

Salivary Cortisol and the Reproductive Endocrine Profile in Infertile Iraqi Men, with Preserved Sperm Chromatin Maturity: A Cross-Sectional Study

Salivary Cortisol
and the
Reproductive
Endocrine Profile
in Infertile

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ABSTRACT

Objective: To examine the associations of salivary cortisol with the reproductive hormone profiles and with sperm chromatin maturity across semen-quality phenotypes in infertile men.

Study Design: Cross-sectional study

Place and Duration of Study: This study was conducted at the Andrology Clinic, Najaf Iraq from 1st September 2025 to 30th November 2025.

Methods: One hundred infertile men were classified by WHO criteria into normozoospermia, oligozoospermia, asthenozoospermia and oligoasthenoteratozoospermia were enrolled. Salivary cortisol, serum luteinizing hormone, follicle-stimulating hormone, total testosterone, prolactin and estradiol, and sperm chromatin maturity (assessed by aniline blue staining) were measured.

Results: Salivary cortisol was inversely associated with follicle-stimulating hormone ($\rho = -0.28$, $p=0.005$; false discovery rate $q=0.026$; 95% CI -0.45 to -0.09) and this association remained independent in multivariable regression (standardized beta $= -0.20$, $p=0.029$). Cortisol showed a weaker positive association with estradiol ($\rho = +0.22$, $p=0.029$) that did not survive false discovery rate correction, and no significant association with luteinizing hormone, testosterone or prolactin. Follicle-stimulating hormone and testosterone differed across phenotypes ($p<0.001$), being highest in normozoospermia. Sperm chromatin immaturity was low (median 6% aniline-blue-positive), did not differ across phenotypes ($p=0.60$), and was not associated with cortisol or the endocrine profile, apart from a weak correlation with prolactin ($\rho = +0.28$, $p=0.011$).

Conclusion: In infertile men, higher salivary cortisol is independently associated with lower follicle-stimulating hormone, consistent with an inhibitory influence of hypothalamic-pituitary-adrenal activity on the gonadotropic axis. Sperm chromatin maturity was largely preserved and unrelated to stress or the endocrine profile.

Key Words: Salivary cortisol, Psychological stress, Follicle-stimulating hormone, Reproductive hormones, Sperm chromatin maturity, Aniline blue, Male infertility

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INTRODUCTION

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Male infertility is a major global health concern, contributing to about half of all cases of couple infertility.¹ Semen analysis remains the cornerstone of evaluation², but it does not capture the hormonal and molecular factors that shape testicular function, and there is growing interest in additional markers.

The hypothalamic-pituitary-gonadal (HPG) axis directs spermatogenesis, with circulating FSH, LH and testosterone serving as recognised determinants of semen quality.³ Stress perturbs this system: poorer male fertility has been tied to psychological stress⁴, and the hypothalamic-pituitary-adrenal (HPA) axis restrains the HPG axis through both central and peripheral actions.⁵ HPA activity can be tracked non-invasively through salivary cortisol⁶, whereas salivary alpha-amylase indexes sympathetic tone.⁷ Mechanistically, glucocorticoids suppress gonadotropin secretion and testicular steroidogenesis and can trigger Leydig-cell apoptosis^{8,9}, and clinical studies have associated stress

with altered reproductive hormones and reduced semen quality.^{10,11} FSH in particular drives Sertoli-cell function and the early stages of spermatogenesis.¹²

Sperm chromatin maturity, established during spermiogenesis through the replacement of histones by protamines that compacts and protects the paternal genome, is a recognised marker of sperm quality, and defective protamination detectable by aniline blue staining has been linked to reduced fertilisation and pregnancy outcomes.^{13,14} Poorly condensed, protamine-deficient chromatin is also more vulnerable to oxidative injury, and impaired chromatin condensation predicts assisted-reproduction outcomes.¹⁵ Because systemic stress can raise oxidative load and perturb spermiogenesis, a relationship between HPA activity and sperm chromatin maturity is biologically plausible but has rarely been tested directly in men.

We therefore measured salivary cortisol, the reproductive hormone profile and sperm chromatin maturity in infertile men, and examined how cortisol relates to the endocrine profile and to chromatin maturity, and how these markers vary across semen-quality phenotypes. The study characterises associations within an infertile population; it does not include a fertile control group and makes no claim that the measured concentrations are abnormal.

METHODS

This cross-sectional study was conducted at Andrology Clinic, Najaf Iraq 1st September 2025 to 30th November 2025 No. 159/UOA dated 15-8-2025 and 100 men of infertility care were enrolled. Each contributed a semen sample, a venous blood draw and a saliva sample on the same day, together with his recorded clinical and demographic details. Infertility was defined at the couple level as failure to achieve a pregnancy after at least 12 months of regular unprotected intercourse; men with a normal semen analysis (the normozoospermic group) were therefore included as the male partners of infertile couples, in whom female or unexplained factors may contribute, since a normal ejaculate does not exclude a male partner from an infertile couple.

