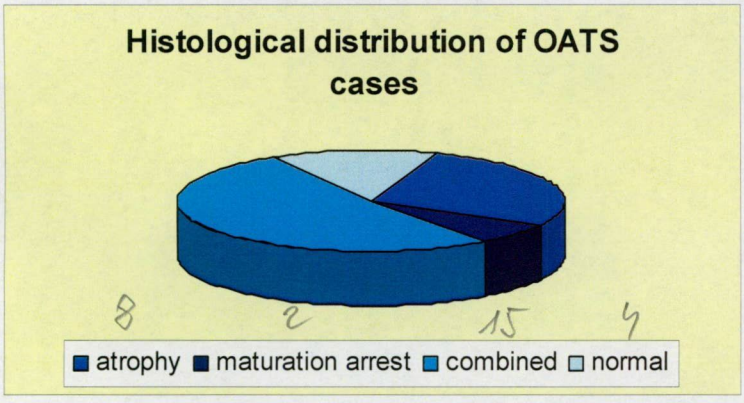


Figure 5. Preserved spermatogenesis. In thin-walled tubules normal amount of precursors and mature spermia is seen in regular arrangement. HE, 120x.

Etiology according to histological appearance of **OATS** patients is shown in table 5.

Table 5.

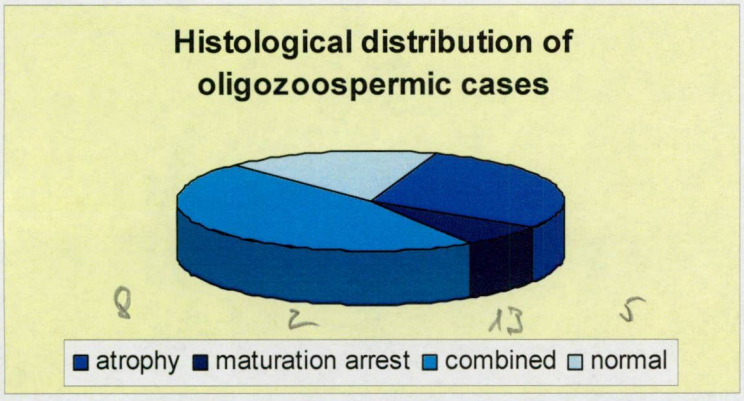


29

In this group atrophy combined with maturation arrest was found in 15 men, 8 patients presented only atrophy. Pure maturation arrest was detected in two cases while preserved spermatogenesis in four.

Histological distribution of **oligozoospermic** group is summarized in table 6.

Table 6.



28

The majority of the group (13) also showed the combination of atrophy and maturation arrest. Pure atrophy was detected in eight patients. Only maturation arrest was present in the biopsies of two cases. The remaining five men showed normal spermatogenesis.

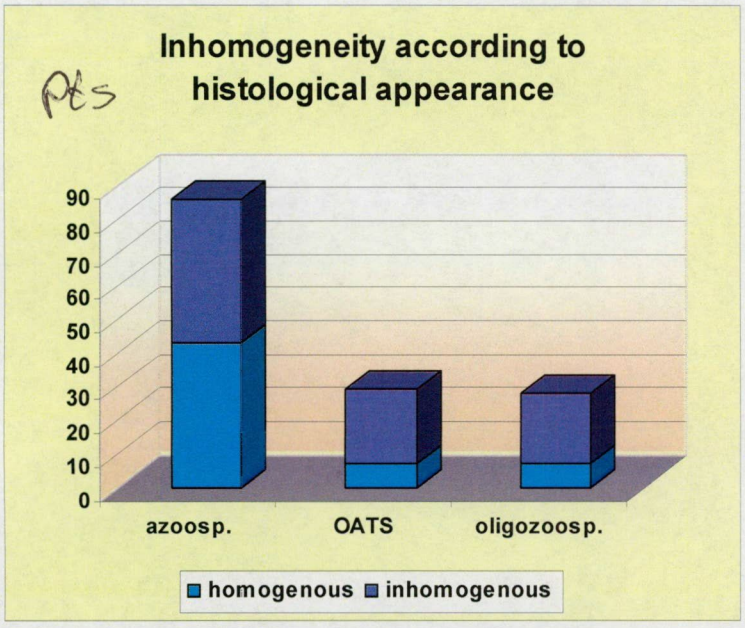
Two of the **ATS** patients presented preserved spermatogenesis, the third developed atrophic changes. The only man suffering of **anejaculation** showed a combination of atrophy and maturation arrest. In the testis of the single **normozoospermic** patient preserved spermatogenesis was detected.

The question concerning inhomogeneity

To prove the usefulness of multiple biopsy method inhomogeneity of appearance of the different location biopsy samples was examined using more methods.

First **etiological homogeneity** was checked - do different samples of the same patient show the same histological alteration (maturation arrest, atrophy, combination, SCOS or normal) or not. Results are shown in table 7.

Table 7.



