

THEORETICAL AND EXPERIMENTAL MEDICINE

Vorontsova L.L., Kovalenko V.A., Dub M.I., Zhuravlova M.Ye.

EFFECT OF ENDOTOXIN AGGRESSION ON THE DEVELOPMENT OF IMMUNE HOMEOSTASIS DISORDERS IN INFERTILE MEN

State establishment “Zaporizhzhya Medical Academy of Post Graduate Education of the Ministry of Health of Ukraine”,
Ukraine

Abstract: The article deals with the study of endotoxin-antiendotoxin system state showing that endotoxin aggression occurs in infertile men and transforms acute character (in considerable decrease in ejaculate fertility) into chronic one (in absolute deficiency of fertile properties in ejaculate). Changes in the indices of the impaired immune system link indicate an increase in tension, that, apparently assumes alternative available agents (in particular, gram-negative bacteria), which further disrupts spermatogenesis. It is possible to assume that endotoxin aggression, chronic in particular, is an important link of pathogenesis of male infertility, though high LPS concentrations potentiate immune phagocytic link, presumably, as a compensator.

KeyWords: endotoxin, innate immune system, male infertility.



INTRODUCTION

Infertility in marriage is a considerably widespread disorder. It is not only a medico-biological problem, but rather a social and demographic one [1]. Male infertility has been established to be the cause of infertile marriages in approximately 50% cases [2].

Spermogram is the most important investigation for diagnosis of male infertility. However, the results of diagnosis for male infertility are not always reliable: the obtained data demonstrate that fertility may be retained even in case when spermogram demonstrates deviations from the norm, and in contrast, infertility may be observed in men with normospermia [3]. The causes of deterioration for fertile characteristics in ejaculate are not known yet; thus, male infertility still presents challenges in diagnosis [4].

Some authors consider that inflammatory infections in different parts of urogenital tract trigger infertility [5].

Infections are known to develop under certain conditions when the main links of immune system are impotent, but their influence on the development of pathology is often underestimated [6, 7]. Recently there has been an increase in studies estimating the pivot role of the immune system in the pathology of male urogenital organs, but the state of phagocytic link in the immune system depending on the type of fertility impairment is under-investigated, moreover the obtained data are quite contradictory [8, 9].

In fact, spermatogenesis is one of the most dynamic process in the body and thus, it is utterly susceptible to injuring agents of both endogenic and exogenic origin [10]. Endotoxin (ET) of gram-negative bacteria is one of the most important agents in forming endogenic intoxication syndrome, as it exerts exceptional biologic activity and is one of the most potent exogenic modulators for immune reactivity [11]. The main pathophysiologic effect of ET consists in induction of ejecting some endogenic mediators for inflammation that are mainly synthesized by myelomonocytic cells [12]. Neutrophils and macrophages activated by ET release considerable amount of free radicals resulting in further destabilization of biologic membranes and, consequently, in potential cytostatic effect for bacterial toxins [13].

Thus, the so-called “vicious circle” is formed, where

Corresponding Author:

Victoria Kovalenko - Candidate of Biological Sciences, Senior lecturer, Department of Clinical Laboratory Diagnostics, State establishment “Zaporizhzhya Medical Academy of Post Graduate Education Ministry of Health of Ukraine”; e-mail: kovalenkovika0809@gmail.com

disrupted biocenosis promotes local immunity suppression determining conditions of pathogenic influence of commensals secondary to immunosuppression that potentiates immune impotence [14,15].

However, the mechanism of bacterial LPC or endotoxins effect on the immune system is rather complicated and is still under-investigated.

2 PURPOSES, SUBJECTS and METHODS:

2.1 Purpose

Having regard to the above, the aim of our study is to assess the influence of endotoxin in gram-negative bacteria on the state of phagocytic link of the immune system in men with fertility impairment in ejaculate.

