ORIGINAL ARTICLE

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Testosterone levels after treatment with urofollitropin in infertile patients with idiopathic mild reduction of testicular volume

Sandro La Vignera [1] · Rosita A. Condorelli[1] · Rossella Cannarella[1] · Laura Cimino[1] · Laura Mongioi'[1] · Ylenia Duca[1] · Filippo Giacone[1] · Aldo E. Calogero[1]

Abstract

Introduction A reduction of testicular volume (TV) represents an important clinical sign, which may hide sperm abnormalities and predispose to hypogonadism.

Aim The primary purpose of this study was to evaluate the serum levels of total testosterone after treatment with urofollitropin in selected patients with male infertility and idiopathic mild reduction of testicular volume.

Methods In this 1-year-long prospective design, patients with abnormal sperm parameters, mild reduction in TV (8–12 mL) and normal gonadotropin, and total testosterone (TT) serum levels were recruited in this study. Patients treated for 4 months with urofollitropin were included in group A, those treated with intracytoplasmatic sperm injection due to a female-factor infertility were included in group B. Hormone values, sperm parameters, and TV were detected at baseline (T0), after 4 (T1) and 12 months (T2) in group A and at T0 and T2 in group B.

Results Group A ($n = 80$) showed increased follicle-stimulating hormone (FSH) at T1 and sperm morphology at T1 and T2 compared to T0 (all $p < 0.05$). Group B ($n = 50$) had lower TT and higher FSH levels at T2 compared to T0 (all $p < 0.05$). At T2, TT, VT, total sperm count, progressive motility, total motility, and sperm morphology were higher in group A compared to group B (all $p < 0.05$).

Conclusion Reduced TV may predispose to infertility and hypogonadism. FSH treatment may improve Sertoli and Leydig cell function and prevent the development of hypogonadism.

Keywords Testiculopathy · Total testosterone · Infertility · Hypogonadism

Abbreviations

FSH	follicle-stimulating hormone;
hCG	human chorionic gonadotropin;
LC	Leydig cell;
LH	luteinizing hormone;
O	oligozoospermia;
OA	Oligo-asthenozoospemia;
OAT	oligo-astheno-teratozoospermia;
SC	Sertoli cell;
TT	total testosterone;
TV	testicular volume.

✉ Sandro La Vignera
   sandrolavignera@unict.it

[1] Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

Introduction

Testicular volume (TV) is an important clinical parameter. Normal TV values range between 12 and 25 mL. A reduced volume may hide hypogonadism and sperm abnormalities in adult men of reproductive age [1]. Ultrasound should be always adopted to detect TV in the clinical practice, since orchidometry may overestimate it [2].

On a physiological point of view, the increase in TV is mainly due to the follicle-stimulating hormone (FSH)-dependent Sertoli cell (SC) proliferation in childhood. On the contrary, the achievement of a normal TV in the adulthood relies on the germ cell pool expansion and differentiation [2]. Clinically, when a primary testiculopathy occurs, a progressive and compensatory increase in serum gonadotropins usually arise [3].

Primary testiculopathy is widespread in our society [4], thus alarming International Societies [5]. Basing on the clinical practice, three different phenotypes mainly occur: (i) patients with a prevailing abnormality in the germ and/or

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SC compartment, featured by sperm abnormalities, isolated rise in FSH levels but having normal luteotropic hormone (LH), and total testosterone (TT) levels [suggesting no alteration in Leydig cell (LC) function]; (ii) those with idiopathic sperm abnormalities but serum gonadotropin and TT levels in the low quartile of the reference range (functional hypogonadism); (iii) patients with unexplained reduced TV (which is a sign of germ cell compartment damage), sperm abnormalities and normal serum gonadotropin, and TT levels (meaning normal LC function).

