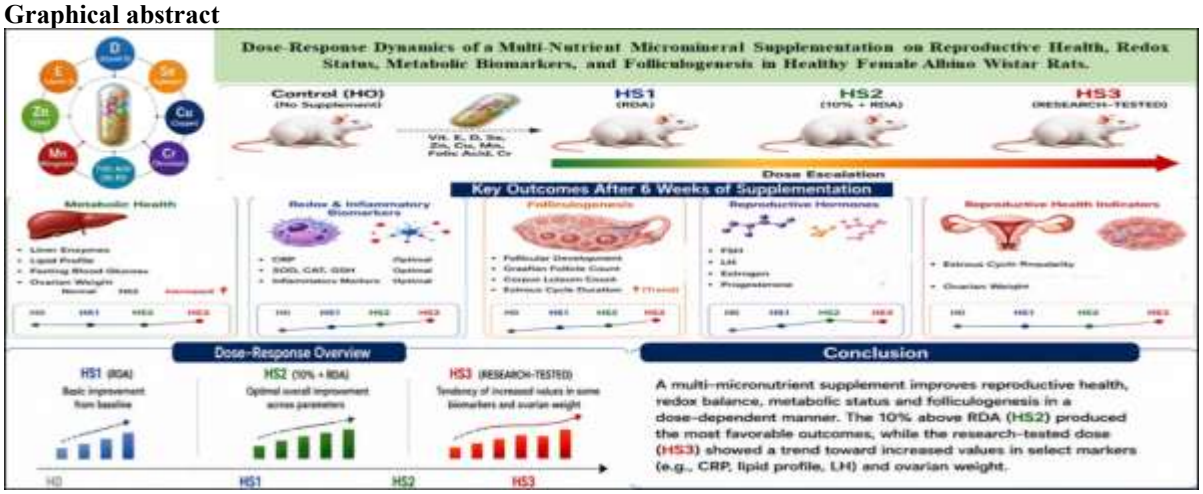
GMR
The Original

DOSE-RESPONSE DYNAMICS OF A MULTI-MICRONUTRIENT SUPPLEMENTATION ON REPRODUCTIVE HEALTH, REDOX STATUS, METABOLIC BIOMARKERS, AND FOLLICULOGENESIS IN HEALTHY FEMALE ALBINO WISTAR RATS

Kanwal Hafeez Khan[1]. Prof. Dr Mahr-Un-Nisa[2], Dr Nazir Ahmad[3], Dr Huma Umbreen[4]

[1]Ph.D. Scholar, Department: Nutritional Sciences, Faculty: Medical Sciences, Government College University, Faisalabad,
Email: kanwalhafeezkhan@gmail.com, ORCID ID: https://orcid.org/0009-0007-6339-7622
[2]Department: Nutritional Sciences, Faculty: Medical Sciences, Government College University, Faisalabad, Email: linknisa@gcuf.edu.pk,
ORCID ID: https://orcid.org/0000-0001-7723-8744
[3]Department: Nutritional Sciences, Faculty: Medical Sciences, Government College University, Faisalabad, Email: drnazirahmad@gcuf.edu,
ORCID ID: https://orcid.org/0000-0003-3151-0276
[4]Department: Nutritional Sciences, Faculty: Medical Sciences, Government College University, Faisalabad,
Email: huma_umbreen@yahoo.com, ORCID ID: https://orcid.org/ 0000-0001-8728-9289

Graphical abstract



ABSTRACT

Background: Female reproductive health in all species is significantly influenced by oxidative stress and metabolic dysregulation, which compromise fertility outcomes. Micronutrient supplementation holds therapeutic promise, but optimal dosages and combined effects remain uncertain. This study investigated the impact of a multinutrient supplement (containing vitamins E and D, selenium, zinc, copper, manganese, folic acid, and chromium picolinate) at varying doses on reproductive, metabolic, and oxidative parameters in female albino Wistar rats.

Methodology: Thirty-six female albino Wistar rats (7–8 weeks; 137 ± 10 g) were allocated to four groups (n = 9): control (H0, basal diet) and three supplemented groups (HS1: RDA; HS2: 10% +RDA; HS3: research-tested doses). Over 6 weeks, nutrient intake/digestibility, body and ovarian weight, abdominal circumference, estrous cyclicity, reproductive hormones (LH, FSH, testosterone, estrogen, progesterone), metabolic indices (glucose, insulin, HbA1c, lipid profile), hepatic enzymes (ALT, AST), and oxidative/antioxidant markers (MDA, TOS, TAC, SOD, CAT, GSH, CRP, uric acid) were assessed. Data were analysed by ANOVA ($p \leq 0.05$).