2.2 Subjects & Methods

The study involved 54 men aged from 20 to 45. They signed a written agreement to participate in investigations. This agreement was submitted to the approval of Bioethics committee at SE "Zaporizhzhya Medical Academy of Post- Graduate Education of the Ministry of Health of Ukraine" in accordance with ethic, moral and legal requirements by the order of the Ministry of Health of Ukraine No. 281of 01.11.2000.

All men were divided into three groups. The first group (control one) consisted of 20 healthy men without any impairments of the reproductive system; they had 1-2 children aged 1-2. The second group comprised 19 men with decreased fertility in ejaculate. The third group included 15 men whose ejaculate was found to lack fertile properties. The men of the second and third groups were in childless marriages from one to fifteen years.

Each patient was referred to microbiologic examination of ejaculate according to the Order of the Ministry of Health of USSR No. 535 of 22.04.1985 "Unification for microbiologic (bacteriologic) examinations used in clinical and diagnostic laboratories

at medical institutions". Bacteriologic investigation of ejaculate in men of the second and third groups showed bacteriospermia caused by gram-positive and gram negative flora.

All the men were referred to a comprehensive examination including spermogram analysis according to the WHO recommendations [16] and assessment of the state of phagocytic link of immune and endotoxin-antiendotoxin systems.

The assessment on phagocytic activity of neutrophils and monocytes in blood was carried out by the technique based on identification of their absorbing and digestive capacity 30-120 minutes after preincubation with 24 hours culture of *Staphylococcus epidermis* strain [17]. Neutrophils oxygen-depending metabolism (NBT-test) and functional cell reserve (NBT-test stimulated) were assessed by the cell ability to restore nitro-blue tetrazolium after M.E.Wiksman and A.N. Mayansky [18].

The assessment for total concentration of endotoxin (ET) in systemic blood circulation was performed using "Micro-LAL-test". To assess humoral link of antiendotoxin immunity antibodies titers to glycolipid (At to GLP) and general enterobacterial antigen (At to GEA) were identified by "SOIS- IFA" technique. Statistical data processing was performed with STATISTICA (StatSoftStatistica v. 6. 0.) software using Wald-Volkovits test. The difference was considered reliable when the achieved value was <0.05 . The data under investigation were presented as median (ME) and interquartile swing (RQ), presenting the difference between the values of 75-th and 25-th percentiles ($RQ = 75\% UQ - 25\% LQ$) where UQ is the upper quartile and LQ is the lower one.

Conflict of interests

There is no conflict of interests.

3 RESULTS AND DISCUSSION

Our microbiologic examination of ejaculate showed that microorganisms isolated from the samples of infertile men had both gram-positive and gram negative bacteria (*E. Coli*, *Ent. faecalis*, *S.*, *Str.*, as well as coagulo-negative and coagulo-positive and *Candida* fungi. Comparative test for microbiocenosis of ejaculate in infertile men showed that in 80% men of the second group (decreased ejaculate fertility) conventionally pathogenic flora was presented by *Ent. faecalis*, *S. haemolyticus* in concentrations from 10 to 10 UFC/ml, *C. albicans* - 10 UFC/ml and *S. epidermis* had poor growth. Whereas in the third group (ejaculate without fertile properties) pathogenic flora was presented by *Str. Mitis* - 10 UFC/ml, *S. epidermis* - 10 UFC/mg, *S. haemolyticus* - 10 UFC/ml, *E. coli* - 10 UFC/ml, *Klebsiella pneumonia* - 10 (4) UFC/ml.

Microbiologic findings did not provide any undisputable information concerning the influence of changes in microbiocenosis on ejaculate fertile properties. They only confirmed the presence of changes. Taking into account that bacterial associations revealed the ability to decrease unspecific reactivity that often leads to chronic inflammation, further dissemination of mixed infection and obviously, infertility, it is necessary to conduct extended studies regarding the influence of bacterial infection on male infertility and particularly, the impact of the endotoxin-antiendotoxin system state on male reproductive performance.

