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The content of four immunomodulatory steroids and major androgens in human semen☆

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Abstract

Seminal fluid fulfils a dual role: it provides optimal conditions for fertilization and protects male germ cells from infections. Besides both major sexual hormones and cortisol it contains a considerable amounts of dehydroepiandrosterone (DHEA), known to counteract the excessive actions of glucocorticoids. From this point of view of importance may be our recent finding of both 7-hydroxy-dehydroepiandrosterone epimers (7-OH-DHEA) in semen, believed to be in some instances the locally active immunoprotective agents. The concentrations of these steroids were of the same range or even higher than in blood. Here further data on 7-OH-DHEA in semen, along with other relevant steroid hormones, are given in 79 samples, either from healthy males or from patients with various sexual disorders. A method has been developed enabling us a simultaneous determination of DHEA, 7-OH-DHEA epimers, testosterone, dihydrotestosterone and cortisol in seminal fluid. It was based on ether extraction, solvent partition and HPLC separation, followed by specific radioimmunoassays in the respective fractions. In addition, the steroids were measured in serum and the concentrations in both fluids were compared. The concentrations of 7-OH-DHEA in seminal fluid varied from 1.8 to 15.7 nmol/l, while those of DHEA were about five times higher.

Keywords: Human semen; Serum; Dehydroepiandrosterone metabolites; Androgens; Immune environment

1. Introduction

From the immunological point of view seminal fluid fulfils a dual role: it provides optimal conditions for fertilization including a break of the ovum membrane and suppressing the host immune response and, at the same time, protects vulnerable male germ cells from infection [1–3]. These requirements are enabled by an interplay of biologically active factors present in semen, as cytokines, prostaglandins, various peptide hormones, other peptides, enzymes, transport proteins and also steroids. In addition, both spermatozoa and seminal leukocytes generate reactive oxygen species (ROS), which are necessary for key biological events such as acrosome reaction and hyperactive sperm motility [4,5].

As mentioned above, seminal fluid contains various hormonal steroids and their precursors or metabolites, together with their major regulatory pituitary hormones [6]. Most studies addressing this topics dealt with androgens and to a less extent with estrogens, in relation to pathology of the male reproductive system, such as sperm abnormalities, disorders of sexual function and fertility. The recent survey of the literature on steroids in semen and their origin is given elsewhere [7].

With respect to immunomodulatory and immunoprotective effects of dehydroepiandrosterone (DHEA), believed to act at least in some instances as antiglucocorticoid at non-genomic level [8], the detection of DHEA and its precursor, DHEA sulfate, in seminal fluid [9–12] together with cortisol [13,14], may be of particular interest. Recent studies in vitro as well as in vivo demonstrated that not only DHEA itself, but some of its 7-hydroxylated metabolites, formed in various tissues and cell types and until recently believed to lack any biological activity, are more potent in enhancing immune response and counteracting immunosuppressive effects of glucocorticoids than parent steroid, and are considered local immunomodulatory agents [15–20]. The literature on this topics have been reviewed [21,22].

We have detected both 7-hydroxy-DHEA isomers in human semen [23]. Here, further data on these steroids together with its precursors, cortisol and the major androgens in human ejaculates are presented. Steroid concentrations in semen were compared with the corresponding levels in plasma.

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2. Materials and methods

2.1. Steroid and chemicals

7α-OH-DHEA and its 7β-isomer were purchased from Steraloids (Newport, RI, USA), other steroid standards and chemicals were from Sigma–Aldrich (Czech Division, Praha), the solvents for high performance liquid chromatography (HPLC) were from Merck (Darmstadt, FRG). Radioactive steroids, [1,2,6,7-^{3}H]cortisol, [1,2,6,7-^{3}H]DHEA, specific radioactivities 1.95 and 2.05 TBq/mmol, respectively, and carrier free [Na^{125}I] were from Radiochemical Center, Amersham (UK). [^{3}H]11β-hydroxyandrostendione was prepared by oxidation of [^{3}H]cortisol with sodium bismuthate [24].

2.2. Subjects and sample collection

A number of 79 semen and blood samples were obtained by masturbation from 40 men in the age of 19–53 years, who attended the Out-Patient Fertility Centre at the Clinic of Obstetric and Gynaecology, 1st Faculty of Medicine, Charles University, Prague. With one exception, two semen samples were obtained from each subject within 1-month period, either without, or after 3–5 days sexual abstinence. In the subject group there were 26 healthy men from infertile couples where the fertility failure was at the female partner side. Remaining 14 men were the patients with various sexual disorders (decreased sexual apetency, erectile dysfunction) or with spermiogram abnormalities. All subjects gave an informed consent with the use of their semen samples for research purposes. The Ethical Committee of the 1st Faculty of Medicine has approved the study. A conventional semen analysis was performed in the samples after liquefaction according to the recommendation of the World Health Organization. The seminal plasma was collected by separation of the cells by centrifugation at 2000 × g for 20 min, and it was stored frozen at −20 °C until analyzed. Blood was obtained by venipuncture of the cubital vein between 8 and 9 a.m. After centrifugation, the sera were collected, frozen and stored at −20 °C until analyzed.

2.3. Steroid analysis

Seminal plasma (1 ml), spiked with [^{3}H]11β-hydroxyandrostendione (40 000 dpm), was extracted at first with 4 ml and then 2 ml of diethyl ether. Separation of organic phase was done by freezing of the water phase in solid carbon dioxide. The combined ether extracts were evaporated to dryness and the dry residues were partitioned between methanol (2.5 ml), water (2 ml) and *n*-hexane (0.5 ml). Following mixing the upper phase was carefully sucked off with a Pasteur pipette and discarded, while the lower phase was evaporated in a speed-vac centrifuge. The dry residue was dissolved again in ethanol (1 ml), and in an aliquot (200 ml) the radioactivity of ^{3}H was measured to determine the losses during extraction and solvent partition, while the rest was evaporated again and subjected to high performance liquid chromatography (HPLC), as given further.