Results: HS2 significantly reduced b.w, abdominal circumference, testosterones and LH as compared to HS3 where an increased body weight gain (+48.46% vs. H0), abdominal circumference (+12.32%), LH (+45.33%), testosterone (+31.24%), serum glucose (+44.62%), and LDL (+3.70%), with elevated oxidative stress (MDA +12%; TOS ↑), reduced CAT (−9.08%) was observed. HS2 enhanced TAC (+20.57%), SOD (+8.42%), HDL (+4.68%), and maintained regular estrous cyclicity. HS1 improved protein digestibility (+5.59%) and GSH (+2.15%). H0 exhibited a baseline status characterised by higher NFE intake (+15.85% vs. HS3) and shorter oestrous cycles.

Conclusion: Higher-dose supplementation (HS3) promoted growth and reproductive activity but induced oxidative and metabolic strain. Moderate doses (HS1/HS2) provided antioxidant and metabolic benefits without adverse hormonal shifts, highlighting a dose-dependent threshold for micronutrient efficacy in reproductive health.

KEYWORDS: Female Reproductive Metabolic Axis, Antioxidant Supplementation, Dose-Response Relationship, Oxidative Stress, Nutrient Thresholds, Ovarian Histopathology

INTRODUCTION

Female reproductive and metabolic health are increasingly challenged by oxidative stress and nutritional imbalances, underscoring the need for supportive strategies, such as micronutrient supplementation. Diets often fail to provide optimal intake due to sedentary lifestyles, poor dietary habits, and increased physiological demands, creating barriers to maintaining fertility and reproductive wellbeing (Sonawdekar et al., 2024).

Female reproductive health issues, most commonly known now as, Polyendocrine Metabolic Ovarian Syndrome (PMOS), affecting 5–20% of females from humans as well as animals of reproductive age, exemplify this challenge. It is a heterogeneous endocrine and metabolic disorder characterised by oligo-anovulation, hyperandrogenism, polycystic ovarian morphology, obesity, insulin resistance, and oxidative stress (Helvaci & Yildiz, 2025). Beyond reproductive dysfunction, female reproductive health issues are linked to low-grade inflammation, elevated oxidative markers such as MDA and Uric acid, and metabolic derangements including insulin resistance and dyslipidemia (Tatone et al., 2021). Physiological ROS support follicular growth, angiogenesis, and steroidogenesis. However, their excess impairs oocyte maturation, accelerates follicular atresia, and induces oxidative DNA damage, as reflected by elevated 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels (a biomarker found to be considerably higher in the PMOS groups than healthy female controls(Panti et al., 2018; Zeng et al., 2024). This oxidative milieu contributes to disrupted menstrual cyclicity, altered liver enzymes, and increased adiposity(Papalou et al., 2016).

Conventional therapies such as oral contraceptives and metformin improve symptoms but are often limited by gastrointestinal side effects (Wahid et al., 2024). Nutraceuticals, particularly antioxidants, have therefore emerged as promising adjuncts to target oxidative and metabolic dysfunction. Non-enzymatic antioxidants from diet or supplementation play a central role in maintaining ROS-antioxidant balance essential for ovulation and ovarian function (Amirkhizi et al., 2024); (Chen et al., 2025).

Among these, vitamin D deficiency, prevalent in up to 85% of women, exacerbates insulin resistance and hyperandrogenism by impairing aromatase activity and IRS expression (Kazeminia et al., 2024); (Trummer et al., 2019). Selenium and vitamin E synergistically safeguard ovarian function by neutralising lipid peroxyl radicals and detoxifying peroxides via glutathione peroxidase (Farahavar et al., 2020; Karami et al., 2025). Copper–zinc balance regulates hepatic and insulin-related processes, while folic acid counteracts hyperhomocysteinemia-induced oocyte damage(Mohmmed et al., 2019). Manganese, as a cofactor for Mn superoxide dismutase, further supports glucose–lipid metabolism and oxidative defence(Li & Yang, 2018). Chromium picolinate activates PPAR-γ and GLUT-1, reducing IL-1β expression, which alleviates inflammation-related insulin resistance and hyperandrogenism (Amiri Siavashani et al., 2018).

