Agenesia of deferential duct and the others congenital malformations of mesonephronical duct are often encountering condition in andrological practice. This study presents the possibilities of reproductive medicine to restore the male fertility and concentrates on biological and clinical aspects of malformations of mesonephronic duct like factors for excretory male infertility.

The investigations including 104 patients (mean age 30.25±1.91 years old) with congenital anomalies of mesonephronic duct with: Agenesia of mesonephronical derivates – case with unilateral agenesia of the deferent duct, kidney and left urether (n=1); Dysgenesia of the epididymis (n=5); Cysts of the epididymis (n=47); Agenesia of the deferential duct (n=48); Aplasia of the eja-culatorial duct (n=3).

The following methods were used: open testicular biopsy by Vilar; objective findings in situ operation; bilater epididyim and deferentovesiculography; morphological specimens of the testicular and epididymal tissues; enzymo-histochemical analysis of testicular specimens; the ejaculates and morphological analysis of spermatozoa were assessed for quality parameters by the standard protocol; enzymocytological research of spermatozoa in seminal fluid; biochemical analysis of seminal fluid fructose and citric acid concentrations; radioimmunoassay for measurements of blood FSH and testosterone concentration.

Results: Most often observed localization of the malformations of mesonephronic canal is bilateral at 54.81%. The testicular specimens proved preserved testicular architecture and spermatogenesis, and the epididymal slides showed dilatation of the epididymal tubules, which were overfilled with a lot of spermatozoa. The strongest expression of the enzymo-histochemical NADH-TR reactions is in the Sertoli cells. We observed spermatozoa with disrup-ted configuration among which the abnormalities in sperm head and acrosome were predominated. The biochemical analysis of seminal fluid proves normal citric acid and low or lack of fructose concentration in seminal fluid. There were no breaches in endocrine regulation of the reproductive process. Patients with congenital anomalies of mesonephronic canal have preserving fertility in 31.73%. There are morpho-functional disorders of the testes, epididymis and seminal ducts in 68.27%.

Conclusion: There are normal spermatogenesis but definite sterility. These results necessitate an application of plastic reconstructive operation for recovery of fertilizing ability of the patients, or their involvement in ICSI programme.

Key words: malformations of mesonephronic duct, sperm, LH, FSH, Testosterone, NADH-TR, fructose, citric acid, infertility, men.

INTRODUCTION

Post testicular reasons of male sterility are 10-20% of the pathology of congenital malformations of the male reproductive system (Amelar & Hotchkiss, 1963; Klosterhalfen&Wagenknecht, 1972; Tzvetkov, 1981; Tzvetkov&Tzvetkova, 1999). It is very important to know that there a fundamental cause for excretory sterility. The excretory and incretory functions of the testes are well preserved (Wagenknecht, 1994; Tzvetkov&Tzvetkova, 1999; Schroeder-Printzen et al., 2000; Tzvetkov&Tzvetkova, 2001).

Agenesia of deferential duct and the others congenital malformations of mesonephronical duct are often encountering condition (1-2%) in andrological practice (Amelar, 1975; Gert et al., 1997). This study presents the possibilities of reproductive medicine to restore the male fertility and concentrates on biological and clinical aspects of malformations of mesonephronic duct like factors for excretory male infertility.
MATERIAL AND METHODS

The clinical and experimental analyses were performed in the Andrological Clinic at the Medical University and in the Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Science, including 104 patients (mean age 30.25 ± 1.91 years old) with congenital anomalies of mesonephronical duct with:

- Agenesia of mesonephronical derivates – case with unilateral agenesia of the deferent duct, kidney and left urether (n = 1)
- Dysgenesia of the epididymis (n = 5)
- Cysts of the epididymis (n = 47)
- Agenesia of the deferential duct (n = 48)
- Aplasia of the ejaculatorial duct (n = 3)

The following methods were used:
- Open testicular biopsy by Vilar O. (1976)
- Objective findings in situ operation.
- Bilateral epididymo – and deferentovesiculography.

Morphological specimens of the testicular and epididymal tissues.

Enzymohistochemical analysis of testicular specimens: The histochemical analysis of NADH – diaforazal and LDH activity was made in incubation tissue by referative protocol (Yonkov, 1986). The reaction were evaluated by means of 5 exponential scale.

The ejaculates and morphological analysis of spermatozoa were assessed for quality parameters by the standard protocol according to the requirements of WHO (1996).

Enzymocytological research of spermatozoa in seminal fluid.

Morphological analysis of NADH-TR activity in spermatozoa by the method of Kiernan (1981), and the different components of the developing medium were in concentrations validated by Yonkov (1986).

