

RESEARCH ARTICLE

Evaluation of sex-related hormones and semen characteristics in reproductive-aged male COVID-19 patients

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Abstract

In the past several months, the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-associated infection (coronavirus disease 2019 [COVID-19]) developed rapidly and has turned into a global pandemic. Although SARS-CoV-2 mainly attacks respiratory systems, manifestations of multiple organs have been observed. A great concern was raised about whether COVID-19 may affect male reproductive functions. In this study, we collected semen specimens from 12 male COVID-19 patients for virus detection and semen characteristics analysis. No SARS-CoV-2 was found in semen specimens. Eight out of 12 patients had normal semen quality. We also compared the sex-related hormone levels between 119 reproductive-aged men with SARS-CoV-2 infection and 273 age-matched control men. A higher serum luteinizing hormone (LH) and a lower ratio of testosterone (T) to LH were observed in the COVID-19 group. Multiple regression analysis indicated that serum T: LH ratio was negatively associated with white blood cell counts and C-reactive protein levels in COVID-19 patients. It's the first report about semen assessment and sex-hormone evaluation in reproductive-aged male COVID-19 patients. Although further study is needed to clarify the reasons and underlying mechanisms, our study presents an abnormal sex hormone secretion among COVID-19 patients, suggesting that attention should be paid to reproductive function evaluation in the follow-up.

KEYWORDS

COVID-19, male gonadal function, SARS-CoV-2, semen analysis, sex-related hormones

1 | INTRODUCTION

Since the first report in December 2019, a novel coronavirus-associated infection (called coronavirus disease 2019 [COVID-19]) spread rapidly and triggered a global pandemic. As of 15 June 2020, a total of 200 countries or regions were affected and more than 7 970 000 cases have been reported in the whole world.

COVID-19 is caused by beta-coronavirus which is currently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) due to its high sequence similarity (~80%) with SARS-CoV.¹ The common symptoms of COVID-19 include fever, dry cough, fatigue and dyspnea, but manifestations of multiple organs or systems have been reported, such as cardiovascular, urinary, gastrointestinal and liver injury.²⁻⁴

It was suggested that SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) and the cellular serine protease (TMPRSS2) for entry into host cells.⁵ Based on small conditional RNA-seq profiling of human testes, Wang et al⁶ reported that ACE2 is predominantly enriched in spermatogonia, Leydig and Sertoli cells. In a retrospective study involving 1099 cases, it's shown that the percentage of male COVID-19 patients was nearly 60% and around 55% of them were reproductive-aged (15~49 years old).⁷ Therefore, concern was raised about whether SARS-CoV-2 may affect the male reproductive system.

There are controversial opinions on whether SARS-CoV-2 can be present in semen. A recent study indicated that SARS-CoV-2 was detectable in semen specimens of male COVID-19 patients (6 out of 38, 15.8%), including the recovering patients (2 out of 23, 8.7%).⁸ But the other two teams reported the absence of SARS-CoV-2 in semen from male COVID-19 patients in the recovery stage.^{9,10} Besides, it should be noted that virus-associated febrile illness may exert a negative influence on male reproductive function via multiple mechanisms. Apart from the direct invasion and damage by a virus, many other factors such as fever, inflammation, hypoxia, or drugs may also affect male hypothalamic–pituitary–gonadal (HPG) axis and spermatogenesis. Based on the autopsy of six patients who died of SARS, Xu et al¹¹ presented the evidence of orchitis although no SARS virus was found in testes. Therefore, to better understand the impact of COVID-19 on fertility, testicular endocrine function and spermatogenesis should also be assessed.

In this study, we compared the sex-related hormones between 119 reproductive-aged male COVID-19 patients and 273 age-matched uninfected men. Moreover, semen samples from 12 patients were collected for SARS-CoV-2 virus detection and semen characteristics analysis. Our study provides valuable clinical evidence which will facilitate further evaluation of the effect of COVID-19 on male reproductive function.

