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Biomarkers of Stress and Male Fertility

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Abstract

OBJECTIVE: To study if stress, as measured by salivary alpha-amylase and cortisol, negatively impacts male fertility, as measured by semen parameters, pregnancy and live birth rates.

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Ethics approval: Approval for the study was obtained from the University of Pennsylvania, which served as the single institutional review board for each site, with additional local site review.

Consent to participate: Written informed consent was obtained from all male and female participants.

Consent for publication: Written informed consent was obtained from all male and female participants.

Code availability: Not applicable

DESIGN: Prospective, cohort study of men enrolled in the Males, Antioxidants, and Infertility (MOXI) Trial.

MATERIALS AND METHODS: 112 infertile men provided first morning salivary and semen samples at baseline. Salivary samples were analyzed for alpha-amylase and cortisol. Couples attempted to conceive naturally (months 1-3) and with clomiphene citrate/intrauterine insemination (months 4-6). The association between stress related biomarkers and semen parameters including DNA fragmentation were assessed using linear regression models adjusting for male age. Salivary levels were dichotomized at the 80th percentile. Pregnancy/live birth rates in couples in the upper quintile were compared to remaining subjects using chi-square testing.

RESULTS: Salivary levels of alpha-amylase were not associated with semen parameters or DNA fragmentation. Salivary cortisol levels were not correlated with DNA fragmentation or normal morphology. For every 1-unit increase in salivary cortisol, total sperm count increased by 13.9million (95%CI: 2.5, 25.3) and total motile sperm count increased by 9.9million (95%CI: 3.2-16.6). Couple pregnancy rates and live birth rates did not differ for males in the highest quintile of alpha-amylase (27% and 28%, p=0.96; 23% and 21%, p=0.87) or cortisol (40% and 26%, p=0.22; 35% and 19%, p=0.12), compared to males with lower values.

CONCLUSION: Physiologic measures of high stress may not harm but actually improve semen parameters among men with male-factor infertility.

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Keywords

Amylase; Cortisol; Male Infertility; Semen parameters

Introduction

When a chronic stimulus is perceived as stressful, physiologic signals to the hypothalamus activate two different pathways: (1) the hypothalamic-pituitary-adrenal axis (HPA), which results in increased blood cortisol followed by elevations in salivary cortisol, and (2) the sympathetic adrenal medullary axis (SAM), which increases blood norepinephrine followed by salivary alpha-amylase [1]. Recent work suggests that psychological stressors produce a more pronounced alpha-amylase response than physical stressors [2]. Stress levels tend to increase as infertility treatment intensifies and as the duration of treatment continues [3]. For the infertile couple, the perception of stress is not equally distributed, with female partners reporting a higher level of stress [4–7].

Research to date has focused on the impact of psychological stress in the female partner on fertility and pregnancy outcomes. The LIFE study demonstrated that women in the highest tertile of salivary alpha-amylase had a 29% reduction in fecundability (a longer time-to-pregnancy [TTP]). This reduction in fecundity translated into a greater than 2-fold risk of infertility among these women (RR = 2.07, 95% CI = 1.04, 4.11) [1]. Another prospective study of 809 women pursuing IVF reported that women's number of previous negative life-events was a significant predictor of pregnancy [8].

This study sought to determine the impacts of stress, as measured by salivary alpha-amylase (which more accurately represent psychological stress) and cortisol (representing physical stress), on male fertility, as measured by semen parameters and couple's pregnancy and live birth rates. Based on the female infertility literature, we hypothesized that men with higher levels of stress, as indicated by higher salivary alpha-amylase and cortisol, would have worse semen parameters and higher DNA fragmentation than those with lower levels.

Material and methods

This study is a secondary analysis of men enrolled in the Males, Antioxidants, and Infertility (MOXI) clinical trial. 112 men aged 18 years and older who enrolled in the MOXI Trial and provided a saliva sample and semen analysis at baseline were included. MOXI was conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Cooperative Reproductive Medicine Network [9]. The Collaborative Center for Statistics in Science at Yale University served as the data coordinating center. The trial was conducted at nine clinical sites throughout the United States. A full description of the trial with inclusion and exclusion criteria is listed on Clinicaltrials.gov (NCT02421887) [10]. Approval for the study was obtained from the University of Pennsylvania, which served as the single institutional review board for each site, with additional local site review [11]. Written informed consent was obtained from all male and female participants.