The specificity of the cytochemical reaction was controlled with aid of smears incubated in the developing medium without NADH-TR. The intensity of cytochemical reaction expressed tetrazolium reductase in sperm mid-piece as evaluated by 5 exponential scale (negative, slight, moderate, strong and very strong reaction). The following parameters were estimated by positively reacting spermatozoa (PRS) and mean cytochemical coefficient (MCC). Mean cytochemical coefficient was calculated by using the following formula:

\[ MCC = \frac{A.X1+B.X2+C.X3+D.X4+E.X5}{200} \]

Where,
A-0, absence of reaction
B-+, weak positive reaction
C-++, moderate reaction
D-++++, intensive positive reaction
E-+++++, very intensive positive reaction
X – number of analysed cells

Biochemical analysis of seminal fluid fructose and citric acid concentrations by WHO (1996).
The standard radioimmunoassay (RIA) for measurements of blood FSH and testosterone concentration by Kanchev et al. (1976).

All results are statistically analysed by the Student T-test and presented as MEAN ± SE. All data were compared with a control group of 10 healthy men (mean age 21.86 ± 1.33 years old) with preserved fertilizing ability.

RESULTS

I. Clinical study

The evaluated patients were in reproductive age (23 to 33.5 years old), 35 of them were not married, and 53 married without children with prolongation of infertility marriage 3.70 ± 1.48 years (Tabl. 1).

The implemented epididymo- and deferentovesiculography proved the presence of agenesis of the deferential duct, dysgenesia of the epididymis and aplasia of the ejaculatorial duct. The patient with unilateral agenesis of the deferential duct combine the disease with agenesis of the kidney and urether.

Most often observed localization of the malformations of mesonephronic canal is bilateral at 54.81% (Tabl. 2).

II. The morphological research

The testicular specimens proved preserved testicular architecture and spermatogenesis (Fig. 1). The analysis of the epididymal slides showed dilatation of the epididymal tubules, which were overfilled with a lot of spermatozoa. (Fig. 2).

III. Enzymohystochemical research of the testicular slides:

The results from the enzymichistochemical reactions of the testicular specimens gave an account that NADH – daforazal and LDH – activity in the curved seminal tubules decreases from the basal membrane to the luminal compartment. The strongest expression is in the Sertoli cells (Fig. 3). All interstitial cells (Leidig cells) demonstrate high expression of both enzymes. The control slides were negative. There were not significant difference in both enzymic expression in the slides of patients with aspermia and normospermia.

IV. Spermatological analysis of ejaculate.

In cases with disgenesia of the epididymis, agenesia of the deferential duct and aplasia of the ejaculatory duct spermatological analyses was proved aspermia with 1.2 ± 0.12 ml volume, ph 7.2 ± 0.09 and normal viscosity of the ejaculate. The small volume of ejaculate is corresponded with the presence of the fluid of the prostate and bulbourethral glands. The patient with unilateral lack of deferential duct had also disrupted fertility – 25 mil/ml with 30% motile spermatozoa and low power security of the germ cells (Tabl. 3).

In other cases with epididymal cysts in 70.21% patients had preserving fertility. There were oligoastenospermia in 19.15% of cases and azoospermia in 10.64%.

Patients with congenital anomalies of mesonephronic canal have preserving fertility in 31.73 %. There are morpho-functional disorders of the testes, epididymis and seminal ducts in 68.27 %.

V. The morphological research of the gametes

We observed spermatozoa with disrupted configuration (Fig 4) among which the abnormalities in sperm head and acrosome were predominated.

The patients with bilateral lack of the deferential duct and aplasia of the ejaculatory duct possessed conglomerate of spermatozoa in large part destructed from deadlock.

VI Cytochemical enzyme activity of spermatozoa:

The enzyme activity of spermatozoa were expressed cytochemically in patients with unilateral agenesis of the deferential duct and epididymal cysts. They were visual-
ized by diformazane sediment in the sperm midpiece in the form of blue diformazane deposits different in amounts, size and staining intensity displaying single enzyme active mitochondria arranged in the shape of a helix. (Fig. 5).

Spermatozoa with disrupted configuration did not differ from the morphologically normal cells in NADH-TR activity.

The percentage of positively reacting spermatozoa in these cases and the average values of the mean cytochemical coefficient are given in Table 4. It is seen that the values of both parameters were lower in patients of trial group than healthy men of control group. The relative proportion of spermatozoa with expressed intensity of the reaction in ejaculates shown higher percentage of cells with good motility. They were greater than the same degree of reaction for NADH-TR in ejaculates containing higher percentage of slightly motile and unmotile spermatozoa. In control smears incubated in developing medium without NADH, the reaction in the spermatozoa was negative for the enzyme system studied here.

**VII Biochemical analysis of seminal fluid**

The analysis was proves normal citric acid and low or lack of fructose concentration in seminal fluid.

**VIII Plasma LH, FSH and Testosterone concentration and congenital malformations of the mesonephronic canal**

There were no breaches in endocrine regulation of the reproductive process.