The samples were dissolved in methanol (70 ml), of which 25 ml corresponding to 286 ml of seminal plasma was injected into the HPLC system GILSON (Villiers le Bel, France). It consisted of a programmable pump 305 with a manometric module 805, a slave pump 306, dynamic mixer 811 C, autoinjector 234 and a programmable sample collector FC 203B. UV detector LCD 2082 and a column thermostat LCO 100 were from Ecom (Czech Republic). Reverse phase C18 column ET 250/4 NUCLEOSIL® 100-5 was from MACHEREY-NÄGEL (FRG). The software CSW APEX system (DataApex, CR) was used for processing and evaluation of the data. Elution was performed using binary gradient system consisting of methanol (A) and water–acetonitrile mixture (85:15 (v/v)) containing 100 mg/l ammonium hydrocarbonate (B). The temperature was kept constant at 40 °C, the flow rate was 1 ml/min, standards were detected at 205 nm. The following fractions were collected with the respective retention times (in minutes): (1) 7β-OH-DHEA (8.4–8.9), (2) cortisol (8.9–9.4), (3) 7α-OH-DHEA (9.4–9.9), (4) testosterone (14.0–14.5), (5) DHEA (14.7–15.2), and (6) dihydrotestosterone (15.2–15.8). No further losses during HPLC were recorded, as controlled by the chromatography of tritiated standards, namely 11β-hydroxyandrostenedione (co-eluted with 7α-OH-DHEA), DHEA and cortisol.

The solvent was evaporated and each fraction, following re-dissolving in the assay buffer, the respective steroids were determined by radioimmunoassay in duplicates as follows: 7α- and 7β-OH-DHEA by the recently described methods [25,26]. Testosterone, dihydrotestosterone (after oxidation of cross-reacting testosterone with potassium permanganate) and cortisol, respectively, were determined by the methods developed in the author's laboratory [27–29]. DHEA was determined by the commercial kit from IMMUNOTECH (France and Czech Republic). The results were corrected to losses during extraction and solvent partition by measuring the recovered [^{3}H]11β-hydroxyandrostenedione. Steroids in serum were determined by the same methods [25–29].

2.4. Statistical treatment of the data

The age dependence of the ratio 7α-OH-DHEA/7β-OH-DHEA in seminal plasma was evaluated using simple two-parameter linear regression. Due to non-Gaussian data distribution, the dependent variable was subjected to power transformation [30,31] to a minimum skewness of studentized residuals. To lower the number of influential points, the independent variable was transformed to minimum skewness as well. In both cases, the data with absolute studentized values grater than three were excluded from further calculations to eliminate an influence of severe univariate outliers on the shape of the data distribution. The points

excluded from the calculations of the model were retained in the figures for completeness. The calculations were performed using the software Statgraphics Plus, version 3.3 (Manugistics Inc., Rockville, MA, USA).

To evaluate the mutual correlations between the steroids, Pearson's correlations were used. To stabilize the variance, improve the data distribution towards Gaussian and to erect a simple monotonically curved relationship, the data were transformed by power transformations to minimum skewness in both dimensions [30]. The data with absolute studentized values grater than 3 were excluded from further calculations as the multivariate outliers detected with *F*-distributed Mahalanobis distance. Principal axes and 95% confidence ellipsoids were computed using the software Excel 2000 and the computing method published elsewhere [32]. The results obtained were retransformed to the original scale and plotted.

3. Results

3.1. Steroid concentrations in blood serum and in seminal plasma

Table 1 summarizes the basic statistical data (means, medians, upper and lower quartiles) on the concentrations of studied steroids in blood serum and seminal plasma, along with the semen volumes and sperm counts, from 26 healthy men and 14 patients with various sexual and sperm disorders. Since, with the exception of ejaculate volume and sperm count in the control group, there was no difference between the results obtained without and after sexual abstinence, only the former data are presented. The concentrations of cortisol, DHEA, 7α-OH-DHEA, 7β-OH-DHEA, testosterone and dihydrotestosterone are shown in the right part of the table, while the corresponding levels in blood (without dihydrotestosterone but with DHEAS) are given in the left part. The only significant difference between control subjects and patients in the seminal plasma was found in testosterone concentration, in contrast to serum levels, where no significant differences were revealed.

3.2. Correlation studies of blood serum

All the data (without, as well as after sexual abstinence) were included in the calculations. Mutual correlations of the serum levels of six steroids investigated are shown in the Table 2. The cells above and below the diagonal represent simple pair and partial correlations (with adjustment to constant levels of other variables except the studied pair). The values in upper, middle and lower part of each cell show the correlation coefficients, number of pairs and the levels of significance, respectively. Grey-highlighted cells show the significant correlations ($P < 0.05$). A significant positive correlations revealed by both methods were found between two steroids of mainly adrenal origin, i.e. cortisol and DHEA, 7-OH-DHEA isomers, and between both 7-OH-DHEA isomers and their precursor, DHEA, while a negative correlation was found between 7α-OH-DHEA and testosterone. A positive correlation between DHEAS and unconjugated DHEA and its 7α-hydroxylated metabolite was revealed by a simple paired test only.