Males enrolled in the MOXI trial had 12 or more months of infertility and at least one abnormality on their semen analysis (sperm concentration ≤15M/ml, motility ≤ 40%, or normal morphology ≤4%) at least 6 months prior to study initiation [9]. The female partner was ovulatory with at least one patent fallopian tube. At the baseline visit, self-reported information including basic demographics, smoking and alcohol consumption, herbal use, existing clinical depression and erectile dysfunction were collected. At this visit, men provided a first-morning salivary sample and semen sample in addition to completing a fertility-specific quality of life (FertiQoL) questionnaire [12]. Couples attempted to conceive naturally during the first 3 months. If not pregnant, this was followed by clomiphene citrate induced intrauterine insemination for further three (3) cycles.

Salivary samples were passively self-collected the morning of participants' baseline study visit, prior to initiation of antioxidant or placebo treatment. Men were instructed not to consume food, liquid or medication, brush their teeth, or perform physical exercise 60 minutes prior to providing a sample. Ten minutes prior to salivary sample collection, men rinsed their mouths with water to remove any remaining residue. Salivary samples were obtained by passive collection utilizing SalivaBio Oral Swabs (Salimterics LLC, Item #5001.02, State College, PA, USA) placed under the tongue and not displaced for 1-2 minutes, until saturated. Swabs were inserted securely in a protective tube. Samples were stored at −20° C prior to processing.

Salivary samples were analyzed and the concentrations of cortisol and alpha-amylase (NIH SCCPIR Ligand Core, University of Virginia, Charlottesville, VA, USA) were determined. Salivary cortisol was measured by radioimmunoassay (MP Biomedical, Cat #072211020, Solon, OH; USA) using a protocol validated for salivary samples according to Endocrine

Society's Sex Steroid Assays Reporting Task Force recommendations [13]. Salivary cortisol assay sensitivity was 0.5 ng/ml, with intra- and inter-assay coefficient of variation (CV) of 4.3% and 6.8%, respectively. Salivary alpha-amylase was measured according to a validated glucose oxidase/quantitative colorimetric method (EnzyChrom, Cat # ECAM-100; BioAssay Systems; Hayward, CA, USA). Salivary alpha-amylase assay sensitivity was 3.0 U/L, with intra- and inter-assay CV 2.3% and 7.1%., respectively.

The semen analysis was performed on the baseline semen sample assessing volume, motility, total motile sperm count, and sperm morphology using World Health Organization 5.0 criteria as previously described [9]. Three aliquots of neat semen stored at 4°C were shipped to the Utah Andrology Laboratory for DNA fragmentation analysis by SCSA [14]. In order to assure the quality of DFI testing, control samples for each assay with known low, moderate and high sperm DNA damage were analyzed after the reagents were prepared. If variation in DNA fragmentation was low among test samples, the samples were reanalyzed.

The SCSA assay was performed as described by Simon et al [15]. In summary, a small portion of the semen sample was diluted, treated with an acid detergent solution and stained with purified acridine orange (Polysciences, Inc.,Warrington, PA, USA) in a phosphate-citrate buffer, pH 6.0. A flow cytometer (Accuri C6, Accuri Cytometers, Inc., Ann Arbor, MI, USA), equipped with an air-cooled argon-ion laser, was utilized for cell analysis.

Participant quality of life relating to infertility and treatment was assessed with the Fertility-Related Quality of Life (FertiQoL) survey. The FertiQoL is a 34-item behavioral instrument specifically designed to assess the emotional, mind/body, relational and social burden of infertility in men and women experiencing fertility problems [12]. The survey is scored 0-100, with higher scores indicating better quality of life. All males and female partners completed FertiQoL questionnaires upon entry into the MOXI trial.

