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Serum and Intratesticular Sex Steroids in Azoospermic Men: How Do They Correlate?

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Summary

Five intratesticular sex steroids (testosterone, dihydrotestosterone, androstenedione, estradiol and epitestosterone) along with six serum hormones (LH, FSH, prolactin, SHBG, testosterone and estradiol) were determined in 84 non-obstructive azoospermic men, in order to evaluate to what extent serum and testicular tissue as well as individual hormones in the same material mutually correlate. With exception of androstenedione, tight correlations were found among tissue content of sex steroids, while only weak correlation was recorded between serum and testicular concentrations of major sex steroids testosterone and estradiol. It points to importance of measurement of intratesticular steroids in combination with examination of sperm parameters for assessment of testicular function and spermatogenesis.

Key words

Intratesticular tissue • Serum sex steroids • Azoospermia

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Introduction

As many as 15 % of couples do not achieve pregnancy within 1 year of unprotected sexual intercourse and turn to infertility clinics. Since male causes of infertility are found in about half of involuntarily childless couples, it can be assumed that approximately 7 % of all men are confronted with fertility problems. In 50 % a male infertility associated factor is found together with abnormal semen parameters (World Health Organization 2000). Another factor represents endocrine disorders, as a potentially reversible cause of some cases of male-factor infertility. Therefore, a complete investigation of infertility should include endocrinological evaluation and semen and hormonal analysis. This is especially true where assisted reproductive techniques are becoming more advanced and more widely used (Sussman *et al*. 2008, Vitku *et al.* 2015, Vitku *et al*. 2018).

High levels of intratesticular testosterone secreted by the Leydig cells are necessary for spermatogenesis (Dohle *et al*. 2003, Lombardo *et al*. 2005). A number of studies have been published on intratesticular sex steroid concentrations in normal men as well as in men with various fertility disorders, including gonadotropin administration, for successful *in vitro* fertilization (Roth *et al.* 2010, Page 2011). From the point of view of endocrine/paracrine regulation, testes represent a microenvironment characterized by its own hormone metabolism and regulation mechanisms (Lin *et al*. 2007, Page 2011, Tremblay 2015). Therefore, circulating steroid hormones need not fully reflect the situation in the testis. In addition, it is well known that high levels of testosterone, needed inside the testis, cannot be achieved by oral or parenteral administration of androgens (Lin *et al*. 2007).

To what extent serum hormones in healthy men correlate with intratesticular ones has been addressed by Roth *et al*. (2010). Here we provide further data on five intratesticular sex steroids together with levels of six relevant hormones in serum and their mutual correlations in a group of azoospermic men.

Materials and Methods

Study group

The testicular tissue was obtained from 84 non-obstructive azoospermic men aged 21-58 years (mean ± SD: 35.7±9.7) by surgical retrieval. Patients were evaluated by physical examination, comprehensive history, measurement of testicular volume, at least two semen analysis and hormonal parameters. All patients underwent screening for cystic fibrosis, Y chromosome microdeletion and karyotype analysis and showed normal results. Blood was removed from cubital vein and serum stored at -70 °C until processed. Patients underwent microsurgical testicular sperm extraction (M-TESE) using optical x20-25 magnification (microscope OPMI Pico/S100, Carl Zeiss, Jena, Germany). M-TESE involves "bivalve" opening of the testicle by means of an equatorial or longitudinal incision under general or spinal anesthesia and removal of single tubules observed to have the largest diameter under an operating microscope and testicular pulp (Schlegel 1999). The material was immediately frozen in dry carbon dioxide and stored frozen at -70 °C until processed.

A written informed consent was obtained from all patients and the study was approved by the Ethical Committee of the Institute of Endocrinology.

Methods

Serum hormones, LH, FSH, prolactin, testosterone and SHBG were measured by immunoassay using commercial kits from Immunotech (Czech division of Beckman Coulter, Marseille, France), estradiol by radioimmunoassay kit Spectria Estradiol RIA (Orion Diagnostica Oy, Espoo, Finland). Intratesticular hormones, testosterone, dihydrotestosterone, androstenedione, estradiol and epitestosterone were determined by a method originally used for measurement of sex steroids in prostatic tissue (Heracek *et al.* 2007). In brief, the procedure involved homogenization of the tissue, extraction with diethyl ether, solvent partition between n-hexane and aqueous methanol and high performance liquid chromatography for separation of the analytes, which were finally determined by specific radioimmunoassays (see above). The details of the method were described elsewhere (Zamrazilova *et al*. 2012).

Statistical analysis

Data were reported as mean ± standard deviation (SD), medians and upper and lower quartiles. The correlations among variables were analyzed using Spearman's correlation coefficients. A p value <0.05 was considered to indicate significance. Statistical analysis was performed by the STATISTICA version 8 for Windows (Statsoft, Tulse, USA) software package.

Results

Serum gonadotropins, major sex steroids – testosterone and estradiol and also prolactin and SHBG were determined in a group of 84 azoospermic men. Along with plasma hormones, intratesticular sex steroids were measured including dihydrotestosterone and testosterone precursor androstendione. In addition, epitestosterone was determined with respect to its role as potential antiandrogen (Starka 2003). The data on hormone concentrations are given in Table 1. Mutual correlations were further calculated between pairs of analyzed hormones in serum and intratesticular tissue, as summarized in Table 2.