This was a 1-year-long prospective study carried out in patients with sperm abnormalities, having a mild reduction of TV (from 8 to 12 mL) but normal serum gonadotropin and TT levels, aimed at assessing the effects of FSH treatment, if any, on TV, and TT by the comparison with a not-treated group.

Methods

Patient selection

This was a 1-year-long longitudinal study performed on men referring to the Unit of Andrology and Endocrinology, University of Catania for infertility.

Inclusion criteria were: (i) oligozoospermia (O), oligo-asthenozoospermia (OA) and/or oligo-astheno-teratozoospermia (OAT); (ii) mild reduction of TV (between 8 and 12 mL); and (iii) normal serum gonadotropin and TT levels.

Exclusion criteria were: (i) gonadotropin and TT serum values outside the reference range; (ii) TV < 8 mL; (iii) TV > 12 mL.

Intervention

Among the enrolled patients, those treated with uro-follitropin (150 IU three times a week for 4 months) were included in group A. Group B patients were not given urofollitropin and started with intracytoplasmic sperm injection due to a concomitant female-factor infertility. Patients underwent sperm analysis (WHO, 2010), FSH, LH and TT serum dosage, and scrotal ultrasound for TV detection at baseline (group A and B) (T0), after 4 months (group A) (T1) and after 12 months (group A and B) (T2).

Sperm analysis

Semen samples were collected by masturbation into a sterile container after 2–7 days of sexual abstinence and were analyzed immediately after liquefaction. According to the 2010 WHO guidelines, each sample was evaluated for seminal volume, pH, sperm count, progressive motility, morphology, and round cell concentration (WHO, 2010).

Hormonal measurements

Each man underwent to blood testing for the measurement of LH, FSH, and TT serum levels. The hormone evaluation was performed by electrochemiluminescence (Hitachi-Roche equipment, Cobas 6000, Roche Diagnostics, Indianapolis, IN, USA). The reference values were as follows: LH 1.14–8.75 mIU/mL, FSH 0.95–11.95 mIU/mL, and TT 3.5–9.8 ng/mL.

Scrotal ultrasound evaluation

The ultrasound examination was performed with a GX Megas Esaote (Esaote SpA, Genoa, Italy) device, equipped with linear, high-resolution, and high-frequency (7.5–14 MHz) probes dedicated to the study of soft body areas, with color Doppler for detecting slow flow and a scanning surface of at least 5 cm. The TV was calculated using the ellipsoid formula (length × width × thickness × 0.52). The testis was considered normal in size when it had a volume between 15 and 25 cm^3, low normal when it had a volume between 10 and 12 cm^3, and hypotrophic when it had a volume of less than 10 cm^3 [6, 7]. TV was evaluated by adding the volumes of the right and left testes.

Statistical analysis

Results are reported as mean ± SD throughout the study. The normality of the variables was evaluated with the Shapiro—Wilks test. Data collected from group A and B were analyzed by Student′s t-test. Statistical analysis was performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was accepted as statistically significant. A trend was assumed for p values ranging from 0.05 to 0.099.

Ethical approval

This study was conducted at the Division of Andrology and Endocrinology of the teaching hospital "G. Rodolico", University of Catania (Catania, Italy). The protocol was approved by the internal Institutional Review Board, and informed written consent was obtained from each participant after full explanation of the purpose and nature of all procedures used. The study has been conducted in accordance with the principles expressed in the Declaration of Helsinki.

Results

Group A included 80 patients (mean age 30.6 ± 10.0 years). Group B included 50 patients (mean age 34.0 ± 14.0 years). Hormone values, sperm parameters, and TV did not differ in group A and group B at baseline (Table 1).

Subgroup analysis revealed an increase in FSH serum levels and sperm morphology in group A at T1 compared to T0 ($p < 0.05$). At T2, sperm morphology was improved compared to T0 ($p < 0.05$). No other differences were observed (Table 2). Group B showed a reduction in TT and a raise in FSH serum levels in T2 compared to T0. No other differences were detected (Table 3).

