STEROID HORMONES IN HUMAN SEMEN WITH PARTICULAR RESPECT TO DEHYDROEPIANDROSTERONE AND ITS IMMUNOMODULATORY METABOLITES

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Objective. In addition to many components, seminal fluid contains also hormonal steroids. So far, the studies were focused mostly to androgens, their precursors, estrogens and also cortisol, in most instances in relation to sperm abnormalities. Our group detected for the first time in the ejaculate 7-hydroxylated metabolites of dehydroepiandrosterone, believed to function as immunomodulatory and immunoprotective agents. Occurrence of steroids in seminal fluid and their possible role is reviewed in the first part of the article.

Methods. In a group of 34 men (age 21-46 years) **with various problems of fertility** the presence of 7-hydroxydehydroepiandrosterone isomers and dehydroepiandrosterone in seminal fluid was found.. The concentrations of steroids in seminal fluid and plasma were determined by recently developed radioimmunoassay. In brief, the ejaculate was extracted twice with diethyl ether and the water phase was separated by freezing in solid carbon dioxide and evaporating the solvent to dryness. The dry residue was dissolved in methanol:water:light petroleum ether (1.3:1.0:0.3 ml) and mixed. After separation the water-methanolic phase was evaporated, the dry residue was re-dissolved in phosphate buffer and aliquots were used for RIA. The results were corrected for losses by counting the residual radioactivity of [^{3}H] testosterone added initially to the sample of seminal fluid.

Results. The concentrations of 7-hydroxydehydroepiandrosterone varied from 1.08 to as much as 15.7 nmol/l, while those of dehydroepiandrosterone were about five times higher. The concentrations of both 7-hydroxydehydroepiandrosterone isomers were close to or even higher than recently reported levels of these substances in blood serum.

Conclusion. The presence of steroids derived from dehydroepiandrosterone by 7-hydroxylation with recently reported immunomodulatory activity was for the first time demonstrated in seminal fluid of normal men**.**

Key words: Seminal fluid – Steroid hormones – 7-Hydroxydehydroepiandrosterone – Dehydroepiandrosterone –Immunomodulatory activity

Introduction

Seminal fluid is a complex milieu surrounding spermatozoa. To its composition contribute testicular tissue, epididymis, seminal vesicles and prostate. Semen fluid fulfils a dual role: it provides optimal conditions for fertilization including a break of the zona pellucida of the oocyte and suppressing the host immune response and, at the same time, protects vulnerable spermatozoa from infect. These, often contradictory requirements are enabled by interplay of biologically active factors and mechanisms operating in semen.

From the immunological point of view of particular importance are cytokines released by various cell subsets in the male urogenital tract including spermatozoa and seminal leukocytes. Both TH1 (e.g. IL-12) and TH2 (e.g. IL-10) lymfocytes subpopulations

producing interleukins and their receptors have been found in the ejaculate (Within 1988; Gruschwitz et al. 1996; Dousset et al. 1997; Kelly et al. 1997), along with a number of other compounds such as prostaglandins, peptide hormones, growth factors, enzymes, transport proteins and also steroids (Asch et al. 1984). High concentrations of prostaglandins can affect a cytokine-mediated switch away from a cell-mediated immune response, resulting in a state of non-responsiveness to sperm antigens in the female reproductive tract (Kelly and Critchley 1997)

In addition, both spermatozoa and leukocytes in semen generate reactive oxygen species (ROS), which are necessary for key biological events such as acrosome reaction and hyperactive sperm motility (Depuydt et al. 1996, Sharma and Agarwal 1996). In consistence with their biological role the short half-life of these compounds is enabled by concert action of the enzymes responsible for their formation and scavenging (Sharma and Agarwal 1996). The excessive generation of ROS, usually associated with infections of male urogenital tract (Ochsendorf 1998; 1999), may result in an oxidative stress, known to be one of the causes of male infertility (Aitken and Fischer 1994; Aitken 1994;1995; Henkel et al. 1997; Conte et al. 1999; Pasqualotto et al. 2000).