2 | METHODS

2.1 | Study design and patients

This study was reviewed and approved by the Medical Ethical Committee of Zhongnan Hospital of Wuhan University (approval number 2020033-1). We recruited 119 reproductive-aged (median age 39 years, range 20–49) male patients for sex-related hormones analysis, who were admitted to Wuhan Leishenshan Hospital from 5 to 31 March 2020. A convenient sampling strategy was employed, in which all consecutive patients meeting inclusion criteria were recruited. The blood samples were collected for routine medical purposes. After the required laboratory tests were completed, the residual serum was collected for male hormone profiles detection. Since the residual serum samples were usually discarded as a medical waste otherwise, and the procedure exerted no additional burden nor harm on the patients, written informed consent was waived in this part of the study. The patients were in stable clinical status when

the study was conducted and all of them had been discharged from the hospital as of 15 April. The control group came from the men who received sex hormone measurement as a part of fertility evaluation before marriage or when their partners were planning to get pregnant. 273 age-matched men (median age 39 years, range 24–49) were randomly selected and the data of their sex-related hormones were obtained.

From 26 March 2020 to 23 April 2020, another 12 male COVID-19 patients (median age 31.5 years, range 25–46) were recruited in the reproductive medical center of Zhongnan hospital for semen analysis. Neither preexisting illness nor the other virus infection was complicated. Among them, only case 12 received corticosteroid therapy and no other steroids were used. The SARS-CoV-2 virus in semen was detected using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and semen characteristics were analyzed. Written informed consent was obtained from each participant.

The diagnosis of COVID-19 and the degree of severity (mild, moderate, severe, critical) were determined according to the New Coronavirus Pneumonia Prevention and Control Program (7th ed.) published by the National Health Commission of China.¹² SARS-CoV-2 infection was confirmed by qRT-PCR on nasal and pharyngeal swab specimens or by serum virus antibody (IgM or IgG) detection using the colloidal gold test. In this study, the recovery stage refers to the condition with two continuous negative SARS-CoV-2 nucleic acid tests, or with lessened symptoms and substantially resolved lesion shown by chest computed tomography scan.

2.2 | Procedures

Clinical information, laboratory findings, radiological features, and outcome data of COVID-19 patients were obtained from medical records. In the control group, the data of serum testosterone (T), estradiol (E₂), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels were obtained from the database kept in our reproductive medical center. All the men in the control group received hormone detection in the morning. The researcher in charge of data collection was blinded to their fertility status to minimize the selection bias. All information was obtained and curated with a standardized data collection form, which was double-checked by two researchers independently.

Considering the diurnal pattern of sex-related hormones, in the COVID-19 group, only blood samples collected at the same time of the day as the control group were selected. Residual serum was collected and kept at –20°C. Serum T, E₂, FSH, and LH were detected by electrochemiluminescent immunoassays according to the protocol from the manufacturer (cobas e411; Roche, Switzerland). The ratios of T to LH, T to E₂ and FSH to LH were calculated.

Semen samples were obtained by masturbation after an abstinence period of 2–7 days (with a median of 4 days) and processed within 1 hour of ejaculation for analysis. Semen assessment was performed according to the World Health Organization (WHO) laboratory manual

for the examination and processing of human semen (5th ed.). The characteristics such as total sperm count, sperm motility, vitality, and morphology were detected. Low motility is defined as progressive sperm (PR) <32% or PR + nonprogressive (NR) <40%. Sperm DNA fragmentation was assessed by sperm chromatin dispersion (SCD) test (sperm DNA fragmentation assay kit, Wright-Giemsa stain method; Ankebio, Anhui, China) as described previously with minor modifications.¹³ A minimum of 500 spermatozoa per samples were scored under the $\times 100$ objective of the microscope. DNA fragmentation index (DFI, %) equals to the percentage of sperm cells with fragmented DNA in the total sperm cells. A semen aliquot was centrifuged at 600 g to isolate seminal plasma from whole semen cells. The SARS-CoV-2 virus in both seminal plasma and spermatozoa was detected by qRT-PCR. The primers and the cycling parameters were as reported previously.¹⁴

qRT-PCR of SARS-CoV-2 was performed using the Chinese Center for Disease Control and Prevention (CDC) recommended Kit (BioGerm, Shanghai, China). Serum antibodies (IgM and IgG) were detected by a commercial colloidal gold test kit (Wondfo Biotechnology, Guangzhou, China). Sample collection, processing, and laboratory testing complied with WHO guidance.