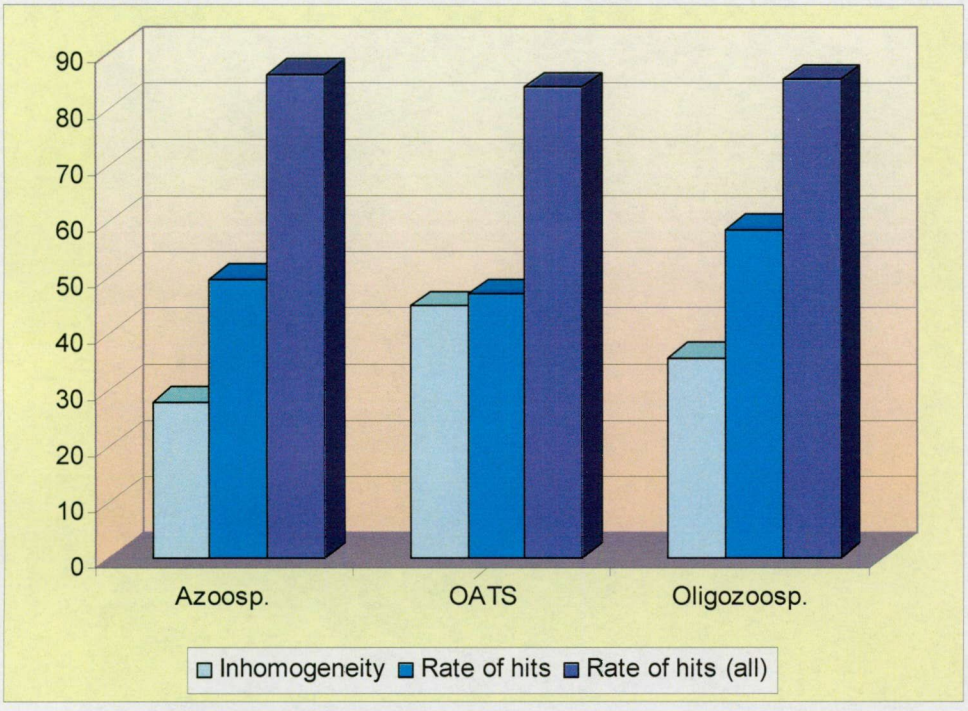
43 of the 86 azoospermic patients presented etiological homogeneity while 43 not. In the OATS group 22 of the 29 patients showed inhomogeneity and in seven cases the etiology was same in all of the examined samples. By the oligozoospermic individuals 21 was proved etiologically inhomogeneous while seven ones of the 28 showed homogeneity. Among the three ATS cases two homogenous and one inhomogeneous was detected. The only anejaculatory patient showed etiological inhomogeneity while the one normal not.

It was examined in which cases histology proved inhomogeneous etiology using the first method. The results were the following: All SCOS, most of the marked atrophy and some of the cases showing preserved spermatogenesis had homogenous histological appearance. Most maturation arrest and mild atrophy cases presented inhomogeneous occurrence.

The second method for testing inhomogeneity is summarized in table 8.

Table 8.

Inhomogeneity of spermatogenetic activity



The number of biopsies containing mature spermia was given in relation to the total number of biopsies of the patient. Homogeneity ment either there was no evidence of spermatozoa in any of the samples or all of the biopsies contained spermia. If only some of the biopsies showed evidence of spermatozoa the case was interpreted as inhomogeneous. The percentage of inhomogeneous cases of the particular spermatogram group is shown in the first column. The second column gives the chance of „hits" looking all of the patients of the group. If the examined single biopsy showed the same result as the examination of all of the biopsies of the same patient (e.g. there was evidence or absence of spermatogenesis in both), it was interpreted as a „hit". On the third column the chance of „hits" of the inhomogeneous cases (subgroup) of the group is presented, the homogenous patients were excluded. In the azoospermic group by 72 persons four samples were evaluated. In four cases only two, in two cases three, in further two cases five and in one case six biopsies were taken. 33 men had no evidence of spermia in any of his

samples. All biopsies of 29 patients contained mature spermatozoa. 33+29=62 patients presented homogenous distribution. In six cases only one, in 13 cases two, in four cases three of the samples showed evidence of spermia. By one azoospermic patient six biopsies were taken and five of six contained spermatozoa. In the azoospermic group 6+13+4+1=24 cases presented inhomogeneous distribution. The chance of hits in the entire group was 86% while in the inhomogeneous subgroup it was only 49,7%

In the OATS group a single patient had no spermia in any samples while 15 presented spermia in all of the biopsies so 16 men was found homogenous. Four patients showed spermatozoa in one, seven in two and two in three samples. The inhomogeneous group consisted of 13 people. Taking the whole group an 83,6% chance of „hits" was detected while in the inhomogeneous subgroup the chance was only 47%.

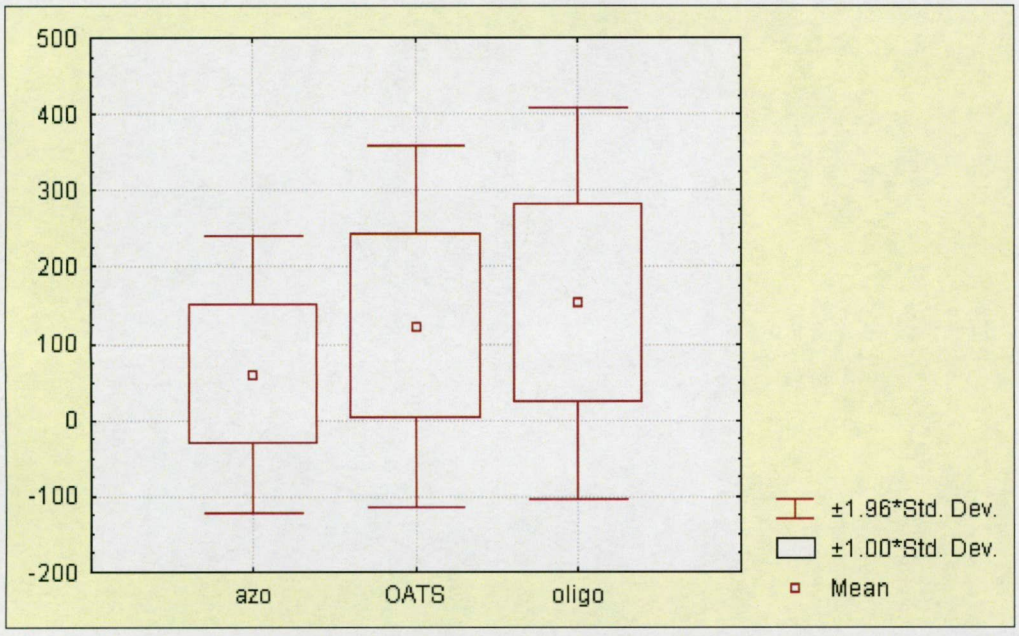
Every oligozoospermic patient showed spermatogenesis at least in one sample. The homogenous group consisted thus of 18 men individuals who presented mature spermia in all of the biopsies. One patient presented spermatozoa in one, four in two and four in three of his four samples. By one man five biopsies were taken and three of them contained spermia. In ten cases inhomogeneous distribution was detected. The chance of „hits" in the entire group was detected 85,2% while in the inhomogeneous subgroup in appeared 58,5%.