For this analysis, all men who enrolled in the MOXI Trial and provided saliva sample and semen analysis at baseline were included. Amylase was measured in all samples, but cortisol could only be measured in 104 (93%) samples due to insufficient salivary volume. Salivary alpha-amylase and cortisol levels were categorized into quintiles and dichotomized at the 80^{th} percentile of alpha-amylase (64.6 U/L) and cortisol (3.5ng/mL). Examination of additional thresholds (i.e., quartiles, continuous variables) did not change the conclusions. Conception was defined by a rising serum hCG level on two consecutive tests 48 hours apart. Pregnancy loss was defined as a nonviable pregnancy prior to 20 weeks gestational age. Live birth was defined as delivery of a viable infant after 20 weeks' gestation.

Initially, baseline patient demographics and semen analysis parameters were compared among men with and without elevated alpha-amylase and men with and without elevated cortisol using Wilcoxon rank-sum test or Student's t-test for the continuous variables, and Chi-square or Fisher's exact test for categorical variables, where appropriate. Subsequently, prediction modeling was conducted using a multi-variable linear regression model with the dependent variables of semen parameters or DNA fragmentation. The models included either salivary amylase or cortisol levels modeled as continuous variables and male age. Pregnancy and live birth rates in couples of those males in the upper quintile were

compared to remaining subjects using chi square testing or Fisher's exact test, where appropriate. Analyses were conducted using SAS 9.4 (SAS Institute). P <0.05 was considered statistically significant.

Results

No differences in demographics such as age, BMI, or ethnicity were noted between men with high salivary amylase levels compared to men with normal amylase levels (Table 1). In addition, the severity of depression, presence of erectile dysfunction, and fertility-related stress levels as measured by FertiQoL scores, did not differ between men with high or lower levels of salivary amylase (Table 1). There were no differences in age, BMI, or ethnicity between men with high salivary cortisol levels compared to normal levels (Table 2). Males within the highest quintile of salivary cortisol levels reported feeling more sad and depressed as well as angry due to fertility problems than those with lower salivary cortisol levels. (FertiQol scores 2.9 vs. 3.3, p 0.013; and 3.1 vs 3.5, p 0.035, respectively; Table 2)

Semen parameters did not differ between men with high cortisol levels (≥80th percentile) versus men with lower cortisol levels (Table 3) nor between men with high amylase levels (≥80th percentile) versus men with lower amylase levels (Table 3). Salivary levels of alpha-amylase did not correlate with changes in semen parameters or the degree of DNA fragmentation (Table 4). Additionally, salivary levels of cortisol were not correlated with semen volume, normal morphology, or degree of DNA fragmentation (Table 4). Interestingly, total sperm count and total motile sperm count increased as salivary cortisol levels increased. For every 1 ng/mL increase in salivary cortisol, there was an increase of 13.89 million total sperm count (95% CI: 2.49, 25.29) and an increase in total motile sperm count of 9.88 million (95% CI: 3.16, 16.61), after adjusting for male age. (Table 4)

Despite this increase in sperm quantity, couple pregnancy rates did not differ for males in the highest quintile of cortisol (40% and 26.2%, p=0.22) or alpha-amylase (27.3% and 27.8%, p= 0.96) compared to males with lower values. Couple live birth rates did not significantly differ for males in the highest quintile compared to males with lower alpha-amylase values (22.7% and 21.1%, p=0.87) or cortisol values (35% and 19%, p=0.12). (Table 5)

Discussion

In this study of infertile males, no negative association was found between increased stress, as evidenced by elevation in salivary cortisol [physical] and alpha-amylase [emotional], and semen parameters or couple pregnancy and live birth rates. Increasing salivary cortisol levels were associated with higher total sperm count and total motile sperm count but did not correlate with higher pregnancy rates.

Our study did not find an association between male stress and couple pregnancy rates. Findings agree with previously reported studies that have not found a significant association between pregnancy outcomes and self-reported psychological stress, depression or anxiety in the male partner [16–18]. However, our findings are contrary to one previous report utilizing a 12-item General Health Questionnaire [GHQ-12] (not utilizing stress-related biomarkers), in which the odds of pregnancy were reduced by 30% in cycles with a male

stress score in the highest compared to lowest quartiles. The effect was confined to men with low sperm concentration (<20M/mL), which could constitute a unique stress-vulnerable group [19]. If indeed a more stress-vulnerable group exists, one physiologic explanation may be linked to lower serum total testosterone levels, higher serum LH and FSH levels [20], and not stress biomarkers amylase and cortisol. Our results did indicate that men in our study were experiencing stress by worsened FertiQOL scores of sadness and anger in those men in the highest salivary cortisol levels. Despite the presence of stress, fertility was not affected in our study.

