Hormone levels in serum and seminal plasma of men with different types of azoospermia

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Summary. Hormone concentrations in the serum and seminal plasma of 15 normozoospermic, 17 excretory azoospermic and 14 secretory azoospermic men were measured. The results indicate that: (a) serum FSH and LH levels are markedly elevated in secretory azoospermia, as compared with excretory azoospermia and normozoospermia; (b) serum 17α -hydroxyprogesterone levels are somewhat raised in secretory azoospermia as compared with excretory azoospermia and normozoospermia; (c) serum testosterone levels are lower in both types of azoospermia with respect to normozoospermia; (d) in secretory azoospermia the oestradiol serum levels are relatively high and dihydrotestosterone serum levels relatively low, whereas the serum levels of these hormones in excretory azoospermia are similar to those in normozoospermic men; (e) in the seminal plasma of azoospermic patients the levels of prolactin, progesterone, testosterone, dihydrotestosterone and oestradiol were depressed, but only dihydrotestosterone levels could be of value in differentiating types of azoospermia because they are lower in secretory azoospermia.

We suggest that the measurement of FSH, LH, 17α -hydroxyprogesterone, dihydrotestosterone and oestradiol in serum and dihydrotestosterone in seminal plasma may be used in the differential diagnosis between secretory and excretory azoospermia when invasive tests are unavailable.

Introduction

Male infertility can usually be diagnosed by testicular biopsy, spermiography and sometimes vasography. However, the first and the last of these methods are not always suitable, safe or acceptable to the patient. We have therefore been looking for biochemical markers in serum and seminal fluid which could help in the diagnosis of azoospermia (Guerin *et al.*, 1981; Gonzalez Buitrago & García Díez, 1982).

Several authors have already reported data on hormone levels related to secretory or excretory azoospermia, with conflicting results. For example, serum levels of the peptide hormones FSH, LH and prolactin have been found to be higher (Franchimont *et al.*, 1972; de Kretser, Burger & Hudson, 1974; Purvis, Landgren, Cekan & Diczfalusy, 1975a; Segal, Polishuk & Ben-David, 1976; Nankin, Castaneda & Troen, 1977; Bain, 1979; García Díez, Gonzalez Buitrago, Corrales & Miralles, 1981), lower (Pierrepoint, John, Groom, Wilson & Gow, 1978) or unmodified (Sheth, Joshi, Moodbidri & Rao, 1973) in azoospermic patients as compared to normal controls.

The same can be said for serum concentrations of steroid hormones. Some authors report normal levels (Purvis *et al.*, 1975a; Nankin *et al.*, 1977; Bain, 1979) while others find lower levels

(Tea, Castanier, Grenier & Scholler, 1972; Luga *et al.*, 1977; Pierrepoint *et al.*, 1978; Abdalla *et al.*, 1979a, b). For seminal plasma the findings are equally inconclusive; FSH, LH and prolactin have been found to be low (Sheth, Mugatwala, Shah & Rao, 1975; Sheth, Shah & Mugatwala, 1976; Biswas *et al.*, 1978) or normal (De Aloysio, Busacchi, Bolelli, Vecchi & Flamingni, 1974; Schoenfeld, Amelar, Dubin & Numeroff, 1978). However, there seems to be some agreement with respect to the steroid hormone levels, which most authors find to be low compared with those of normozoospermic subjects (Tea *et al.*, 1972; Purvis *et al.*, 1975b; Nieschlag, Wickings & Mauss, 1978; Bain, 1979; Bain, Duthie & Keene, 1979).

This paper reports the result of a study carried out to investigate the serum and semen levels of various hormones in relation to different types of azoospermia.

Materials and Methods

Patients

The study was carried out on 46 men, classified as normozoospermic ($N = 15$), excretory azoospermic ($N = 17$) and secretory azoospermic ($N = 14$). Azoospermia was diagnosed when 3 semen samples contained no spermatozoa in the sediment after centrifugation. The two types of azoospermia were differentiated by testicular biopsy using a standard haematoxylin-eosin staining. Subjects with normal spermatogenesis were classified as excretory and those with alterations in the seminiferous epithelium as secretory. These alterations could be classified in one of three groups: (1) hypospermatogenesis associated with maturation arrest ($N = 8$); (2) maturation arrest at the spermatocyte or spermatid stage ($N = 4$); and (3) Sertoli-cell-only syndrome (absence of germ cells) ($N = 2$).

Normozoospermic men had sperm densities of $> 40 \times 10^6/\text{ml}$, more than 70% motile spermatozoa (with at least 30% with forward progressive motility), $< 20\%$ non-viable cells and a minimum of 65% normal forms in the ejaculate. Fertility had been confirmed in 11 normozoospermic subjects within the year before the study.