2.3 | Statistical analysis

All statistical analysis was performed using Graphpad Prism 6.04 (San Diego) and SPSS 16.0 (Chicago, IL). Continuous variables were expressed as means \pm standard deviations (SD) or medians and interquartile ranges (IQR) as appropriate. Categorical variables were summarized as the counts and percentages (%). The distribution of data was analyzed by Kolmogorov-Smirnov test. Differences between two groups were analyzed by Student *t* test (parametric) or the Mann-Whitney *U* test (nonparametric). Multiple linear regression was performed to analyze the relationship between serum T: LH ratio

and the clinical characteristics of the COVID-19 patients. Statistical significance was defined as *P* value of <.05.

3 | RESULTS

Semen samples were obtained from 12 male COVID-19 patients. Among them, one man (case 1) was regarded as a mild type with a positive virus nucleic acid test on 21 March. His semen sample was collected on 26 March. The other 11 men (case 2-11) were diagnosed as moderate COVID-19, whose nucleic acid test was all negative when the semen specimens were obtained. The time between semen collection and disease onset ranged from 56 days to 109 days (with a median of 78.5 days). Their clinical characteristics were presented in Supplemental Table 1. For all the 12 patients, no SARS-CoV-2 virus was found in semen specimens.

Semen analysis showed that eight patients (66.7%, 8/12) had normal sperm parameters and low DFI (7.6 ± 2.2). Four patients (33.3%, 4/12) had low sperm motility (defined as PR + NP <40%) with higher sperm DFI (20.05 ± 3.80 vs 7.6 ± 2.2 in the other eight patients), among whom two participants also had poor sperm morphology (defined as normal morphology <4%). Three cases (case 8, 9, 11) reported a loss of libido after the COVID-19 attack. Case 8 also complained about the loss of morning erection. Case 8 and 9 had normal semen quality. The details can be seen in Table 1.

Three patients (case 3, 4, 11) received semen assessment(s) in our center before SARS-CoV-2 infection (Seen in Table 2). Case 11 showed a decrease in sperm motility (31.1%, PR + NP) after COVID-19. However, since there is a biological variation in semen parameters over time, repeated detections in the follow-up may be needed to interpret this change. Case 4 was diagnosed as asthenospermia before COVID-19, and no much alteration was seen in his current semen characteristics. Semen parameters of case 3 were defined

TABLE 1 Semen characteristics of 12 reproductive-aged men after COVID-19 infection

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12
Volume, mL	3.5	1.5	4.2	5.9	1.5	4.9	2.0	2.4	4.1	4.1	3.8	5.1
Sperm concentration ($\times 10^6$ /mL)	50.2	112.8	54.1	7.0	41.9	20.7	98.7	135.8	101.4	15.3	98.2	49.9
Total mobile sperm count ($\times 10^6$ per ejaculate)	70.28	77.66	97.93	7.02	6.35	38.54	131.07	181.21	302.66	7.09	116.05	154.48
Motility (%)												
Progressive (PR, %)	38.0	38.9	37.5	15.0	8.7	36.3	63.1	50.1	69.7	9.5	27.4	53.3
Nonprogressive (NR, %)	2.0	7.0	5.6	2.0	1.4	1.7	3.3	5.5	3.1	1.8	3.7	7.4
Immotility (IM, %)	60.0	54.1	56.9	83.0	89.9	62.0	33.6	44.4	27.2	88.7	68.9	39.3
Vitality (%)	76	72	70	52	24	68	82	75	87	32	58	82
Morphology												
Normal sperm (%)	6.3	4.8	4.1	3.4	3.5	5	6.1	6.9	5	4.6	7.5	5.5
DNA fragmentation index (DFI, %)	6.7	5.4	10.1	17.4	23.7	7.8	5.7	7.5	11.6	16.2	22.9	6.0

Abbreviation: COVID-19, coronavirus disease 2019.