The physiological impact of stress on the male reproductive system is complex and reports are conflicting. Contrary to the hypothesis, our study noted increased total sperm count and total motile sperm count with increasing salivary cortisol levels. Aside from the known effect of testosterone on the upregulation of nitric oxide synthase and the permissive role that androgens play in sexual desire and function, surprisingly little is known on the clinical effects that hypothalamic-pituitary-adrenal hormones have on sexual function (including erectile dysfunction) [21]. Histological examination of testicular tissue from men with chronically elevated cortisol (Cushing syndrome) show tubular atrophy, disorganization of seminal epithelium and decreased number of Leydig cells [22–23]. However, in men with low cortisol levels (Addison disease), short-term hormonal replacement has been reported to improve sexual function (including erectile dysfunction) [24]. These results suggest that in the absence of chronically elevated cortisol, acute increases in cortisol levels may have positive results on male reproductive systems, although its mechanism of activity is not entirely clear. Chronic stress has been shown to elicit a wide variety of hypothalamic-pituitary-adrenal responses [25], likely explaining the reported differences in downstream reproductive effects.

Previous studies reporting adverse effects of stress on testicular function highlight the supportive roles of gonadal hormones estrogen and testosterone in spermatogenesis [26]. We did not identify an association between total sperm count or any other sperm parameters and salivary alpha-amylase levels. A post hoc power analysis was performed. Despite our small sample size, the study was adequately powered to detect a 5M/mL difference in sperm concentration between the dichotomized Amylase groups. Given that our semen parameters between groups were similar (Table 3), thousands of men would be required to achieve adequate power to detect a statistical difference. The positive relationship between cortisol and sperm count has not previously been reported and warrants further study. In the present investigation, perceived fertility-related stress levels measured by FertiQoL scores did not differ between men with high compared to normal levels of salivary amylase (Table 1). This finding supports a recent conclusion reached by Loa Nordkap et al. regarding the association between various stress scales and semen parameter results in young men. The study of 1,362 men indicated that *perception* of stressful events rather than the specific stressful event most affects fertility parameters [27]. Potentially, fertility-related stress was not *perceived* as a significant life stressor in our cohort than other life stressors. Therefore, FertiQOL scores did not correlate with biomarkers of stress.

FertiQOL scores have been studied in infertile couples with male-factor, female-factor and unexplained infertility [28,29]. Males have been shown to have higher overall FertiQOL

scores, suggesting better functioning and lower stress levels, compared to their female partners. Compared to males with unexplained infertility, men with a partner with assumed female-related infertility had higher FertiQOl scores [29].

One of our study's strengths includes the multisite, randomized, double-blind, placebo-controlled nature of its study cohort. All men enrolled in the MOXI trial had male factor infertility, with at least one abnormal semen parameter and a normal fertility testing partner. Salivary alpha-amylase and cortisol measurements and FertiQoL survey results are verified and reliable methods to assess psychological and physical stressors. This study is one of few to analyze the effects of stress on male-specific fertility factors.

While homogeneity is a strength of the cohort, the use of an infertile male cohort may also represent a limitation. By enrolling men with infertility and abnormal semen parameters, our results cannot be generalized to men without an infertility diagnosis. As with any subgroup analysis, we are limited by the original study parameters. Additionally, our findings should be applied with caution to those in extreme stress situations, as our cohort of men had a limited range of stressors. Another limitation of the study was the inability to measure all participants' salivary cortisol samples. Due to insufficient volume, cortisol could only be measured in 104 (93%) samples. While this difference in number of measurable alpha-amylase and cortisol samples is small, it is possible that the discrepancy skewed our results.

Conclusions

This study offers a unique assessment of stress-related changes in salivary cortisol and alpha-amylase levels and their associations with male fertility. Stress, as measured by salivary biomarkers, in infertile men does not appear to negatively impact semen parameters or male fertility. Instead, our study suggests that higher levels of cortisol are associated with higher total sperm count in men with male-factor infertility. Future studies should investigate whether stress impacts semen parameters among men in the general population.