3.3. Correlation studies of seminal plasma

Mutual correlations of steroid concentrations in seminal plasma, together with the main semen characteristics, namely the semen volume and sperm count and also with age, are shown in Table 3. The values have the same meaning as in the Table 2. Positive correlations found by both methods were recorded between the pairs DHEA-7α-OH-DHEA, 7α-OH-DHEA-7β-OH-DHEA, 7β-OH-DHEA-testosterone and testosterone-dihydrotestosterone. A positive correlation was also found between DHEA and cortisol by a simple paired method. 7α-OH-DHEA (but not its 7β-isomer) negatively correlated by both methods with age, while a negative correlation of DHEA with age was found only with the simple pair method. A negative correlation found by both methods occurred also between DHEA and a semen volume, while a positive partial correlation was found between the latter parameter and 7α-OH-DHEA.

3.4. Correlation of the steroid concentrations in blood serum and in seminal plasma

The concentrations of five steroids (cortisol, DHEA, both 7-OH-DHEA isomers and testosterone) in blood serum and seminal plasma were mutually correlated. The correlation matrix is shown in Table 4. Significant correlations are highlighted. The only positive correlation between serum levels and concentration in seminal plasma was found in cortisol. On the other hand, positive correlations were found between serum DHEA and cortisol and 7α-OH-DHEA in the seminal plasma.

3.5. Dependence of 7α-OH-DHEA/7β-OH-DHEA ratio in the seminal plasma on age

As shown in the Table 3, 7α-OH-DHEA in the seminal plasma decreased significantly with age, while its 7β-isomer remained unchanged or even increased. Therefore, the ratio of both isomers was plotted as a function of age. As demonstrated on Fig. 1, a significant decrease of the ratio with age was recorded.

3.6. Correlation graphs

Figs. 2–7 show two-dimensional scatter plots of mutual relationships between the steroid pairs, either in seminal plasma where a significant correlation was found by both

Table 1
Summary statistics of the concentrations of steroids in serum and in seminal plasma

Status	Statistics	Volume of ejaculate (ml)	Concentration of sperm ((cells/ml) × 10^6)	Serum (nmol/l)						Ejaculate (nmol/l)					
				Cortisol	DHEA	DHEAS (mmol/l)	7α-OH-DHEA	7β-OH-DHEA	Testosterone	Cortisol	DHEA	7α-OH-DHEA	7β-OH-DHEA	Testosterone	DHT
Controls	Count	22	23	23	22	23	23	23	23	22	22	22	22	22	22
	Mean	1.56	38.5	574	24.3	6.29	1.41	1.23	14.9	56.6	23.4	1.67	1.45	0.91	2.47
	S.D.	0.78	23.3	144	10.3	2.48	0.77	0.6	3.72	20.3	10.9	0.66	0.67	0.4	0.79
	Median	1.4	30	557	24.4	6.17	1.35	1.09	14.2	61.9	22.8	1.54	1.35	0.85	2.5
	Lower quartile	1.1	20	483	16.4	4.01	0.72	0.8	12.1	39.4	14.7	1.24	0.95	0.67	1.88
	Upper quartile	2.15	60	695	33.6	7.35	1.77	1.69	17.8	71.6	25.8	2.17	1.92	1.09	3.31
Patients															
	Count	11	12	12	12	12	12	12	12	11	11	11	11	11	11
	Mean	1.97	33.6	519	21.8	7.16	1.26	1.15	16.9	48.4	18.7	1.57	1.11	0.66	2.2
	S.D.	1.08	33.2	131	9.1	1.92	0.9	0.51	5.93	16.3	8.3	1.04	0.52	0.26	0.36
	Median	2.15	24	492	21.3	6.72	0.87	1.08	16.4	44	17.1	1.51	0.9	0.51	2.23
	Lower quartile	1.1	7.5	407	15	6.05	0.66	0.72	11.7	39.2	11.3	0.63	0.76	0.48	2.03
	Upper quartile	2.85	55	638	30.3	8.62	1.61	1.42	22	50.8	25.5	2.04	1.5	0.78	2.46
Differences (patients − controls)*		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.04	NS

*($P<\ldots$) Student's *t*-test or Mann–Whitney test (in the case of severe non-homogenity in the data, or if the data distribution can not be improved by transformation to Gaussian).

Table 2
Correlations among age, serum cortisol, DHEA, DHEAS, 7α-OH-DHEA (DHEA7a), 7β-OH-DHEA (DHEA7b) and testosterone (T)

Age	-0.1437 73 0.2251	-0.1721 73 0.1455	-0.1892 73 0.1090	0.0187 73 0.8749	0.0604 72 0.6143	0.2818 74 0.0150
-0.1046 72 0.4108	**Cortisol**	0.3840 76 0.0006	0.1531 76 0.1866	0.2439 76 0.0337	0.1589 75 0.1734	-0.0298 77 0.7972
-0.1359 72 0.2843	0.3113 75 0.0103	**DHEA**	0.3597 76 0.0014	0.6958 76 0.0000	0.5546 75 0.0000	-0.2113 77 0.0651
-0.1463 72 0.2487	-0.0812 75 0.5136	0.2330 75 0.0578	**DHEAS**	0.2604 76 0.0231	0.2645 75 0.0218	-0.2228 77 0.0514
0.1938 72 0.1249	0.0626 75 0.6148	0.4408 75 0.0002	0.0674 75 0.5879	**DHEA7a**	0.6504 76 0.0000	-0.2654 77 0.0197
0.0315 71 0.8064	-0.0727 74 0.5618	0.2676 74 0.0298	0.0547 74 0.6627	0.3808 75 0.0015	**DHEA7b**	-0.1942 76 0.0927
0.2938 73 0.0175	0.1000 76 0.4172	0.0224 76 0.8561	-0.0636 76 0.6064	-0.2478 76 0.0416	0.0224 75 0.8572	**T**

