LETTER TO THE EDITOR

Relationships between biological aging and male reproductive monitors

Giorgio Cavallini‡, Fabrizio Scroppo

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Dear Editor,

Advanced paternal age affects testicular function, reproductive hormones, and sperm parameters, but it is unclear whether chronological age or biological age exercises a greater sway on reproductive function. Thus, the authors carried out this prospective, multicenter, observational study, in which sperm concentration; motility and morphology; ejaculated volume; testicular volume; and the blood concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone (T) were related to chronological and biological age.†

The study was authorized by the Institutional Review Board (IRB) of Gynepro Medical Team. Patient recruitment began on January 2, 2015, and ended on November 30, 2017. A power analysis was carried out to estimate the number of observations needed to have a reliable chance of detecting the effect sought; the power (π) of our tests used π = 0.90 as a standard for adequacy;‡ an online sample-size calculator was used: http://www.sample-size.net/correlation-sample-size/.

All asymptomatic fertile males over 40 years of age who presented at the outpatient clinics of the authors requesting an andrological checkup were invited to participate in the study; they were a part of the existing andrological clinic cohort.

The exclusion criteria were the presence of any ejaculation disorder (42 patients), clinical varicocele (38 patients), previous genital surgery/trauma (15 patients), ongoing medical treatment (any type of antibiotic use, antitumoral treatments, nonsteroidal anti-inflammatory drugs, gonadotropins, and steroids; 12 patients), and any previous treatment for couple infertility (28 patients).

Clinical history collection and physical examination were carried out on each participant. Biological age was assessed using the Aging Males’ Symptoms Scale Score (AMSSS) (Italian version),§ the Charlson Comorbidity Index (CCI), and the body adiposity index (BAI).¶ Testicular volume (TV) was assessed and measured using an automated formula provided by the ultrasound machine: TV = width × height × depth × 10⁻³ × 0.523.¶ The FSH, LH, and T levels were assessed using the same methods and reference values in Bologna and Varese.¶ Semen volume, sperm concentration, motility, and morphology were also assessed for each patient in two consecutive sperm analyses according to the World Health organization (WHO).∥

The importance of the relationships regarding the CCI, the BAI, and the AMSSS and age with respect to reproductive monitors were assessed using multiple regression analysis. Each patient delivered two semen samples; thus, the analysis was corrected for the number (two) of sperm samples with Bonferroni correction to guard against the bias of repeated testing effects (i.e., because each regression was computed twice). There were five assumptions of linear regression, and all were fulfilled: absence of any multicollinearity, linearity, statistical independence of the errors, homoscedasticity, and normality of the error distribution.¶ Two hundred and fifty-four patients were eligible for participation in the study. Twenty patients refused to participate in the study and 14 patients dropped out for unknown reasons; thus, 220 patients were assessed. Their characteristics were as following median (range): age (year) = 49.0 (40.3–63.4), BAI = 25.3 (22.0–30.1), CCI = 1.0 (0.0–4.0), AMSSS = 42.0 (17.5–70.3), left testicular volume (cm³) = 16.0 (10.5–20.0), right testicular volume (cm³): 17.0 (11.1–20.0), FSH (mIU ml⁻¹) = 7.7 (2.1–56.3), LH (mIU ml⁻¹) = 6.6 (1.5–48.7), prolactin = 8.4 (4.7–38.9), T (nmol l⁻¹)= 4.9 (2.9–18.2), ejaculated volume (ml, 1st sample) = 2.5 (1.0–7.5), sperm concentration (10⁶ ml⁻¹, 1st sample) = 38.5 (15.1–110.3), progressive motile sperm (% 1st sample) = 46.3 (21.2–85.2), typical forms (% 1st sample) = 10.4 (6.0–21.2), ejaculated volume (ml, 2nd sample) = 2.7 (1.0–8.0), sperm concentration (10⁶ ml⁻¹) (2nd sample) = 42.2 (16.3–120.4), progressive motile sperm (% 2nd sample) = 50.0 (22.3–88.4), and typical forms (% 2nd sample) = 9.1 (6.3–22.4). The results of the multivariate analysis are presented analytically in Table 1. The same results are briefly indicated in decreasing order of their importance in influencing the reproductive monitors: AMSSS, BAI, CCI, and, finally, chronological age. These relationships were positive in the case of FSH and LH whereas they were negative in the other cases (left/right testicular volume, sperm volume, testosterone, sperm concentration, motility, and morphology).

The results in the present study indicated that clinical monitors of reproduction were strongly influenced by the monitors of biological age (CCI, AMSSS, and BAI) in patients over 40 years of age whereas the importance of chronological age was lower.

The final pathways of biological aging are programmed cell death (apoptosis) or permanent cell-cycle arrest (cellular senescence).¶ These data explain the relationship between the AMSSS and sperm count and testicular volume (90% of testicular volume is made up of gametes).¶ The AMSSS assesses the symptoms of aging, independently of those which are disease related; it was developed in response to the lack of standardized scales which

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1. Private Outpatient Clinic, Via Mascherario 46, Ferrara 44121, Italy; 2. Department of Urology and Andrology, Ospedale di Circolo and Macchi Foundation, Viale L. Borri 57, Varese 21100, Italy.

2. Passed away in 2019.

3. Correspondence: Dr. F Scroppo (fabrizio.scroppo@asst-settelaghi.it)

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§ Private Outpatient Clinic, Via Mascherario 46, Ferrara 44121, Italy; ¶ Department of Urology and Andrology, Ospedale di Circolo and Macchi Foundation, Viale L. Borri 57, Varese 21100, Italy.