At T2, inter-group analysis showed higher TT (5.3 ± 0.5 ng/mL versus 3.84 ± 0.61, $p < 0.05$) and TV (12.8 ± 2.39 mL versus 9 ± 1.58 mL; $p < 0.05$) in group A compared to group B (Fig. 1a). Similarly, group A had higher total sperm number (41.6 ± 18.89 mil/ej. versus 16.2 ± 12.97 mil/ej.; $p < 0.05$), progressive motility (20.8 ± 7.05 versus 9 ± 5.1; $p < 0.05$), total motility (26.8 ± 7.66 versus 15.2 ± 8.04; $p < 0.05$), and morphology (7.2 ± 2.28 versus 2.4 ± 1.52; $p < 0.05$). They did not differ for the sperm concentration (15.2 ± 7.69 mil/mL versus 11.6 ± 3.78 mil/mL; $p > 1$) (Fig. 1 b).

Finally, the spontaneous pregnancy rate recorded among couples of the group A after one year from the start of treatment was 20/80 cases (25%). The ICSI success rate among couples of the group B was 11/50 cases (22%).

Discussion

This longitudinal study provide evidence for the existence of a possible paracrine function of SCs on LC function. Accordingly, 1 year after the 4-month-long SC stimulation with urofollitropin, the treated group showed increased TT levels compared to the non-treated one, thus suggesting an impact of SC stimulation on LC function. Treated group also showed higher total sperm count, progressive motility, total motility, morphology, and TV compared to the non-treated one.

The two major functions of the testis, androgen production, and spermatogenesis, take place in two different compartments, the vascularized interstitial compartment containing LCs and the avascular seminiferous tubule which is made exclusively of SCs and germ cells. Under physiological conditions, the initiation and maintenance of these two highly specialized functions of the testis are absolutely under the endocrine input provided by pituitary gonadotropins (LH and FSH).

In addition to the well-established endocrine regulation of testicular functions by gonadotropins, many data accumulated in the last years indicate that a local control is

Table 1 Baseline hormonal values, sperm parameters, and testicular volume in group A and group B

Parameter	Group A	Group B	p
FSH (mIU/mL)	3.08 ± 0.8	3.14 ± 0.7	NS
LH (mIU/mL)	2.96 ± 0.68	2.72 ± 0.64	NS
TT (ng/mL)	5.24 ± 0.62	5.24 ± 0.67	NS
Sperm concentration (mil/mL)	12.2 ± 10.06	13 ± 5.39	NS
Total sperm count (mil/ej.)	21.8 ± 7.95	28.4 ± 11.52	NS
Progressive motility (%)	14.2 ± 5.12	14.4 ± 3.85	NS
Total motility (%)	19.8 ± 6.94	20 ± 6.78	NS
Normal forms (%)	3.8 ± 1.48	3.8 ± 1.48	NS
TV (mL)	10.2 ± 1.48	10.4 ± 1.52	NS

FSH follicle stimulating hormone, *LH* luteinizing hormone, *TT* total testosterone, *TV* testicular volume

Table 2 Hormonal values, sperm parameters, and testicular volume evaluated at baseline (T0), after the 4-month-long urofollitropin treatment (T1) and after 12 months (T2) in group A

Parameter	T0	T1	T2
FSH (mIU/mL)	3.08 ± 0.8	4.68 ± 0.89*	4.02 ± 0.89
LH (mIU/mL)	2.96 ± 0.68	3.02 ± 0.36	2.78 ± 0.58
TT (ng/mL)	5.24 ± 0.62	5.7 ± 0.75	5.3 ± 0.5
Sperm concentration (mil/mL)	12.2 ± 10.06	18.2 ± 7.95	15.2 ± 7.69
Total sperm count (mil/ej.)	21.8 ± 7.95	44.8 ± 25.16	41.6 ± 18.89
Progressive motility (%)	14.2 ± 5.12	22.6 ± 7.27	20.8 ± 7.05
Total motility (%)	19.8 ± 6.94	30 ± 9.38	26.8 ± 7.66
Normal forms (%)	3.8 ± 1.48	7.4 ± 2.41*	7.2 ± 2.28*
TV (mL)	10.2 ± 1.48	12.8 ± 2.39	12.8 ± 2.39

FSH follicle-stimulating hormone, *LH* luteinizing hormone, *TT* total testosterone, *TV* testicular volume

*$p < 0.05$ versus T0

required for a normal production of androgens and spermatogenesis [8–13].