The cells above and below the diagonal represent pair and partial correlations (adjusted for constant values of remaining variables except the pair evaluated). The numbers in upper, central and lower part of the cells represent correlation coefficients, number of subjects and significance levels, respectively. Data were transformed to minimum skewness and uni- and multivariate outliers were excluded prior correlation analysis (see statistical data treatment). Shaded cells highlight significant corrrelations.

simple paired and partial correlations, or between their concentrations in serum and in seminal plasma. In each graph, the principal axis reflecting the linearized correlation and a 95% confidence elipsoid are given. The left pannels (A) show the transformed, the right (B) untransformed data. Symbols *r*, *p* and *n* represent the correlation coefficients, the level of significance of the model, and the number of paired values, respectively. The list of figures: Fig. 2: DHEA versus 7α-OH-DHEA in seminal plasma, Fig. 3: serum DHEA versus 7α-OH-DHEA in seminal plasma, Fig. 4: 7α-OH-DHEA versus its 7β- isomer in seminal plasma, Fig. 5: serum cortisol versus cortisol in seminal plasma, Fig. 6: testosterone versus dihydrotestosterone in seminal plasma, Fig. 7: testosterone versus 7β-OH-DHEA in seminal plasma.

Table 3
Correlations among age, the concentrations of cortisol, DHEA, 7α-OH-DHEA (DHEA7a), 7β-OH-DHEA (DHEA7b) testosterone (T) and dihydrotestosterone (DHT) in seminal plasma

Age	-0.1354 75 0.2468	-0.2398 74 0.0396	-0.3246 75 0.0045	0.0680 75 0.5624	-0.1812 75 0.1198	0.1151 75 0.3255
-0.0072 75 0.9539	**Cortisol**	0.2756 78 0.0146	0.2004 79 0.0766	0.1186 79 0.2978	0.2334 79 0.0384	-0.1327 79 0.2436
0.0289 74 0.8178	0.0853 78 0.4826	**DHEA**	0.4593 78 0.0000	0.1773 78 0.1205	0.2973 78 0.0082	-0.0412 78 0.7202
-0.3810 75 0.0015	0.0683 79 0.5714	0.4046 78 0.0005	**DHEA7a**	0.4808 79 0.0000	0.5025 79 0.0000	0.2774 79 0.0133
0.3056 75 0.0119	0.0183 79 0.8796	-0.0944 78 0.4370	0.3987 79 0.0006	**DHEA7b**	0.4991 79 0.0000	0.4042 79 0.0002
-0.1696 75 0.1700	0.2093 79 0.0798	0.1333 78 0.2713	0.1326 79 0.2703	0.2567 79 0.0307	**T**	0.4725 79 0.0000
0.2367 75 0.0538	-0.2459 79 0.0387	-0.2127 78 0.0771	0.1864 79 0.1196	0.0966 79 0.4229	0.4093 79 0.0004	**DHT**

The cells above and below the diagonal represent pair and partial correlations (adjusted for constant values of remaining variables except the pair evaluated). The numbers in upper, central and lower part of the cells represent correlation coefficients, number of subjects and significance levels. Data were transformed to minimum skewness and uni- and multivariate outliers were excluded prior correlation analysis (see statistical data treatment). Shaded cells highlight significant corrrelations.

Table 4
Correlations between the concentrations cortisol, DHEA, 7α-OH-DHEA (DHEA7a), 7β-OH-DHEA (DHEA7b) and testosterone (T) in serum and in seminal plasma

		EJACULATE				
		Cortisol	**DHEA**	**DHEA7a**	**DHEA7b**	**T**
SERUM	**Cortisol**	**0.3344 77 0.0030**	-0.0277 76 0.8125	0.1172 77 0.3099	-0.0475 77 0.6819	0.0124 77 0.9149
	DHEA	0.2777 77 0.0145	**0.1038 76 0.3724**	0.3423 77 0.0023	0.0541 77 0.6405	0.0072 77 0.9501
	DHEA7a	0.0281 77 0.8086	-0.0920 76 0.4293	**0.2048 77 0.0739**	0.0444 77 0.7016	-0.0985 77 0.3941
	DHEA7b	0.1545 76 0.1828	-0.0820 75 0.4841	0.0967 76 0.4060	**-0.0545 76 0.6402**	-0.0939 76 0.4198
	T	0.1266 78 0.2693	0.0402 77 0.7285	-0.1432 78 0.2109	0.1196 78 0.2970	**-0.0826 78 0.4723**

The cells above and below the diagonal represent pair and partial correlations (adjusted for constant values of remaining variables except the pair evaluated). The numbers in upper, central and lower part of the cells represent correlation coefficients, number of subjects and significance levels. Data were transformed to minimum skewness and uni- and multivariate outliers were excluded prior correlation analysis (see statistical data treatment). Shaded cells highlight significant corrrelations.

4. Discussion

For the first time 7α-OH-DHEA and its 7β-isomer, together with other immunomodulatory steroids and with major androgens, were determined at the same time in seminal plasma and blood in a representative group of men.