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Conflicts of Interest/Competing Interests:

T.L.S. reports no conflicts of interest and is a military service member. This work was prepared as part of her official duties. Title 17 U.S.C. 105 provides that "Copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. 101 defines a United States Government work as a work prepared by a military service member or employee of the United States Government as part of that person's official duties. H.Z. reports grants from NIH during the conduct of the study. J.C.T., R.M.C., K.T.B., M.I.C., F.S. and N.S. report no conflicts of interests/disclosures. M.P.D. reports grants from NIH/NICHD and NIH/NICHD Yale subcontract during the conduct of the study, is on the Board of Directors and stockholder in Advanced Reproductive Care, and has grants from AbbVie, ObsEva and Bayer outside of submitted work. K.R.H. reports grants from NIH/NICHD, Roche Diagnostics and Ferring International Pharmascience Center US, in addition to personal fees from Ablacare, outside of the submitted work. S.A.K. reports grants from NICHD during the conduct of the study, personal fees from EIC Systems Biology in Reproductive Medicine, grant from Merck and book royalties from

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Availability of data and material:

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Table 1.

Baseline characteristics for all enrolled male subjects - Alpha-amylase group

	3.0 ≤ alpha-amylase ≤ 64.5 Units/L (n=90)	64.6 ≤ alpha-amylase ≤ 120.6 Units/L (n=22)	Overall P value*
Median Age (IQR)-yr	34.0 (31.0-38.0)	32.5 (29.0-36.0)	0.340
Median BMI (IQR)	27.8 (24.5-31.3)	27.8 (24.4-30.2)	0.592
Ethnicity			0.824
Hispanic or Latino	5/90 (5.6)	0/22 (0.0)	
Non-Hispanic	81/90 (90.0)	21/22 (95.5)	
Unknown	4/90 (4.4)	1/22 (4.5)	
Race			0.883
White	71/90 (78.9)	19/22 (86.4)	
Black	6/90 (6.7)	2/22 (9.1)	
Asian	5/90 (5.6)	0/22 (0.0)	
American Indian or Alaska Native	1/90 (1.1)	0/22 (0.0)	
Unknown	6/90 (6.7)	1/22 (4.5)	
Mixed Race	1/90 (1.1)	0/22 (0.0)	
Herbal supplements in past 6 months	42/90 (46.7)	10/22 (45.5)	0.919
History of smoking			0.517
Never	56/90 (62.2)	11/22 (50.0)	
Current	10/90 (11.1)	4/22 (18.2)	
Former	24/90 (26.7)	7/22 (31.8)	
History of alcohol use			0.402
Never	3/90 (3.3)	2/22 (9.1)	
Current (in the past year)	82/90 (91.1)	19/22 (86.4)	
Former (not in the past year)	5/90 (5.6)	1/22 (4.5)	
FertiQoL			
Feeling drained or worn out	3.1 ± 0.9, n=89	3.4 ± 1.0, n=21	0.051
Feeling able to cope with fertility problems	1.2 ± 1.2, n=89	1.1 ± 1.3, n=21	0.399
Fertility problems cause jealousy and resentment	3.4 ± 0.9, n=89	3.1 ± 0.9, n=21	0.128
Experience grief about not having children	3.0 ± 0.8, n=89	3.1 ± 0.9, n=21	0.605
Fertility problems strengthen commitment to partner	1.8 ± 1.1, n=88	2.3 ± 1.2, n=20	0.099
Feeling sad and depressed	3.1 ± 0.8, n=88	3.3 ± 0.7, n=21	0.274
Fertility problems cause anger	3.3 ± 0.9, n=89	3.5 ± 0.7, n=21	0.597
Feeling pain and physical discomfort	3.9 ± 0.4, n=89	3.9 ± 0.4, n=21	0.753
Depression Severity			0.415
Minimal or none	70/89 (78.7)	16/21 (76.2)	
Mild	16/89 (17.9)	3/21 (14.3)	
Moderate	2/89 (2.3)	1/21 (4.8)	
Moderately severe	1/89 (1.1)	1/21 (4.8)	
Erectile Dysfunction			0.688

	3.0 ≤ alpha-amylase ≤ 64.5 Units/L (n=90)	**64.6 ≤ alpha-amylase ≤ 120.6 Units/L (n=22)**	**Overall P value***
No erectile dysfunction	76/86 (88.4)	20/21 (95.2)	
Mild erectile dysfunction	10/86 (11.6)	1/21 (4.8)	

* IQR, interquartile range; categorical variables are expressed as no. (%). Student's t test or Wilcoxon's rank-sum test was used for continuous variables, and Chi-square or Fisher's exact test was used for categorical variables.