Local regulators can be divided into several classes [14]. A first class include factors which are secreted by one cell and move through the interstitial space to act on neighboring target cells in a paracrine fashion. Two other types of cell communication can be included in this group of regulation: cryptocrine and juxtacrine. In cryptocrine communication, one cell is surrounded by the other cell, as with lymphocytes and nurse cells in the thymus, and spermatids, and SC in the seminiferous tubules [15]. In the other type, juxtacrine communication between cells can be mediated by the binding of a membrane-anchored factor to the receptor for that factor on an adjacent cell [16]. This group includes epidermal growth factor, transforming growth factor-α, amphiregulin, colony-stimulating factor 1, and tumor necrosis factor. All of these factors are synthesized as membrane-bound glycoproteins which can be cleaved in a

Table 3 Hormonal values, sperm parameters, and testicular volume evaluated at baseline (T0) and after 12 months (T2) in group B

Parameter	T0	T2
FSH (mIU/mL)	3.14 ± 0.7	3.94 ± 0.24*
LH (mIU/mL)	2.72 ± 0.64	3.3 ± 0.64
TT (ng/mL)	5.24 ± 0.67	3.84 ± 0.61*
Sperm concentration (mil/ml)	13 ± 5.39	11.6 ± 3.78
Total sperm count (mil/ej.)	28.4 ± 11.52	16.2 ± 12.97
Progressive motility (%)	14.4 ± 3.85	9 ± 5.1
Total motility (%)	20 ± 6.78	15.2 ± 8.04
Normal forms (%)	3.8 ± 1.48	2.4 ± 1.52
TV (mL)	10.4 ± 1.52	9 ± 1.58

FSH follicle-stimulating hormone, *LH* luteinizing hormone, *TT* total testosterone, *TV* testicular volume
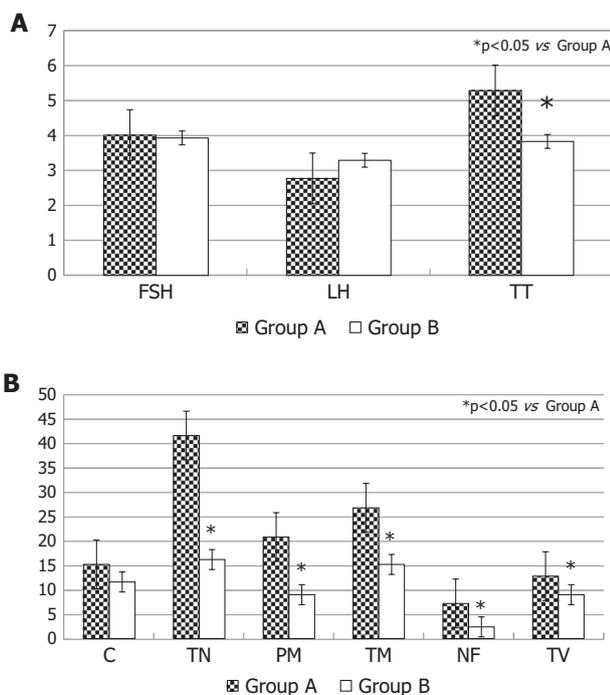
*$p < 0.05$ versus T0

Fig. 1 Hormone values, sperm parameters, and testicular volume in group A and Group B. **a** Hormone values from group A and group B are showed at T2. FSH, follicle-stimulating hormone; LH, luteinizing hormone; and TT, total testosterone. **b** Sperm parameters and testicular volume from group A and group B are showed at T2. C, concentration; NF, normal forms; PM, progressive motility; TN, total number; TM, total motility; and TV, testicular volume

regulated fashion. Therefore, these factors can act as juxtacrine or paracrine regulators.

