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SEMINAL-PLASMA KISSPEPTIN AND THE SEMINAL/SERUM KISSPEPTIN RATIO IN RELATION TO SPERM MOTILITY, MORPHOLOGY AND OXIDATIVE STRESS IN INFERTILE IRAQI MEN

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ABSTRACT

Kisspeptin regulates the reproductive axis centrally, but it is also present in seminal plasma, where its meaning is unclear. We compared the two compartments and asked whether their ratio tracks semen quality better than either value alone. One hundred and fifty men were studied: 40 fertile controls, 40 asthenozoospermic, 30 teratozoospermic and 40 with oligoasthenoteratozoospermia (OAT). Semen was assessed by WHO 2021 criteria. Kisspeptin was measured in serum and seminal plasma by ELISA, and seminal malondialdehyde (MDA), total antioxidant capacity (TAC), total oxidant capacity (TOC), superoxide dismutase, glutathione and the oxidative stress index (OSI) were measured in the same samples. Seminal kisspeptin fell stepwise, from 136.64 ± 32.67 pg/mL in controls to 56.88 ± 17.17 pg/mL in OAT ($F = 76.59$, $P < 0.001$), while serum kisspeptin moved the other way (81.53 ± 20.95 to 109.09 ± 29.36 pg/mL, $P < 0.001$). The seminal/serum ratio followed the same gradient, falling from 1.79 ± 0.60 to 0.56 ± 0.26 . Seminal kisspeptin correlated positively with total motility ($r = 0.725$) and normal morphology ($r = 0.643$), and negatively with MDA ($r = -0.670$) and OSI ($r = -0.604$); all $P < 0.001$. By ROC analysis it separated infertile from fertile men with an AUC of 0.941 (95% CI 0.902–0.981) at a cut-off of 99.4 pg/mL, and the ratio gave 0.935 at a cut-off of 1.24. Serum kisspeptin reached only 0.732. Seminal kisspeptin, taken directly or as a ratio, appears to be a redox-linked marker of sperm function.

Keywords: Kisspeptin; seminal plasma; oxidative stress index; sperm motility; sperm morphology; male infertility; Iraq.

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1. INTRODUCTION

Infertility affects roughly 15% of couples, and a male factor contributes to about half of those cases [1]. The global burden has not eased; more than 55 million prevalent cases were recorded in 2021 [2], and national analyses show the same upward drift in absolute case numbers [3]. In Iraq the clinical picture is dominated by varicocele, obesity and smoking, and oligoasthenozoospermia is the commonest seminal abnormality seen in andrology clinics [4]. Routine semen analysis, standardised by the sixth WHO manual [5], remains the first-line test. It is also a blunt one. The 2021 reference limits reclassified only a small fraction of samples [6], and morphology in particular is a weak predictor on its own [7]. Something beyond the conventional count-motility-morphology triad is needed.

Oxidative stress is the mechanism most often invoked to fill that gap. Spermatozoa carry polyunsaturated membranes, little cytoplasm and therefore little antioxidant reserve, so a modest excess of reactive oxygen species is enough to impair motility

and damage DNA [8, 9, 10]. In asthenozoospermia the link is especially well documented [11].

Kisspeptin, the KISS1 gene product acting through KISS1R, is the gatekeeper of GnRH release and hence of the whole reproductive axis [12, 13]. What is less widely appreciated is that the kisspeptin system is expressed locally in the male tract: KISS1R is present on spermatozoa and is repositioned on the sperm head during epididymal transit [14], and kisspeptin signalling is active at the gonadal level [15]. Seminal-plasma kisspeptin has only recently been examined in men, with results that do not yet agree [16, 17].

We therefore measured kisspeptin in serum and seminal plasma from the same men, alongside a six-marker seminal redox panel, and tested one specific idea: that the ratio of seminal to serum kisspeptin, by normalising the local pool against the systemic one, carries more information about sperm function than either value taken alone.

2. MATERIALS AND METHODS

2.1. Study design and participants

This was a case-control, cross-sectional study carried out at the Andrology Laboratory, Al-Diwaniyah Teaching Hospital, Al-Diwaniyah, Iraq, between 2024 and 2025. One hundred and fifty men were enrolled. Forty were fertile controls, defined as men who had fathered a child within the previous two years and whose semen parameters met the WHO 2021 lower

reference limits. The remaining 110 attended for primary or secondary infertility of at least twelve months and were classified from their semen analysis into as the nozoospermic ($n = 40$), teratozoospermic ($n = 30$) and oligoasthenoteratozoospermic (OAT, $n = 40$) groups.

Men with azoospermia, clinical varicocele of grade II or III, leukocytospermia ($> 1 \times 10^6$ peroxidase-positive cells/mL), a history of orchitis, cryptorchidism, testicular surgery, chemotherapy or radiotherapy, endocrine disease, or antioxidant or hormonal treatment within the preceding three months were excluded. Age, body mass index, smoking status and duration of infertility were recorded at enrolment.

2.2. Ethical approval

The protocol was approved by the Research Ethics Committee of Al-Diwaniyah Teaching Hospital (approval number QU-0330-2024-6-21, dated June 21, 2024) and was conducted in accordance with the Declaration of Helsinki. Every participant gave written informed consent before any sample was taken.

2.3. Semen collection and analysis

Semen was produced by masturbation into a sterile container after two to seven days of sexual abstinence, and was allowed to liquefy at 37 °C for 30 minutes. The analysis was performed according to the sixth edition of the WHO handbook [5]. Volume, concentration, progressive motility and total motility were recorded. Morphology was examined on stained smears using rigorous Tygerberg criteria, with 200 spermatozoa counted per slide by a single investigator who was blind to group allocation.

After analysis, the leftover semen was centrifuged at $300 \times g$ for 10 min at 4 °C. The supernatant was removed from the cells and aliquoted and kept at -80 °C until assayed. Samples were thawed only once.