Semen was assessed following WHO protocols.¹⁸ Using the WHO 2010 thresholds (concentration 15 million per millilitre; progressive motility 32%), each man was placed in one of four groups: normozoospermia, oligozoospermia, asthenozoospermia or OAT. Sperm chromatin maturity was assessed by aniline blue staining, which discriminates lysine-rich histones (retained in immature chromatin, stained) from arginine-rich protamines (mature chromatin, unstained), and expressed as the percentage of aniline-blue-positive (immature-chromatin) spermatozoa

Salivary cortisol was measured by a competitive-inhibition ELISA for which the manufacturer validates saliva (ELK Biotechnology, ELK8526; working range 3.13 to 200 ng/mL; sensitivity 1.02 ng/mL; intra- and

inter-assay CV under 8% and 10%). Serum LH, FSH, total testosterone, prolactin and estradiol were each run on matched ELISA kits per the manufacturers' instructions, with values read from the relevant plate standard curve. Sampling of saliva and blood was confined to a fixed evening window (17:00 to 21:00; median 18:00) to dampen diurnal variation. Salivary alpha-amylase was additionally assayed but discarded as unreliable, the recorded values (observed range approximately 12 to 244 U/mL) falling outside the manufacturer's validated range for the kit

All computations were carried out in Python using SciPy, stats models and scikit-learn. Group differences were tested by the Kruskal-Wallis test, and associations by the Spearman rank-correlation coefficient, with magnitudes interpreted by conventional thresholds as weak (absolute rho below 0.3), moderate (0.3 to 0.5) or strong (above 0.5). For the family of cortisol-hormone correlations, p values were adjusted by the Benjamini-Hochberg procedure to control the false discovery rate (FDR), and 95% confidence intervals were obtained by nonparametric bootstrapping (percentile method, 5000 resamples). To assess whether the cortisol-FSH association was independent of demographic and gonadal factors, a rank-based multiple regression was fitted with FSH as the dependent variable and cortisol, age, body-mass index, testosterone and LH as predictors. The post-hoc sensitivity analysis indicated 80% power (alpha = 0.05) to detect a correlation of absolute rho of at least 0.28, so the study was only borderline-powered for the observed effect and weaker associations would not have been detectable.

RESULTS

Among the 100 men, the four groups numbered 26 (normozoospermia), 25 (oligozoospermia), 22 (asthenozoospermia) and 27 (OAT). Group-mean age fell between 27.7 and 33.5 years and group-mean body-mass index between 26.2 and 28.5 kg/m² (Table 1).

Table No. 1: Demographic characteristics by semen-quality group

Group	No.	Age (years)	BMI (kg/m ²)
Normozoospermia	26	33.5±9.3	28.2±7.7
Oligozoospermia	25	27.7±4.4	27.2±3.0
Asthenozoospermia	22	32.0±5.6	26.2±4.2
OAT	27	30.2±6.6	28.5±6.7

Salivary cortisol was inversely associated with FSH at a weak-to-moderate magnitude (Spearman rho = -0.28, p=0.005), the only association to survive FDR correction (q=0.026; bootstrap 95% CI -0.45 to -0.09) (Table 2, Fig. 1). A weaker positive association with estradiol (rho=+0.22, p=0.029) did not survive correction (q=0.072), and cortisol was not significantly associated with LH, total testosterone or prolactin.

FSH and total testosterone differed significantly across phenotypes (both Kruskal-Wallis $p < 0.001$), being highest in normozoospermia and lower in the abnormal

groups, and estradiol also differed ($p = 0.005$). LH and prolactin did not differ significantly across phenotypes (Table 3).

Table No. 2: Spearman correlations of salivary cortisol with reproductive hormones

Hormone	Rho	P	q (FDR)	95% CI
FSH	-0.28	0.005	0.026	-0.45 to -0.09
Estradiol	+0.22	0.029	0.072	+0.01 to +0.42
Testosterone	-0.14	0.175	0.291	-0.34 to +0.07
LH	-0.07	0.514	0.514	-0.25 to +0.12
Prolactin	+0.08	0.410	0.513	-0.12 to +0.26

Table No. 3: Reproductive hormones by semen-quality group, median (interquartile range)

