Androgen Receptor Expression Relationship with Semen Variables in Infertile Men with Varicocele

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Purpose: Androgen receptor, a member of the nuclear receptor superfamily, has important roles in male reproductive function. It is required for sexual differentiation, pubertal development, spermatogenesis regulation, meiosis completion and spermatocyte transition to haploid round spermatids. We assessed the association of androgen receptor expression and semen variables in infertile men with varicocele.

Materials and Methods: A total of 299 men were grouped into healthy, fertile controls, infertile men without varicocele and men with infertility associated with varicocele. A history was obtained, clinical examination and semen analysis were done and reproductive hormones were estimated. Androgen receptor expression and the acrosome reaction were determined in recovered spermatozoa.

Results: Androgen receptor expression was significantly decreased in infertile men with varicocele more than in infertile men without varicocele compared to fertile controls. Androgen receptor correlated positively with sperm count, motility, normal forms, velocity, linear velocity, acrosome reaction and α-glucosidase. It correlated negatively with serum follicle-stimulating hormone and estradiol. Multiple stepwise regression analysis of androgen receptor expression revealed that the sperm acrosome reaction and linearity index were the most affected independent variables.

Conclusions: Androgen receptor expression was significantly decreased in infertile men with varicocele more than in infertile men without varicocele compared to fertile men. Androgen receptor expression correlated positively with sperm count, motility, normal forms, velocity, linear velocity and acrosome reaction.

Key Words: testis; infertility, male; spermatozoa; varicocele; receptors, androgen

Abbreviations and Acronyms

AR = androgen receptor
E2 = estradiol
FSH = follicle-stimulating hormone
LH = luteinizing hormone
PCR = polymerase chain reaction
PRL = prolactin
T = testosterone
Vx = varicocele

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GENETIC factors, including Y chromosome microdeletions and AR gene mutations, are responsible for some cases of male infertility.[1] The local action of androgen on testicular function demonstrated that T alone could support spermatogenesis in the absence of the gonadotropins LH and FSH.[2] AR was detected in Sertoli, Leydig and peritubular myoid cells, and spermatids, in which androgens mediate a wide range of physiological responses and developmental processes by signaling through AR.[3,4]

AR, which is encoded by a single copy gene located in the long arm of the X chromosome (Xq11-12), is a member of the nuclear receptor superfamily that acts as a ligand inducible transcription factor to modulate the expression of target genes.[5] The AR gene com-

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prises 8 exons that encode a 110 kDa protein containing an N-terminal transactivation domain, a central DNA binding domain and a C-terminal ligand binding domain. T and its more potent metabolite dihydrotestosterone can bind AR, and the ligand AR forms homodimers and interact with many co-regulators to modulate androgen target genes.[6] AR also has dual post-meiotic roles during male germ cell differentiation, consisting of terminal differentiation of spermatids and their release from the seminiferous epithelium. Furthermore, the steps of spermatid progression to elongation are sensitive to Sertoli cell AR function.[5] Failure of the mutated receptor to activate its target genes causes a spectrum of hereditary disorders of androgen insensitivity syndrome or testicular feminization mutation.[7]

Vx has long been considered the most treatable cause of male infertility with multiple pathophysiological theories addressing low sperm count, decreased sperm motility and increased abnormal sperm morphology.[8,9] Various mechanisms are suggested to account for the testicular dysfunction and negative impact on semen parameters associated with Vx, for example hyperthermia, retrograde flow of toxic metabolites, venous stasis with hypoxia, alterations in the hypothalamic-pituitary-gonadal axis and increased oxidative stress.[10–13]

We assessed the association of AR expression and semen variables in infertile men with Vx.