2.4. Blood sampling

Venous blood samples (5 mL) were collected on the same morning (between 08:00 and 10:00), after an overnight fast. Blood was clotted for 20 min, centrifuged at $3000 \times g$ for 15 min and serum kept at -80 °C parallel to the seminal aliquots.

2.5. Kisspeptin assay

Kisspeptin was measured in serum and in seminal plasma with a commercial sandwich ELISA kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA, Cat. No. EEL080), used strictly according to the manufacturer's instructions. The stated detection range was 31.25–2000 pg/mL with a sensitivity of 18.75 pg/mL. Intra- and inter-assay coefficients of variation were $<10\%$ and $<10\%$. Seminal plasma was diluted 1:2 in the supplied buffer, and the dilution factor was applied before analysis. All samples were run in duplicate on the same plate lot, and serum and seminal aliquots from one man were always assayed side by side. The seminal/serum ratio was then computed for each participant as seminal kisspeptin divided by serum kisspeptin.

2.6. Oxidative stress markers

All redox markers were measured in the same seminal-plasma aliquots. TAC and TOC were measured with automated colorimetric kits from Rel Assay Diagnostics (Gaziantep, Turkey): Cat. No. RL0017 for TAC and Cat. No. RL0024 for TOC. These kits use the methods described by Erel for TAC [18] and TOC [19]. TAC was expressed in mmol Trolox equivalent/L and TOC in $\mu\text{mol H}_2\text{O}_2$ equivalent/L. OSI was calculated for each sample as TOC ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) divided by TAC (mmol Trolox equivalent/L). MDA, SOD activity and reduced glutathione (GSH) were measured with commercial colorimetric kits. MDA was determined by the thiobarbituric acid reactive substances method and expressed in nmol/mL. SOD activity was expressed in U/mL and GSH in mg/dL. Assay details and calibration ranges for each kit are given in the manufacturers' data sheets. For all assays, the intra-assay and inter-assay coefficients of variation were below 5% and 8%, respectively.

2.7. Statistical analysis

Data were analysed in Python 3.11 (SciPy, statsmodels and scikit-learn). Distributions were checked before testing. Results are given as mean $\pm$ standard deviation. Differences across the four groups were tested by one-way ANOVA with Tukey's HSD for pairwise comparison; the smoking distribution was tested by χ^2 . Associations were examined by Pearson correlation, and Spearman's rho was used as a sensitivity check for the ratio. Multivariable linear regression was used to test whether seminal kisspeptin predicted motility and morphology independently of age, BMI, smoking and MDA. Diagnostic performance was assessed by receiver operating characteristic (ROC) analysis; the area under the curve (AUC) and its 95% confidence interval were obtained by the DeLong method, paired AUCs were compared by the DeLong test, and the optimal cut-off was taken at the maximum Youden index. A two-sided P value below 0.05 was treated as significant.

3. RESULTS AND DISCUSSION

3.1. Baseline characteristics and semen parameters

The four groups were comparable in smoking prevalence (30–50%, $\chi^2 = 3.04$, $P = 0.386$) but not in age or BMI (Table 1). OAT men were about five years older than controls (36.31 ± 5.50 vs 31.54 ± 4.82 years) and carried roughly four extra BMI units (29.37 ± 3.22 vs 25.17 ± 3.05 kg/m²); both differences were highly significant. Duration of infertility was also longest in the OAT group (4.51 ± 2.20 years, $P < 0.001$). This is the expected clinical gradient, and it matches what is reported from Iraqi andrology clinics, where obesity and smoking cluster with the more severe seminal phenotypes [4]. Because age and BMI both track semen quality in their own right [20, 21], we returned to them later in the regression models.

Semen parameters behaved as the group definitions require. Total motility fell from $58.29 \pm 7.95\%$ in controls to $22.41 \pm 7.52\%$ in OAT, and normal forms from $7.60 \pm 2.29\%$ to $1.80 \pm 0.89\%$ (both $P < 0.001$). Sperm concentration stayed above the WHO lower reference limit in every asthenozoospermic and teratozoospermic man, although the teratozoospermic mean was already about a quarter below the control mean (45.15 ± 15.07 versus $59.31 \pm 16.43 \times 10^6/\text{mL}$, Tukey $P < 0.001$). Only in OAT did it collapse outright ($7.63 \pm 4.38 \times 10^6/\text{mL}$). Semen volume differed only modestly ($P = 0.011$), which is usual.

Table 01. Demographic, lifestyle and conventional semen parameters of the four study groups. Values are mean $\pm$ SD unless stated. P values are from one-way ANOVA, except smoking (χ^2) and infertility duration (ANOVA across the three infertile groups only).

Parameter	Control (n = 40)	Astheno-zoospermic (n = 40)	Terato-zoospermic (n = 30)	OAT (n = 40)	F	P
Age (yr)	31.54 $\pm$ 4.82	32.16 $\pm$ 5.85	32.38 $\pm$ 4.74	36.31 $\pm$ 5.50	6.70	<0.001
BMI (kg/m ²)	25.17 $\pm$ 3.05	26.60 $\pm$ 4.32	27.19 $\pm$ 3.33	29.37 $\pm$ 3.22	9.78	<0.001
Smokers, n (%)	18 (45.0)	20 (50.0)	9 (30.0)	16 (40.0)	$\chi^2 = 3.04$	0.386
Infertility duration (yr)	—	2.95 $\pm$ 1.52	2.67 $\pm$ 1.20	4.51 $\pm$ 2.20	12.26	<0.001
Semen volume (mL)	3.51 $\pm$ 0.69	3.28 $\pm$ 1.14	2.96 $\pm$ 0.83	2.88 $\pm$ 0.90	3.88	0.011
Sperm concentration (M/mL)	59.31 $\pm$ 16.43	56.83 $\pm$ 17.72	45.15 $\pm$ 15.07	7.63 $\pm$ 4.38	110.50	<0.001
Progressive motility (%)	41.46 $\pm$ 6.15	23.46 $\pm$ 8.13	33.49 $\pm$ 8.12	12.43 $\pm$ 4.30	135.49	<0.001
Total motility (%)	58.29 $\pm$ 7.95	34.00 $\pm$ 6.79	46.01 $\pm$ 9.16	22.41 $\pm$ 7.52	154.57	<0.001
Normal morphology (%)	7.60 $\pm$ 2.29	6.25 $\pm$ 2.17	2.48 $\pm$ 1.07	1.80 $\pm$ 0.89	98.61	<0.001