Serum and semen analyses

Blood samples were obtained after overnight fasting from a venous catheter inserted 15 min before sampling. Serum was stored at -20°C until use. Semen samples were collected into sterile containers by masturbation after 5 days of sexual abstinence. Sperm density and motility were assessed by the technique of Makler (1978). Viability of spermatozoa was evaluated by nigrosin-eosin staining (Parrish, Polakoski & Zaneveld, 1979). Sperm morphology was classified according to the criteria of David, Bisson, Czyglik, Jouannet & Gernigon (1975). After liquefaction, the seminal plasma was separated by centrifugation of semen at 800 g at 4°C and stored at -20°C until hormone analysis.

Hormone levels were determined by specific radioimmunoassays using commercial reagents: Amersham (FSH, LH, testosterone and dihydrotestosterone), Serono-Biodata (prolactin), CIS-CEA Sorin (17α -hydroxyprogesterone), Nordiclab (progesterone and oestradiol) and Wien-Labs (androstenedione and dehydroepiandrosterone sulphate). All samples were processed in one assay to avoid interassay error. Standards for FSH and LH were calibrated against the 2nd IRP HMG (correlation, 1 mi.u. 2nd IRP HMG/ml = 23 ng LER 907/ml for FSH and 1 mi.u. 2nd IRP HMG/ml = 3.7 ng LER 907/ml for LH). Prolactin was calibrated against the standard WHO 75/504 preparation (correlation, 1 ng = 32.5 mi.u. WHO 75/504).

Hormone levels are expressed as mean $\pm$ s.d. Student's *t* test was used to compare the means of the different groups.

Results

We first tested the validity of the hormone immunoassays in seminal plasma by means of dose-response curves. Table 1 shows the sensitivities, intra-assay variations and recoveries for the radioimmunoassay techniques used for serum and seminal plasma.

Table 1. Characteristics of the radioimmunoassays

Hormone	Sensitivity		Intra-assay variation (%)		Mean $\pm$ s.d. % recovery	
	Serum	Seminal plasma	Serum	Seminal plasma	Serum	Seminal plasma
FSH	1.6 mi.u./ml	1.6 mi.u./ml	8.4	11.5	96 $\pm$ 8	99 $\pm$ 5
LH	1.1 mi.u./ml	1.1 mi.u./ml	6.6	9.5	96 $\pm$ 10	98 $\pm$ 8
Prolactin	1 ng/ml	1 ng/ml	7.1	8.0	96 $\pm$ 7	96 $\pm$ 8
Progesterone	0.2 ng/ml	0.2 ng/ml	8.1	8.6	96 $\pm$ 5	94 $\pm$ 7
17 α -Hydroxyprogesterone	0.6 ng/ml	0.07 ng/ml	8.4	9.5	95 $\pm$ 8	94 $\pm$ 6
Androstenedione	0.1 ng/ml	0.1 ng/ml	8.5	9.0	97 $\pm$ 6	95 $\pm$ 8
Testosterone	0.1 ng/ml	0.1 ng/ml	7.2	7.5	95 $\pm$ 7	95 $\pm$ 8
Dihydrotestosterone	0.2 ng/ml	0.2 ng/ml	6.5	6.8	80 $\pm$ 10	80 $\pm$ 12
Oestradiol	10 pg/ml	10 pg/ml	8.1	8.5	100 $\pm$ 6	100 $\pm$ 6
Dehydroepiandrosterone sulphate	0.05 μ g/ml	0.1 μ g/ml	7.1	7.6	95 $\pm$ 7	95 $\pm$ 9

There were no differences between the 3 groups of men in the serum concentrations of prolactin, progesterone, androstenedione and dehydroepiandrosterone sulphate (Text-fig. 1a). FSH, LH and oestradiol levels were significantly higher ($P < 0.001$) in the secretory azoospermic men than in those in the other 2 groups. In secretory azoospermic patients the levels of 17 α -hydroxyprogesterone were higher than in normozoospermic ($P < 0.01$) and excretory azoospermic ($P < 0.001$) subjects. Azoospermic individuals had significantly lower testosterone levels ($P < 0.02$) when compared to the normozoospermic controls. The dihydrotestosterone levels of the secretory azoospermic patients were significantly lower than those of normal ($P < 0.005$) and excretory azoospermic ($P < 0.001$) men.

There were no differences in the concentrations of FSH, LH, 17 α -hydroxyprogesterone, androstenedione and dehydroepiandrosterone sulphate in seminal plasma (Text-fig. 1b). The amounts of FSH, LH and prolactin were higher than the values in serum in the normozoospermic subjects; all the steroid hormones except oestradiol were lower. In azoospermic subjects the levels of prolactin ($P < 0.001$), progesterone ($P < 0.01$), testosterone ($P < 0.05$) and oestradiol ($P < 0.001$) were lower than in normozoospermic individuals. Dihydrotestosterone levels were significantly lower in secretory azoospermic men than in excretory azoospermic ($P < 0.005$) and normozoospermic ($P < 0.001$) men.