Assessment of endotoxin-antiendotoxin system in men of the second group showed a considerable increase in ET concentration in general blood circulation in 1079 % as compared to the control group (Table 1).

The study showed a tendency to increasing titers At to GLP and At to GEA in 11% and 8% as compared to control indices, respectively. Thus, an increase in ET occurred secondary to an increase in activity for humoral link of antiendotoxin immunity that proves acute EA in this group of men. Evaluation of endotoxin-antiendotoxin system in men of the third group revealed a considerable increase in ET concentration almost 25-fold (in 2389%) as compared to the control group.

Table 1.
The state of endotoxin-antiendotoxin system in men suffering from different types of ejaculate fertility impairment, Me (75%Q - 25%Q = RQ)

Index, unit of measure	ET concentration, UFC/ml	Antibodies titer to GLP, c.u.	Antibodies titer to GEA, c.u.
Group 1 (n = 20)	0.19 (0.21 - 0.18 = 0.03)	195.2 (196.1 - 193.9 = 2.2)	389.4 (389.8 - 388.9 = 0.9)
Group 2 (n = 19)	2.24* (2.34 - 1.98 = 0.36)	216.0 (218.4 - 213.8 = 4.6)	421.1* (424.7 - 419.3 = 5.4)
Group 3 (n = 15)	4.73* (4.80 - 4.43 = 0.37)	116.9* (120.6 - 112.2 = 8.4)	291.2* (300.4 - 289.6 = 10.8)

Note: * statistically significant difference ($p < 0.05$) as compared to the control group.

The rate At to GLP and At to GEA was decreased in 40% and 25% as compared to the control group, respectively. Thus, a considerable increase in ET rate in blood circulation in men of the third group was observed secondary to a sharp decrease in the activity of humoral link of antiendotoxin immunity which indicates the development of chronic EA in this group.

Assessment of indices for functional and metabolic status of neutrophils and monocytes (Table 2) in men with decreased fertility in ejaculate (second group) as compared to the control values of a decrease in functional activity of neutrophils was revealed both at the 30th minute and at the 120th minute in 13% and 9%, respectively, but the latter case was statistically unreliable though clinically significant. Absorptive capacity of neutrophils at the 30th minute coincided with the control indices, but at the same time digestive capacity of neutrophils was increased in 27%, respectively to the values of the control group.

Functional activity of monocytes (NPI) at the 30th minute in the second group corresponded to the control group. Functional activity of monocytes at the 120th minute exceeded the control values in 22%. Absorbing and digestive capacity of monocytes was increased in 7% (statistically unreliable, but clinically significant) and they were increased in 18% as compared to the control values, respectively.

Table 2.

The state of metabolic and functional status of neutrophils and monocytes in men with different impairments of ejaculate fertility, Me (75%Q - 25%Q = RQ)

Index, unit of measure	1 st group (n = 20)	2 nd group (n = 19)	3 rd group (n = 15)
NPI at 30 min., %	58 (74 - 29 = 45)	50.5 (59.0 - 41.0 = 18)	48* (52 - 38 = 14)
NPN at 30 min., c.u.	1.8 (6.5 - 1.3 = 5.2)	1.85 (3.0 - 1.4 = 1.6)	1.5*. ^{**} (1.8 - 1.3 = 0.5)
NPI at 120 min., %	50 (68 - 29 = 39)	45.5 (52.0 - 40.0 = 12)	54 (57 - 39 = 18)
NPN at 120 min, c.u.	1.3 (3.1 - 1.1 = 2.0)	1.7* (2.2 - 1.2 = 1.0)	1.5** (1.8 - 1.3 = 0.5)
MPI at 30 min., %	28 (30 - 20 = 10)	29 (45 - 24 = 21)	30 (30 - 22 = 8)
MPN at 30 min., c.u.	1.4 (1.7 - 1.0 = 0.7)	1.5 (1.9 - 0.9 = 1.0)	1.7 (1.7 - 1.0 = 0.7)
MPI at 120 min, %	20 (25 - 16 = 9)	24.5 (54.0 - 20.0 = 34)	28* (35 - 24 = 11)
MPN at 120 min., c.u.	1.1 (1.5 - 0.8 = 0.7)	1.3 (3.1 - 0.7 = 2.4)	1.5* (1.7 - 1.0 = 0.7)
NBTsp., c.u..	1.2 (1.3 - 1.0 = 0.3)	1.5 (1.9 - 0.7 = 1.2)	1.1 (1.5 - 1.0 = 0.5)
NBTsp., c.u..	1.4 (1.5 - 1.0 = 0.5)	1.8* (2.2 - 0.7 = 1.5)	1.1 (1.4 - 0.9 = 0.5)