Nearly 40% of reproductive health cases remain misdiagnosed until infertility, exposing limitations in early management(Helvaci & Yildiz, 2025). Current treatments rarely improve oxidative stress, appetite dysregulation (characterised by ghrelin/leptin imbalance), or hepatic lipid metabolism, all of which contribute to female reproductive health problems(Papalou et al., 2016). To date, no study has systematically evaluated the combined dose response effects of these micronutrients, all with dual relevance to antioxidant defense and female reproductive function — on integrated reproductive, metabolic, and oxidative endpoints in a controlled in vivo model. Additionally, effective dose regimens and synergistic effects of certain micronutrients are unknown. To fill these gaps, this study examined the effects of a multinutrient supplement on hormonal profiles (LH, FSH, testosterone), metabolic parameters (glucose tolerance, lipid profile, ALT/AST), oxidative stress markers (CRP, UA), and reproductive outcomes (follicular development, estrous cycle duration) in healthy female rat model.These results define a previously unrecognized dose-dependent therapeutic window for multinutrient nutraceutical use, cautioning that higher doses may incur oxidative and metabolic costs even while stimulating reproductive hormones.

MATERIAL AND METHOD

Research area and ethical approval

The in vivo experiment was conducted at the Department of Pharmacology, Biochemistry, and Zoology of Government College University, Faisalabad, Pakistan, in accordance with the guidelines established by the National Research Council in 1995 for the care and use of animals. The ethics committee of Government College University, Faisalabad, Pakistan, approved the experimental procedure under Ref. **No. GCUF/ERC/224** by applying the standards of Laboratory Animal Care. The study's objectives were explained to all committee members, and their consent was received (Council et al., 1995).

Animal Selection and care, and trial duration

Thirty-six female Wistar rats (7–8 weeks old; 137 ± 10 g) were kept in a controlled environment with unlimited food and water. They were given an iso-caloric, iso-nitrogenous diet based on the AIN-93 formulation. The trial consisted of a six-week data collection period.

Estrous cycle evaluation

To evaluate the regulation of the oestrous cycle, regular vaginal smears were analysed during the anovulatory oestrous cycle across the first and last 12 days of the study period.

Weekly serum glucose, body weight changes, and abdominal circumference

Weekly blood glucose levels were measured using an AccuChek Active glucometer (Roche Diagnostics GmbH, Germany), and the body weight of all rats was recorded using a weighing balance. Abdominal circumference was measured by gauging the girth at the midpoint between the forelegs and hindlegs.

Daily Feed Intake and Water Intake Calculations

The daily water and feed intake were noted down, and FCR and FER were computed as a ratio between feed consumed and the gain of body mass.

Growth and Digestibility

The nutritional digestibility assay assessed intake and excreta. Nutritional intake was documented weekly until the study concluded, with the final 10 days for feces collection. 500 (g) of excreta were homogenised for disposal. Nutrient digestibility, nutrient content, dry matter, crude protein, ash, crude fiber, ether extract, and gross energy were measured (Nisa et al., 2006). The method used to measure growth, weight changes, FCR, and FER, according to Eqs. 1-3.

Feed Conversion Ratio (FCR):

$$\text{FCR (\%)} = \frac{\text{Feed Intake}}{\text{Body Weight Gain}} \times 100$$

Feed Efficiency Ratio (FCR):

$$\text{FER} = \frac{\text{Body Weight Gain}}{\text{Feed Intake}} \times 100$$

Digestibility:

$$\text{Digestibility (\%)} = \frac{\text{Nutrient Intake} - \text{Nutrient in Feces}}{\text{Nutient Intake}} \times 100$$

Blood collection:

At the end of the trial, rats were euthanised under anesthesia, blood was collected from the cervical region into EDTA tubes, centrifuged at 5,000 rpm, and serum was stored at -20 °C for lipid, insulin, and hormone analysis; ovaries were preserved in 10% formalin and 90 % distilled water solution for histopathology.