**DISCUSSION**

In our experience the malformations of the mesonephronic duct were found in 9.23% of 184 cases with azoospernia (Tzvetkov, 1981). This relevance is in agreement with that observed in other recent clinical studies reported in the literature (Borrelli et al., 1978; Fonzo et al., 1982; Donat et al., 1997; Irvine, 1998).

There is a normal spermatogenesis but definite sterility in these cases (Tzvetkov, 1989; Sharif, 2000). We found by histomorphological studies of the testicular and epididyms tissue that bilateral d. deferens agenesis, disgenesis of the epididyms and aplasia of the ejaculatorial duct were combined with preserved spermatogenesis. We observed in the epididyms a conglomerate of spermatozoa. It is an evidence for the different origin of testes and ejaculate duct, respectively from the genital comb and from the mesonephronic duct (Klotz, 1973; Linarabh et al., 1975). Frequently we observed epididyms changes and probably d. deferens agenesis secondary influences the epididyms morphology. The observed changes were reported previously (Tzvetkov, 1981;1989; Tzvetkov&Tzvetkova,1999; Tzvetkov&Tzvetkova,2001; Tzvetkova&Tzvetkov, 2004).

According to the Fonzo et al. (1982) the hormonal investigations in cases with congenital malformations of the mesonephronic duct have shown a normal function of the pituitary - gonadal axis. This again confirmed the presence of preserved incretory and excretory testes functions and in this case the disease is typical example for post testicular infertility.

The low ejaculate volume and the absence of fructose in the seminal plasma were explained post-testicular cases of infertility. We observed fertility changes in the ejaculate in the case with unilateral d. deferens agenesis too. The presence of oligoastenospermia in that case was because of the accompanied disease of contralateral right testis - varicoce. That’s why in addition to the classical spermatologtical analyses of the ejaculate, the application of enyzmocytochemical methods make it possible to study the metabolism of male gametes in normal and pathological states. More comprehensive cytochemical investigations published by, Edvinsson et al. (1981) and Yonkov (1986) on NADH-TR activity revealed that spermatozoa in normospermic ejaculates manifested stronger enzyme activity in comparison with those in astenospermic ejaculates.

The discussed above results necessitate an application of plastic reconstructive operation for recovery of fertilizing ability of the patients, or their involvement in ICSI programme (Gil-Salom et al., 1998; Steele et al., 1999).

**CONCLUSION**

There are normal spermatogenesis but definite sterility. These results necessitate an application of plastic reconstructive operation for recovery of fertilizing ability of the patients, or their involvement in ICSI programme.

**SUMMARY**

**KONGENITALNE ANOMALIJE MEZONEFROSNOG KANALA I FERTILITET**

Agenezija duktusa deferensa i druge urodjene anomalije mezonefrosnog kanala su često sukovljeni uslovi u andro-loškoj praksi. Ova studija predstavlja mogućnosti reproduktivne medicinc da restauriraju muški fertilitet. Istraživanja uključuju 104 bolesnika (srednje godine starosti 30.25±1.91) sa kongenitalnim anomaliama mezonefrosnog kanala, kao što su istostrane agenezija deferensijalnog kanala, bubrega i levog uretera, disgenija epididima (n-5), cista epididima (n-48), agenezija d. deferens (n-48), aplazija ejakulatornog kanala (n-3). Za ova ispitivanja su korišćene sledeće metode, kao što su otvorena biopsija te stisa, objektivni nalazi na operacijama, enzimocitoška ispitivanja spermatozoida u semenoj tečnosti, biohemijadska analiza fruktoze i limunske kiseline i dr.

**REZULTATI:** Najčešće postomatna lokalizacija malformacija mezonefrosnog kanala je obostrana u 54,81% slučajeva. Uzorci tkiva testisa su sačuvali arhitekturu i spermatogenzu, a tkivo epididima je pokazalo dilataciju tubula koji su ispunjeni masom spermatozoida. Najjača ekspresija enzimhistrohemijskih NADAH-tr reakcije je na Sertolijeve celije. Uočili smo spermatozoide sa poremećenom konfiguracijom i te promene su predominirale na glavi i akrozomu. Biohemijadske analize semene tečnosti pokazuju normalan nivo limunske kiseline i smanjen nivo fruktoze. Nije bilo prekida u endokrinoj regulaciji repro-
ZAKLJUČAK: Postoji normalna spermatogeneza ali odredjen sterilitet. Ovi rezultati zahtevaju primenu rekonstruktivnih operacija za oporavak fertilitne sposobnosti pacijenata ali i njihovo uključenje u ICSI program.

Ključne reči: malformacije mezonefrosnog kanala, sperma, LH, FSH, testosteron, NADAH-TR, fruktoza, limunska kiselina, infertilitet, muški

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