Hormonal steroids in seminal plasma. As mentioned already, seminal fluid contains various hormonal steroids and their precursors or metabolites, together with their major regulatory pituitary hormones (Asch et al. 1984). Most studies addressing these topics dealt with androgens and to a less extent with estrogens, in relation to pathological markers of the male reproductive system, such as sperm abnormalities, disorders of sexual function and fertility (Asch et al. 1984); Shirai et al. 1975; Purvis et al. 1975; DeAloysio et al. 1978; Pazzagli et al. 1979; Castaneda-Pena et al. 1979; Le Lannou et al. 1980; Moreno-Escallo et al. 1982; Hudson et al. 1983; Ando et al. 1983; Kurniawan et al. 1983; Garcia Diez et al. 1983; Facchinetti et al. 1987; Singer et al. 1987; Kwan et al. 1992; Bujan et al. 1993; Zalata et al. 1995; Laudat et al. 1998).

Since there is no recent survey of biologically active steroids in human semen in the literature, we add a table of representative digest of the literature on this topics (Table 1).

The origin of steroid hormones in human seminal plasma. The main source of androgens (testosterone, dihydrotestosterone) and their direct precursors (androstenedione, 17α-hydroxyprogesterone and apparently also pregnenolone and dehydroepiandrosterone) is the testis, namely Leydig cells. Rete testis fluid carrying testicular sperm into the epididymis is rich in dehydroepiandrosterone and testosterone, while plasma from cauda epididymis surrounding sperm, which have completed maturation, contains high levels of progesterone, dihydrotestosterone, testosterone, 5-androstene-3β,17β-?diol, and dehydroepiandosterone. Substantial amount of steroids enter the epididymis in rete testis fluid, but little is found in plasma from cauda epididymis. The balance presumably is the result of absorption of steroids by epithelium in the proximal caput epididymis and of the 5α-reduction of testosterone to dihydrotestosterone. Thus the prevailing androgen in seminal plasma fluid is dihydrotestosterone (Ganjam and Amann 1976). It is also the only sexual steroid hormone, which differs so far in its concentration that it can be of value in differentiating secretory and excretory azoospermia (Garcia Diez et al. 1983). Of special interest are the experiments using reverse-isotope dilution technique that brought evidence for metabolic transformation of pregnenolone via dehydroepiandrosterone to testosterone (Gunasegaram et al. 1997) in sperm.

Relatively high (in μmol/l) are the concentrations of dehydroepiandrosterone sulphate followed by pregnenolone sulphate and dihydrotestosterone sulphate (Purvis et al. 1976; Steeno et al. 1966; Ying et al. 1983). DHEAS is quantitatively the most important steroid present in seminal plasma. It is at least partially of adrenal origin (Steeno et al. 1969). The concentration of dehydroepiandrosterone in the seminal plasma is not substantially reduced by vasectomy in contrast to dihydrotestosterone (40 % decrease) and testosterone (23 % decrease) and to lower degree also to estradiol, pregnenolone sulphate and dihydrotestosterone sulphate, which exhibited a smaller reduction following vasectomy (Ying et al. 1983).

Estrogens are normal constituents of seminal plasma and a substantial source of them is the secretory activity of prostate. The fundamental discovery of estrogens in seminal fluid was realised as early in 1969 in Prague Sexuological Institute (Raboch et al. 1969). There is an association of free estrogens, par-