Table 2.

Baseline characteristics for all enrolled male subjects – Cortisol group

	0.34 ≤ Cortisol ≤ 3.4 ng/mL (n=84)	3.5 ≤ Cortisol ≤ 12.3 ng/mL (n=20)	Overall P value*
Median Age (IQR)-yr	34.0(31.0-38.0)	34.0(29.0-37.0)	0.511
Median BMI (IQR)	27.9(24.5-31.3)	27.5(22.8-29.5)	0.122
Ethnicity			0.824
Hispanic or Latino	5/84 (6.0)	0/20 (0.0)	
Non-Hispanic	75/84 (89.3)	19/20 (95.0)	
Unknown	4/84 (4.8)	1/20 (5.0)	
Race			0.615
White	63/84 (75.0)	19/20 (95.0)	
Black	8/84 (9.5)	0/20 (0.0)	
Asian	5/84 (6.0)	0/20 (0.0)	
American Indian or Alaska Native	1/84 (1.2)	0/20 (0.0)	
Unknown	6/84 (7.1)	1/20 (5.0)	
Mixed Race	1/84 (1.2)	0/20 (0.0)	
Herbal supplements in past 6 months	39/84 (46.4)	9/20 (45.0)	0.908
History of smoking			0.962
Never	50/84 (59.5)	12/20 (60.0)	
Current	11/84 (13.1)	3/20 (15.0)	
Former	23/84 (27.4)	5/20 (25.0)	
History of alcohol use			0.014
Never	5/84 (6.0)	0/20 (0.0)	
Current (in the past year)	77/84 (91.7)	16/20 (80.0)	
Former (not in the past year)	2/84 (2.4)	4/20 (20.0)	
FertiQoL			
Feeling drained or worn out	3.2 ± 1.0, n=82	3.0 ± 0.8, n=20	0.198
Feeling able to cope with fertility problems	1.2 ± 1.2, n=82	1.2 ± 1.1, n=20	0.733
Fertility problems cause jealousy and resentment	3.3 ± 0.9, n=82	3.3 ± 0.8, n=20	0.431
Experience grief about not having children	3.0 ± 0.8, n=82	2.8 ± 0.9, n=20	0.227
Fertility problems strengthen commitment to partner	1.9 ± 1.2, n=80	1.7 ± 0.9, n=20	0.425
Feeling sad and depressed	3.3 ± 0.7, n=81	2.9 ± 0.7, n=20	0.013
Fertility problems cause anger	3.5 ± 0.9, n=82	3.1 ± 0.9, n=20	0.035
Feeling pain and physical discomfort	3.9 ± 0.4, n=82	3.9 ± 0.4, n=20	0.973
Depression Severity			0.651
Minimal or none	66/82 (80.5)	15/20 (75.0)	
Mild	12/82 (14.6)	4/20 (20.0)	
Moderate	2/82 (2.4)	1/20 (5.0)	
Moderately severe	2/82 (2.4)	0/20 (0.0)	
Erectile Dysfunction			0.682

	0.34 ≤ Cortisol ≤ 3.4 ng/mL (n=84)	**3.5 ≤ Cortisol ≤ 12.3 ng/mL (n=20)**	**Overall P value***
No erectile dysfunction	71/79 (89.9)	19/20 (95.0)	
Mild erectile dysfunction	8/79 (10.1)	1/20 (5.0)	

* IQR, interquartile range; categorical variables are expressed as no. (%). Student's t test or Wilcoxon's rank-sum test was used for continuous variables, and Chi-square or Fisher's exact test was used for categorical variables.

Table 3.

Semen parameters by stress related biomarker groups.

