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Associations of serum estradiol level, serum estrogen receptor-alpha level, and estrogen receptor-alpha polymorphism with male infertility

A retrospective study

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Abstract

Estradiol regulates spermatogenesis partly via estrogen receptor-alpha (ESRα). This study aimed to analyze the associations of serum estradiol level, serum ESRα level, and ESRα gene polymorphisms with sperm quality.

This retrospective study included infertile men attending the Reproductive Center, Affiliated Hospital of Youjiang Medical University for Nationalities, and a control group without a history of fertility (October, 2016 to March, 2017). Data regarding sperm quality, serum levels of estradiol and ESRα, and rs2234693C/T genotype were extracted from the medical records. Pearson/Spearman correlations (as appropriate) between estradiol level, ESRα level, and sperm quality parameters were evaluated.

The analysis included 215 men with infertility and 83 healthy controls. The infertile group had higher serum levels of estradiol (147.57 ± 35.3 vs 129.62 ± 49.11 pg/mL, $P < .05$) and ESRα (3.02 ± 2.62 vs 1.33 ± 0.56 pg/mL, $P < .05$) than the control group. For the infertile group, serum estradiol level was negatively correlated with sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology ($r = 0.309$, 0.211, and 0.246, respectively; all $P < .05$). Serum estradiol and ESRα levels were lower in infertile men with normozoospermia than in those with azoospermia, oligozoospermia, mild azoospermia, or malformed spermatozoa (all $P < .05$). Sperm concentration, percentage of progressively motile sperm, serum ESRα level, and serum estradiol level did not differ significantly among the rs2234693 CC, CT, and TT genotypes.

Elevated serum levels of estradiol and possibly ESRα might have a negative impact on sperm quality and fertility, whereas single nucleotide polymorphisms at rs2234693 of the ESRα gene had little or no effect.

Abbreviations: ESRα = estrogen receptor-alpha, ESRβ = estrogen receptor-beta, IQR = interquartile range, SD = standard deviation, SNPs = single nucleotide polymorphisms.

Keywords: estrogen receptor alpha, estrogens, infertility, male, semen analysis, single nucleotide polymorphism, sperm count, sperm motility

1. Introduction

Infertility is a global health problem affecting up to 15% of couples worldwide.[1] Male factors are believed to contribute to 50% of cases of infertility.[2] Recognized causes of male infertility include medical conditions, medications, and genetic factors, although around 30% of cases are associated with idiopathic sperm abnormalities.[3] The diagnosis of male infertility mainly depends on the routine analysis of semen samples, which provides 3 main parameters: sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology. Notably, Huang et al reported that the semen quality of young Chinese men declined during the 15-year period between 2001 and 2015, with decreases in sperm concentration, total sperm count, sperm progressive motility, and normal morphology.[4]

Spermatogenesis is a complex process involving a series of cellular activities such as proliferation, meiosis, and differentiation. The process of spermatogenesis is regulated by a variety of factors, with changes in gene expression levels in spermatogenic cells playing a particularly important role.[5] Disturbances in semen quality, sperm count, and reproductive function are

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closely related to environmental estrogen and endocrine disorders.[6] Aromatase cytochrome P450 synthesizes estrogen from testosterone in the testes of males.[7] Estrogen plays an important role in the differentiation, maturation, and function of the human reproductive system has a negative feedback effect on gonadotropins, and adversely affects reproductive parameters through estrogen receptors.[8] Studies have shown that estrogen exerts various effects on testicular development and homeostasis, including germ cell apoptosis, sperm activation, and spermatogenesis, through its receptor signaling pathways.[9,10] Estradiol influences testicular cells, germ cells, and mature sperm by binding to classical intracellular estrogen receptors or membrane estrogen receptors to trigger genomic and nongenomic signal transduction pathways.[11]

The effects of estrogen are mediated by 2 main receptors, estrogen receptor-alpha(ESRα) and estrogen receptor-beta (ESRβ).[12] ESRα is a 595-amino acid protein encoded by *ESR1* on chromosome 6q25. ESRα is thought to influence semen quality through its effects on the early stage of spermatogenesis and late maturation of spermatogenic cells.[13] The role of genetic polymorphisms in human infertility has received great interest in recent years, and there is evidence that polymorphisms in ESRα might be associated with semen quality and male infertility.[14–16]

One of the main single nucleotide polymorphisms (SNPs) of *ESR1* studied to date is rs2234693 (397T→C).[14–16] Still, there is little information regarding the associations of rs2234693 polymorphisms with ESRα expression, serum estrogen concentration, semen quality, and infertility. Therefore, the aim of this study was to examine the relationship between rs2234693 SNPs and ESRα expression level, estradiol level, sperm quality, and male infertility.