Biochemical analysis:

Serum Lipid Profile

Serum samples were assessed for cholesterol and triglycerides through spectrometric analysis, specifically the CHOD-PAP method provided by Human Diagnostic Worldwide, Netherlands. DiaSys Diagnostic System GmbH, Germany, enzymatically determined HDL cholesterol levels in serum. The LDL cholesterol was calculated using the Friedewald Equation as follows:

LDL-c (mg/dL) = Total cholesterol (mg/dL) - (Triglycerides (mg/dL)/5 - HDL (mg/dL)

(Manzoor et al., 2020).

Serum Hormonal Profile

Serum follicular stimulating hormone (FSH; mlμ/ml) (Cat: ELK4808), serum estrogen (pg/ml) (Cat: ELK8407), progesterone (ng/dl), Cat: ELK8854), and testosterone (ng/dl) (Cat: ELK8854) were determined by Radioimmunoassay (RIA; Gamma Counter) using the ELK Biotechnology USA, kits. The Enzyme-Linked Immunosorbent Assay (ELISA) Method was used to determine serum luteinizing hormone (LH) levels (μg/ml) using the ELK Biotechnology USA kit (Cat: ELK2368). Serum insulin (μlU/mL), Calbiotech Inc. USA (CAT: ISI30D), and HbA1c (Glycated Hemoglobin A1c) ELISA Kit(μg/mL) (CAT: ELK5108) were determined by using the kit of Calbiotech Inc. USA,as instructed.

Serum Antioxidant and Oxidative Stress Biomarker Analysis

For MDA (Malondialdehyde, U/ml, Cat. No. BC0020), GSH (Reduced Glutathione, μg/ml, Cat. No. BC1175), SOD (Superoxide Dismutase, U/ml, Cat. No. BC0170), CAT (Catalase, U/ml, Cat. No. BC0205), kits were used for serum analysis by Solarbio Beijing, China kits) as instructed. The methodology adhered to the principles outlined by(Chance & Maehly, 1955). CRP (ng/mL) levels in serum were measured using the ELK Biotechnology CRP (C-Reactive Protein) ELISA Kit (Cat.ELK1056), USA.The methodology adhered to the principles outlined by Chance and Maehly (1955). For the detection of Total antioxidant capacity (TAC; mmol of Trolox $_{\text{equiv/L,}}$ Total oxidative status (TOS; μmol of $H_2O_{2\text{equiv.}}$·L-1) was measured in serum samples by the Erel Method (Erel, 2004) as explained by (Manzoor et al., 2020). ALT (U/L) and AST (U/L) were measured using the Cobas E311 method, which employs a spectrophotometer and commercially available kits: Alanine Transaminase (WHO) Ray Biotech Activity Assay Kit (Code: MA-ALT-1) and Aspartate Aminotransferase (AST) Ray Biotech Activity Assay Kit (Code: MA-AST-1). Serum uric acid (mg/dL) was measured using a colorimetric assay, utilising the Ray Biotech uric acid kit, Code: MA-UA-1, method adopted as instructed.

Histological Examination of Ovarian and Intestinal Tissues and Ovarian Weight

The histological examination of the ovarian tissues in female rats was performed using the method. After careful dissection, the ovary weight was recorded using an electronic scale. The ovaries were fixed in 10% neutral buffered formalin, and 7 μm-thick serial sections were cut and stained with hematoxylin and eosin.

Statistical analysis

Statistical software (Statistics 8.1) was used to analyse the data using a completely randomised design. A two-way ANOVA was used to analyse the weekly feed, water, and body intake.

Weight was analysed using one-way ANOVA, and a post hoc test was used (Tukey, followed by LSD). The level of significance was $p \leq 0.05$.

Experimental Protocol

From day one, female rats were randomly allocated (CRD) into four groups (n = 9) with three subgroups, which were given a basal diet and assigned specific nutraceutical supplements. The body weights of the participants were noted at the start of supplementation.

Experimental supplement

Commercially obtained macro- and micronutrient salts were supplied via gavage with the conventional AIN-93G diet to evaluate their impact on female reproductive health. Merck KGaA (Germany) provided vitamin E, D3, Zn-methionine, Copper, selenium selenite, folic acid, and manganese, while JIAHERB Phytochem (China) provided chromium picolinate.