The anejaculatory patient had no spermia in his biopsies. The ATS and normozoospermic men showed evidence of spermatogenesis in all of the samples so chance of „hits" was 100%.

For third the inhomogeneity of spermium numbers counted in 10 round shaped tubules was examined using **statistical methods**. The data are summarized in table 9.

Table 9.

Average, range and standard deviation of spermatozoa numbers counted in 10 round shaped tubules in our patient groups



The ATS group consisted of 12 samples. Spermatozoa numbers ranged 59-626 with an average of 13,0+-/165,0. 376 biopsies from azoospermic patients were examined. In this group the average was only 59,4+/-92,3. Data ranged 0-512. The number of OATS biopsies was 121, Spermatozoa numbers in 10 round shaped tubules ranged 0-588 with an average of 122+/-120.4. The oligozoospermic group consisted of 112 samples. Data ranged 0-582 with an average of 152.7+/-130.5.

Effect of varicocele on spermatogenesis

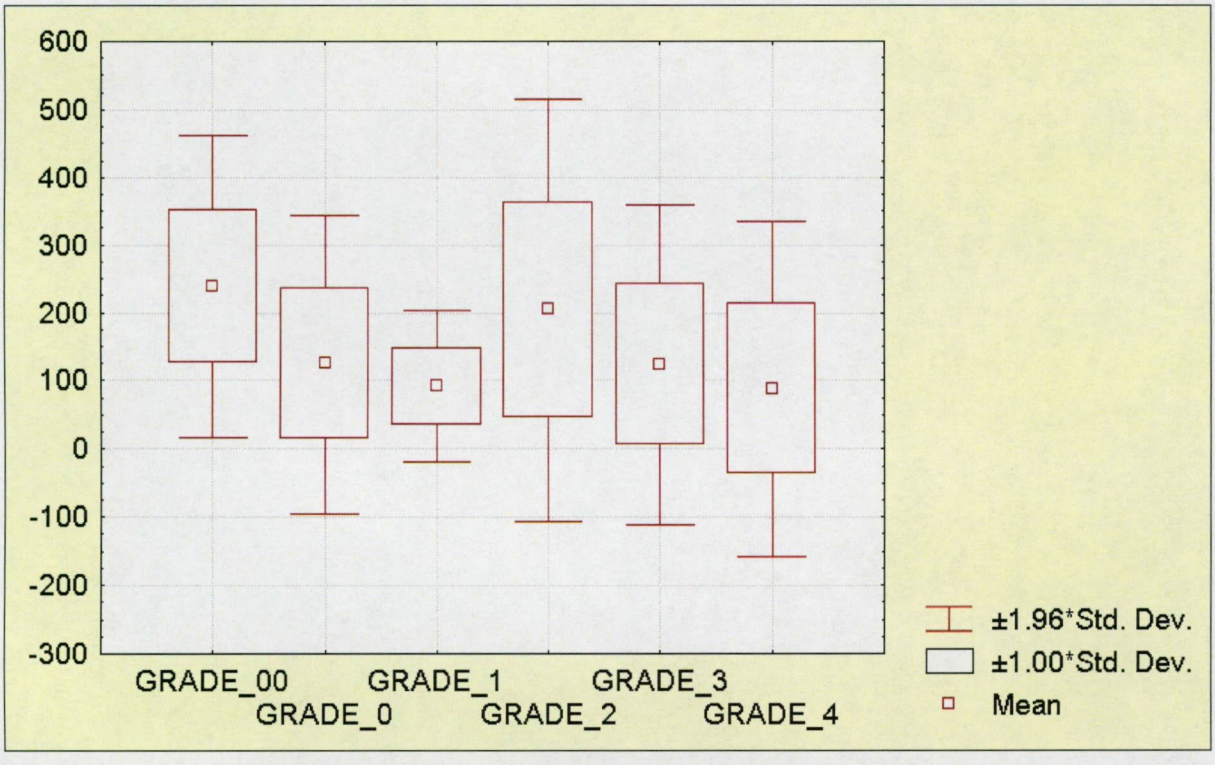
The spermatogenetic activity of the varicocelic cases was evaluated in correlation with the grade of the varicocele. By all of these men four biopsies were taken. A control group of five obstruction azoospermic patients was also examined. In all of their samples preserved spermatogenesis was detected. Spermatozoa numbers counted in 10 round shaped tubules were evaluated statistically.