2. Methods

2.1. Study subjects

This retrospective study included men with infertility who were treated at the Reproductive Center of the Affiliated Hospital of Youjiang Medical University for Nationalities between October, 2016 and March, 2017, as well as a control group of men without infertility. The inclusion criteria for the patients with infertility (infertile group) were (1) male, (2) residing in the Guangxi Zhuang region of China, and (3) infertile for more than 1 year after a regular sexual life despite having well-developed secondary sexual characteristics and normal testicular volume. The exclusion criteria for the infertile group were (1) sexual dysfunction, (2) urogenital infection, (3) sperm production disorder, (4) sperm-egg binding disorder, (5) endocrine disorder, (6) immunologic disorder, (7) abnormality of physical development, (8) overweight or obesity, (9) female factor infertility, and (10) environmental or occupational exposures that could affect semen quality or fertility outcomes (including consumption of alcohol or strong tea, smoking, use of saunas, wearing tights, regular use of a computer on the lap, use of electric blankets, use of medications such as gentamicin, erythromycin, nitrofurantoin, certain hormones, steroids and chemotherapy drugs, drug abuse, long-term consumption of crude cottonseed oil, long-term work in a high-temperature environment, employed as a nuclear power plant worker, steelworker, welder, printer or fuel worker, and long-term exposure to radioactive substances, herbicides, fertilizers, insecticides, pesticides, toxic plastic products, or other chemical poisons).

A healthy control group was enrolled during the same period using the following criteria: (1) male, (2) residing in the Guangxi Zhuang region, and (3) a history of fertility with at least 1 normal child born without the use of assisted reproductive technology. The exclusion criterion for the healthy control group was occupational exposures that could affect semen quality or fertility outcomes (as for the infertile group). There was no blood relationship between the 2 groups.

2.2. Ethics

All healthy subjects voluntarily donated experimental samples after providing informed written consent. This study was approved by the Ethics Committee of Youjiang Medical University for Nationalities [YYFY-LL-2018-006]. The subjects did not receive any support/money for their participation.

2.3. Collection of baseline clinical data

Baseline demographic and clinical characteristics were extracted from the medical records.

2.4. Assessment of semen quality

The semen examinations were performed according to the WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th ed.[17] The subjects were asked to provide semen samples after 2–7 days of ejaculation abstinence. Each specimen was collected in a clean tube and incubated at 37°C. All samples were analyzed by full-time staff at the Reproductive Medicine Center. An SCA automatic semen analysis system (Microptic, Barcelona, Spain) was used to evaluate semen characteristics, including liquefaction time, viscosity, appearance, volume, and pH. Sperm kinetic parameters, including density, motility, and survival rate, were analyzed using a CX41-32C02 optical microscope (Olympus, Tokyo, Japan). Sperm morphology was assessed using the Diff-Quik staining procedure. At least 200 spermatozoa were counted using oil immersion microscopy, and the percentages of sperm with normal morphology, head defects, neck defects, and midsection defects were calculated.[17] Semen quality was classified as normal, azoospermia, oligozoospermia, oligoasthenozoospermia, or malformed spermatozoa. Normozoospermia: the percentage of forward-motile sperms was 32% or higher, while the percentage of normal sperms was 4% or higher. Azoospermia: there were no sperms in semen. Oligozoospermia: sperm count was lower than 15×10^6/mL. Oligoasthenozoospermia: the total number or concentration of sperms was lower than 15×10^6/mL, but the percentage of forward-motile sperm was lower than 32%. Asthenoteratozoospermia (malformed spermatozoa): the percentage of normal sperm was less than 4%.

2.5. Determination of serum estradiol levels

The serum levels of estradiol were measured by enzyme-linked immunosorbent assay (ELISA) using an E2 ELISA test kit (Cusabio Biotech Co. Ltd., Wuhan, China). Briefly, 50-μL volumes of test samples and standards were added to a microtiter plate, and to each well was added 50 μL of conjugate (excluding the blank control well) and 50 μL of antibody. The plate was incubated at 37°C for 1 hour. After 3 washes, 50 μL of developer A solution and 50 μL of developer B solution were added

Table 1

Baseline characteristics of the study participants.