3.2. Kisspeptin in the two compartments

The central result is summarised in Table 2 and Figure 1. Seminal-plasma kisspeptin declined in a stepwise fashion across the groups: 136.64 $\pm$ 32.67 pg/mL in controls, 88.89 $\pm$ 19.44 in asthenozoospermia, 83.34 $\pm$ 23.35 in teratozoospermia and 56.88 $\pm$ 17.17 in OAT (F = 76.59, P < 0.001). Tukey's test confirmed that every infertile group differed from the control group and that OAT differed from the other two; only the asthenozoospermic and teratozoospermic groups were statistically indistinguishable from one another (P = 0.772), which is unsurprising given that they overlap in everything except the defining defect.

Serum kisspeptin moved the other way, rising from 81.53 $\pm$ 20.95 pg/mL in controls to 109.09 $\pm$ 29.36 pg/mL in OAT (F = 10.43, P < 0.001). The two compartments therefore diverge, and this divergence is the point of the ratio. In controls the seminal value exceeded the serum value in 37 of 40 men (92.5%), with a median ratio of 1.68. In OAT only two men out of forty had a ratio above 1.0, and the median was 0.50. Expressed as means, the ratio fell from 1.79 $\pm$ 0.60 to 0.56 $\pm$ 0.26 (F = 68.56, P < 0.001).

How does this sit with what little literature exists? Parkpinyo and colleagues reported, as we did, that serum kisspeptin is higher in infertile than in fertile men, with means of 85.18 $\pm$ 20.47 versus 109.37 $\pm$ 28.64 pg/mL [16]. Their seminal values, however, did not differ significantly between groups, and their seminal kisspeptin correlated negatively rather than positively with motility. Korkmaz and co-workers, working with kisspeptin-1 and kisspeptin-54 in seminal plasma, found the opposite: a progressive decline with increasing severity of infertility, together with inverse associations with TOC and OSI [17]. Our data align with the second report. The discrepancy is worth stating plainly rather than explaining away. The two studies used different antibodies, different dilutions and different units, and kisspeptin circulates as several fragments of unequal immunoreactivity; an assay raised against kisspeptin-54 and one raised against kisspeptin-10 are not measuring the same thing. Until a reference method exists, absolute seminal concentrations from different laboratories should not be compared directly. Ratios and within-study gradients are safer.

Table 02: Serum kisspeptin, seminal-plasma kisspeptin and the seminal/serum ratio across the four groups.

Parameter	Control (n = 40)	Astheno-zoospermic (n = 40)	Terato-zoospermic (n = 30)	OAT (n = 40)	F	P
Serum kisspeptin (pg/mL)	81.53 $\pm$ 20.95	95.33 $\pm$ 19.65	104.37 $\pm$ 22.17	109.09 $\pm$ 29.36	10.43	<0.001
Seminal kisspeptin (pg/mL)	136.64 $\pm$ 32.67	88.89 $\pm$ 19.44	83.34 $\pm$ 23.35	56.88 $\pm$ 17.17	76.59	<0.001
Seminal/Serum ratio	1.79 $\pm$ 0.60	0.97 $\pm$ 0.27	0.85 $\pm$ 0.35	0.56 $\pm$ 0.26	68.56	<0.001

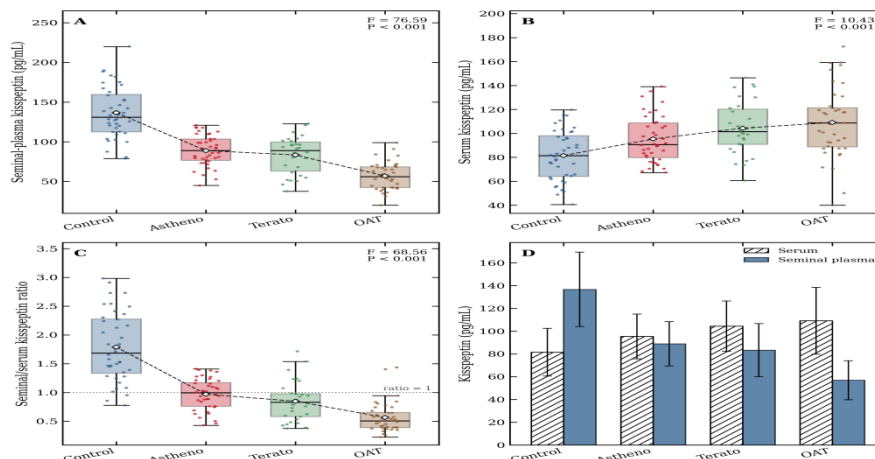


Figure 01: Kisspeptin across the four study groups. (A) Seminal-plasma kisspeptin. (B) Serum kisspeptin. (C) Seminal/serum kisspeptin ratio; the dotted line marks a ratio of 1. (D) Paired group means for the two compartments (mean $\pm$ SD). Boxes show median and interquartile range, whiskers the 1.5 $\times$ IQR limits, open diamonds the group means, and individual points every participant.

3.3. Seminal oxidative stress

The redox panel moved exactly as the kisspeptin data would predict (Table 3, Figure 2). MDA rose from 1.18 ± 0.31 nmol/mL in controls to 3.09 ± 0.73 in OAT, and TOC from 8.64 ± 1.84 to 18.46 ± 4.08 μ mol/L. On the defence side, TAC fell from 1.72 ± 0.34 to 0.80 ± 0.23 mmol/L, SOD from 27.70 ± 6.11 to 14.09 ± 3.30 U/mL and GSH from 4.02 ± 0.86 to 2.00 ± 0.61 mg/dL. OSI, which combines both arms, showed the widest separation of all: 5.25 ± 1.76 in controls against 25.84 ± 11.45 in OAT, a five-fold increase ($F = 53.29$, $P < 0.001$).