TABLE 2 Semen characteristics of three male COVID-19 patients before and after SARS-CoV-2 infection

	Case 3			Case 4			Case 11	
	Before-1	Before-2	After	Before-1	Before-2	After	Before	After
Volume, mL	3.4	5.1	4.2	3.5	5.1	5.9	2.8	3.8
Sperm concentration ($\times 10^6$ /mL)	63.9	52.4	54.1	12.2	17.4	7.0	78.0	98.2
Total mobile sperm count ($\times 10^6$ per ejaculate)	111.67	122.40	97.93	7.64	19.97	7.02	134.75	116.05
Motility (%)								
Progressive (PR, %)	48.9	44.7	37.5	16.7	20.0	15.0	26.7	27.4
Nonprogressive (NR, %)	2.5	1.1	5.6	1.2	2.5	2.0	35.0	3.7
Immotility (IM, %)	48.6	54.2	56.9	82.1	77.5	83.0	38.3	68.9
Vitality (%)	75	73	70	65	67	52	70	58
Morphology								
Normal sperm (%)	6.3	5.0	4.1	2.9	3.7	3.4	7.3	7.5

Notes: Before: Before COVID-19; After: After COVID-19.

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

as normal before and after COVID-19, but a decrease in total mobile sperm number was observed.

Among 119 male COVID-19 patients whose sex-hormones were detected, 2.52% (3/119) were diagnosed as "mild type", 84.03% (100/119) as "moderate type", 11.76% (14/119) as "severe type" and 1.68% (2/119) as "critical type." The usage of corticosterone, arbidol, oseltamivir, or intravenous antibiotics was 14.81% (16/119), 45.38% (54/119), 33.61% (40/119) or 56.30% (67/119) respectively. 36.97% (44/119) of the patients had elevated serum alanine transaminase (ALT) and/or serum aspartate transaminase (AST), indicating the impaired liver function. Four patients reported coexisting chronic diseases, including liver transplantation history, type II diabetes, kidney dysfunction, and lymphoma. The clinical characteristics of the 119 patients were presented in Table 3.

Compared to the control group, COVID-19 patients had significantly higher serum LH ($P < .0001$). Although there was no statistical difference in serum T ($P = .1886$) or FSH ($P = .5585$) between the two groups, the ratios of T: LH ($P < .0001$) and FSH: LH ($P < .0001$) were decreased in the COVID-19 group (seen in Table 4). In the control group, only 150 out of 273 men had the data of serum E_2 . Since they were also age-matched with the COVID-19 patients ($P = .3295$), the comparison of E_2 or T: E_2 ratio was performed between them and the COVID-19 group. No significant difference was observed in either E_2 ($P = .9364$) or T: E_2 ratio ($P = .7096$). (seen in Table S2)

By multivariable linear regression analysis, it can be seen that serum T: LH ratio in the COVID-19 group was negatively associated with white blood cell counts ($P = .0069$) and CRP level ($P = .0285$). (Seen in Table 5)

4 | DISCUSSION

The main functions of the testes are spermatogenesis and steroidogenesis. It is known that a broad range of virus families, including human immunodeficiency virus (HIV), mumps virus,

TABLE 3 Clinical characteristics of 119 male COVID-19 patients for sex hormone detection

COVID-19 patients, n = 119	
Age, median (range), y	39 (20–49)
Age group (%)	
<30 y	7.56% (9/119)
30–39 y	46.22% (55/119)
40–49 y	46.22% (55/119)
Symptoms (%)	
Fever	94.96% (113/119)
Cough	52.10% (62/119)
Sore throat	6.72% (8/119)
Myalgia	12.61% (15/119)
Dyspnea	14.29% (17/119)
Chest pain	3.36% (4/119)
Diarrhea	8.40% (10/119)
CT evidence of viral pneumonia (%)	96.64% (115/119)
Corticosteroid therapy (%)	15.97% (19/119)
Laboratory characteristics (median, range)	
White blood cell count (WBC, $\times 10^9$ /L)	6.24 (2.25–12.02)
Lymphocyte count ($\times 10^9$ /L)	1.87 (0.22–3.77)
Lymphocyte percentage (%)	31.80 (2.70–60.80)
C-reactive protein (CRP, mg/L)	2.42 (0.50–125.1)
Alanine transaminase (ALT, IU/L)	40 (7–710)
Aspartate transaminase (AST, IU/L)	23 (7–257)
Serum albumin (g/L)	41.40 (23.8–48.1)
Creatinine (μ mol/L)	74.85 (39.7–1113.0)
Urea nitrogen (mmol/L)	4.40 (2.60–36.1)
D-dimer (mg/L)	0.22 (0.10–7.00)

Abbreviations: COVID-19, coronavirus disease 2019; CT, computed tomography.