The nonvaricocelic side of unilateral varicocele patients was compared to the control cases. In the 20 biopsies of the nonvaricocelic control group the average of spermatozoa numbers ranged 60-512 with an average of 239,4+/-114,09. In the contralateral side of the 17 unilateral varicocele cases spermatozoa count varied 0-464 with an average of 124,71+/-111,53.

The average and standard deviation values of different grades are summarized in table 10.

Table 10.

Average and standard deviation of spermatozoa numbers in correlation with varicocele grades.

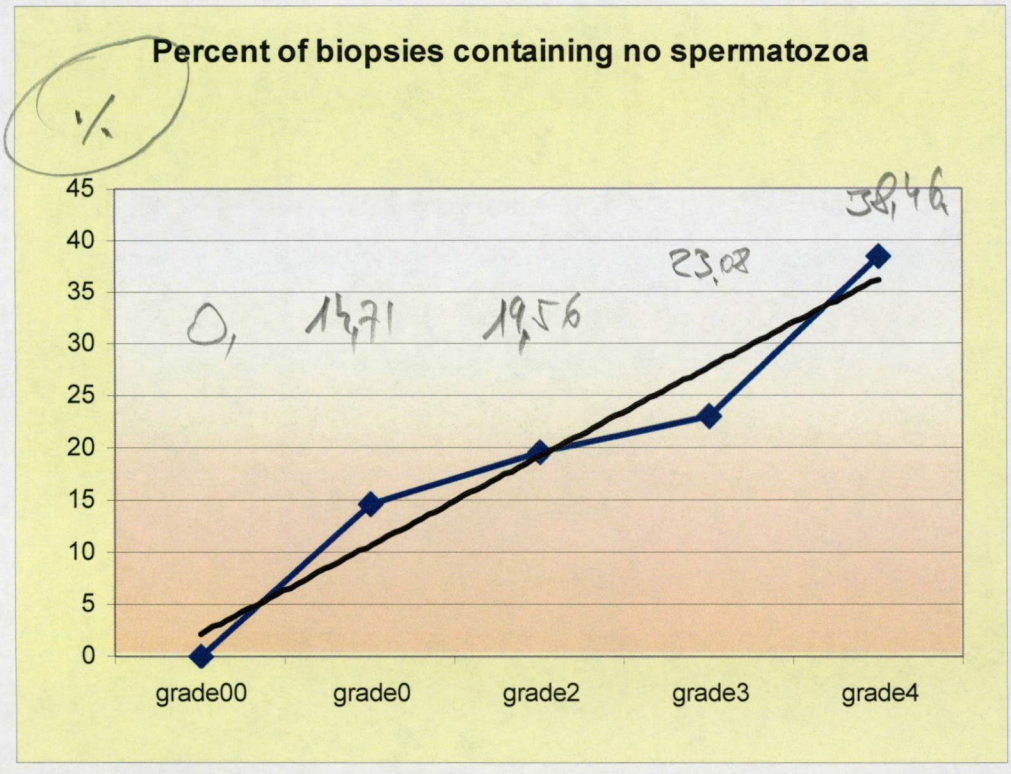


By grade 1 varicocele spermatozoa numbers ranged 0-179 with an average of 91,75+/-56,73. However, this data might not be realistic because of the low number of grade 1 cases (8). In the grade 2 group spermatozoa numbers ranged 0-626 with a surprisingly high average of 204,87+/-158,51. However, the standard deviation is the highest in this group. By grade 3 cases spermatozoa numbers ranged 0-525 with an average of 124,19+/-119,62.

Grade 4 group showed the lowest spermatozoa numbers (0-558, with an average of 88,75+/-125,29.

The percentage of biopsies containing no mature spermatozoa in the different grade groups is shown on table 11.

Table 11.