These values are well within the published range. The similar five-group approach was used by Ben Rhouma et al. who identified MDA lowest in controls and highest in OAT with catalase, SOD and glutathione peroxidase all decreasing with higher severity [22]. The mechanical story underlying those numbers is now fairly settled: excess ROS peroxidise the sperm plasma membrane, impair the mitochondrial machinery supplying flagellar ATP, and fragment nuclear and mitochondrial DNA [10, 23]. The mitochondrial membrane potential per se has been shown to correlate with traditional seminal parameters [24]. In idiopathic asthenozoospermia, ROS are likely the underlying cause not a bystander [25]. All of this is centrally involved in the Nrf2-regulated arm of the antioxidant response [26].

One detail is worth a pause. The distribution of OSI rose significantly with severity, from a standard deviation of 1.76 in controls to 11.45 in OAT. Thus, means alone underestimate the heterogeneity of the OAT group. Out of the OAT men, just two were within the control range of 2.68 to 11.20, and the highest was 53.27, some ten times the control mean. That heterogeneity is consistent with the view that OAT is a common endpoint of several different lesions rather than a single disease, and it argues for stratifying such patients by redox phenotype before any antioxidant intervention is judged [27, 28]. The same caution applies to the antioxidant enzymes. Rodak and colleagues found no difference in seminal SOD1 or GPX1 protein concentration between fertile and infertile men and concluded that functional activity, not abundance, is what distinguishes them [29]. Our SOD figures are activity measurements, which may be why they separated the groups so clearly where their protein levels did not.

Table 03: Seminal-plasma oxidative stress and antioxidant markers across the four groups.

Parameter	Control (n = 40)	Astheno-zoospermic (n = 40)	Terato-zoospermic (n = 30)	OAT (n = 40)	F	P
MDA (nmol/mL)	1.18 ± 0.31	2.16 ± 0.49	2.31 ± 0.59	3.09 ± 0.73	81.69	<0.001
TAC (mmol/L)	1.72 ± 0.34	1.33 ± 0.32	1.14 ± 0.37	0.80 ± 0.23	59.24	<0.001
SOD (U/mL)	27.70 ± 6.11	18.46 ± 5.11	17.67 ± 5.04	14.09 ± 3.30	53.51	<0.001
TOC (μ mol/L)	8.64 ± 1.84	12.81 ± 2.98	13.68 ± 3.53	18.46 ± 4.08	63.75	<0.001
GSH (mg/dL)	4.02 ± 0.86	3.08 ± 0.75	2.86 ± 0.91	2.00 ± 0.61	45.06	<0.001
OSI (TOC/TAC)	5.25 ± 1.76	10.52 ± 4.59	14.39 ± 8.92	25.84 ± 11.45	53.29	<0.001

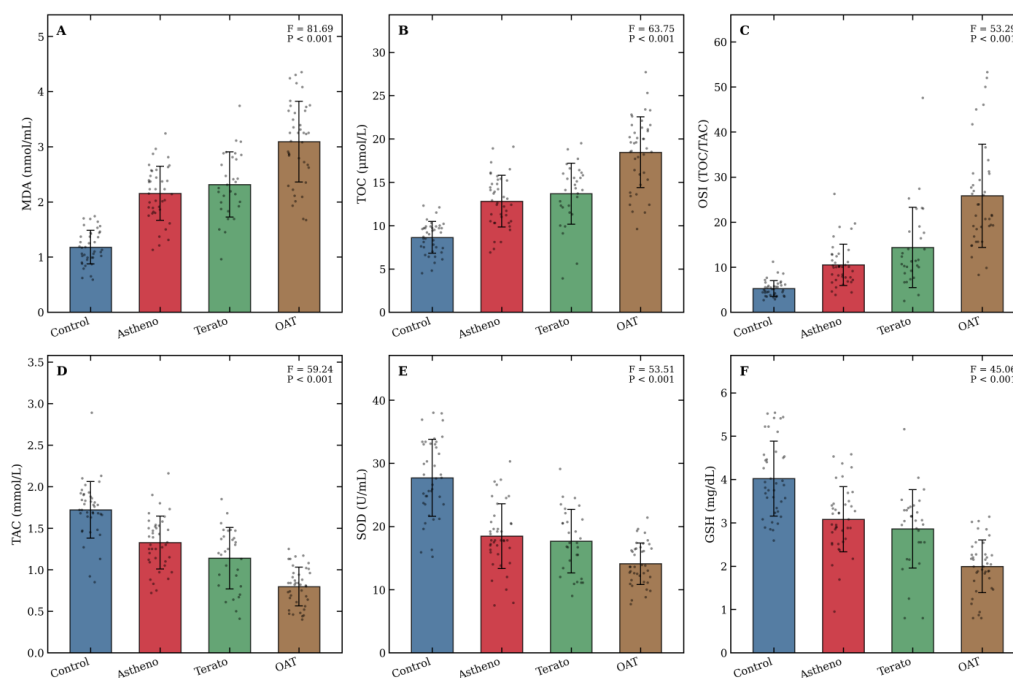


Figure 02: Seminal redox markers by group. (A) MDA. (B) TOC. (C) OSI. (D) TAC. (E) SOD. (F) GSH. Bars are mean $\pm$ SD; individual observations are overlaid.