Dosage Calculation Method for Micronutrient Supplement

For different levels of selected micronutrients, dose preparation and calculation methods were used for each rat based on body weight, following the formula adopted by (Manzoor et al., 2020). Calculate the Dosage in mg for a 137g Rat: The formula used was: Dosage in mg:

- (Body weight of the rat in g / 1000 g) × dose in mg
- **Volume Selection as per OECD Guidelines for a 137g Rat:**
- Rat weight/1000 × 10mL

Nutraceutical Supplement Dose Levels for Healthy Female Albino Rats.

Micro Nutrients	NTS-1	NTS-2	NTS-3	References
Vitamin E(mg)	0.46	0.506	0.75	(Evans & Emerson, 1943)
Vitamin D3(IU)	19.2	21.1	600	(Refaat & El-Boshy, 2021)
Chromium Picolinate(µg)	3.9	4.29	200	(Amiri Siavashani et al., 2018)
Zinc Methionine(mg)	0.16	0.176	75	(Fazel Torshizi et al., 2020)
Copper Sulphate(mg)	0.066	0.0726	2	(Galhardi et al., 2005)
Selenium Selenite(µg)	1.3	1.43	2.8	(Elmalkey, 2018)
Folic Acid (mg)	3	3.3	5	(H.Al-Mosawi & Hamood, 2020)
Manganese Chloride(mg)	0.66	0.726	1.37	(Chen et al., 2000)

NST-1(Nutraceutical Supplement Treatment -1 (RDA)) NST-2(Nutraceutical Supplement Treatment -2(10% extended from RDA) and NST-3(Nutraceutical Supplement Treatment -3(Research tested)

Basal Diet and Nutrient Composition for Healthy Female Albino Rats

INGREDIENTS	BD g/1000g	Kcal/kg	Nutritive Value of Experimental Diet	
Corn starch	200	762	**Nutrient /day**	**BS(P0)**
Dextrose	150	880	**Dry matter**	89.93
Sucrose	100	400	**Crude protein**	18.00
Soya Bean Meal	250	848	**Ether extract**	7.00
Canola Meal	183	750	**Crude fiber**	7.83
Crude Oil	70	630	**Crude ash**	8.01
AIN-93-VX – Vitamin mix	10	-	**Moisture**	10.07
AIN-93G-MX- Mineral mix	35	-	**NFE**	49.09
Nutraceutical Supplement	-	-	On Dry Matter Basis (g/1000g) (BD: basal diet (*isocaloric and isonitrogenous*); for all groups.)*Nutrient composition and nutritive value of the basal diet for all groups.	
Choline Bitrate	2	-		
Methionine	3	-		
Total	1000g	4270kcl		

Experimental Protocol of Groups

GROUPS	Experimental Protocol	LABELLED
Group 1	Negative control healthy female rats were fed a basal diet and distilled water.	**H0**
Group 2	**Healthy Female Albino Wistar Rats** were fed a basal diet and distilled water +NTS-1 (Zn, Se, Vitamin E and D, Cr, Cu, Mn, Folate according to rat RDA)	**HS1**
Group 3	**Healthy Female Albino Wistar Rats were** fed a basal diet and distilled water + NTS-2 (Zn, Se, Vitamin E and D, Cr, Cu, Mn, Folate 10% increase from rat's RDA)	**HS2**

Group 4	**Healthy Female Albino Wistar Rats** were fed a basal diet and distilled water + NTS-3(Zn, Se, Vitamin E and D, Cr, Cu, Mn, Folate, research-tested dose)	**HS3**

RESULTS INTERPRETATIONS

Nutrient intake: Figure 1(Graph(a); Nutrient intake was significantly affected by supplementation levels ($p \leq 0.05$). HS3 showed increased intake of dry matter (18.76 g), protein (3.78 g), fat (1.41 g, +8.46% vs. H0), fiber (1.51 g), and ash (1.71 g), while HS1 recorded the highest protein intake (3.80 g, +5.56% vs. H0). In contrast, nitrogen-free extract intake was greatest in H0 (9.82 g) and lowest in HS3 (7.77 g), indicating a shift toward higher protein and fat intake with increasing supplementation.