Note: * statistically significant difference ($p < 0.05$) as compared to the control group; ** statistically significant difference ($p < 0.05$) as compared to the 2nd group.

The study also involved evaluation of peculiarities of the development of phagocytizing cells with NBT-test as an index. Spontaneous NBT-test displays a degree in functional irritation of phagocytizing cells. Stimulated NBT-test characterizes potential activity of phagocytizing cells and is considered to be a biochemical criterion for their readiness to complete phagocytosis. It mainly concerns blockade for producing oxygen-depending bactericidal agents. The second group was shown to have increased activity of phagocytizing cells both in spontaneous NBT-test in 25% and in stimulated NBT-test in 29% as compared to healthy men.

Thus, in men with decreased fertility phagocytic number of neutrophils and monocytes both at absorbing and digestive stage indicated completed phagocytosis. Indices increased through NBT-test suggested tension in unspecific link of the immune system.

4 CONCLUSIONS

1. Evaluation of endotoxin-antiendotoxin system state showed that endotoxin aggression occurring in infertile men transforms acute character (in considerable decrease in ejaculate fertility) into chronic one (in absolute deficiency of fertile properties in ejaculate).
2. Changes in indices of the impaired immune system link indicate increased tension, which definitely assumes alternative available agents (in particular, gram-negative bacteria) with further impairment of spermatogenesis.
3. It is possible to assume that endotoxin aggression and chronic in particular, is an important link for pathogenesis of male infertility, though high LPS concentrations potentiate immune phagocytic link, presumably, as a compensator.
4. Assessment of endotoxin-antiendotoxin system in men with reproductive function impairment is an indispensable component of comprehensive examination aimed to improve diagnosis of male infertility and promote planning of therapeutic management based on pathogenesis.

REFERENCES

1. Gorpy`nchenko I.I., Nurimanov K.R., Sajdakova N.O. (2012) Choloviche bezplidnya v Ukrayini: staty`sty`ka ta tendencyi [Male infertility in Ukraine: statistics and trends]. Zdorov`e muzhchiny, 4, 132-141.
2. Chadaev V.E., Kozub N.I., Mironenko M.V. (2007) Muzhskoe besplodie: sovremennyye aspekty [Male infertility: contemporary aspects]. Mezhdunarodnyiy meditsinskiy zhurnal, 4, 79-82.
3. Simon L., Brunborg G., Stevenson M. (2010) Clinical significance of sperm DNA damage in assisted reproduction outcome. Human Reproduction, 25, 1594-1608.
4. Ciporenko S.Yu., Loskutova I.V. (2012) Subpopulyacijnij sklad lejkocytiv spermy` ta jogo vplyv na morfogenez spermatozoyidiv u cholovikiv z urogenital`noyu infekciyeyu zalezno vid fertyl`nosti [Leukocyte subpopulation composition of sperm and its impact on morphogenesis of

sperm in men with urogenital infections depending on the fertility]. *Ukrayins'kyj zhurnal dermatologiyi, venerologiyi, kosmetologiyi*, 4 (47), 120-123.