MATERIALS AND METHODS

A total of 299 semen samples obtained from men attending University Hospital were included in this study after receiving institutional review board approval and informed consent. Men were grouped by semen parameters and concurrent unsuccessful efforts at pregnancy during more than 1 year of unprotected coitus into 107 infertile men without Vx, 125 infertile men with left Vx and 67 healthy, fertile controls. Fertile, healthy controls had fathered a child within the previous year and were normozoospermic and devoid of Vx. Infertile men had a sperm count of less than 20×10^6 per ml, sperm motility less than 50% and less than 14% sperm normal forms. Study exclusion criteria were smoking, leukocytospermia, nonpalpable Vx, bilateral Vx and hormonal treatment. Participants provided a history and underwent clinical examination, semen analysis and estimation of reproductive hormones. After a clinical varicocele was palpated, Vx was clinically confirmed by scrotal ultrasound using a Tellus UF-850XTD (Fukuda Denshi, Tokyo, Japan) equipped with color flow.

Semen samples were collected by masturbation after 4 to 5 days of abstinence and allowed to liquefy completely for 30 minutes, according to WHO guidelines.[14] Computer assisted semen analysis was performed using AutoSperm (FertiPro, Beernem, Belgium). Sperm morphology was evaluated by phase contrast microscope and sperm Mac stain (FertiPro). The sperm acrosome reaction was assessed before and after stimulation with calcium ionophore A23187 using the Pisum sativum (Sigma®) fluorescence method with simultaneous vitality staining (Hoechst, Frankfurt, Germany).

Spermatozoa were isolated from pooled normal semen samples by centrifuge on a discontinuous Percoll density gradient (80:40 volume per volume). The pellet was washed twice with bovine serum albumin-free Ham's F-10 medium (Sigma). Cells were used immediately for total RNA isolation according to the Chomczynski method[15] using Tri-Fast™ reagent. The AR gene oligonucleotide primer designed GenBank® sequences were forward 5′-TGC CCATTGACTATTACTTTCC-3′ and reverse 5′-TGTCCAGCACACACTACACC-3′. They were chosen to amplify the gene region from 1648 to 2055 bp, corresponding to the DNA binding domain plus the hinge region of human AR. The GAPDH oligonucleotide primer designed GenBank sequences were forward 5′-CGG AGT CAA CGG ATT TGG TCG TAT-3′ and reverse 5′-AGC CTT CTC CAT GGT GGT GAA GAC-3′.

Semiquantitative reverse transcriptase-PCR was performed using Ready-to-Go (Amersham®) according to the Berchtold method.[16] cDNA was amplified by PCR using a thermal cycler with 40 cycles of denaturation at 95C for 1 minute, annealing at 55C for 1 minute and extension at 72C for 2 minutes with final extension at 72C for 10 minutes.[17] Products were subjected to agarose gel electrophoresis, visualized by ultraviolet light transilluminator and photographed. Results were analyzed with Image®, release Alpha 4.0.3.2s (Scion, Frederick, Maryland) to detect bands and convert to peaks. The area under each peak was calculated in pixel^2. AR gene expression was determined by calculating the ratio between pixel^2 values of target bands related to control bands.

For serum hormones 5 ml blood were drawn after overnight fasting. Serum was separated to estimate T, LH, FSH, E2 and PRL by enzyme-linked immunosorbent assay.

Statistical Analysis

Statistical analysis was done with SPSS®, version 17. Data are shown as the median and range. The Mann-Whitney test was used as a test of significance to compare 2 groups. The Spearman rank correlation coefficient was used to study relationships of variables. Multiple stepwise regression analysis was done to analyze the relationship between a dependent variable and 1 or more independent variables with $p < 0.05$ considered statistically significant.

RESULTS

There was a significant decrease in AR expression, and sperm motility, linear velocity, linearity index and acrosome reaction in infertile men with Vx compared with infertile men without Vx and controls (see table and figure). AR expression significantly correlated positively with sperm count ($r = 0.382$, $p = 0.001$), motility ($r = 0.476$, $p = 0.0001$), normal morphology ($r = 0.470$, $p = 0.001$), velocity ($r = 0.362$, $p = 0.001$), linear velocity ($r = 0.454$, $p = 0.001$), linearity index ($r = 0.321$, $p = 0.003$) and acrosome