Discussion

Investigation of sperm parameters along with measurement of basal or stimulated plasma sex hormones belong to classical tests for evaluation of male fertility. More recent studies point to importance of intratesticular steroids with respect to their role in spermatogenesis. Concerning the concentrations of intratesticular tissue testosterone, our results are very close to those reported by Roth *et al*. (2010) for healthy men (2.2 nmol/g tissue compared to 2.2 nmol/ml), but our data on dihydrotestosterone and estradiol are slightly higher, pointing to an excess of estrogens in azoospermia. In the latter study (Roth *et al*. 2010) intratesticular androgens correlated well with serum gonadotropin levels and also with serum testosterone, while in our study of azoospermic men only weak correlation was found between serum and tissue concentrations of testosterone (r=0.2622, p=0.0196) and between serum and tissue concentrations of estradiol (r=0.2306, p=0.0409). The serum levels of gonadotropins, prolactin and SHBG did not correlate with tissue concentrations of sex hormones at all.

On the other hand, there were very tight correlations among all intratesticular sex steroids, namely testosterone, dihydrotestoterone, estradiol and even epitestosterone (Table 2). The correlation of the latter

Table 1. Hormones analyzed in serum and testicular tissue.

	Serum						Testicular tissue				
	FSH (IU/l)	**LH (IU/l)**	**Prolactin (ng/ml)**	**Estradiol (nmol/l)**	**SHBG (nmol/l)**	**Te (nmol/l)**	**Te (nmol/g)**	**DHT (nmol/g)**	**A-dione (nmol/g)**	**Estradiol (nmol/g)**	**EpiTe nmol/g)**
Mean	4.18	5.41	13.8	0.0762	20.9	0.0142	2.09	0.34	0.08	70	0.26
SD	13.9	4.07	6.50	0.0312	7.22	0.0050	2.37	0.50	0.13	90	0.27
Median	6.50	4.20	11.4	0.0648	19.3	0.0140	1.23	0.21	0.04	30	0.18
Lower quartile	3.20	3.30	8.50	0.0543	15.6	0.0106	0.51	0.10	0.02	10	0.08
Upper quartile	15.6	6.05	16.9	0.0936	25.6	0.0164	2.99	0.42	0.10	90	0.36

FSH – folitropin, LH – lutropin, SHBG – sex hormone-binding globulin, Te – testosterone, DHT – dihydrotestosterone, A-dione – androstenedione, EpiTe – epitestosterone, SD – standard deviation.

Table 2. Mutual correlations of analyzed hormones in serum and testicular tissue.

	FSH in serum	**LH in serum**	**PRL in serum**	**SHBG in serum**	**Estradiol in serum**	**Te in serum**	**Te in tissue**	**DHT in tissue**	**A-dione in tissue**	**Estradiol in tissue**	**EpiTe in tissue**
FSH in serum		**0.5256**	0.1399	**0.2407**	0.0301	-0.0941	0.1100	0.0689	0.1014	0.0654	-0.0479
LH in serum	**0.0000**		-0.1319	0.0156	-0.07321	-0.0886	0.0994	0.0719	-0.1134	0.1730	-0.0225
PRL in serum	NS	NS		-0.0160	0.2007	-0.0697	-0.0122	-0.0444	0.0784	0.0760	0.1122
SHBG in serum	**0.0326**	NS	NS		0.0410	0.0666	0.1341	0.0720	0.1464	-0.0881	0.0442
Estradiol in serum	NS	NS	NS	NS		0.1498	0.1321	0.1061	0.0892	**0.2306**	0.2119
Te in serum	NS	NS	NS	NS	NS		**0.2622**	-0.0220	-0.1706	0.1876	0.1371
Te in tissue	NS	NS	NS	NS	NS	**0.0196**		**0.3699**	0.2019	**0.4326**	**0.6309**
DHT in tissue	NS	NS	NS	NS	NS	NS	**0.0005**		**0.3462**	**0.5205**	**0.6002**
A-dione in tissue	NS	NS	NS	NS	NS	NS	NS	**0.0012**		**0.2629**	**0.2924**
Estradiol in tissue	NS	NS	NS	NS	**0.0409**	NS	**0.0000**	**0.0000**	**0.0157**		**0.6761**
EpiTe in tissue							**0.0000**	**0.0000**	**0.0070**	**0.0000**	

Values above the diagonal show correlation coefficients of the respective pairs, below the diagonal significance as **p** values. FSH – folitropin, LH – lutropin, PRL – prolactin, SHBG – sex hormone-binding globulin, Te – testosterone, DHT – dihydrotestosterone, A-dione – androstenedione, EpiTe – epitestosterone. NS – not significant.

with all three sex hormones supports the idea of autonomous role of the testis in endocrine regulations and metabolism (Tremblay 2015). In conclusion, the results confirm the close relationship among intratesticular steroid hormones, which better reflect the situation in the testis than estimation of circulating hormones. Therefore, we suggest that measurement of intratesticular steroids, along with examination of sperm parameters may be a suitable tool for assessment of testicular function and spermatogenesis.

Conflict of Interest

There is no conflict of interest.

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