3.4. Kisspeptin, sperm function and redox state are linked

Across all 150 men, seminal kisspeptin correlated positively with total motility ($r = 0.725$), progressive motility ($r = 0.643$), normal morphology ($r = 0.643$) and sperm concentration ($r = 0.586$), and negatively with MDA ($r = -0.670$), TOC ($r = -0.667$) and OSI ($r = -0.604$). Every one of these reached $P < 0.001$ (Table 4, Figure 3). On the antioxidant side the signs reverse as they should: TAC $r = 0.707$, GSH $r = 0.646$, SOD $r = 0.609$. The seminal/serum ratio behaved almost identically, with coefficients between 0.52 and 0.66 in

absolute value, and Spearman's rho reproduced the Pearson values closely (for example $\rho = -0.710$ for the ratio against OSI), so the associations are not being driven by a few extreme observations.

Serum kisspeptin told a rather different, weaker story. Its relationships were reversed and modest: -0.278 with overall motility, $+0.330$ with MDA, $+0.198$ with OSI. Figure 4A shows a heat map of the contrast between the two compartments and constitutes the fundamental reason for measuring kisspeptin where the spermatozoa really are.

Obvious argument is that the whole pattern is an artefact of membership. It isn't. When the 40 controls were excluded and the study repeated in the 110 infertile men exclusively, seminal kisspeptin correlated with overall motility ($r = 0.525$, $P < 0.001$), morphology ($r = 0.440$, $P < 0.001$), TAC ($r = 0.527$, $P < 0.001$) and OSI ($r = -0.504$, $P < 0.001$). Thus the link is not only categorical but ongoing. Partial correlation correcting for MDA substantially maintained the association with overall motility ($r = 0.484$, $P < 0.001$), indicating that lipid peroxidation explains some of the association but not all of it.

More technically, the multivariable models make the same point (Table 5). Kisspeptin in semen was an independent predictor of total motility ($B = +0.193$ per pg/mL, $P < 0.001$; model $R^2 = 0.617$), progressive motility ($B = +0.109$, $P < 0.001$) and normal morphology ($B = +0.027$, $P < 0.001$) after adjusting for age, BMI, smoking and MDA in the model. MDA was consistently unfavourable independently. Upon inclusion of kisspeptin and MDA, age and BMI lost relevance. Smoking had a small detrimental effect on progressive motility only ($B = -3.18$, $P = 0.037$), consistent with the described effects of smoke on sperm stress-response pathways [30].

Is there a reasonable mechanism? The evidence has two threads. First, KISS1R is expressed on spermatozoa and trafficked to the perforatorium as sperm mature in the epididymis, a trafficking pattern consistent with a role in fertilisation competence [14]; kisspeptin binding increases intracellular calcium, the second messenger that drives hyperactivation and capacitation. Second, and more directly, treatment of hypothyroid rats with kisspeptin-10 reduced testicular apoptosis and peroxynitrite generation and increased SOD1 and GPX1/2 expression and activity of catalase [31]. If kisspeptin is part of the local antioxidant system, then a decrease in seminal kisspeptin and an increase in seminal oxidants are two sides of the same coin, rather than independent observations. The seminal compartment is increasingly viewed in precisely these terms, as an integrated redox-endocrine environment rather than a passive transport medium [32]. We should be careful, though: a cross-sectional association cannot establish direction. Low kisspeptin may impair the antioxidant response, or an oxidant-rich environment may degrade the peptide, or an upstream lesion in the seminiferous epithelium may reduce both. Our data cannot separate these.

Table 04: Pearson correlations between the three kisspeptin measures and clinical, seminal and redox variables in all 150 participants. P values in parentheses.

Variable	Seminal kisspeptin r (P)	Seminal/serum ratio r (P)	Serum kisspeptin r (P)
Semen volume (mL)	+0.260 (0.001)	+0.170 (0.038)	-0.009 (0.913)
Sperm concentration (M/mL)	+0.586 (<0.001)	+0.515 (<0.001)	-0.263 (0.001)
Progressive motility (%)	+0.643 (<0.001)	+0.592 (<0.001)	-0.282 (<0.001)
Total motility (%)	+0.725 (<0.001)	+0.656 (<0.001)	-0.278 (<0.001)
Normal morphology (%)	+0.643 (<0.001)	+0.569 (<0.001)	-0.281 (<0.001)
MDA (nmol/mL)	-0.670 (<0.001)	-0.636 (<0.001)	+0.330 (<0.001)
TAC (mmol/L)	+0.707 (<0.001)	+0.625 (<0.001)	-0.267 (<0.001)
SOD (U/mL)	+0.609 (<0.001)	+0.556 (<0.001)	-0.249 (0.002)
TOC (μ mol/L)	-0.667 (<0.001)	-0.616 (<0.001)	+0.347 (<0.001)
GSH (mg/dL)	+0.646 (<0.001)	+0.619 (<0.001)	-0.345 (<0.001)
OSI (TOC/TAC)	-0.604 (<0.001)	-0.516 (<0.001)	+0.198 (0.015)
Age (yr)	-0.165 (0.044)	-0.121 (0.140)	+0.035 (0.669)
BMI (kg/m^2)	-0.355 (<0.001)	-0.271 (<0.001)	+0.075 (0.363)