Marker	Normo	Oligo	Asthen	OAT
FSH (mIU/mL)	1.70 (1.57-1.95)	0.77 (0.47-1.07)	1.16 (0.62-1.37)	0.97 (0.70-1.40)
Testosterone (ng/mL)	3.75 (2.70-4.83)	1.80 (1.60-2.10)	2.30 (2.10-2.30)	2.10 (1.70-2.35)
LH (mIU/mL)	7.15 (4.78-10.5)	7.50 (5.70-10.5)	9.65 (6.22-14.4)	7.50 (5.75-10.0)
Prolactin (ng/mL)	46.2 (29.5-70.4)	39.5 (20.0-61.1)	59.9 (38.4-81.5)	47.2 (39.5-68.8)
Estradiol (pg/mL)	237 (73-387)	257 (188-350)	190 (103-253)	123 (65-197)

Kruskal-Wallis p: FSH < 0.001; Testosterone < 0.001; LH 0.320; Prolactin 0.119; Estradiol 0.005

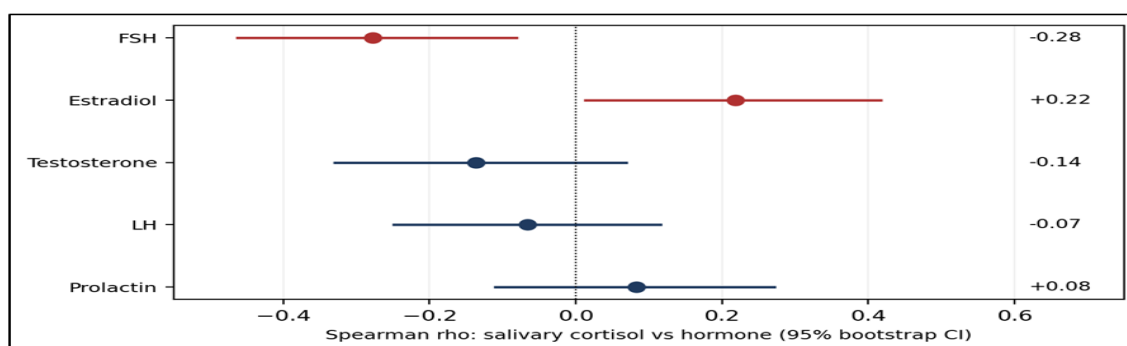


Figure No. 1: Spearman correlations (with 95% bootstrap confidence intervals) of salivary cortisol with the reproductive hormones. Only the inverse association with FSH excludes zero. Cool colour, negative; warm colour, positive

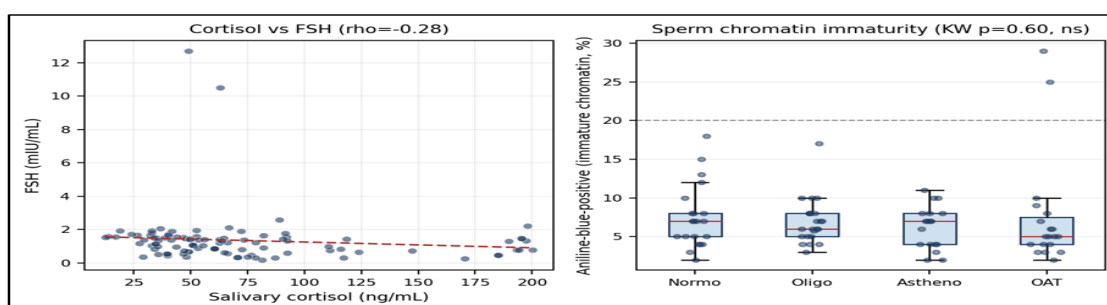


Figure No. 2: Left: Salivary cortisol (ng/mL) versus FSH (mIU/mL); the dashed line shows the linear trend for visualisation only. Right: sperm chromatin immaturity (aniline-blue-positive spermatozoa, %) across semen-quality phenotypes (n = 81); boxes show median and interquartile range. Chromatin immaturity did not differ across phenotypes (Kruskal-Wallis $p = 0.60$, not significant)

Table No. 4: Rank-based regression model predicting FSH

Predictor	Beta	SE	P value
Testosterone	+0.48	0.090	<0.001
Cortisol	-0.02	0.089	0.029
BMI	+0.10	0.090	0.247
LH	+0.06	0.088	0.472
Age	-0.02	0.090	0.840

To test whether the cortisol-FSH association was independent of demographic and gonadal factors, a rank-based multiple regression was fitted with FSH as the dependent variable (Table 4). The model was significant (adjusted R-squared = 0.26, F-test $p < 0.001$). Salivary cortisol remained an independent inverse predictor of FSH (standardized beta = -0.20, $p = 0.029$) after adjustment for age, BMI, testosterone and LH;

testosterone was the strongest predictor ($\beta = +0.48$, $p < 0.001$).