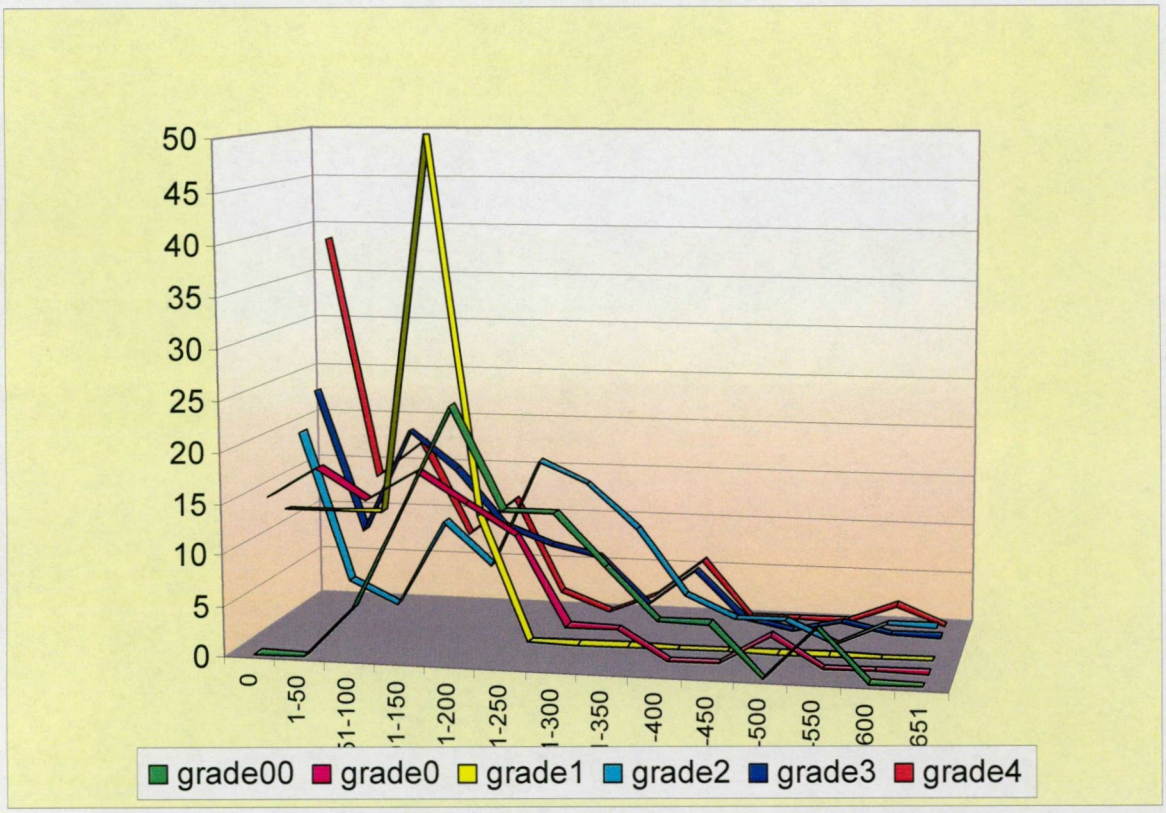


By grade 00 (control group) all samples showed evidence of spermatozoa. By grade 0 in 14,71%, by grade 2 in 19,56%, by grade 3 in 23,08%, by grade 4 in 38,46% of the cases no mature spermatozoa were detected.

The distribution of spermatozoa numbers was examined in every group; frequency tables and histograms were created. Data are summarized in table 12.

Table 12.

Histogram of different grade varicoceles.



Control group (grade 00) cases showed normal distribution with a peak of 150-200. Normal distribution (Gauss curve) is characteristic to a statistically homogenous group. There was evidence of spermatozoa in each biopsy sample. Unilateral varicocele cases (grade 0) also presented normal distribution but the peak was shifted to left (around 100) and in some samples no mature spermatozoa could be found. Grade 1 cases also showed a Gauss curve characteristic to normal distribution. The peak was between 100 and 150. In this group some biopsies showed no evidence of spermatogenesis. In categories grade 2, 3 and 4 histograms presented more than one peak, which represent a statistically inhomogeneous group. A second peak appeared at zero and the height of this peak showed a clear increase. In the grade 2 group two other peak are located at 100-150 and 200-250. The number of data is to low to decide if these are really two separated peaks or just one peak. By the

grade 3 cases this second peak was shifted to the left (100-150). In this group a small third peak appeared by 400. In the grade 4 category even more peaks were seen. The highest is represented by cases showing no evidence of spermatozoa (at 0), the others at 91-120, 211-240, 391-420, and 571-800, respectively.

The homogeneity of spermatogenesis was examined using statistical methods. A percent difference was calculated from the spermatozoa number counted in 10 round shaped tubules using the formula (a-b)/(a+b)x100. If the testis shows homogenous spermatogenesis, the two biopsies contain approximately same amount of mature spermia in 10 round shaped tubules, the difference a-b (the numerator) is a small number, the percent difference is close to zero. In an inhomogeneous case only one of the biopsies contains spermatozoa, the value of the percent difference is 100. These are the two extremities of the percent difference.

Percent differences were grouped by the grade of the varicocele, average and standard deviation were calculated. In the nonvaricocelic control group an average of 21,32+/-12,36 was found. The contralateral side of unilateral cases an average of 36,55+/-33,18 was seen. Grade 1 cases were excluded because of low case number. The percent difference of grade 2 varicoceles was surprisingly low (28,46+/-31,31). By grade 3 and 4 an average of 38,41+/-35,61 and 43,40+/-41,40, respectively.

Discussion

Histology gives detailed additional information to spermatogram. In the background of the clinically detected azoospermia, OATS or oligozoospermia different pathological entities can be encountered [34]. In some of them mature spermia are found in testicular tissue, in others not. The therapy of different alterations is also diverse. Histological evaluation of testicular biopsy can elucidate the etiology of male infertility and helps the andrologist to choose the appropriate way of treatment. Before irreversible histological alterations develop, ipsi- and contralateral varicocelectomy can improve semen quality [5, 7]. Some obstructions of the seminal vasa remained hidden on ultrasound. In these histologically detected cases reconstruction of the seminal vasa or TESE can be tried. Some of the maturation arrests may be treated by hormones [23]. Patients who show mature spermia or at least round spermatides in the testicular sample are candidates for TESE and IVF or ICSI [29, 35]. By others who have no spermia in any biopsy at all (like late stage atrophy or SCOS) there is no reason to apply the time- and money consuming techniques of ART [33].

Which method of ART is to choose? By most of the cases the andrologist may decide for ICSI but by some cases it is not recommended. Genetic disorders (Klinefelter's syndrome or microdeletions of chromosome Y) also lead to fertility problems. By some deletions of the region AZFc patients may show focal spermatogenesis. Their genetic abnormalities may be inherited by the children [8, 11, 24]. By the use of ICSI a single ovum is fertilized by a single spermium so the chance of natural competition is excluded. By genetic disorders the use of IVF is a better choice because of the presence of natural selection possibility.