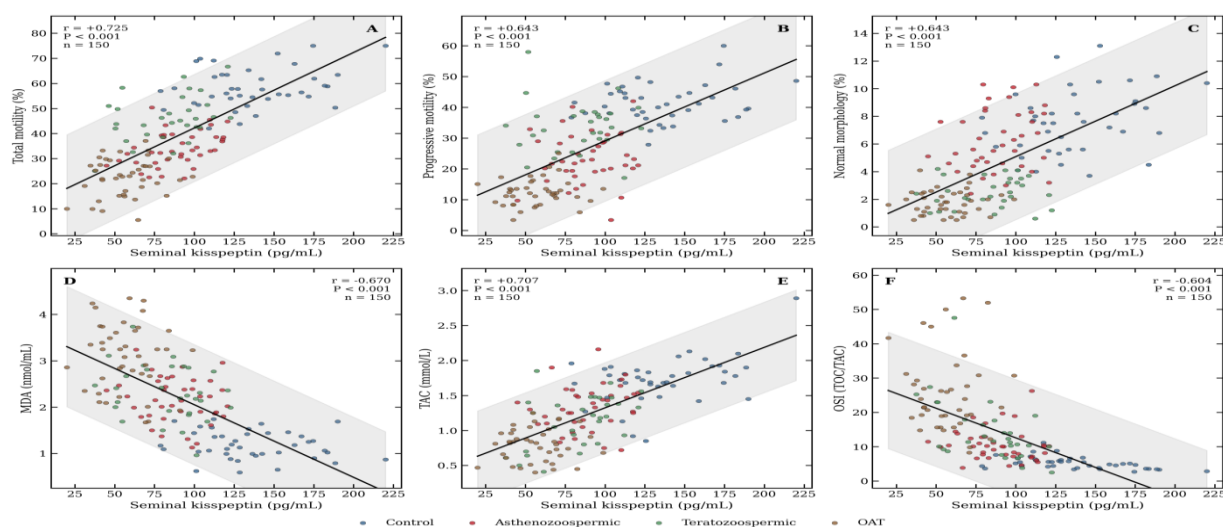


Figure 03: Seminal-plasma kisspeptin against sperm function and redox markers in all 150 men. (A) Total motility. (B) Progressive motility. (C) Normal morphology. (D) MDA. (E) TAC. (F) OSI. Solid lines are least-squares fits, shaded bands the 95% prediction intervals, and point colour indicates group.

Table 05: Multivariable linear regression models with seminal kisspeptin, age, BMI, smoking and MDA as predictors of sperm motility and morphology.

Dependent variable	Predictor	B	SE	t	P	Model R ²
Total motility (%)	Seminal kisspeptin (pg/mL)	+0.1926	0.0290	+6.63	<0.001	0.617
	Age (yr)	-0.2191	0.1515	-1.45	0.150	
	BMI (kg/m ²)	+0.2481	0.2461	+1.01	0.315	
	Smoking (yes vs no)	-1.8078	1.6892	-1.07	0.286	
	MDA (nmol/mL)	-7.2076	1.3748	-5.24	<0.001	
Progressive motility (%)	Seminal kisspeptin (pg/mL)	+0.1089	0.0259	+4.20	<0.001	0.552
	Age (yr)	-0.1666	0.1353	-1.23	0.220	
	BMI (kg/m ²)	+0.0802	0.2197	+0.37	0.716	
	Smoking (yes vs no)	-3.1782	1.5081	-2.11	0.037	
	MDA (nmol/mL)	-7.1374	1.2274	-5.82	<0.001	
Normal morphology (%)	Seminal kisspeptin (pg/mL)	+0.0274	0.0062	+4.44	<0.001	0.531
	Age (yr)	-0.0459	0.0322	-1.43	0.156	
	BMI (kg/m ²)	-0.0279	0.0523	-0.53	0.595	
	Smoking (yes vs no)	-0.0511	0.3587	-0.14	0.887	
	MDA (nmol/mL)	-1.3920	0.2919	-4.77	<0.001	

3.5. Diagnostic performance

ROC analysis quantified what the group comparisons imply (Table 6, Figures 4B and 5). For all infertile men against fertile controls, seminal kisspeptin gave an AUC of 0.941 (95% CI 0.902–0.981) at a cut-off of 99.4 pg/mL, with 81.8% sensitivity and 92.5% specificity. The seminal/serum ratio gave an AUC of 0.935 (0.895–0.976) at a cut-off of 1.24, with 89.1% sensitivity and 82.5% specificity. Serum kisspeptin managed only 0.732 (0.643–0.821). The paired DeLong test confirmed that both seminal measures outperformed the serum measure ($P = 1.3 \times 10^{-5}$ and $P = 5.5 \times 10^{-8}$), while the two seminal measures did not differ from each other ($P = 0.695$). Performance varied with phenotype in an interpretable way. Against controls, seminal kisspeptin reached an AUC of 0.905 for asthenozoospermia, 0.920 for teratozoospermia and 0.994 for OAT. The ratio behaved similarly, and in OAT it achieved 92.5% sensitivity at 97.5% specificity with a cut-off of 0.84. The marker is therefore at its strongest where the seminal lesion is most severe, which is both reassuring and a limitation: the harder clinical question is usually the borderline case, and there the AUC of about 0.90 is good but not decisive. It is also worth noting how differently the two seminal measures trade sensitivity against specificity. At its Youden point, raw seminal kisspeptin was the more specific test (92.5%) and the ratio the more sensitive one (89.1%). For a screening application the ratio would be preferable; for confirming an abnormal seminal environment before counselling a couple, the absolute concentration would be. Because the ratio divides out much of the between-assay variation in the kisspeptin measurement, it should also travel better between laboratories, and that may prove its main practical advantage over time. For context, seminal oxidation reduction potential, currently the best investigated redox test in andrology, pools to an AUC of roughly 0.80 with 81% sensitivity and 66% specificity [33]. In a smaller set [34] the antioxidant capacity of sperm, measured as TEAC, reaches 0.97. Our seminal kisspeptin figures are intermediate and a combined redox-plus-peptide panel is a logical next step, especially as seminal-plasma proteomic indicators are being developed in similar directions [35]. Two cautions apply. The cut-offs reported here are internal, derived by the Youden index in this sample, and they will shift with any change of assay; external validation in an independent cohort is mandatory before clinical use. And AUCs computed in a design that contrasts well-defined cases against well-defined controls are optimistic by construction.

Table 06: ROC analysis of the three kisspeptin measures against fertile controls. AUC 95% confidence intervals by the DeLong method; cut-offs at the maximum Youden index.