Characteristic	Control group (n = 83)	Infertile group (n = 215)	P
Age (years), mean ± SD	32.6 ± 6.32	31.05 ± 5.82	.145
Semen pH, mean ± SD	7.15 ± 0.34	7.17 ± 0.41	.883
Abstinence time (days), mean ± SD	4.75 ± 2.26	4.45 ± 1.88	.385
Semen volume (mL), mean ± SD	3.18 ± 1.14	3.08 ± 1.55	.652
Sperm concentration ($\times 10^6$/mL), median (IQR)	71.60 (42.00, 99.10)	22.90 (10.50, 60.20)	<.01
Progressively motile sperm (%), median (IQR)	50.80 (42.70, 60.40)	24.80 (15.30, 47.05)	<.01
Normal sperm morphology (%), median (IQR)	5.00 (4.00, 6.00)	2.00 (1.00, 3.00)	<.01
Estradiol (pg/mL), mean ± SD	129.62 ± 49.11	147.57 ± 35.36	.007
ESRα (pg/mL), mean ± SD	1.33 ± 0.56	3.02 ± 2.62	<.01

ESRα = estrogen receptor α, IQR = interquartile range, SD = standard deviation.

sequentially to each well, and the plate was incubated in the dark for 15 minutes at 37°C. The optical density value at 450 nm (OD_{450}) was measured with a microplate reader within 10 minutes of the reaction being terminated by the stop solution. The estradiol concentration in the sample was calculated using a standard curve.

2.6. Determination of serum ESRα levels

The serum levels of ESRαwere measured using an ESRα ELISA test kit (Cusabio Biotech Co. Ltd., Wuhan, China). Test samples and standards (100 μL) were added to the wells and incubated at 37°C for 2 hours. After removing the liquid and drying without washing, 100 μL of biotin-labeled antibody working solution was added to each well, and the plate was incubated at 37°C for 1 hour. Following 3 washes, 100 μL of horseradish peroxidase-labeled avidin working solution was added to each well, and the plate was incubated at 37°C for 1 hour. After 3 washes, 90 μL of substrate solution was added to each well, and the color was developed in the dark (15 minutes at 37°C). The reaction was terminated with the stop solution, and the ESRα level in each test sample was quantified by measurement of the OD_{450} and comparison with a standard curve.

2.7. Detection of rs2234693 polymorphism in the ESR1 gene

Genomic DNA was extracted, and the polymerase chain reaction-restriction fragment length polymorphism method was used to detect rs2234693C/T polymorphism in the *ESR1* gene. The polymerase chain reaction primers used for detection of the SNP were designed according to the reference sequence provided in the GenBank database using Primer 5 software. The primer sequences were forward GGCTCAAACTACAGGGCT and reverse TTTCAGAACCATTAGAGACCA. The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

2.8. Statistical analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Quantitative data were tested for normality by the Kolmogorov–Smirnov test. Variables that met the normal distribution were expressed as mean ± standard deviation and compared between groups using the *t* test or analysis of variance. Variables that were non-normally distributed are presented as median and interquartile range and were compared between groups using the Mann–Whitney *U* test or Kruskal–Wallis test. Correlations between quantitative data were evaluated using Pearson correlation analysis (normally distributed variables) or Spearman rank correlation analysis (non-normally distributed variables). All reported *P* values are two-tailed and were considered statistically significant when <.05.

3. Results

3.1. Baseline characteristics of the study participants

A total of 215 patients were included in the infertile group and 83 healthy subjects in the control group. There were no significant differences between groups in age, number of abstinence days, semen pH, or semen volume (all $P > .05$; Table 1). Sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology were significantly lower in the infertile group than in the control group (all $P < .01$). Serum estradiol and ESRα levels were significantly higher in the infertile group than in the control group (both $P < .05$).

3.2. Correlation of semen quality parameters with serum estradiol and serum ESRα levels for patients in the infertile group

Ten patients in the infertile group had azoospermia and so were excluded from the correlation analyses; therefore, the correlations of semen quality with serum estradiol and serum ESRα levels were evaluated in the remaining 205 patients in the infertile group (Table 2). Serum estradiol levels were significantly negatively correlated with sperm concentration, percentage of

Table 2

Correlations of semen quality parameters with serum estradiol and serum estrogen receptor α.