Alpha-amylase			
	3.0 ≤ alpha-amylase ≤ 64.5 (n=90)	**64.6 ≤ alpha-amylase ≤ 120.6 (n=22)**	**p value**
Sperm concentration (million/ml)	18.5 (11.0, 48.0), n=90	19.0 (10.4, 28.0), n=22	0.39
Normal morphology (Kruger) (%)	5.0 (2.0, 9.0), n=67	5.0 (3.0, 11.0), n=14	0.76
Total motility (%)	40.0 (35.0, 54.0), n=90	49.0 (38.0, 53.0), n=22	0.30
DNA fragmentation (SCSA, DNA fragmentation index) (%)	19.1 (14.5, 28.4), n=79	23.1 (15.2, 29.0), n=17	0.57
Total sperm count (million)	52.5 (31.0, 89.8), n=90	43.4 (25.3, 58.5), n=22	0.20
Semen Volume (mL)	2.5 (1.7, 3.5), n=89	2.4 (1.8, 4.0), n=22	0.68

Table 4.

Semen parameters by stress related biomarker groups.

	Cortisol		
	0.34 ≤ Cortisol ≤ 3.4 (n=84)	**3.5 ≤ Cortisol ≤ 12.3 (n=20)**	**p value**
Sperm concentration (million/ml)	17.5 (11.0, 39.5), n=84	19.5 (10.7, 61.5), n=20	0.57
Normal morphology (Kruger) (%)	5.0 (2.0,10.0), n=61	5.0 (3.0, 9.0), n=14	0.96
Total motility (%)	42.3 ± 17.1, n=84	46.9 ± 14.1, n=20	0.41
DNA fragmentation (SCSA, DNA fragmentation index) (%)	20.9 (14.8, 28.4), n=72	17.6 (16.4, 29.3), n=16	0.97
Total sperm count (million)	48.0 (26.3, 75.5), n=84	58.5 (23.4, 167.3), n=20	0.44
Semen Volume (mL)	2.5 (1.7, 3.4), n=83	3.3 (2.1, 4.0), n=20	0.12

Table 5.

Association between salivary alpha-amylase and cortisol and semen parameters *(adjusted by male age)*

	Sperm concentration (million/ml)	Normal morphology (Kruger) (%)	Total motility (%)	DNA fragmentation (SCSA, DNA fragmentation index) (%)	Total sperm count (million)	Total motile sperm count (million)	Semen Volume (ml)
Alpha-amylase Beta * (95% CI)	0.10 (−0.21, 0.40)	−0.01 (−0.06, 0.05)	−0.05 (−0.17, 0.08)	0.04 (−0.05, 0.13)	0.48 (−0.50, 1.47)	0.30 (−0.29, 0.88)	0.01 (−0.004, 0.02)
Cortisol Beta * (95% CI)	2.10 (−1.55, 5.75)	0.04 (−0.54, 0.63)	0.89 (−0.59, 2.38)	0.12 (−0.86, 1.09)	13.89 * (2.49, 25.29)	9.88 ** (3.16, 16.61)	0.04 (−0.08, 0.15)

* p= 0.018;

** p=0.004

Table 6.

Fertility and Pregnancy Outcomes

	3.0 ≤ alpha-amylase ≤ 64.5 Units/L (n=90)	**64.6 ≤ alpha-amylase ≤ 120.6 Units/L (n=22)**	**Overall P value**
Achieved Pregnancy	25/90 (27.8)	6/22 (27.3)	0.96
Live birth	19/90 (21.1)	5/22 (22.7)	0.87
Pregnancy Loss	5/25 (20.0)	1/6 (16.7)	1.00
Time to Pregnancy (months)	117.3 ± 54.6, n=24	123.8 ± 57.4, n=6	0.80

Table 7.

Fertility and Pregnancy Outcomes

	0.34 ≤ Cortisol ≤ 3.4 ng/mL (n=84)	3.5 ≤ Cortisol ≤ 12.3 ng/mL (n=20)	Overall P value
Achieved Pregnancy	22/84 (26.2)	8/20 (40.0)	0.22
Live birth	16/84 (19.0)	7/20 (35.0)	0.12
Pregnancy Loss	5/22 (22.7)	1/8 (12.5)	1.00
Time to Pregnancy (months)	116.6 ± 55.1, n=21	118.9 ± 57.0, n=8	0.92