TABLE 4 Sex-related hormone profiles in the COVID-19 group and the control group

	Men with COVID-19 (n = 119)	Age-matched control group (n = 273)	P value
Age, y	39 (35.0~44.0)	39 (35.0~42.0)	.1585
Testosterone, ng/mL	3.97 (3.14~5.74)	4.43 (3.53~5.24)	.1886
FSH, mIU/mL	4.57 (3.15~6.67)	4.64 (3.51~6.38)	.5585
LH, mIU/mL	6.36 (4.63~8.37)	3.38 (2.48~4.52)	<.0001*
T: LH	0.68 (0.43~0.96)	1.24 (0.92~1.84)	<.0001*
FSH: LH	0.80 (0.53~1.04)	1.40 (1.04~1.94)	<.0001*

Note: Data are presented as medians (IQR).

Abbreviations : COVID-19, coronavirus disease 2019; FSH, follicle stimulating hormone; LH, luteinizing hormone.

*Statistically significant.

influenza, Zika virus, and so forth, may attack testes and affect male reproductive function.¹⁵ Except for the direct damage of testes by viruses, many other factors such as fever, inflammation, and dysregulation of the HPG axis may also impair testosterone secretion or sperm production.^{15,16} Therefore, to better

TABLE 5 Multiple linear regression for association of clinical characteristics of COVID-19 patients with serum T: LH ratio

	95% CI		P value
Age	-0.0200	~ 0.0040	.1871
Severity ^a	-0.2414	~ 0.1919	.8213
White blood cell count (WBC, ×10 ⁹ /L)	-0.1669	~ -0.02729	.0069*
Lymphocyte count (×10 ⁹ /L)	-0.1327	~ 0.2941	.4549
Lymphocyte percentage (%)	-0.02297	~ 0.006507	.2707
C-reactive protein (CRP, mg/L)	-0.01190	~ -0.0006757	.0285*
Alanine transaminase (ALT, IU/L)	-0.003524	~ 0.002383	.7025
Aspartate transaminase (AST, IU/L)	-0.008145	~ 0.009696	.8635
Serum albumin, g/L	-0.03309	~ 0.02713	.8449
Creatinine, μmol/L	-0.001961	~ 0.001260	.6674
Urea nitrogen, mmol/L	-0.05946	~ 0.04560	.7943
D-dimer, mg/L	-0.1740	~ 0.06645	.3771

Note: The degree of severity is determined according to the New Coronavirus Pneumonia Prevention and Control Program (7th ed.) published by the National Health Commission of China.

Abbreviations: 95% CI, 95% confidence interval; COVID-19, coronavirus disease 2019.

^aseverity: 1- mild type, 2-moderate type, 3-severe type, 4-critical type.

*Statistically significant.

understand the effect of COVID-19 on the male reproductive system, not only the possibility of sexual transmission but also the status of reproductive endocrine and spermatogenesis should be evaluated.

Using qRT-PCR, we did not find SARS-CoV-2 virus in semen samples from twelve male COVID-19 patients. This finding is consistent with two recent reports.^{9,10} The protection by a blood-testes barrier and the low incidence of viremia in COVID-19 are both possible reasons.¹⁷ Besides, since most of the semen specimens in this study were obtained from patients in the recovery stage, the virus (if ever existed in semen) may be cleared up by the time of detection.