Nutrient in Feces: Figure 1(Graph(b); Nutrient excretion in feces differed significantly among groups ($p \leq 0.05$). Dry matter excretion was highest in HS1 (4.08 g, +7.94% vs. H0), while crude protein (0.81 g, +12.5% vs. H0) and ether extract (0.49 g, +48.48% vs. H0) were significantly increased in HS3. Ash excretion was lowest in HS3 (0.26 g), reflecting improved mineral absorption, whereas nitrogen-free extract excretion was highest in HS1 (2.45 g, +20% vs. H0).

Nutrient Digestibility: (Figure 1(Graph(c); **displays the digestibility data, which shows**Significant ($p \leq 0.05$) differences were observed in nutrient digestibility among groups. Dry matter digestibility was similar between H0 (78.61%) and HS3 (78.57%, $p > 0.05$). Crude protein digestibility significantly increased in HS1 (84.47%), while ether extract (78.99%), crude ash (84.62%), and nitrogen-free extract digestibility (73.33%) were significantly higher in HS2 compared to H0. In contrast, HS3 showed a declining trend with the lowest values for crude protein, ether extract, and nitrogen-free extract.

FCR and FER Ratios to Body Weight Changes. (Figure 1(Graph(d);**shows that** different levels of nutraceutical supplements had a significant ($p \leq 0.05$) impact on feed conversion ratio (FCR) and feed efficiency ratio (Pham et al.) relative to weight changes in healthy female albino rats over six weeks ($p \leq 0.05$). HS3 had the highest feed intake (23.01g), 11.72% higher than HS2 (20.12g) and 6.77% higher than H0 (21.55g). HS3 also had the highest weight gain after six weeks (22.12g), 48.46% higher than H0 (14.90g). FCR was lowest in HS3 (1.04), 28.28% lower than H0 (1.45), indicating a decrease in feed conversion efficiency. FER was highest in HS3 (0.961), 39.03% higher than H0 (0.691), reflecting improved feed efficiency with increased supplementation.

Total Water Intake: (Figure 2 (Graph e), **shows that** Water intake was significantly influenced by nutraceutical supplementation over six weeks ($p \leq 0.05$). HS3 showed the highest daily (23.43 mL), weekly (164.03 mL), and total intake (1312.31 mL), averaging 4–10% greater than H0 and HS1. H0 maintained baseline values, HS1 showed the lowest intake, and HS3 consistently exhibited the most significant consumption across the study period.

Serum Glucose Level: (Figure 2 (Graph(f); Serum glucose levels differed significantly among groups over six weeks ($p \leq 0.05$). By Week 6, HS3 showed the highest glucose (134.5 mg/dL, +44.62% vs. H0), followed by HS2 (+22.58% vs. H0), while HS1 remained close to control values. Overall, H0 maintained normal baseline levels, HS1 showed minimal change, HS2 demonstrated a moderate rise, and HS3 exhibited the most pronounced and adverse increase, particularly between Weeks 3–6.

Weekly Weight Changes:(Figure 2(Graph(g); **indicates that** Body weight increased significantly across groups over six weeks ($p \leq 0.05$). By Week 6, HS3 reached the highest weight (158.91 g), 4.34% above H0 and 3.29% above HS1, with the most significant mean gain (22.12 g, +48.46% vs. H0; +21.22% vs. HS2). Significant differences emerged from Week 2 onward, with HS3 consistently showing the highest weight gain compared to all groups.

Serum Lipid Profile: (Figure 2(Graph(h); **shows** that HS3 exhibited significant $p \leq 0.05$, inclined trend in total cholesterol, triglycerides, and LDL, with a significant reduction in HDL compared to other groups. HS2 showed improved lipid regulation, with higher HDL (+4.68% vs. H0) and lower LDL relative to controls. HS1 remained statistically similar ($p > 0.05$) to H0. Overall, HS3 reflected the most adverse lipid profile, while HS2 demonstrated a protective effect.

Reproductive Hormones: Figure 3 (Graph(i);showed thatReproductive hormones varied significantly across groups ($p \leq 0.05$). HS3 showed the highest testosterone (+31.24% vs. H0/HS2), FSH (+16–18% vs. H0/HS2), LH (+40–45% vs. H0/HS2), estrogen (+18–20% vs. H0/HS2), and progesterone (+75–80% vs. H0/HS2). H0, HS1, and HS2 maintained balanced LH: FSH ratios displaying healthier gonadotropin profiles, whereas HS3 reflected consistently elevated but suboptimal values.