	Estradiol		ESRα	
Parameter	r	P	r	P
Sperm concentration ($\times 10^6$/mL)	−0.309	0.001	0.098	.193
Progressively motile sperm (%)	−0.211	0.004	0.068	.367
Normal sperm morphology (%)	−0.246	0.002	0.124	.124
Estradiol	–	–	−0.198	.002
ESRα	−0.198	0.002	–	–

Correlation analysis was performed using data from 205 of the 215 patients in the infertile group (10 patients with azoospermia were excluded).
ESRα = estrogen receptor α.

Table 3

Characteristics of patients in the infertile group stratified according to semen quality.

Parameter	Normal (n = 83)	Azoospermia (n = 10)	Oligozoospermia (n = 24)	Mild azoospermia (n = 46)	Malformed spermatozoa (n = 52)	P
Age (years)	33.00 ± 5.93	27.57 ± 2.59	32.92 ± 4.91	32.68 ± 4.94	32.70 ± 6.20	.170
Semen pH	7.15 ± 0.34	7.18 ± 0.90	7.20 ± 0.22	7.17 ± 0.29	7.14 ± 0.32	.953
Abstinence time (days)	5.43 ± 2.05	5.67 ± 1.90	5.54 ± 2.29	5.15 ± 1.96	5.38 ± 1.99	.900
Semen volume (mL)	3.58 ± 1.22	3.65 ± 1.47	3.82 ± 1.43	3.76 ± 1.39	3.78 ± 1.24	.438
Sperm concentration ($\times 10^6$/mL)	69.75 (41.93, 99.33)	–	10.05 (7.33, 12.13)	26.90 (13.75, 40.30)	39.90 (15.00, 62.30)	<.01
Progressively motile sperm (%)	51.00 (42.58, 60.73)	–	30.45 (15.58, 44.60)	18.30 (12.60, 22.90)	28.50 (15.10, 48.40)	<.01
Normal sperm morphology (%)	5.00 (4.00, 6.00)	–	2.00 (1.00, 3.38)	1.00 (1.00, 2.00)	1.50 (1.00, 2.00)	<.01
Serum estradiol (pg/mL)	129.54 ± 49.79	162.49 ± 25.44*	145.04 ± 28.17*	148.98 ± 33.01*	145.33 ± 39.81*	.090
Serum ESRα (pg/mL)	1.33 ± 0.56	4.22 ± 1.14*	5.01 ± 3.63*	5.03 ± 3.83*	4.75 ± 2.46*	<.01

Data are presented as mean ± standard deviation or median (interquartile range).
ESRα = estrogen receptor α.
* $P < .05$ vs Normal group.

progressively motile sperm, and percentage of sperm with normal morphology ($r = 0.309$, 0.211, and 0.246, respectively; all $P < .05$). Serum ESRα content was not significantly correlated with sperm concentration, percentage of progressively motile sperm, or percentage of sperm with normal morphology (all $P > .05$). However, there was a significant negative correlation between serum estradiol level and serum ESRα content ($r = 0.198$, $P < .05$).

3.3. Characteristics of patients in the infertile group stratified according to semen quality

The patients in the infertile group were divided into 5 subgroups according to semen quality (normal, azoospermia, oligozoospermia, mild azoospermia, or malformed spermatozoa). The characteristics of the patients in each subgroup are shown in Table 3. Serum estradiol and ESRα concentrations were significantly lower in the normozoospermia group than in the other 4 groups (all $P < .05$). Furthermore, pairwise analyses using the Bonferroni method revealed no significant differences among the azoospermia, oligozoospermia, mild azoospermia, and malformed spermatozoa groups ($P > .05$).

3.4. Characteristics of patients in the infertile group stratified according to age

A further subgroup analysis based on age (<30 years old, 30–40 years old, or >40 years old) was performed in 190 of the 215 patients in the infertile group (25 patients were excluded due to missing data). As detailed in Table 4, there were no significant differences among subgroups in any of the characteristics analyzed ($P > .05$).

3.5. Clinical phenotypes of patients with polymorphisms at the rs2234693 locus of ESR1

Genotype results were available for 90 of the 215 patients in the infertile group. Notably, there were no significant differences in sperm concentration, percentage of progressively motile sperm, serum ESRα level, or serum estradiol level among the CC, CT, and TT genotypes of the rs2234693 locus ($P > .05$; Table 5).