The results have proved that the spermatogenetic function of testes shows an inhomogeneous distribution pattern [30]. The rate of inhomogeneity in the different histological groups is not the same. Sertoli cell only syndrome showed homogenous distribution in every case. The distribution of atrophy depends on the severity of the individual case. In mild atrophy, some

locations might show relatively normal histology while others marked thickening of the tubular walls with decreased spermatogenesis. In cases of marked atrophy histological appearance is more likely to be homogenous with a higher degree of tubular fibrosis and no evidence of germ cells. According to our results maturation arrest frequently shows an inhomogeneous distribution pattern. A hypothetical mechanism of pathological changes seen on microscopical examination might be the following:

- Any worsening in the microenvironment (oxygen or nutrition supply, increasing temperature etc) causes first focal damage of germ cells. (Germ cells are more vulnerable for these changes than Sertoli or Leydig cells). In this stage histology detects **inhomogeneous** pattern of mild atrophy and/or maturation arrest (mostly incomplete or late). These alterations are **reversible**, when cause is eliminated, spermatogenetic function recurs. No urgent sperm extraction and cryopreservation is necessary.
- At one stage changes become **irreversible**, germ cells got more and more destructed, the relative number of Sertoli and Leydig cells increases, tubular walls become thickened, lumina obstructed. On histology **homogenous** severe alterations can be seen. If focal spermatogenesis is still present, sperm extraction and cryopreservation is needed.

There is a relatively high number of normal histology in every patient group with mostly homogenous distribution pattern. These men have probably some obstruction of the seminal vasa, which remained hidden for the imaging techniques.

Statistical evaluation of spermatozoa count in 10 round shaped tubules revealed that spermatozoa count of the ejaculate showed correlation to the spermatozoa count in the biopsies. The lowest spermatozoa numbers (in average) was detected in the azoospermic group, the average of OATS, oligozoospermic and ATS biopsies were higher. The homogeneity of data is characterized by standard deviation. This value was highest in the ATS group.

This might be caused by the low data number (12). Standard deviation of oligozoospermic and OATS biopsies appeared lower. The lowest standard deviation (most homogenous) was detected in the azoospermic group.

Taking of multiple testicular biopsies gives more realistic information than single ones [3, 10, 14]. Some patients showed spermatogenesis only in one or two samples. Using the single biopsy method these potential candidates for TESE are missed. By evaluating four samples the number of cases treatable by ART can be increased. By comparing of the histological picture of the different samples the optimal location of the later therapeutic biopsy can be chosen.

Varicocele is one of the mean reasons of male infertility. By worsening of microcirculation the decreased oxygen supply and the disturbed thermoregulation leads to spermatogenetic failure [24, 39]. Data showed that milder varicocele usually causes only late maturation arrest or atrophy. The clinical follow-up proved that these changes might be reversible [5,7]. By some of the patients the three-month control examinations presented better semen quality [19]. High-grade varicocele cases are mostly characterized by marked atrophy (hyalinised tubules, Leydig cell hyperplasia) or early maturation arrest. These changes were often irreversible, later controls showed no clinical improvement [38]. The data suggest that varicocele should be operated the earlier the better.

In unilateral varicocele cases complicated with infertility by varicocelectomy double bilateral testicular biopsy was carried out. The comparison of testicular samples of the non-varicocelic side to obstructive azoospermic (control) males presented interesting data. The significant reduce of spermatogenesis proved that contralateral varicocele also has a negative effect on fertility. This is another reason to perform varicocelectomy even by unilateral cases.

Data of detailed statistical evaluation of the spermatogenetic activity of different grade varicoceles cases proved that high-grade varicocele causes decrease of the number of mature spermia in the testicular sample. According

to our data two grades do not fit in this tendency. Grade 1 cases show surprisingly low spermatogenetic activity. One explanation of this phenomenon is probably the low number of cases. However, grade 1 varicocele presents no clinical signs, it can be detected only accidentally. These patients visit andrologist because of their contralateral higher grade (3 or 4) varicocele, which has negative effect on the spermatogenetic activity of the grade 1 side. Grade 2 cases show surprisingly high spermatozoa numbers. The explanation of this phenomenon can be the aim of another study.

Histograms showed a normal distribution suggesting a statistically homogenous group by unilateral cases and lower grades. By grade 2 a second peak appears by 0. The height of this second peak shows increasing tendency with the grade (the number of biopsies presenting no spermatogenesis tends to be higher). The number of peaks rises with the grade: the histogram of grade 2 cases show a two-or three-peak, grade 3 a three-peak and grade 4 a five-peak appearance. The presence of more than one peak proves statistical inhomogeneity of the group. The etiological reason of this phenomenon is unknown and requires further examinations.

The correlation of the grade of the varicocele and homogeneity of spermatogenesis showed that our control group presented most homogenous spermatogenesis. Grade 2 cases again do not fit in the row - it is the second most homogenous. The relatively high percent difference of contralateral side of unilateral varicocele cases is probably caused by the high-grade contralateral varicocele. Grade 3 and 4 cases showed striking inhomogeneity. With the crescent grade it is more and more difficult to find the most preserved area because local differences of spermatogenesis also increase.

Summarizing the conclusion of the study it can be established that the diagnosis and therapy of male infertility is a complex problem. Different parts of the whole aspect can be elucidated by different examination of specialists (radiologist, clinical pathologist, molecular biologist, genetist and histopathologist) [4, 22].

In the era of new ART techniques histopathological report may become one of the most important keystones of the therapy planning. Pathologists should therefore participate in the work of the reproductive team. The new requirements of andrologists have changed the histological reporting protocol. For this reason specially trained pathologists with great interest in andrology are needed. This training should also be organized.