A second class of local regulators comprises factors which are synthetized in one cell, exit it, bind to membrane receptors on the parent cell and influence it in an autocrine fashion. The last class of local regulation is intracrine, whereby a factor is synthesized and acts within the cell, without exit, by binding to intracellular receptors. This type of regulation has been demonstrated for some growth factors, e.g., platelet-derived growth factor and fibroblast growth factor [17], and might exist in cells which produce steroid hormones (adrenal, Leydig, and granulosa cells) and contain specific intracellular receptors for such hormones. Several of these regulatory mechanisms may exist in the gonads [9, 10, 12, 13, 18].

Data from several experimental approaches have been reviewed and the findings clearly indicate the existence of multiple interactions between testicular cells and the potential role of these interactions in the paracrine control of testicular functions.

The SC is important for endocrine and paracrine control of spermatogenesis. Functions attributed to SC are: (1) supportive and trophic functions for the cells of the seminiferous epithelium, (2) transport of mature spermatids towards the lumen of seminiferous tubules, (3) secretion of androgen binding protein, (4) production of substances with endocrine or paracrine action for spermatogenesis control and (5) interaction with intertubular endocrine LCs [19].

In fact, both testicular interstitial fluid and spent media from cultured SC had an acute steroidogenic effect on LCs. This effect is not species specific but is mediated mainly by diffusible factors. The secretion of this steroidogenic factor (s), which is probably a protein, is enhanced by previous FSH treatment of SC [10]. Co-culture for 2–3 days of pig Leydig cells with homologous or heterologous SC enhances LC specific functions [human chorionic gonadotropin (hCG) receptor number and hCG responsiveness] and induces LC hypertrophy [20].

A similar but less pronounced trophic effect is seen when LCs are cultured with spent media from SC cultured in the presence of FSH and high concentrations of insulin, but the spent media from SC cultured in the absence of these two hormones inhibits LC specific functions. Somatomedin-C might play an important role in the positive trophic effect of SCs on LCs, since this peptide is secreted by SCs and it has trophic effects on the specific function of LCs. Moreover, SCs, probably through a diffusible factor and cell-to-cell contacts, control the multiplication, meiotic reduction, and maturation of germ cells.

Hazra and colleagues have created a mouse model displaying precocious SC and spermatogenic development induced by SC-specific transgenic androgen receptor expression (TgSCAR). Here they reveal that TgSCAR regulates the development, function, and absolute number of LCs without reflex changes in serum LH, indicating a local intratesticular regulatory mechanism [21].

Thus, if a normal SC function (which depends not only on FSH but also on LC and myoid cell secretory products) is an absolute requirement for germ cell multiplication and

maturation, these cells, in turn, cyclically regulate SC function, and through these cells the size and the function of LCs.

Finally, with regard to the pregnancy rate reported in group A, which did not represent the purpose of this study, the utility of urofollitropin treatment in patients with normogonadotropic infertility is confirmed [22]. From this point of view, the pregnancy rate of group B is not comparable, being associated with female factor infertility.

In conclusion, low TV may predispose to both infertility and hypogonadism. Our data suggest that primary testiculopathy featured by abnormal sperm parameters, a mild reduction of TV and gonadotropin, and TT within the normal range may benefit from FSH treatment not only on sperm parameters and TV, but also on TT serum levels, indicating an impact on LC function possibly through a SC-mediated paracrine mechanism. Therefore, the treatment of male infertility may prevent male hypogonadism.

Compliance with ethical standards

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