Table 1
Survey of steroids found in human semen

Steroid	Reference
Testosterone	Asch et al. 1984; Shirai et al. 1975; Purvis et al. 1975; DeAloysio et al. 1978; Pazzagli et al. 1979; Castaneda-Pena et al. 1979; Le Lannou et al. 1980; Moreno-Escallo et al. 1982; Hudson et al. 1983; Ando et al. 1983; Kurniawan et al. 1983; Garcia Diez et al. 1983; Facchinetti et al. 1987; Singer et al. 1987; Kwan et al. 1992; Bujan et al. 1993; Zalata et al. 1995; Laudat et al. 1998).
Testosterone conjugates	Purvis et al. 1975;Purvis et al. 1976
Dihydrotestosterone	Asch et al. 1984; Shirai et al. 1975; Purvis et al. 1975; DeAloysio et al. 1978; Pazzagli et al. 1979; Castaneda-Pena et al. 1979; Le Lannou et al. 1980; Moreno-Escallo et al. 1982; Hudson et al. 1983; Ando et al. 1983; Kurniawan et al. 1983; Garcia Diez et al. 1983; Facchinetti et al. 1987; Singer et al. 1987; Kwan et al. 1992; Bujan et al. 1993; Zalata et al. 1995; Laudat et al. 1998; Purvis et al. 1976; Ying et al. 1983; Schwartz et al. 1997
Dihydrotestosterone sulfate	Purvis et al. 1976; Ying et al. 1983
Androstenedione	Asch et al. 1984; Purvis et al. 1975; DeAloysio et al. 1978; Facchinetti et al. 1987; Purvis et al. 1976
Dehydroepiandrosterone	Purvis et al. 1975; Hudson et al. 1983;Steeno et al. 1966
Dehydroepiandrosterone sulfate	Bujan et al. 1993; Purvis et al. 1976; Ying et al. 1983
5a-Androstene-3a,17b-diol and its 3b-isomer	Kurniawan et al. 1983; Facchinetti et al. 1987
Estradiol and its sulfate	Asch et al. 1984; Purvis et al. 1975; Garcia Diez et al. 1983; Singer et al. 1987; Bujan et al. 1993; Ying et al. 1983
Estrone and its sulfate	Ying et al. 1983; Santiemma et al. 1991
Progesterone	Garcia Diez et al. 1983; Loganath et al.1994
17a-Hydroxyprogesterone	Loganath et al.1994
Pregnenolone and its sulfate	Purvis et al. 1975; Purvis et al. 1976; Ying et al. 1983
Cortisol	Abbaticchio et al. 1981; Brotherton 1990 Loganath et al.1994
5a-Androstan-16-en-3a-ol and its 3b-isomer	Kwan et al. 1992
5,16-Androstadien-3b-ol and its 4-ene- isomer	Kwan et al. 1992

ticularly of estradiol with spermatozoa in the fractionated ejaculate (Dousset et al. 1997; Purvis et al. 1975; Gracia Diez et al. 1983; Singer et al. 1987; Bujan et al. 1993; Ying et al. 1983).

The concentration of cortisol in seminal plasma has been reported to be about 60 % to 10 % (Abbaticchio et al. 1981; Brotherton 1990) of random levels in blood serum. It is noteworthy that in human semen 11β-hydroxysteroid dehydrogenase is present, the enzyme that catalyses the conversion of biologically active glucocorticoids to their less active metabolites. Its association with sperm characteristics has been reported (Nacharaju et al. 1997). It suggests functional role for glucocorticoid exclusion in the sperm maturation process.

It has been concluded that a selective filter for steroid hormones exists between the systemic circulation and the male genital canal (Abbaticchio et al. 1981). The levels of all steroids so far detected in seminal plasma were significantly lower than the corresponding blood levels. The ratio of blood plasma/seminal plasma levels of the various steroids varies from nearly 40 (testosterone) to nearly 1.7 (dihydrotestosterone) (Purvis et al. 1975).

Possible action of steroid hormones in seminal plasma. With respect to immunomodulatory and immunoprotective effects of dehydroepiandrosterone (DHEA), believed to act at least in some instances as antiglucocorticoid at non-genomic level (Kalimi et al. 1994), the detection of DHEA and its precursor, DHEA sulphate, in seminal fluid is of particular interest (Purvis et al. 1975; Hudson et al. 1983; Bujan et al. 1993; Purvis et al. 1976; Steeno et al. 1966; Ying et al. 1983). In various cell systems and also in vivo DHEA influenced cytokine formation by lymphoid cells in favour to „anti-inflammatory" interleukins by antagonizing glucocorticoid induced decrease of IL-2 and increase of IL-4; for the literature see (Kalimi et al. 1994). DHEA may also influence ROS formation by its effect on peroxisomal enzymes and lipid peroxidation (Yamada et al. 1994; Aragno et al. 1994; Swierczynski et al 1996).

Recent reports demonstrated that not only DHEA itself, but some of its 7-hydroxylated metabolites, until recently believed to lack any biological activity, may act as locally active agents (Padgett and Loria 1994; Morfin and Courchay 1994; Hampl et al.; 1997 Lafaye et al. 1999; Chmielewski et al. 2000). Recently we have detected both 7-hydroxy-DHEA isomers in human semen (Hampl et al. 2000). Here, further data on these steroids in human ejaculates are presented.

Materials and Methods

Subjects and semen collecting. Semen was obtained by masturbation from 34 men (21-46 years), among them 26 were normal healthy men and 8 were patients with various sperm disorders attending the Out-Patient Fertility Centre, Clinic of Obstetric and Gynaecology, 1st Faculty of Medicine, Charles University, Prague, for fertility problems. 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. The samples after liquefaction were left frozen in solid carbon dioxide and stored frozen at –20 °C until analysed.