5. Badalyan R.R., Fanarjyan S.V., Aghajanyan I.G. (2003) Chlamydial and ureaplasma infections in patients with nonbacterial chronic prostatitis. *Andrologia*, 35 (5), 263-265.

6. Buharin O.V., Kuzmin M.D., Ivanov Yu.B. (2000) Rol' mikrobnogo faktora v patogeneze muzhskogo besplodiyia [The role of microbial factor in the pathogenesis of male infertility]. *Zhurnal mikrobiologii*, 2, 106-110.

7. Kulakov V.I., Suhii G.T., Vanko L.V. (1999) Immunologiya reproduktivnoy [Immunology of reproduction]. *Vestnik RAMN*, 4, 44-48.

8. Tsiporenko S. (2013) Vpliv imunokorektsii na zapal'nyy proces urogenital'nogo traktu u cholovikiv z bezplodiyam [Influence of immunocorrection on the inflammatory process of the urogenital tract in men with infertility]. *Fiziologichnij zhurnal*, 59 (6), 72-80.

9. Bozhedomov V., Nikolaeva M., Golucheva E. (2007) Problemy diagnostiki immunologicheskoy prichiny muzhskogo besplodiyia [Problems of diagnostic of immunological causes of male infertility]. *Problemy reproduktivnoy*, 2, 81-89.

10. Galimov Sh.N., Amirov Z.K., Galimova E.F. (2005) «Krizis spermatozoida» i tehnogennoye zagryazneniye okruzhayushey sredy: faktory i gipotezy ["Sperm crisis" and technogenic pollution: the factors and hypotheses]. *Problemy reproduktivnoy*, 2, 19-22.

11. Anikhovskaya A., Oparina O.N., Yakovleva M.M. (2006) Intestinal endotoxin as a universal factor of adaptation and pathogenesis of general adaptation syndrome. *Human Physiology*, 32 (2), 200-203.

12. Bierhaus A., Chen J., Liliensiek B. (2000) LPS and cytokine-activated endothelium. *Semin. Thromb. Hemost.*, 26 (5), 571-587.

13. Bizenkova M.N., Chesnokova N.P., Ponukalina E.V. (2007) Sostoyaniye antiradikalnoy zashchity kletok v dinamike bakterialnogo endotoksikoza i vozmozhnosti ih medikamentoznoy korrektsii [Status antiradical defense cells in the dynamics of bacterial endotoxemia and the

possibility of their drug correction]. *Fundamentalnyye issledovaniya*, 11, 55-58.

14. Gabidullin Z.G., Ahtarieva A.A., Tuygunov M.M. (2009) Vzaimodeystvie bakteriy semeystva Enterobacteriaceae c antigenprezentiruyuschimi kletkami immunnogo sistema [The interaction of bacteria of the family Enterobacteriaceae c antigen-presenting cells of the immune system]. *Meditinskiy vestnik Bashkortostana*, 4 (5), 78-86.

15. Tarkovskiy I.S. (2015) Evolyutsiya vzglyadov na problemu oportunisticheskikh infektsiy [Evolution of views on the problem of opportunistic infections]. *Natsionalnyye dni laboratornoy meditsiny Rossii 2015 g.*, Rossiyskiy kongress laboratornoy meditsiny «Laboratornaya meditsina i klinicheskaya praktika», M., 70.

16. WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction [4th ed.]. New York: Cambridge University Press, 1999, 128 p.

17. Frimel N. (1984) Immunologicheskie metody [Immunological methods]. M.: Meditsina, 472

18. Viksman M.E., Mayanskiy A.N. (1979) Sposob otsenki funktsionalnoy aktivnosti neytrofilov cheloveka po reaktsii vosstanovleniya nitrosinogo tetrazoliya: metod. rekomendatsii [A method for evaluating the functional activity of human neutrophils by the reduction reaction of nitro blue tetrazolium: method. recommendations]. Kazan: Kazanskiy NIEM, 21.

Received: 13-Jan. - 2017

Accepted: 24-Mar. - 2017