Abdominal Circumference: Figure 3 (Graph(j); **shows that** Abdominal circumference increased significantly across all groups ($p \leq 0.05$) over six weeks. By Week 6, HS3 had significantly increased value (15.22 cm), 12.32% higher than H0 and 2.08% above HS2, while HS1 was 9.75% greater than H0. Mean values confirmed that HS3 consistently increased significantly (+11.7% vs. H0), with significant differences across groups at all time points.

Antioxidant and Oxidative Stress Biomarkers, liver enzymes: Figure 3 (Graph(k);demonstrates that at $p \leq 0.05$, nutraceutical supplementation significantly influenced antioxidant and oxidative stress biomarkers. HS2 showed the most favourable profile, with higher GSH (+2.15% vs. HS1; +4.30% vs. H0), SOD (+6.81% vs. HS1; +8.42% vs. H0), CAT (+10.33% vs. H0), and TAC (+20.57% vs. H0). Concurrently, HS2 significantly reduced MDA (–9.50%), TOS (–8.53%), UA (–6.56%), CRP (–3.75%), ALT (–9.08%), and AST (–2.19%) compared with H0. In contrast, HS3 demonstrated impaired antioxidant status, with significant reductions in CAT (–10.33%) and TAC (–6.29%), alongside increases in MDA (+12.00%), TOS (+2.81%), UA (+16.39%), CRP (+2.50%), ALT

(+4.58%), and AST (+6.96%) versus H0 ($p \leq 0.05$). No significant differences ($p > 0.05$) were observed between HS1 and H0 across measured biomarkers.

Serum Insulin and HbA1c: Figure 3 (Graph(l);demonstrates that Significant changes were noted for HbA1c, where HS2and H0 were nonsignificant to H0 (5.05), while HS3 showed a 17.02% increase within the optimal range. For insulin, HS3 exhibited the highest level (16.61 uIU/mL), which was 5.39% higher than HS1 (15.76 uIU/mL), 9.85% higher than HS2 (15.12 uIU/mL), and 15.50% higher than H0 (14.38 uIU/mL). HS1 and HS2 showed non-significant results compared to HS3.

Estrous Cycle Length and Ovarian Weight: Figure 3 (Graph(m) shows that oestrous cycle length and ovarian weight were significantly influenced by supplementation ($p \leq 0.05$). HS3 showed ($p \leq 0.05$) extended cycle significantly (5.1 days, +27.18% vs. H0), indicating a negative outcome, while HS1 and HS2 maintained shorter, more stable cycles closer to control values. Ovarian weight was highest in HS3 (+7–9% vs. others), whereas HS1 and HS2 reflected comparatively moderate, healthier values.