The negative effect of more and more environmental factors on the spermatogenesis is confirmed. Mobile phones and computer screens are mentioned. The examination of these can be the aim of further studies. The results of the researches can be used for prevention or development of new diagnostic methods.

By the end of the diagnostic process the andrologist can synthesize the data and choose the most appropriate treatment for the patient. It is a complex, multidisciplinary teamwork where histopathologist plays an important role.

The effective work of reproductive team might help to stop population reduce in the far future.

Summary

Multiple testicular biopsies of 149 young infertile men were examined histologically. The distribution of clinical diagnosis was the following: 86 azoospermic, 29 OATS, 28 oligozoospermic, three ATS, one normozoospermic and one anejaculatory patient.

Etiological background of every group showed in majority combined atrophy and maturation arrest, followed by atrophy, normal spermatogenesis and maturation arrest. In azoospermic individuals a low number of SCOS was also found. The rate of cases presenting normal histology showed an increase with the better spermatograms.

Looking the **etiological inhomogeneity**, half of the azoospermic patients and great majority of the OATS and oligozoospermic men presented different etiology in the samples taken from different locations. All SCOS and most marked atrophy and normal cases showed homogenous, while mild atrophy and maturation arrest cases presented an inhomogeneous appearance.

Inhomogeneity of **spermatogenetic activity** was checked. The chance of missing spermatozoa with taking of only a single biopsy was proven around 15% in all of the patient groups.

Statistical inhomogeneity: examining the average and standard deviation of spermatozoa count in 10 round shaped tubules azoospermic cases presented an average of 59,4+/-92,3, while average and standard deviation values of OATS and oligozoospermic patients was 122+/-120,4 and 152,7+/-130,5 respectively.

61 of the infertile individuals presented with coexisting **varicocele**. 44 of them had bilateral, 17 unilateral varicocele. The grade distribution was: grade 1 four, grade 2 23, grade 3 52, grade 4 26 cases. For control group 4 obstructive azoospermic men were chosen with normal spermatogenesis in both testes. Spermatozoa numbers counted in 10 round shaped tubules were examined using different statistical methods. Contralateral varicocele had a negative effect on the spermatogenesis of the "healthy" side. The spermatogenetic activity showed a decrease with the increase of the grade

(except grade 2 cases). The number of biopsies presenting no evidence of mature spermatozoa increased with the grade. Histograms of higher grades showed a multi-peak appearance characteristic for statistical inhomogeneity of the group. Higher percent differences suggesting inhomogeneity were found by higher grades (except grade 2 cases).

Conclusion: Histological evaluation of testicular biopsy gave additional information for the andrologist about etiology. Concerning inhomogeneity all of the methods used confirmed that etiology and spermatogenetic activity of the testis presented striking inhomogeneity. Using of the multiple biopsy method gives thus more realistic information about testicular function than single. For all these reasons histopathologist should participate in the work of the reproductive diagnostic team.

Acknowledgements

I would like to thank my teacher in pathology prof. Éva Magyar to support my work and help in reporting testicular biopsies. I am very grateful to dr. Edit Erdei for offering me the chance to take part in the work of the andrology team and answer my questions of urology.

I am grateful to prof. Gábor Kendrey for his useful advices.

My great thanks go to prof Giuseppe Tritto and Marie-Odile North for the successful international collaboration.

Many thanks for our histopathological technicians for their excellent work and to my colleagues for their helpful attitude and patience doing our everyday routine work for a time, while I was able to concentrate on my research and complete my thesis.

I am grateful to my family especially to my parents for all of their support. I would like to thank to my husband Béla Schmatz for his help in creating the database, evaluating the statistical data and making the presentation.

Thank to dr. Péter Kádár for his previously reported testicular biopsies, which were included in the study.

References

1. Ald M, Niederberger CS, Ross LS: Surgical sperm retrieval for assisted reproduction. Minerva Ginecol 56, 217-22, 2004

2. Ali S, Hasnain SE: Genomics of the human Y-chromosome. Association with male infertility. Gene 321, 25-37, 2003

3. Amer M, Haggar SE, Moustafa T, Abd El-Naser T, Zohdy W: Testicular sperm extraction: impact of testicular histology on its outcome, number of biopsies to be performed and optimal time for repetition. Hum Reprod 14, 3030-44, 1999

4. Brugh VM, Matschke HM, Lipshultz LI: Male factor infertility. Endocrinol Metab Clin North Am 32, 689-707, 2003

5. Cayan S, Lee D, Black LD, Reijo Pera RA, Turek PJ: Response to varicocelectomy in oligospermic men with or without defined genetic infertility. Urology 57, 530-35, 2001

6. Charny CW: Testis biopsy: its value in male sterility. JAMA 115, 1429-1432, 1940

7. Cozzolino DJ, Lipshultz LI: Varicocele as a progressive lesion: positive effect of varicocele repair. Hum Reprod Update 7, 55-58, 2001

8. Dohle GR, Halley DJ, Van Hemel DJ, van den Ouwel AM, Pieters MH, Weber RF, Govaerts LC: Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod 17, 13-6, 2002

9. Eskew LA, Watson NE, Wolfman N, Bechtold R, Scharling E, Jarow JP: Ultrasonographic diagnosis of varicoceles. Fertil Steril 60, 693-97, 1993

10. Gottschalk-Sabag S, Weiss DB, Folb-Zacharov N, Zuckerman Z: Is one testicular specimen sufficient for quantitative evaluation of spermatogenesis? Fertil Steril 64, 399-402, 1995

11. Gruber CJ, Hengstschlager M, Leipold H, Gruber IM, Ferlitsch K, Gruber DM, Huber JC: Genetic factors in assisted reproduction. Wien Klin Wochenschr 115, 805-11, 2003

