Abstract. Background/Aim: Binding of FAS ligand (FASL) to its physiological receptor FAS, induces the activation of caspase-8, which triggers cell death. The FAS–FASL system regulates germ cell death. In this study, the role of the FAS–FASL system in male infertility was examined. Patients and Methods: 72 samples were used (age=38.76±9.06 years). Basic semen analysis was performed according to the WHO Laboratory Manual. Soluble (s) forms of FAS and FASL were measured in seminal plasma using commercially available immunoassay kits. Results: Among the examined samples, 24 were normal and 48 abnormal, as evaluated by basic semen analysis. sFAS and sFASL levels in abnormal samples were slightly higher than in the normal ones. In all samples, sFAS correlated negatively with pH. In normal samples, sFAS was positively correlated with sperm concentration. In abnormal samples, sFAS strongly correlated with sFASL. Conclusion: Both factors of the FAS system were detected in seminal plasma. Further studies are necessary to shed light into the possible role of FAS-FASL system in male infertility.

FAS receptor (APO-1/CD95) belongs to the tumor necrosis factor receptor superfamily (1, 2). The gene for human FAS is situated on chromosome 10, producing various isoforms, with a mass estimated at 43-48 kDa, which are transmembrane proteins inducing apoptosis (1-5). FASL is a 26-kDa transmembrane type-II protein. Binding of FASL to FAS results in the formation of the death-inducing signaling complex (DISC) and in the subsequent activation of caspase-8 (3, 4). Upon activation, caspase-8 is released from the DISC and triggers the activation of a cascade of caspases, resulting in DNA degradation, membrane destruction and subsequently to rapid cell death.

Both FAS and FASL also exist as soluble isoforms (3-5). Soluble FAS (sFAS) lacks the 21-amino acid transmembrane death domain; it is produced either by alternative splicing or by cleavage of membrane-bound FAS isoforms by metalloproteinases (6-9). Although able to bind with FASL, sFAS cannot initiate apoptosis within the cell. Therefore, sFAS has been proposed as an inhibitory factor of FAS-mediated apoptosis (6, 7).

The FAS–FASL system is known to be implicated in the maintenance of the immune–privileged status of the testes (10). FASL is expressed by Sertolli cells in order to eliminate activated T-cells which express FAS. In this way, gametes are protected from rejection reactions triggered by the immune system. Furthermore, the FAS–FASL system in the testes seems to mediate gamete apoptosis. Spermatogenesis is characterized by a vast and continuous proliferation of germ cells and a subsequent maturation from spermatogonia, which are diploid cells, to haploid spermatozoa. During this process, a large proportion of gametes undergo apoptosis. Apoptosis in the testes is a physiological function aiming to eliminate abnormal gametes, as well as to reduce the population of gametes in order to match the capacity of Sertolli cells which support their maturation. It has been shown that a number of gametes express FAS, which by binding to FASL expressed by Sertolli cells activates apoptosis (10).

Although the FAS/FASL system is recognized as one of the most important factors regulating germ cell death, the status of this system in patients with infertility have not been adequately studied. In this study, sFAS and sFASL were measured in the seminal plasma from infertile men and the association of sFAS and FASL with parameters of basic semen analysis was explored in order to determine the FAS/FASL status in cases of defective spermatogenesis.

Patients and Methods

Seventy-two men were included in this study (age: 38.76±9.06 years). Semen samples were obtained by masturbation after 3-4 days
of abstinence. Informed consent was granted by all study participants. The study was performed in compliance with institutional guidelines approved by the Institutional Review Board of the University Hospital of Alexandroupolis and in accordance with the Declaration of Helsinki. Basic semen analysis was performed according to the WHO Laboratory Manual for the Examination and Processing of Human Semen (11).

Within the first hour after ejaculation, an aliquot from each liquefied semen sample was mildly centrifuged and the seminal plasma was stored at –20˚C until further analysis.

sFAS and sFASL were measured in seminal plasma using commercially available immunoassay kits: sFAS: BE51901, IBL, Hamburg, Germany; sFASL: BE51921, IBL. Taking into consideration that neither kit was validated for seminal plasma samples, we performed validation tests in order to be assured that the kits accurately quantify sFAS and sFASL in seminal plasma. The results showed dose-response curves that were parallel to the standard curves obtained using the recombinant sFAS and sFASL. The recovery rates for sFAS were 98-111% and for sFASL were 84-119%.