The studied group was recruited from healthy men as well as from patients with various sexual disorders. With exception of testosterone in seminal plasma, no differences have been recorded between patients and healthy subjects, but it should be taken into account that the patient's group was not homogenous and more data are needed from well defined patient groups. With respect to immunomodulatory properties of 7-OH-DHEA, the patients where immune disorders are suspected for impairment of sexual function will be the first at stake. It should be mentioned, however, that even in this small patient's group the concentrations of testosterone in

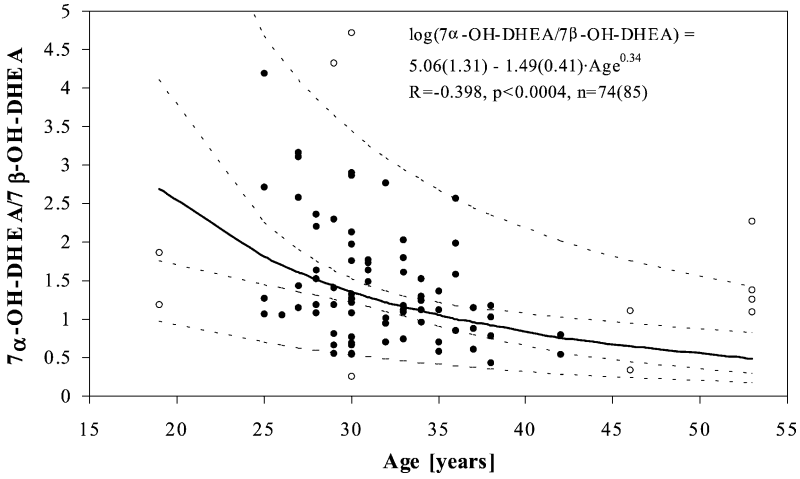

Fig. 1. Age dependence of 7α-OH-DHEA/7β-OH-DHEA ratio in 85 samples of semen plasma from 40 men in the age of 19–53 years. Due to non-Gaussian data distribution, the dependent variable was transformed to minimum skewness of studentized residuals. To lower the number of influential points, the independent variable was transformed as well to its minimum skewness. Regression curve obtained from the transformed data (full line) and its 95% confidence intervals (dashed lines close to regression line) and further 95% confidence intervals of predictions including theoretically 95% of the experimental points were retransformed to the original scale. Both parameters of the regression were significant as confirmed by *t*-tests. *R* represents correlation coefficient of linear regression, *P* is the significance level of the model and *n* is the number of points included in the calculation of the regression parameters. The number in parentheses is the number of all samples including outliers and influential points. Full circles represent the samples included in calculations while the empty circles depict the outliers and high leverage points.

semen were lower than in healthy subjects, while the blood serum levels were undistinguishable.

The analyzed material included samples obtained from men without as well as after 3–5 days lasting sexual abstinence. The only difference between those samples occurred in the ejaculate volume, but not in steroid concentration. Therefore all the data were used for correlation studies.

Though all the subject were adult men in the age covering approximately three decades, there was a clear tendency to an increase of 7β-OH-DHEA to the detriment of

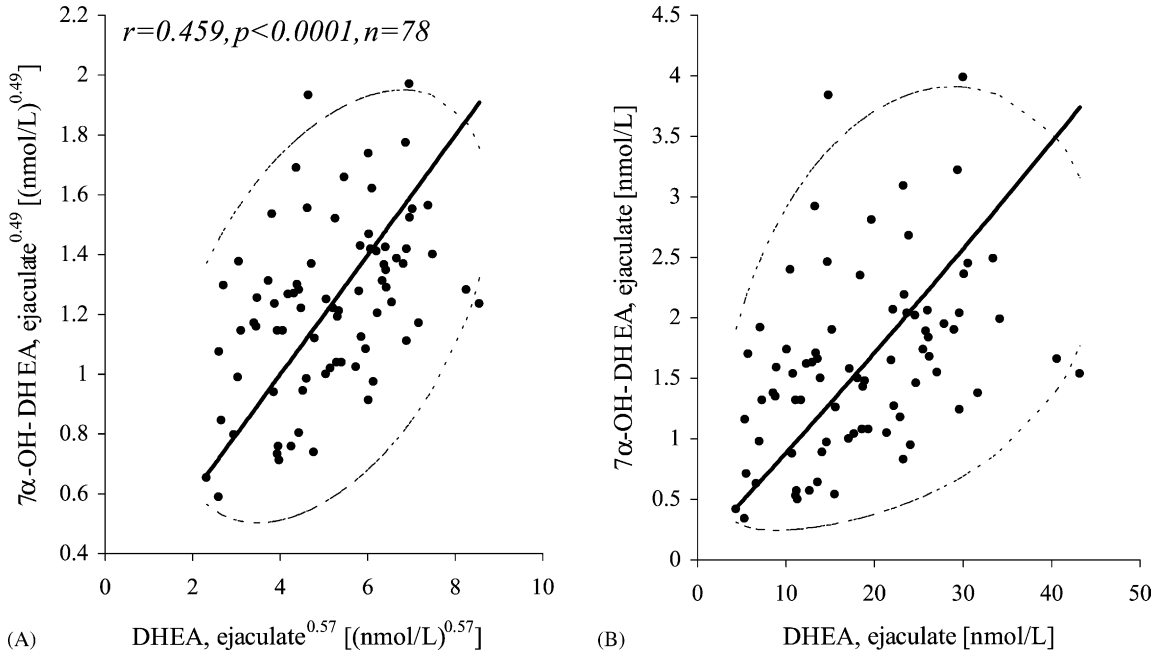

Fig. 2. Correlation between concentration of 7α-OH-DHEA and DHEA in seminal plasma. Due to non-Gaussian distribution, the data in both dimensions underwent the power transformation to minimum skewness. After transformation and elimination of uni- and multivariate outliers, the principal axis (full line) and 95% confidence ellipsoid (dashed line) were calculated and the results obtained were retransformed to the original scale. Symbol *r* represent Pearson's correlation coefficient, *P* is the level of statistical significance of the correlation and *n* is the number of samples. (A) Transformed values; (B) untransformed values.