12. Guarch R, Pesce C, Puras A, Lazaro J: A quantitative approach to the classification of hypospermatogenesis in testicular biopsies for infertility. Hum Pathol 23, 1032-7, 1992

13. Hargreave TB, Liakatas J: Physical examination for varicocele. Br J Urol 67, 328-32, 1991

14. Hauser R, Botchan A, Arnit A, Ben Yosef D, Gamzu R, Paz G, Lessing JB, Yogev L, Yavetz H: Multiple testicular sampling in non-obstructive azoospermia-is it necessary? Hum Reprod 13, 3081-5, 1998

15. Hauser R, Botchan A, Yogev L, Gamzu R, Ben Yosef D, Lessing JB, Amit A, Yavetz H: Probability of sperm detection in nonobstructive azoospermic men undergoing testicular sperm extraction procedures unrelated to clinical parameters. Arch Androl 48, 301-5, 2002

16. Hotchkiss RS: Testicular biopsy in the treatment of sterility in the male. Bull NY Acad Med 18, 600-5, 1942

17.Jarow JP: Effects of varicocele on male infertility. Hum Reprod Update 7, 59-64, 2001

18.Kadioglu A, Tefekli A, Cayan S, Kandirali E, Erdemir F, Tellaloglu S: Microsurgical inguinal varicocele repair in azoospermic men. Urology 57, 328-33, 2001

19.Kamal KM, Jarvi K, Zini A: Microsurgical varicocelectomy in the era of assisted reproductive technology: influence of initial sperm quality on pregnancy rates. Fertil Steril 75, 1013-16, 2001

20.Kisfaludy N, Magyar É, Tóth A, Erdei E: Az ejakulátum elektronmikroszkópos vizsgálata infertilitás esetén. Magyar Andrológia, 9, 57-59, 2004

21. Lellei I, Magyar E, Erdei E: Histological evaluation of multiple testicular biopsies helping assisted reproduction. Pathol Res Pract 197, 727-733, 2001

22. Makar RS, Toth TL: The evaluation of male infertility Am J Clin Pathol 117 suppl., 95-103, 2002

23.North MO, Lellei I, Erdei E, Barbet JP, Tritto J: Meiotic studies of infertile men of nonobstructive azoospermia with normal karyotype and no microdeleted Y-chromosome precise the clinical couple management. Ann Genet 47, 113-23, 2004

24. North MO, Lellei I, Rives N, Erdei E, Dittmar A, Barbet JP, Tritto G (2004): Reversible meiotic abnormalities in azoospermic men with bilateral varicocele after microsurgical correction. Cell Mol Biol (noisy-le-grand) 50, 281-9, 2004

25.Palermo G, Joris H, Devroey P, Van Steirteghem AC: Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340, 17-18, 1992

26.Pare A: Les Oevres d'Ambroise Pare, 1541

27.Schlegel PN, Palermo GD, Goldstein, Menendez S, Zaninovic N, Veeck LL, Rosenwaks Z: Testicular sperm extraction with ICSI for nonobstructive azoospermia. Urology 49, 435-40, 1997

28.Schultze W, Thoms F, Knuth UA: Testicular sperm extraction: a comprehensive analysis with simultanously performed histology in 1418 biopsies from 766 subfertile men. Hum Reprod 14, 82-96, 1999

29.Schwartzer JU, Fiedler K, Hertwig I, Krusmann G, Wurfel W, Schleyer M, Muhlen B, Pickl U, Lochner-Ernst D: Sperm retrieval procedures end intracytoplasmic spermatozoa injection with epididymal and testicular sperms. Urol Int 70, 119-23, 2003

30.Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC: Distribution of spermatogenesis in testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure. Hum Reprod 12, 2422-8, 1997

31.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, 240-49, 2004

32.Skakkebak NE, Heller CG: Quantification of human seminiferous epithelium. J Reprod Fert 32, 379-83, 1993

33. Su LM, Palermo GD, Goldstein M, Veeck LL, Rosenwaks Z, Schlegel PN: Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia: testicular histology can predict success of sperm retrieval. J Urol 161, 112-6, 1999

34. Tchovelidze C, Sibony M, Callard P, Guetta T, Lababidi H, Arvis G: The testicular biopsy and spermatogenesis disturbance of infertile patients with bilateral varicocele. Arkh Patol 66, 40-45, 2004

35. Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, Van Steirteghem AC, Devroey P: Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. Hum Reprod 11, 127-32, 1996

36. Tritto G, Giargia E, Erdei E, Morlier D: The role of testicular biopsy in microsurgical correction of bilateral varicocele. AMBER '95 Paris, Proceedings 274-78, 1995, edited by G Tritto

37. WHO: The influence of varicocele on parameters of fertility in a large group of men presenting to fertility clinics. Fertil Steril 57, 1289-93, 1992

38. Yoshida K, Kitahara S, Chiba K, Horiuchi S, Horimi H, Sumi S, Moriguchi H: Predictive indicators of successful varicocele repair in men with infertility. Int J Fertil Womens Med 45, 379-84, 2000

39. Zorgniotti AW: Testis temperature, infertility and the varicocele paradox. Urology 16, 7-10, 1980

40. Zuckerman Z, Rodriguez-Rigau LJ, Weiss DB, Chowdhury L, Smith KD, Steinberger E: Quantitative analysis of the seminiferous epithelium in human testicular biopsies, and the relation of spermatogenesis to sperm density. Fertil Steril 30, 448-455, 1978