Marker	Group vs controls	AUC	95% CI	Cut-off	Sens. (%)	Spec. (%)	Youden J	P
Seminal kisspeptin	All infertile	0.941	0.902–0.981	99.40	81.8	92.5	0.743	<0.001
Seminal/serum ratio	All infertile	0.935	0.895–0.976	1.24	89.1	82.5	0.716	<0.001
Serum kisspeptin	All infertile	0.732	0.643–0.821	83.40	79.1	55.0	0.341	<0.001
Seminal kisspeptin	Asthenozoospermic	0.905	0.841–0.969	118.20	97.5	70.0	0.675	<0.001
Seminal/serum ratio	Asthenozoospermic	0.900	0.832–0.968	1.41	100.0	70.0	0.700	<0.001
Serum kisspeptin	Asthenozoospermic	0.669	0.551–0.787	67.30	100.0	32.5	0.325	0.005
Seminal kisspeptin	Teratozoospermic	0.920	0.859–0.982	108.20	90.0	82.5	0.725	<0.001
Seminal/serum ratio	Teratozoospermic	0.920	0.859–0.982	1.24	90.0	82.5	0.725	<0.001
Serum kisspeptin	Teratozoospermic	0.759	0.647–0.871	90.80	76.7	65.0	0.417	<0.001
Seminal kisspeptin	OAT	0.994	0.984–1.000	98.90	100.0	92.5	0.925	<0.001
Seminal/serum ratio	OAT	0.982	0.959–1.000	0.84	92.5	97.5	0.900	<0.001
Serum kisspeptin	OAT	0.775	0.672–0.878	108.70	52.5	90.0	0.425	<0.001

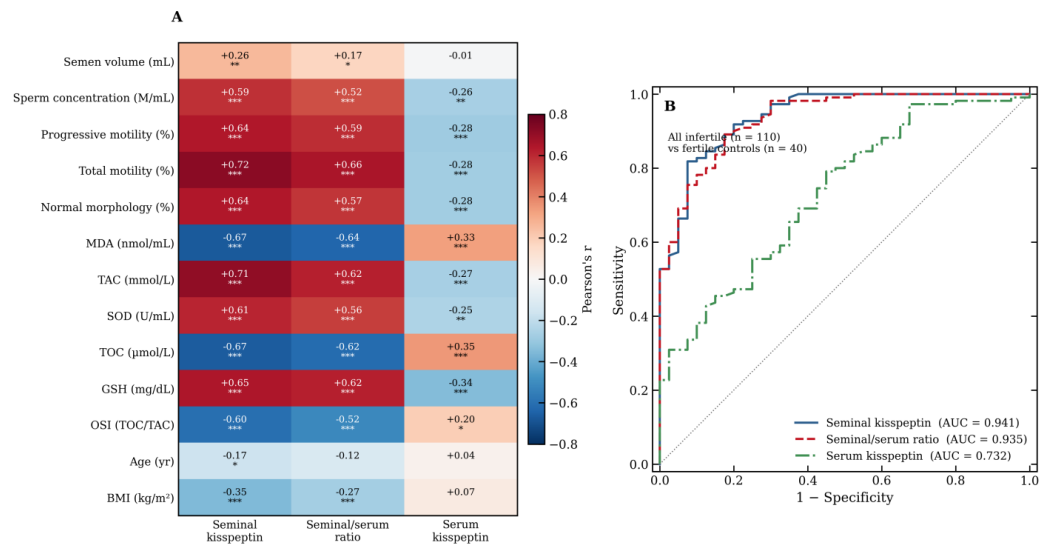


Figure 04: (A) Heat map of Pearson correlations between the three kisspeptin measures and all clinical, seminal and redox variables. Asterisks mark significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (B) ROC curves for all infertile men versus fertile controls.

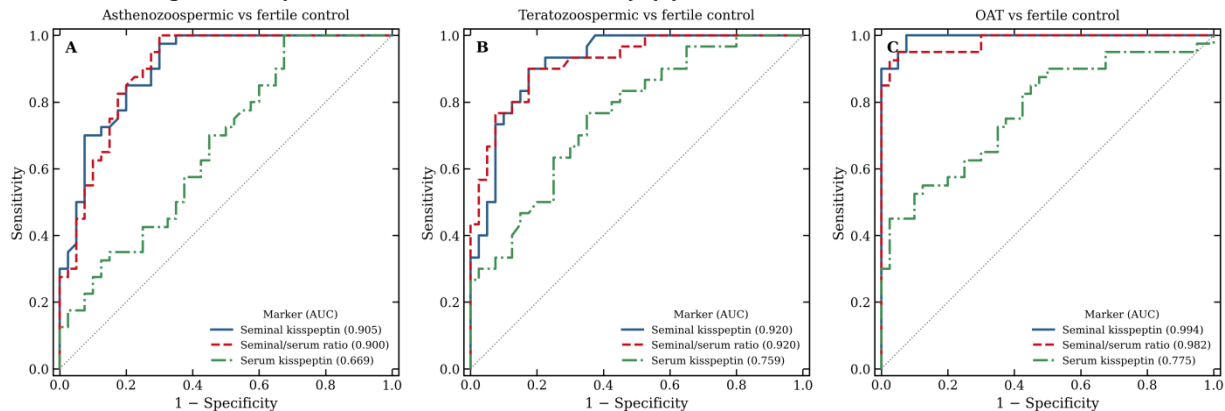


Figure 05: ROC curves by infertility phenotype. (A) Asthenozoospermic. (B) Teratozoospermic. (C) OAT, each against fertile controls.

3.6. Interpretation, strengths and limitations

Taken together, the data support a two-compartment reading of kisspeptin in the infertile male (Figure 6). The central pool, reflected in serum, is under classical feedback control and rises modestly as testicular function declines, much as gonadotrophins do; this is consistent with the reported inverse relation between serum kisspeptin and sperm concentration and its positive relation with FSH [16, 36]. The local pool, reflected in seminal plasma, behaves as a marker of secretory competence of the testis, epididymis and accessory glands, and it falls as that competence fails. The ratio combines both signals. It performed no better than seminal kisspeptin alone in our hands – the two AUCs were statistically indistinguishable – but it does not depend on the absolute calibration of the assay, and that is where its value probably lies.