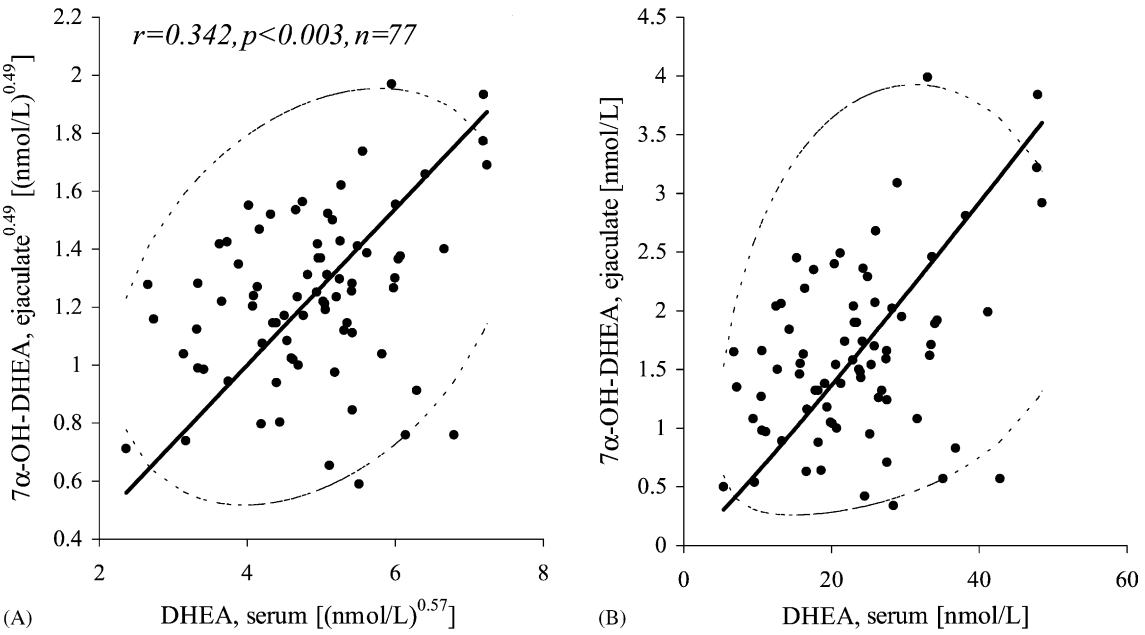

Fig. 3. Correlation between concentration of 7α-OH-DHEA in seminal plasma and serum levels of DHEA. The meaning of the symbols is the same as in the Fig. 2.

its 7α-isomer. The same phenomenon was observed in blood plasma when large population groups were investigated [33]. This points to the changes in the 7-hydroxylating enzyme entities in the life regardless their localization. On the base of correlation analysis, some conclusions can be done concerning the origin of steroids present in semen.

As no correlation was found between 7α- and 7β-OH-DHEA concentrations in semen and in blood, it indicates that these steroids are formed in situ in the male reproductive system (testes and accessory sexual glands) rather than being transported from blood circulation. Indeed, 7α- as well as 7β-hydroxylation of DHEA and other androgens in the human testis, epididymis and also prostate have been reported as early as in 70s [34,35].

The substrate for 7α-OH-DHEA is DHEA, as demonstrated by a correlation of the former with DHEA in seminal

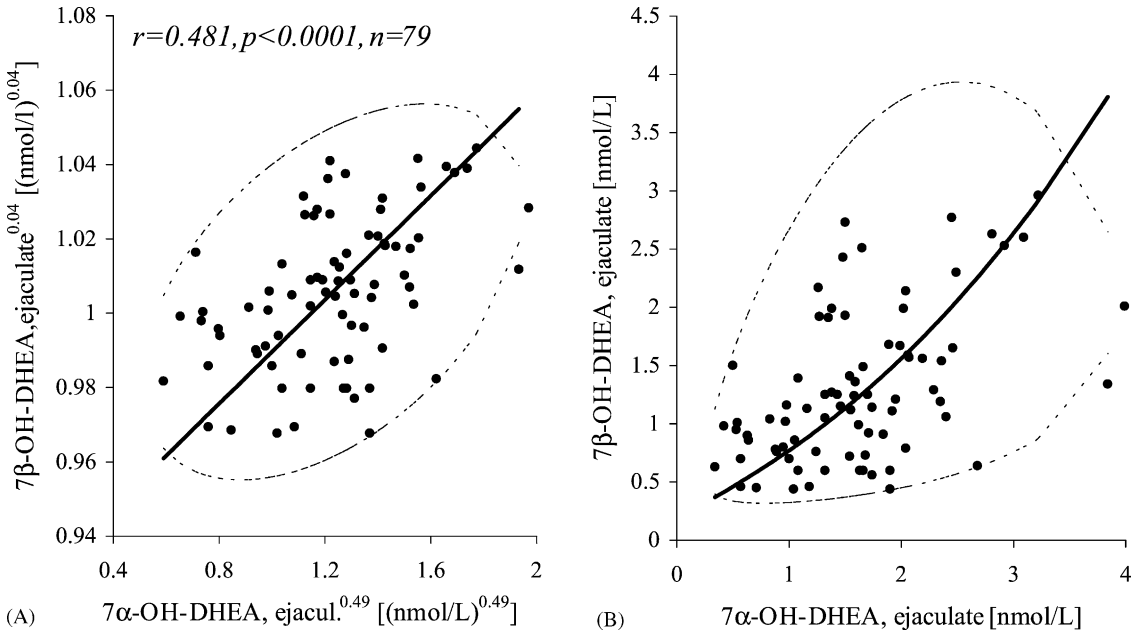

Fig. 4. Correlation between concentrations of 7β- and 7α-OH-DHEA in seminal plasma. The meaning of the symbols is the same as in the Fig. 2.