Sperm chromatin immaturity (aniline-blue positivity, available for 81 of 100 men) was low (median 6%, range 2 to 29%), with only 2 men exceeding 20% and none exceeding 30% immature spermatozoa, indicating predominantly mature chromatin. It did not differ across semen-quality phenotypes (Kruskal-Wallis $p = 0.60$) and was not significantly associated with salivary cortisol ($\rho = +0.14$, $p = 0.22$), LH, FSH, testosterone or estradiol. The only nominal association was a weak positive correlation with prolactin ($\rho = +0.28$, $p = 0.011$), which was not corrected for multiple testing and should be regarded as exploratory (Fig. 2). Overall, sperm chromatin maturity was preserved in this cohort and was independent of stress and the endocrine profile.

DISCUSSION

In this cohort of infertile males, higher salivary cortisol was associated with lower FSH, and this inverse relationship was the only cortisol-hormone association to survive correction for multiple testing and remained independent after adjustment for age, BMI, testosterone and LH. By contrast, sperm chromatin immaturity was low and showed no relationship with cortisol or the endocrine profile. The principal message is therefore an independent inverse cortisol-FSH association set against preserved, stress-independent sperm chromatin maturity. The inverse cortisol-FSH association is biologically coherent. The HPA axis exerts an inhibitory influence on the HPG axis⁵, and glucocorticoids can suppress gonadotropin-releasing hormone and gonadotropin secretion.⁹ A relative reduction in FSH in men with higher cortisol is consistent with this central inhibition, and because FSH is the principal driver of Sertoli-cell function and early spermatogenesis¹², such an effect could provide one route by which stress relates to impaired spermatogenesis.^{11,12} The expected inverse cortisol-testosterone relationship was present in direction but did not reach significance⁸, which may reflect the modest sample, single-sample cortisol measurement, or the low testosterone concentrations characteristic of this infertile cohort. The cortisol-FSH association is of weak-to-moderate magnitude ($\rho = -0.28$; 95% CI -0.45 to -0.09); it is statistically robust but accounts for only a limited share of the variance in FSH, and is therefore best read as one contributory influence on gonadotropic output rather than a dominant determinant.

Sperm chromatin maturity, which reflects the histone-to-protamine transition of spermiogenesis rather than oxidative DNA strand breakage showed no association with salivary cortisol or the reproductive hormones and did not differ across phenotypes.¹⁵ Two features help explain this. First, immaturity values were low (median 6%, only 2 men above 20% and none above 30%), so

the cohort had largely mature chromatin and limited variance, a range-restriction effect that mechanically constrains any correlation involving chromatin immaturity and means the null findings cannot exclude a relationship in populations with greater chromatin defects. Second, HPA-mediated stress, acting largely through the gonadotropic axis, need not perturb the protamination programme of spermiogenesis.¹⁶ The isolated weak correlation with prolactin did not withstand the multiplicity of testing and is best regarded as a chance finding pending replication. These observations indicate that, in this population, sperm chromatin maturity was preserved and largely independent of stress-axis activity, a negative result that is itself informative.¹⁷

Strengths include the use of a salivary stress marker, a full reproductive hormone panel, a direct measure of sperm chromatin maturity, phenotype stratification, rank-based statistics with FDR control and bootstrap confidence intervals, and a multivariable model.

Limitations: First, the study included only infertile men and no fertile control group, so it characterises associations within an infertile population and cannot determine whether the measured concentrations are abnormal. Second, the design is cross-sectional and cannot establish causality. Third, the cohort was modest ($n = 100$; chromatin maturity available for 81) and drew on a pre-existing sample without an a priori power calculation, so weaker associations may have been missed; in particular, sperm chromatin immaturity showed marked range restriction (median 6%, no value above the 30% threshold), which limits the power to detect any chromatin-maturity association and means the negative chromatin findings should be read with that constraint in mind. Fourth, salivary cortisol was sampled once within an evening window, which coincides with the diurnal nadir; a single evening measurement likely attenuates the true association magnitude, and repeated or diurnal sampling, or the cortisol awakening response, would better capture HPA activity and probably yield stronger estimates. Fifth, salivary alpha-amylase was measured but excluded because its values lay outside the validated assay range, and several of the serum endocrine measurements sit well outside commonly cited male reference intervals: estradiol exceeded the typical upper limit of about 40 pg/mL in 92 of 100 men (28 above 300 pg/mL), prolactin exceeded about 25 ng/mL in 81 of 100 men (10 values recorded as right-censored at the assay ceiling of 100 ng/mL), and FSH (median 1.2 mIU/mL) and total testosterone (median 2.3 ng/mL) lay at or below the lower reference limits.

CONCLUSION

In infertile males, higher salivary cortisol was independently associated with lower FSH, supporting an inhibitory link between HPA activity and the

gonadotropic axis, whereas sperm chromatin maturity was largely preserved and unrelated to stress or the endocrine profile.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Layla Kadhum Radeef, Manal Nasih Ahmed Hamdan, Thulfiqar Ibrahim Raheem
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Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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