The study has genuine strengths. Serum and seminal samples were taken from the same men on the same day and assayed in parallel, which removes much of the noise that plagues comparisons across compartments. The redox panel covered both oxidant and antioxidant arms, so OSI could be computed rather than inferred. Statistical treatment was conservative: DeLong confidence intervals rather than normal approximations, post-hoc correction for multiple group comparisons, and confirmation of the key associations within the infertile subgroup alone.

The limitations are equally real. The design is cross-sectional, so causality remains open. Men with clinical varicocele and leukocytospermia were excluded to reduce confounding, but these are common contributors to seminal oxidative stress [37, 38] and their exclusion narrows the applicability of our cut-offs. We did not measure sperm DNA fragmentation, which would have connected the redox findings to a functional endpoint, nor the seminal microbiome, now implicated in the same inflammatory–oxidative axis [27]. Reproductive hormones were not assayed, so the relation between seminal kisspeptin and the FSH–LH–testosterone profile could not be examined. Trace-element and heavy-metal exposure, relevant in industrial and post-conflict settings and known to interact with seminal oxidative markers [39], was not assessed. Sample size, while adequate for the main comparisons, leaves the teratozoospermic subgroup ($n = 30$) underpowered. Finally, all participants were recruited at a single centre, and molecular studies in Iraqi men have shown that local cohorts can differ in their oxidative and genomic profile [40]. Additionally, seminal plasma was separated by centrifugation at $300 \times g$, which may not fully remove spermatozoa and could result in minor contamination of the seminal-plasma fraction; future studies should consider higher-speed or two-step centrifugation protocols. The group-level kisspeptin comparisons were not adjusted for age and BMI, which differed significantly between groups. However, in the multivariable models (Table 5), seminal kisspeptin remained an independent predictor of motility and morphology after adjustment for these covariates.

Clinically, the findings are suggestive rather than actionable. If they hold up, seminal kisspeptin could act as a second-tier test in men whose routine semen analysis is equivocal, and a ratio below about 1.2 would flag a seminal environment already under oxidative strain. That would sit alongside the modifiable factors that are already known to matter: weight [21], physical activity [41, 42], smoking [30] and glycaemic control [43]. Whether antioxidant supplementation, whose evidence base remains uneven [28, 29, 44], would raise

seminal kisspeptin is untested and would be worth a trial. Kisspeptin analogues are already in clinical use for other reproductive indications [13, 45], so the therapeutic question is not far-fetched.

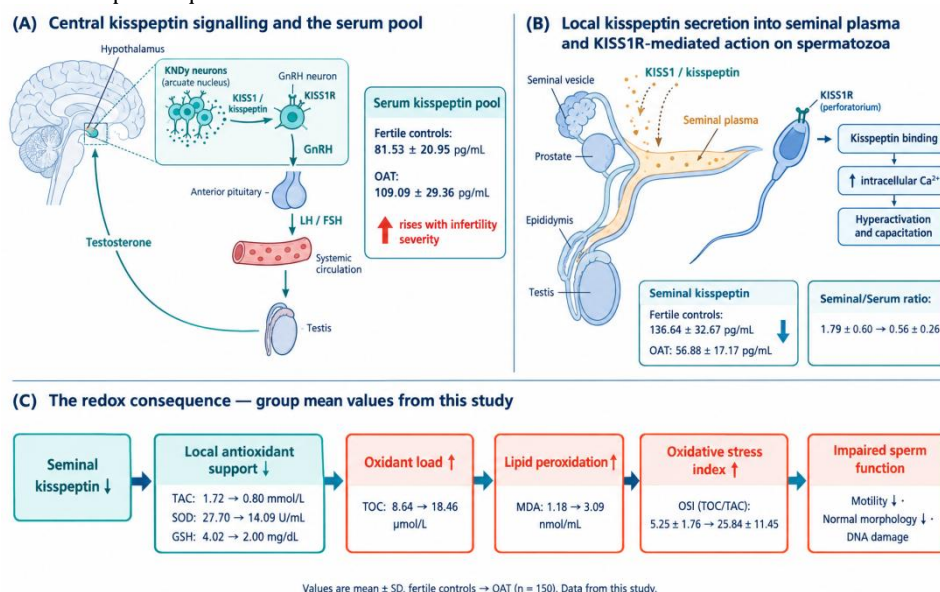


Figure 06: Proposed two-compartment model. (A) Central kisspeptin signalling and the serum pool. (B) Local kisspeptin secretion into seminal plasma and KISS1R-mediated action on spermatozoa. (C) The redox consequence, with group mean values from this study.

4. CONCLUSION

Seminal-plasma kisspeptin fell progressively across increasingly severe seminal phenotypes in this cohort of Iraqi men, while serum kisspeptin rose. The seminal/serum ratio captured both movements and separated fertile from infertile men effectively (AUC 0.935), though not significantly better than seminal kisspeptin alone (AUC 0.941; DeLong $p = 0.695$). The peptide tracked sperm motility and morphology closely and moved inversely with malondialdehyde, total oxidant capacity and the oxidative stress index, and those associations persisted after adjustment for age, BMI, smoking and lipid peroxidation, and also within the infertile men considered on their own. Diagnostically, seminal kisspeptin and the ratio both exceeded an AUC of 0.93, whereas serum kisspeptin reached only 0.73. We interpret this as evidence that the local kisspeptin pool reports on the secretory and redox competence of the male tract, and that measuring it in serum alone misses most of the signal. The study cannot establish direction of causation, and its cut-offs are internal ones that need external validation with a standardised assay. Even so, seminal kisspeptin deserves a place in the next generation of andrological biomarker panels, ideally combined with a redox measure and a test of DNA integrity.

5. AUTHOR CONTRIBUTIONS

Durar Kamil Jabbar: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration.

6. FUNDING

This research received no external funding.

7. CONFLICTS OF INTEREST

The author declares no conflict of interest.

8. DATA AVAILABILITY

The dataset generated and analysed in this study is available from the corresponding author on reasonable request.

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