∥ Passed away in 2019.
Table 1: Results of multiple regression analyses which define the relationships between follicle-stimulating hormone, luteinizing hormone, total testosterone, ejaculated volume, sperm concentration, motility or morphology, and left and right testicular volume and the chronological age, the Charlson Comorbidity Index, the Aging Males’ Symptoms Scale Score, and the body adiposity index

| Relationship | Level of intersection of the Y axis | Regression coefficients | Significance of each regression coefficient of the dependent variables |
|--------------|-------------------------------------|-------------------------|---------------------------------------------------------------|
| **Relationships A** | | | |
| Independent variable | Age | 8.176 | | |
| Dependent variables | | | |
| FSH | 6.287 | 21.199 | 0.009 |
| LH | 8.001 | 21.210 | 0.009 |
| T | −5.334 | 18.221 | 0.010 |
| VOL | −6.445 | 20.300 | 0.009 |
| C | −8.443 | 20.004 | 0.009 |
| Sperm MOT | −7.336 | 21.000 | 0.009 |
| Sperm MOR | −8.667 | 22.008 | 0.009 |
| **Relationships B** | | | |
| Dependent variables | | | |
| FSH | 35.767 | 36.200 | <0.001 |
| LH | 14.770 | 37.180 | <0.001 |
| T | −16.551 | 19.567 | 0.010 |
| VOL | −17.553 | 18.001 | 0.010 |
| C | −21.553 | 22.856 | 0.008 |
| Sperm MOT | −31.433 | 22.402 | 0.008 |
| Sperm MOR | −34.540 | 22.520 | 0.008 |
| LTV | −18.435 | 22.110 | 0.009 |
| RTV | −17.009 | 22.122 | 0.009 |
| **Relationships C** | | | |
| Independent variable | AMSSS | 4.500 | | |
| Dependent variables | | | |
| FSH | 25.333 | 29.200 | 0.005 |
| LH | 17.140 | 27.401 | 0.006 |
| T | −15.667 | 18.989 | 0.009 |
| VOL | −15.117 | 23.300 | 0.008 |
| C | −18.443 | 29.433 | 0.005 |
| Sperm MOT | −31.223 | 27.011 | 0.006 |
| Sperm MOR | −26.667 | 29.120 | 0.005 |
| LTV | −16.334 | 20.010 | 0.009 |
| RTV | −16.009 | 18.922 | 0.010 |
| **Relationships D** | | | |
| Independent variable | BAI | 12.500 | | |
| Dependent variables | | | |
| FSH | 26.287 | 30.509 | 0.004 |
| LH | 28.301 | 29.516 | 0.005 |
| T | −18.009 | 21.165 | 0.009 |
| VOL | −17.121 | 24.009 | 0.008 |
| C | −19.223 | 22.001 | 0.009 |
| Sperm MOT | −26.667 | 24.000 | 0.008 |
| Sperm MOR | −29.134 | 27.904 | 0.006 |
| LTV | −26.388 | 21.010 | 0.009 |
| RTV | −26.129 | 21.922 | 0.009 |

FSH reference value: 1.5-12.4 mIU ml⁻¹; LH reference value: 1.8-12.0 mU ml⁻¹; T reference value: 240-950 ng dl⁻¹; left/RTV reference value: >14 ml. \( t \): \( t \) statistics. Relationships A: relationships between age and FSH, LH, T, VOL, C, MOT or MOR, and left and RTV. Relationships B: relationships between CCI and FSH, LH, T, VOL, C, MOT or MOR, and left and RTV. Relationships C: relationships between AMSSS and FSH, LH, T, VOL, C, MOT or MOR, and left and RTV. Relationships D: relationships between BAI and FSH, LH, T, VOL, C, MOT or MOR, and left and RTV. FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: total testosterone; C: sperm concentration; VOL: ejaculated volume; MOT: motility; MOR: morphology; RTV: right testicular volume; LTV: left testicular volume; BAI: body adiposity index; AMSSS: Aging Males’ Symptoms Scale Score.
measure the severity of aging symptoms and their impact on the quality of life.  

Elevated BAI increases reactive oxygen species (ROS) in human fluids and affects the production of testosterone via a chronic inflammatory status which leads to biological senescence.  

An increase in FSH and LH with increasing age is consistent with the decline in testosterone. This knowledge legitimizes gonadotropin increase with aging. Reactive oxygen species induces subtle changes in intracellular signaling pathways with the result of prostatic function (i.e., secretion) impairment. The final result is a decrease in the sperm volume.  

Actually, the BAI is linked to aging well (i.e., biological age). The CCI affects reproductive monitors as well. In fact, comorbidities are linked to aging and to an increase in biological age.  

The strength of this manuscript lies in the marked distinction between chronological age and biological age. The major limitation of this study is the lack of standardized scales for measuring the severity of chronological aging. To overcome this bias as much as possible, three scales (AMSSS, BAI, and CCI) linked to biological aging were used. These data allowed us to conclude that biological age should be considered as well as chronological age when we are counseling prospective fathers over 40 years of age.

**AUTHOR CONTRIBUTIONS**

GC conceived the study, prepared the research protocol, studied 170 patients, wrote the manuscript, and analyzed the data. FS studied 150 patients, revised the research protocol, and edited the manuscript. Both authors have read and approved the final manuscript.

**COMPETING INTERESTS**

Both authors declared no competing interests.

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