Statistical analysis was performed using the STATISTICA 8.0 software (StatSoft, Tulsa, OK, USA). Comparisons among groups were evaluated with two non-parametric tests: Mann-Whitney U-test and Kolmogorov-Smirnov’s test. Correlations were evaluated with the Spearman’s rank test. The two-tailed significance level was set at p<0.05.

Results

Among the 72 semen samples, 24 were considered normal, having values higher than the lower reference limits proposed by WHO (11). Within the abnormal semen samples, six were oligozoospermic, six asthenozoospermic, one teratozoospermic, five oligoasthenoteratozoospermic, one ashenoasteratozoospermic, fourteen oligoastheno-zoospermic, two oliogoterozoospermic, sixazoospermic and seven with low vitality.

sFAS and sFASL were detected in all semen samples. The results from basic semen analysis, as well as the measurements of sFAS and sFASL, are presented in Table I. sFAS and sFASL measurements in abnormal semen samples were slightly higher than in normal samples, but the difference was not statistically significant.

Correlation analysis showed that, in both normal and abnormal semen samples, sFAS correlated negatively with pH (Table II). In normal semen samples, sFAS was strongly positively correlated with sperm concentration. In abnormal samples, sFAS concentrations were strongly correlated with those of sFASL (Spearman R=0.40; p<0.001).

Discussion

The FAS system is considered to be crucial both in the control of germ cell number in the testes and in the preservation of the immunoprivileged status of testicular in vivo 27: 285-288 (2013)
tissue. Cells expressing FASL induce apoptosis of cells expressing FAS. On the other hand, sFAS blocks apoptosis mediated by cells expressing FASL. Seminal plasma contains both sFAS and sFASL (12, 13). sFASL is produced by proteolytic cleavage of membrane-bound FASL. Matrix metalloproteinase-7 seems to play a key role in the production of sFASL in seminal plasma (13).

In this study, both sFAS and sFASL were detected in all semen samples. Other investigators have failed to detect sFASL in seminal plasma (12). This discrepancy could be due to the different immunoassays used.

Previous studies have also tried to explore the association between the levels of sFAS and sFASL in seminal plasma with sperm parameters. Fujisawa and Ishikawa (12) reported a strong correlation between sFAS levels and sperm concentration in cases with oligozoospermia with varicocele, before and after varicocelectomy. However, they did not find such a relationship in oligozoospermia without varicocele or in men with normal semen. In contrast, in our study, we found a strong positive correlation between sFAS and sperm concentration only in normal semen samples. Riccioli et al. (13) reported higher levels of sFASL in azoospermic samples compared to normal ones. In our study, the levels of sFASL were slightly higher in abnormal than in normal samples but the difference was not statistically significant. Similarly, in the subset of azoospermic samples, the levels of sFAS were also higher (1.54±1.68 pg/ml) than in normal samples, but the difference was not statistically significant (p=0.35).

Statistical analysis showed strong negative correlations between sFAS and pH, both in normal and abnormal samples. The pH ranged between 7.7 and 8.6 in normal samples and 7.4 and 8.6 in abnormal samples. This possibly indicates that pH values closer to neutral favor the proteolytic cleavage of membrane-bound FAS.

In both in normal and abnormal samples, we also found a strong negative correlation between sFAS and time-to-liquefaction. As the separation of seminal plasma took place after liquefaction, this correlation possibly indicates the activity of proteolytic enzymes that degraded sFAS.

In conclusion, the present study showed that both factors of the FAS system are present in seminal plasma in their soluble forms without statistical significant differences between normal and abnormal semen samples. In normal samples, sperm concentration and time-to-liquefaction significantly influence the levels of sFAS. In normal as well as in abnormal samples, alkaline pH is associated with a lower levels of sFAS. Continuation of the investigation of sFAS and sFASL in a larger number of abnormal semen samples may clarify the discrepancies between the present study and previous ones and may reveal new insights for the role of the FAS system in semen pathophysiology.

References

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