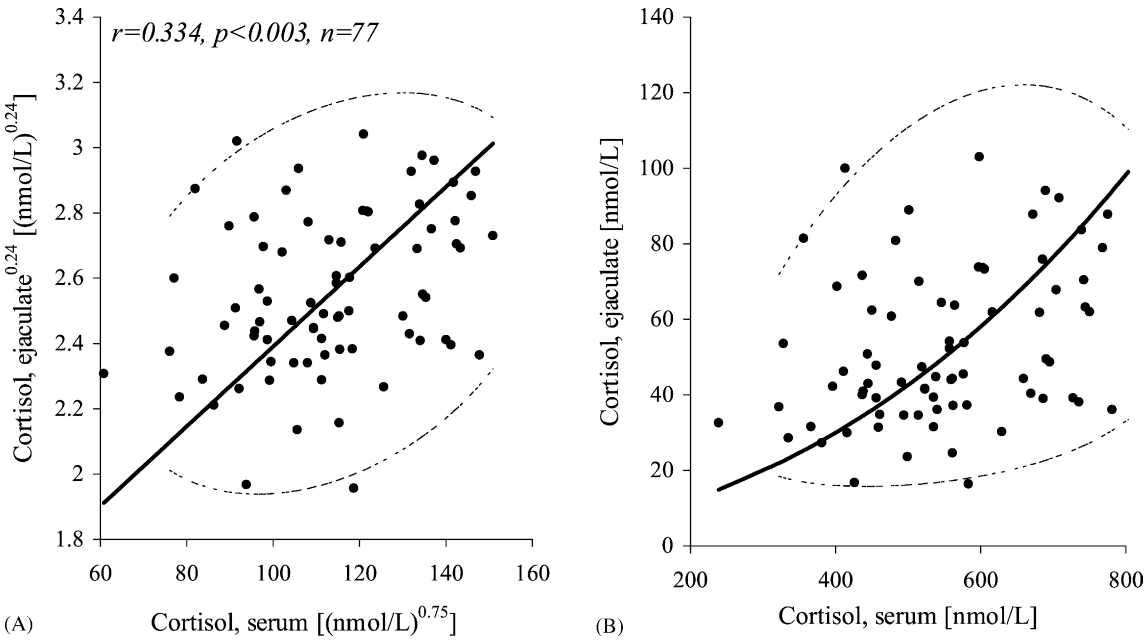

Fig. 5. Correlation between concentrations of cortisol in seminal plasma and in the serum. The meaning of the symbols is the same as in the Fig. 2.

plasma as well as in blood serum. The lack of correlation between serum and seminal DHEA may be explained by a different metabolism in each compartment.

The tight correlation between both 7-OH-DHEA isomers but the poor correlation of 7β-OH-DHEA with DHEA in seminal plasma (in contrast to blood) indicates that at least some portion of 7β-OH-DHEA is formed from its 7α-isomer, probably via 7-oxo-DHEA. We will attempt to detect and quantify this metabolite in semen to confirm this hypothesis.

Cortisol is the only steroid the serum levels of which correlated with its concentrations in seminal plasma, though the latter were almost 10 times lower. It indicates that it enters the testis from blood circulation.

Our results confirmed previous findings of other authors (see e.g. [6] and the literature reviewed in [7]) that the main

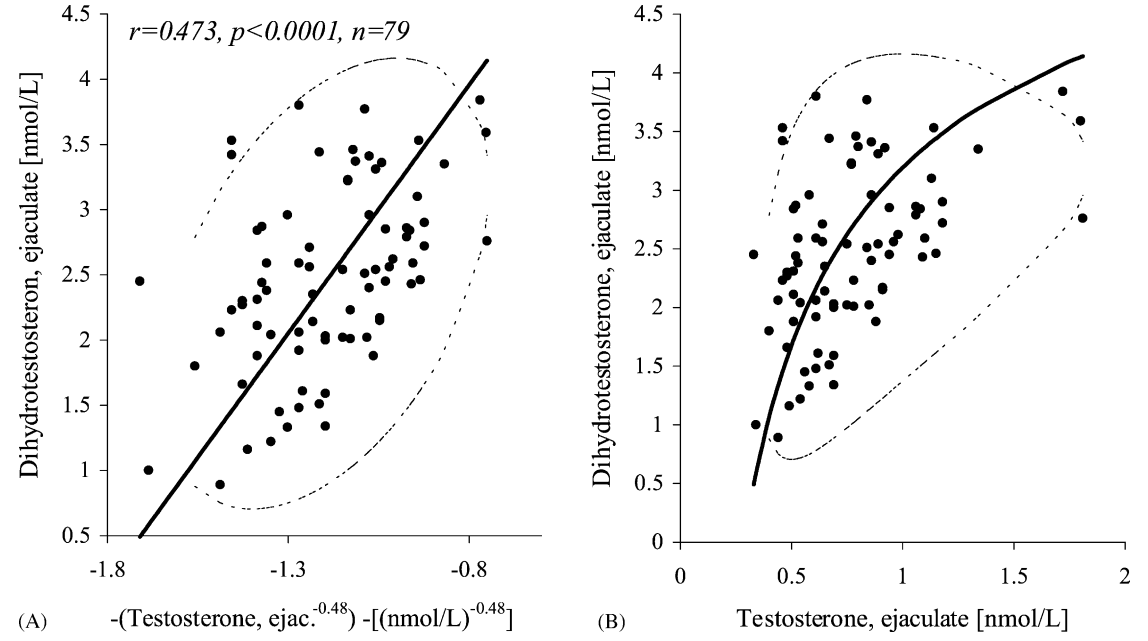

Fig. 6. Correlation between concentrations of dihydrotestosterone and testosterone in seminal plasma. The meaning of the symbols is the same as in the Fig. 2.