4. Discussion

A notable finding of this study was that males with infertility had higher serum levels of estradiol and ESRα, as well as lower sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology than a control group of males without infertility. Furthermore, serum estradiol levels but not serum ESRα levels were significantly negatively correlated with sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology. In addition, serum estradiol and ESRα concentrations were significantly lower in patients with normozoospermia than in patients with azoospermia, oligozoospermia, mild azoospermia, or malformed spermatozoa. Interestingly, there were no signifi-

Table 4

Characteristics of patients in the infertile group stratified according to age.

Parameters	<30 years old (n = 87)	30–40 years old (n = 76)	>40 years old (n = 27)	P
Semen pH	7.20 ± 0.39	7.12 ± 0.37	7.12 ± 0.36	.470
Abstinence time (days)	4.56 ± 1.85	4.42 ± 1.20	4.76 ± 1.86	.779
Semen volume (mL)	3.09 ± 1.45	3.20 ± 1.25	3.01 ± 1.46	.834
Sperm concentration ($\times 10^6$/mL)	44.10 (25.90, 71.60)	39.50 (19.20, 76.90)	40.10 (10.30, 103.2)	.673
Progressively motile sperm (%)	42.90 (22.10, 53.00)	42.80 (21.90, 56.10)	44.40 (38.35, 54.20)	.459
Normal sperm morphology (%)	4.00 (1.00, 5.00)	4.00 (2.00, 5.00)	4.00 (2.00, 5.50)	.985
Estradiol (pg/mL)	139.06 ± 31.25	137.01 ± 34.54	126.56 ± 25.06	.353
ESRα (pg/mL)	2.57 ± 1.85	3.00 ± 1.45	3.50 ± 1.23	.256

The analysis was performed using data from 190 of the 215 patients in the infertile group (25 patients with missing data were excluded). Data are presented as mean ± standard deviation or median (interquartile range).
ESRα = estrogen receptor α.

Table 5

Clinical phenotypes of patients with different polymorphisms at the rs2234693C/T locus of *ESR1*.

Clinical phenotype	CC (n = 25)	CT (n = 47)	TT (n = 18)	*P*
Sperm concentration ($\times 10^6$/mL)	30.85 (12.85, 67.30)	30.90 (11.50, 62.25)	22.90 (9.55, 58.80)	.891
Progressively motile sperm (%)	31.15 (14.75, 47.30)	23.70 (14.85, 43.35)	27.70 (18.15, 55.35)	.456
Estradiol (pg/mL)	146.89 ± 35.05	147.38 ± 33.71	142.34 ± 24.76	.848
ESRα (pg/mL)	3.29 (2.01, 6.67)	4.49 (2.40, 6.18)	3.25 (1.71, 6.99)	.618

The analysis was performed using data from 90 of the 215 patients in the infertile group with genetic testing results. Data are presented as mean ± standard deviation or median (interquartile range). ESRα = estrogen receptor α.

cant differences in sperm concentration, percentage of progressively motile sperm, serum ESRα levels, or serum estradiol levels among the CC, CT, and TT genotypes of the rs2234693 locus. The data suggest that an elevated estradiol level might have a negative impact on sperm quality and thus reduce fertility in men, whereas SNPs at the rs2234693 locus of the gene encoding ESRα have little effect.

It is generally accepted that estrogens are required for the normal functioning of the male reproductive system.[11] Indeed, experiments in rats have shown that estradiol acts via ESRα and ESRβ to stimulate the proliferation of immature sertoli cells, which indicates that estrogens play a key role in the regulation of spermatogenesis and fertility.[18] However, estrogens can also exert detrimental effects on spermatogenesis, sperm maturation, and male fertility.[11] We found that serum estradiol levels were higher in the infertile group than in the control group and higher for men with azoospermia, oligozoospermia, mild azoospermia, or malformed spermatozoa than for men with normozoospermia. Furthermore, serum estradiol levels were significantly negatively correlated with sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology. Taken together, the above observations suggest that elevated estradiol levels might be associated with impaired fertility in men. Experiments in animal models have yielded insights into the possible mechanisms by which estradiol might impair fertility in men. It has been reported that estradiol acts via the ESRα to regulate spermiogenesis and via the ESRβ to influence spermatocyte apoptosis and spermiation.[19] Moreover, estradiol has been shown to downregulate genes involved in cytoskeleton remodeling (*Arpc1b* and *Evl*) and endocytosis (*Picalm*)[20] and impair the formation of tubulobulbar complexes in mature spermatids.[21] Estradiol has also been reported to inhibit testosterone synthesis by Leydig cells both directly[22] and indirectly via the inhibition of luteinizing hormone secretion.[23] Other research has indicated that overproduction of estradiol enhances the expression of growth arrest-specific-6 on Leydig cells, which in turn promotes the engulfment of Leydig cells by estradiol/ESRα-activated macrophages.[24] Interestingly, estradiol was found to upregulate 33 genes and downregulate 67 genes with estrogen-responsive elements in the rat testis,[25] indicating that further research is needed to fully unravel the complex mechanisms underlying the effects of estradiol on male fertility.