Determination of dehydroepiandrosterone and its 7-hydroxylated metabolites in semen. 7α- and 7β- OH-DHEA were determined by recently developed radioimmunoassays (Lapcik et al. 1998; 1999) as described in detail elsewhere (Hampl et al. 2000) with only exception that HPLC pre-purification was omitted. In brief the ejaculate (0.5 ml) was spiked with 20 000 dpm of ethanolic solution of purified [^{3}H] testosterone and extracted twice with diethyl ether (2.5 ml). Freezing in solid carbon dioxide separated the water phase and the solvent was evaporated to dryness. The dry residue was dissolved in methanol (1.3 ml), water (1 ml) and light petroleum ether (0.3 ml) and mixed. Following separation of the phases the upper organic phase was sucked off carefully with a Pasteur pipette and the water-methanolic phase was evaporated in speed-vac centrifuge. The dry residue was re-dissolved in 0.5 ml of buffer for RIA (20 mM Na-phosphate, pH 7.2 containing sodium azide and BSA, 0.1 g/100 ml each, in saline) and the solution was divided as follows: 4x 50 µl were taken for RIA of each 7-OH-DHEA isomer in duplicates, 100 µl for determination of unconjugated DHEA and remaining 100 µl were used for recovery by measuring [^{3}H] testosterone radioactivity by liquid scintillation counting. DHEA was measured by RIA kit from Immunotech (France). The results were corrected for losses during extraction and solvent partition.

Table 2
Concentrations of 7a-OH-DHEA, its 7b-isomer, and DHEA in randomly collected ejaculates from 34 men (in nmol/l)

Statistical parameter	7a-OH-DHEA	7b-OH-DHEA	DHEA
Mean	3.72	3.50	18.0
SD	1.67	2.69	10.9
Median	3.21	2.54	14.2
Maximum	7.88	15.7	48.5
Minimum	1.29	1.08	4.97

Results

Unconjugated DHEA along with 7α-OH-DHEA and its 7β- isomer were determined in a group of 34 randomly collected samples of ejaculate, including samples from healthy men from infertile couples where the cause was on the female side as well as patients with impaired sperm characteristics. The survey of the statistical parameters for 7-OH-DHEA and DHEA concentrations is given in Table 2. The scattered graph and box-and-whiskers graph showing the distribution of the individual values are shown on Figures 1 and 2, respectively. As demonstrated in Figures 3-5, a highly significant correlation has been found between both 7-OH-DHEA isomers, while much less tight, though still significant correlation was found between 7-OH-DHEA isomers and DHEA.

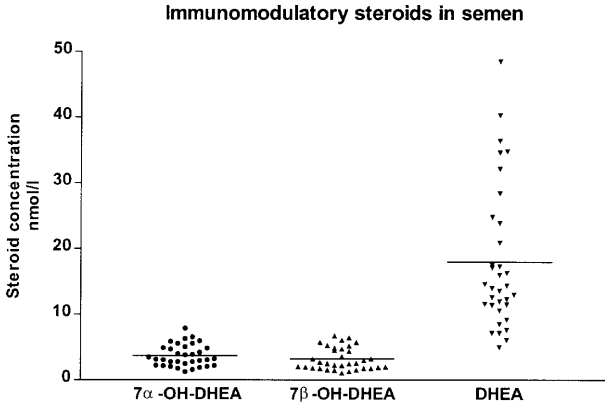

Fig 1 Scattered graph of concentrations of dehydroepiandrosterone and its 7-hydroxylated metabolites in human semen

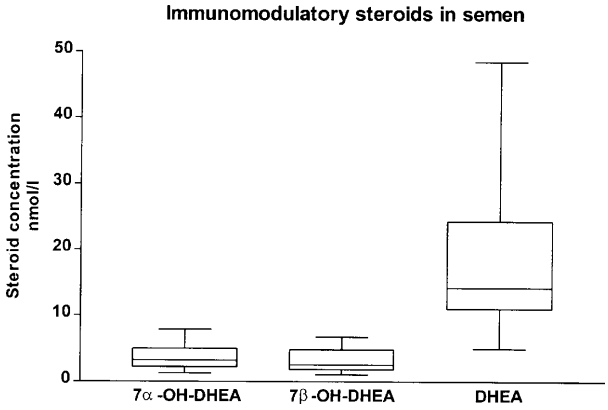

Fig 2 Box-and-whiskers graph of concentrations of dehydroepiandrosterone and its 7-hydroxylated metabolites in human semen