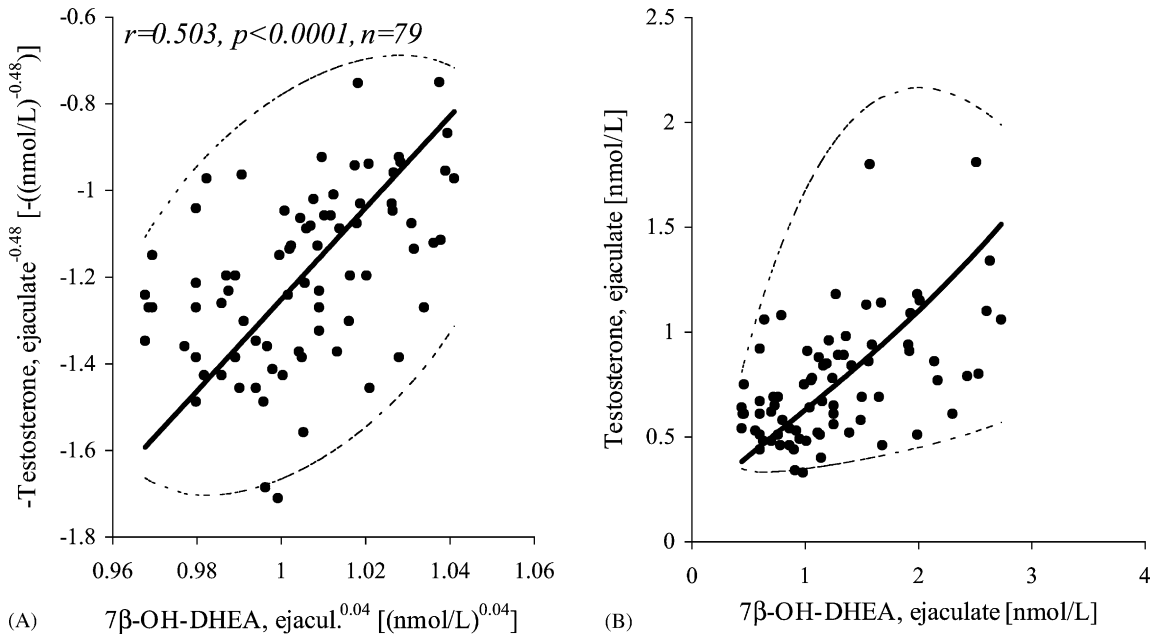

Fig. 7. Correlation between concentrations of testosterone and 7β-OH-DHEA in seminal plasma. The meaning of the symbols is the same as in the Fig. 2.

androgen in seminal fluid is dihydrotestosterone. An excellent correlation between both androgens in seminal plasma but no correlation of serum and seminal testosterone indicates that seminal dihydrotestosterone is formed in the testis and accessory sexual glands by action of local 5α-reductase.

We are not able to explain a significant correlation between testosterone and 7β-OH-DHEA in semen. This points only to the fact that 7β-hydroxylating activity differs from the 7α- one as to their substrate specificity and the genes encoding for the respective enzymes [22].

In an experiment with human tonsils obtained from patients immunized with tetanus oxoid, Morfin and his group [18,22] showed that tonsillar stromal cells, but not T- and B-cells, produced increased amounts of 7α-OH-DHEA when exposed to the antigen. The latter steroid served then as a signal for neighboring T- and B-cells for production of specific IgGs. The authors concluded that by this paracrine mechanism some of the immunomodulatory and antiglucocorticoid actions of locally produced 7-hydroxylated DHEA metabolites may be explained.

Since human semen contains B- and T-cells and their subpopulations [36,37], the next question is whether, and if so, how, do these steroids influence function of immune cells in the male reproductive tract and, especially, whether they are capable to counteract the effect of cortisol, which is present in semen in approximately 50-fold excess. It should be mentioned here that antiglucocorticoid properties of 7-hydroxylated DHEA metabolites are not based on their action on glucocorticoid receptor [21,22] and the ratio cortisol/7-hydroxylated DHEA metabolites in seminal fluid is about 10 times higher than in blood. That means that immunoprotective effect of 7-hydroxylated DHEA metabolites should be considered.

Another question to be addressed is the potential effect of 7-hydroxylated DHEA metabolites on the female reproductive tract. Tremellen et al. [38] demonstrated in mating mice the stimulatory effect of TGF-β from the ejaculate on the inflammation-like reaction in the uterine endometrium. DHEA is known to increase the production of various cytokines including TGF-β in various cell types [39–42]; for the complete survey of the literature see [22]. No report on 7-hydroxysteroid effects in the models investigated so far is available yet.

The mapping of the concentrations of 7-hydroxylated metabolites of DHEA along with other relevant steroids in seminal fluid presented here is the first step to answering these questions.

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