Discussion

Our results show that the serum hormonal profile in secretory azoospermic men is different from those found in normal subjects and excretory azoospermic men. The most striking feature is a significant rise in FSH, LH and oestradiol levels. In addition we found a moderate increase in 17 α -hydroxyprogesterone associated with a lower dihydrotestosterone level, but the diagnostic value of these alterations is doubtful.

The elevation of FSH concentrations in secretory azoospermia seems to be well established (Rosen & Weintraub, 1971; Franchimont *et al.*, 1972; de Kretser *et al.*, 1974) and could be attributed to an inhibin deficiency (Franchimont *et al.*, 1972). Our results for LH are in disagreement with those of Rosen & Weintraub (1971), but confirm the findings of Bain (1979),

Text-fig. 1. Mean $\pm$ s.d. concentrations of FSH, LH, prolactin (PRL), progesterone (P), 17 α -hydroxyprogesterone (17OHP) androstenedione (A), testosterone (T), dihydrotestosterone (DHT), oestradiol (E₂) and dehydroepiandrosterone sulphate (DHEA-S) in (a) serum and (b) seminal plasma of men.

Nankin *et al.* (1977) and Purvis *et al.* (1975a). According to Bain (1979) the elevation of LH and FSH reflects a testicular dysfunction that results in an alteration of the normal feedback relationships between the testis and the hypothalamus/hypophysis. The role of oestradiol as a feedback signal is not at all clear and the reported plasma levels of these hormones are either normal (Purvis *et al.*, 1975a; Nankin *et al.*, 1977), high (Nieschlag *et al.*, 1978; this study) or low (Tea *et al.*, 1972; Abdalla *et al.*, 1979a).

The seminal plasma hormone concentrations in excretory azoospermia do not differ from those found in normal subjects, except for testosterone (decreased) and prolactin (increased). However, these alterations are also seen in secretory azoospermia. Testosterone has been found lowered by some authors (Pierrepont *et al.*, 1978) and elevated by others (Abdalla *et al.*, 1979a, b). This in any

case may reflect a dysfunction of the Leydig cell, but we do not think that the determination of this hormone could be useful to diagnose excretory azoospermia. The role of prolactin in male infertility is far from clear. We sometimes find hyperprolactinaemia concurrent with an azoospermia, but this association is not frequent. Data from other authors do not agree on this point and thus we find reports on normal (Sheth *et al.*, 1973; Bain, 1979; Suchanek & Longhino, 1981), higher (Segal *et al.*, 1976) or lower (Pierrepont *et al.*, 1978) values.

Our results show that in the seminal plasma of azoospermic patients the levels of prolactin, progesterone, testosterone, dihydrotestosterone and oestradiol are significantly lower than in normal controls. However, only dihydrotestosterone may help to differentiate both types of azoospermia, its level being lower in the secretory type, as found by Bain (1979), Bain *et al.* (1979), Biswas *et al.* (1978) and Purvis *et al.* (1975b). On the other hand, seminal plasma levels of the peptide hormones in normal subjects are higher than those detected in serum. These results confirm those of Biswas *et al.* (1978), Segal, Ron, Laufer & Ben-David (1978) and Sheth *et al.* (1975, 1976) but contradict data from De Aloysio *et al.* (1974) and Schoenfeld *et al.* (1978). These higher levels, according to Tolis, Wilson, Kleissl, Naftolin & Posner (1979) suggest the operation of a selective concentration mechanism of the peptide hormones in semen that could be inefficient for prolactin in azoospermic patients. Also, the striking decrease in seminal oestradiol observed in both types of azoospermia, confirming previous reports (Tea *et al.*, 1972; Purvis *et al.*, 1975b; Nieschlag *et al.*, 1978), could indicate a gonadal origin for this steroid in the ejaculate.

In agreement with previous reports (De Aloysio *et al.*, 1974; Nieschlag *et al.*, 1978; Schoenfeld *et al.*, 1978; Bain, 1979) the mean values for steroids in seminal plasma are generally lower than those in serum. Such a decrease could be attributed to a limitation in their transference from plasma (the selective 'blood-testis barrier' proposed by Setchell, Voglmayr & Waites, 1969) or to a faster metabolism and/or conjugation of steroids by the genital tract, its accessory glands or even the spermatozoa (Castaneda *et al.*, 1974; Purvis *et al.*, 1975b; Purvis & Diczfalusy, 1976).

The reasons for the discrepancies found by different authors for the hormone concentrations in serum and semen can be attributed mainly to (a) small numbers of cases; (b) the different criteria used to define the normal condition; (c) the secretory dynamics of hormones; (d) the poor reproducibility of radioimmunoassay data between laboratories; and (e) the fact that most authors do not discriminate secretory from excretory azoospermia. According to our data, this results in a mixed group of azoospermic patients which cannot be considered as homogeneous.

We conclude that the measurement of some hormones in serum and seminal plasma may help in the differential diagnosis of azoospermia when other methods are unavailable.

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