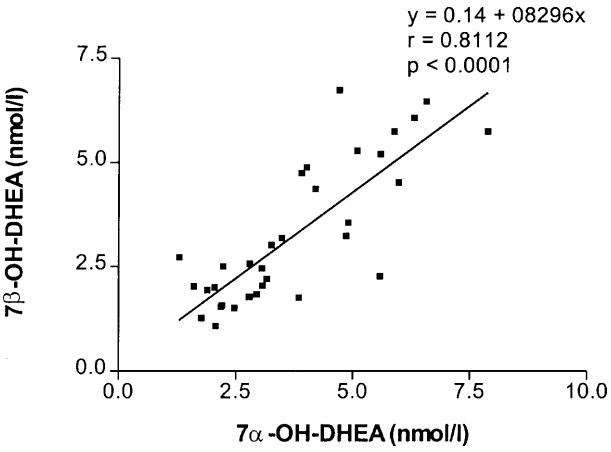

Fig 3 Correlation of 7a-OH-DHEA with its 7b-isomer in human semen

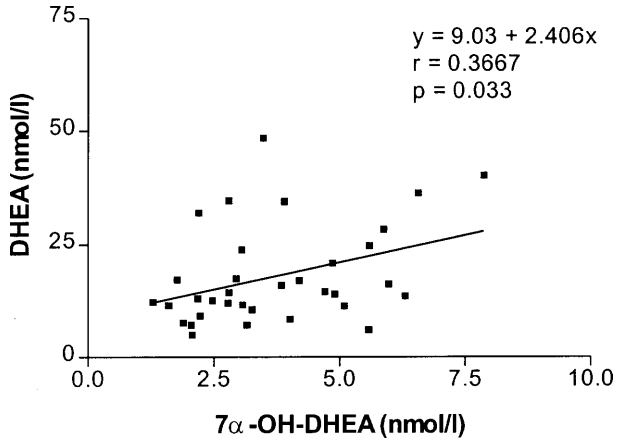

Fig 4 Correlation of 7a-OH-DHEA with DHEA in human semen

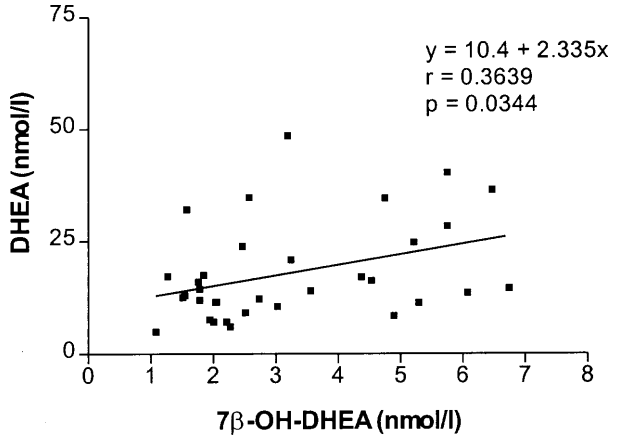

Fig 5 Correlation of 7b-OH-DHEA with DHEA in human semen

Discussion

As demonstrated in Table 1, the reports on steroids in semen published so far, dealt mostly with the major androgens, testosterone and dihydrotestosterone, their conjugates, and to a less extent, estrogens and their common precursor androstenedione. As DHEA/S concerns, with a few exceptions (Bujan et al. 1993), only reports from seventies and early eighties are available.

Here, for the first time 7-OH-DHEA isomers along with its parent steroid DHEA have been measured in a representative group of human semen samples. The concentrations of both 7-OH-DHEA isomers were close to- or even higher than those occurring in serum, and by almost one order of magnitude lower than in saliva, as measured recently by our group (Hampl et al. 2001; Hill et al. 2001). The origin of these metabolites in semen is unknown. The fact that their levels correlate excellently with each other but not with DHEA indicates only that semen is not the site of their formation and further study is needed to clarify this question.

In the light of the plausible immunomodulatory role of DHEA and its 7-hydroxylated metabolites, their detection in semen, opens a new topic on their role in this fluid.

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