We further compared the sex hormone profiles between 119 COVID-19 patients and 273 age-matched control men. Although serum T levels did not statistically change in the COVID-19 group, a higher serum LH level, and a lower serum T: LH ratio were observed in comparison to the control group. The basal T level in the population varies widely, thus the ratio between hormones, such as T: LH ratio was considered as a better parameter for male gonad function evaluation.¹⁸ Abundant IgG precipitation and leukocyte infiltration have been observed in testes from patients who died of SARS.¹¹ Therefore, the authors propose that immunological injury may also play a role in COVID-19. Virus-induced inflammation triggered the systematic or local release of cytokines, such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), interferon (IFN), and monocyte chemoattractant protein 1(MCP-1), etc.¹⁹ Those cytokines may do harm to testicular cells. For example, IL-6 has been implicated to impair Leydig cell differentiation.²⁰ And despite the antiviral function, IFN- γ has been shown to inhibit testosterone production by suppressing the expression of rate-limiting enzyme steroidogenic acute regulatory protein (StAR).²¹ Impaired T secretion induces LH release which can maintain T level temporarily. By multiple linear regression analysis, we demonstrated that T: LH ratio was negatively associated with WBC count and CPR level of COVID-19 patients. As an acute-phase protein, elevated CRP is accompanied by cytokines release and can reflect the severity of inflammation.²² Therefore, further study about the effect of immunological disturbance in COVID-19 on testicular cells is suggested.

Besides, the manifestations of the central nervous system in COVID-19 patients have been reported, including increased antidiuretic hormone secretion by the hypothalamus.²³ Emotional, physical, or psychological stresses and pain associated with infections can stimulate the hypothalamohypophyseal axis. Hence the disturbance of the hypothalamic-pituitary and resulted abnormality in LH secretion rhythm may also be a reason. Moreover, the role of sex-hormone-binding globin (SHBG) should also be considered. By binding to SHBG, the active free testosterone decreases and LH secretion may be induced. Although it remains unclear whether and how SHBG production is affected by COVID-19, elevated SHBG level has been reported in many pathological conditions, such as fatty liver, hepatitis, or stress after anesthesia and surgery.²⁴ Finally, it should be pointed out that the basal LH level in the patient group before the COVID-19 attack also matters. Though only 4 of 119 patients in

this study reported coexisting chronic diseases, the influence of unreported preexisting illness on male gonad function cannot be completely excluded due to the retrospective nature of the study.

Unlike LH, FSH is mainly suppressed by inhibin B secreted by Sertoli cells and reflects the function of Sertoli cells to a certain extent. Estradiol in man normally comes from the peripheral aromatization of androgens. In this study, no significant difference was present in serum FSH, serum E2, and the ratio of T: E2 between the two groups. Thus, the altered LH secretion might not be caused by a single reason but a combination of several factors.

Semen parameters were described in twelve patients. 8 participants had normal semen characteristics after COVID-19. Increased sperm DNA fraction percentage was only seen in four patients with poor semen quality. Among three men receiving semen analysis before the COVID-19 attack, the total mobile sperm count in two cases showed a slight decrease in comparison to their previous records. However, due to the limited sample size and the large biological variation in semen parameters, a further well-designed prospective cohort study is needed to clarify the effect of COVID-19 on spermatogenesis.

There are some limitations in this study. First, all the semen samples came from non-severe patients and most of them were in the recovery stage. Whether SARS-CoV-2 can invade testis in severe patients or during the incubation period is still unknown. Second, the sample size for semen assessment is limited. A prospective cohort involving more cases is required. Third, people with chronic diseases are more vulnerable to the COVID-19 attack. Since we do not know the sex hormone baseline in the patient group before infection, it should be quite cautious to interpret the influence of COVID-19 on male endocrine function.

Our study also has some strengths. First, it is the first study describing the semen characteristics of male COVID-19 patients. Second, it adds more evidence that SARS-CoV-2 was undetectable in semen and there was no risk of sexual transmission after a certain interval since symptom onset (at a median time of 78.5 days in this study). Finally, it describes the phenomenon of increased LH secretion in COVID-19 patients. Further studies should be performed to determine whether it is a transient dysregulation or has prolonged effects and whether it is a preexisting condition or a result of the COVID-19 attack. The underlying mechanisms should also be explored. Gonadal function evaluation including semen examination is necessary for the follow-up of the men who recovered from COVID-19. And appropriate interval time should be determined for those who have a plan of procreation.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

MZ contributed substantially to the study design, manuscript draft, and revision. LM was in charge of the manuscript draft and sample collection. WX took responsibility for sample processing and laboratory tests. DL helped in hormone detection. LS, YX, YM, and ZC were in charge of data collection and analysis. LS, JQ, and JL contributed to semen analysis. GY, HS, and FZ performed qRT-PCR detection. YZ provided suggestions for results interpretation.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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