The present study found that ESRα levels were significantly elevated in the infertile group compared to the control group. The ESRα levels were also higher in men with azoospermia, oligozoospermia, mild azoospermia, or malformed spermatozoa than in men with normozoospermia. The findings for the ESRα levels are similar to those for the estradiol level described above, and this is not unexpected given that ESRs mediate the physiological effects of estradiol. However, it was notable that ESRα levels were not significantly correlated with sperm concentration, percentage of progressively motile sperm, and percentage of sperm with normal morphology, suggesting that estradiol levels might have more important effects on sperm quality than ESRα levels. Interestingly, ESRα levels showed a significant positive correlation with estradiol levels, which might be unexpected given that estradiol is known to reduce the levels of ESRα protein in mammalian cells.[26] Further research is needed to establish the mechanism underlying the positive correlation between estradiol and ESRα levels.

We found no significant effects of SNPs at the rs2234693 locus on sperm concentration, percentage of progressively motile sperm, serum ESRα levels, or serum estradiol levels in men in the infertile group. Safarinejad et al reported that the 397T/T genotype was associated with a higher risk of infertility, lower sperm density, lower sperm motility, poorer sperm morphology, and higher estradiol levels than the 397T/C or 397C/C genotypes.[15] However, Lazaros et al found no significant differences in rs2234693 genotypes between normozoospermic and oligozoospermic men or between asthenozoospermic men and those with normal sperm motility.[14] Although the 397T/T genotype had an adverse effect on sperm motility (compared with the 397T/C or 397C/C genotypes) in oligozoospermic men, this was not observed for men with a sperm concentration $>20 \times 10^6$ spermatozoa/mL.[14] Furthermore, among men with a normal sperm count, the 397T/T genotype was associated with a higher sperm concentration than the 397T/C or 397C/C genotypes, but this difference was not observed for men with a low sperm count.[14] Thus, the 397T/T genotype had both beneficial and detrimental associations with semen quality depending on the subgroup and parameter evaluated. Interestingly, a meta-analysis by Ge et al concluded that the risk of male infertility was highest for the 397T/T genotype in Asians and for the 397C/C genotype in Caucasians.[16] Thus, the apparent inconsistencies between published studies might in part be due to differences in ethnicity. Further investigations are needed to definitively establish the relevance of SNPs at the rs2234693 locus to semen quality and fertility.

Previous research has established that semen volume, percentage of progressively motile sperm, and percentage of sperm with normal morphology decline with age.[27] Furthermore, there are also age-associated increases in sperm DNA fragmentation.[28] By contrast, the present study found no significant variations in sperm quality parameters with age. However, it should be noted that our analysis was performed specifically in men with infertility and reduced semen quality (as compared to controls), whereas previous studies have reported data for a general population. In addition, the infertile men enrolled in our study were quite young (mean age, 31.1 ± 5.8 years), and there were relatively few men aged > 40 years (only 27 of the 190 subjects

included in the analysis). Previous research has indicated that substantial reductions in semen quality in infertile men tend to occur after the age of 50 years,[29] which may explain the lack of an effect of age in our study.

This study has some limitations. First, this was a retrospective analysis, so our findings may be prone to selection bias or information bias. Second, this was a single-center study, so the generalizability of the results is not known. Third, the sample size was quite small, so the analysis may have been underpowered to detect some real differences between groups. In particular, the controls had to be recruited. Fourth, we studied SNPs at only 1 locus of the *ESR1* gene, and the effects of polymorphisms at other loci of the *ESR1* gene or other genes, including *ESR2*, were not evaluated. Large-scale prospective studies are needed to further clarify the roles of estradiol and ESRs in male infertility.

In conclusion, elevated serum levels of estradiol and possibly ESRα might have a negative impact on sperm quality and thus reduce fertility in men. However, SNPs at the rs2234693 locus of the gene encoding ESRα appear to have little effect on semen quality or the serum levels of estradiol and ESRα.

Author contributions

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