DISCUSSION

In terms of nutrient intake and digestibility of dry matter (DM), ether extract EE), and crude ash, the HS1 and HS2 groups showed improved nutrient intake and digestibility of dry matter (DM), ether extract (EE), and crude ash, with the HS1 group achieving a 5.56% increase in crude protein (CP) intake compared to the control. HS3, though higher in total intake, exhibited increased fecal protein loss (12.5%), suggesting less efficient nutrient utilisation. Notably, HS2 showed increased digestibility for DM, EE, and crude ash, suggesting improved gastrointestinal assimilation. Such improvement may stem from the antioxidant modulation of intestinal epithelial integrity and microbiota, leading to enhanced absorption of micronutrients essential for hormone biosynthesis (Pham et al., 2021). Research supports this, with antioxidants such as selenium and zinc shown to upregulate glutathione peroxidase and other gut-associated enzymes that improve mucosal function (Alsulami & El-Saadony, 2023). This mechanistic link highlights how improved nutrient bioavailability can promote hormonal balance by facilitating the biosynthesis of sex steroids and metabolic regulators(Basnet et al., 2024). **Regarding feed intake and body weight**, HS3 recorded the most substantial increases ($p < 0.05$). Although it can be attributed to enhanced feed intake and estrogen-mediated adipogenesis, the accompanying reduction in feed efficiency ratioimplies metabolic inefficiency. This finding aligns with observations by (Mohamed et al., 2023), who reported that zinc and chromium enhanced muscle accretion. However, over-supplementation may dysregulate AMPK (**AMP-activated protein kinase**) and insulin signalling pathways, leading to inefficient nutrient utilisation and a shift in cellular metabolism toward lipogenesis over β-oxidation (Coughlan et al., 2013). Conversely, HS2 exhibited reduced FCR with improved FER and modest weight gain, indicating balanced metabolic activity. This response may be related to the antioxidant-induced modulation of insulin receptor substrate-1 (IRS-1) and phosphoinositide 3-kinase (PI3K) pathways, which regulate energy homeostasis(Yao et al., 2021). Thus, optimising nutrient intake may enhance ovulatory function by maintaining balanced adiposity and energy regulation, both of which are essential for the proper functioning of the hypothalamic-pituitary-ovarian axis(Amiri Siavashani et al.). **Water intake results** over six weeks were significantly higher in HS3 (1312.31 mL), 8.53% higher than H0 (1256.51 mL), and 8.89% higher than HS1 (1205.13 mL). This might be due to high doses of vitamins and minerals; the combination may disturb the redox balance and impose metabolic stress, increasing osmotic load and urine output, which drives compensatory thirst in female rats. Estrogen further modulates this response by influencing fluid regulation, while renal adjustments sustain elevated intake(Kisley et al., 1999)h. Dehydration impacts fertility and menstruation. Low water intake raises cortisol, which lowers estrogen and progesterone(Silvestris et al., 2019). Antioxidants regulate oxidative stress response by modulating signalling via Nrf2 and NF-κB transcription factors. Balanced redox signalling improves vascular health, capillary permeability, and tissue water distribution(Hrelia & Angeloni, 2021). Enzymatic antioxidants like superoxide dismutase, catalase, and glutathione peroxidase prevent lipid peroxidation and stabilise cell membranes, controlling water and electrolyte flow. This enhances cell water retention and tissue hydration (Vašková et al., 2023). (Umer et al., 2023)Umer et al. observed that administering quercetin (an antioxidant supplement) to hyperuricemic rats led to enhanced water intake due to the antioxidant effect, increased nutrient availability, improved regulation of ALT and AST enzymes, and reduced oxidative stress by lowering UA in healthy rats.**Weekly fasting blood glucose** showed a rising trend, especially in HS3, potentially due to hepatic gluconeogenesis stimulated by estrogen and high-dose antioxidant supplementation; meanwhile, HS1 and HS2 maintained stable glucose levels. Several studies have confirmed the role of selenium, zinc, chromium, and vitamin D in enhancing insulin sensitivity by activating the AKT (also known as Protein Kinase B) signalling pathway and suppressing endoplasmic reticulum (ER) stress (Han et al., 2024). Improved glycemic control facilitates ovulation by reducing oxidative stress in pancreatic β-cells and ovarian granulosa cells, thereby enhancing hormonal feedback and menstrual regulation. Folic acid, another component of the nutraceutical regimen, has demonstrated efficacy in reducing the risk of gestational diabetes through methylation pathways that influence insulin sensitivity (Baykara, 2023). **Body weight changes** mirrored nutrient intake trends, with HS3 showing the highest gain, and HS1 3.29% lower than HS3. Estrogen-induced adipogenesis, mediated by the upregulation of PPARγ (Peroxisome Proliferator-Activated Receptor Gamma) and SREBP-1c (Sterol Regulatory Element-Binding Protein 1c), may explain this trend (Kuryłowicz, 2023). Zinc and vitamin E have been shown to influence leptin regulation and energy metabolism, reducing obesity-induced inflammation and improving menstrual regularity (Villa et al., 2017). These findings suggest that nutraceutical-induced modulation of adipose-derived cytokines may restore insulin and androgen sensitivity, crucial for resuming ovulatory cycles in PMOS.

Hormonal profiling revealed an increase in all reproductive hormones in HS3, though within physiological ranges. HS1 and HS2 demonstrated optimal LH, FSH, and estrogen profiles, supporting improved ovarian activity. This supports prior work by(Li et al., 2024) , who reported that vitamin D plays a role in reducing testosterone and promoting follicular growth. Vitamin E, acting via the protein kinase C and melatonin pathways, enhances INHBA expression (**Inhibin Subunit Beta A),** a gene encoding a subunit of activin A in the TGF-β superfamily (growth factor-beta), which regulates reproductive hormone synthesis, granulosa cell proliferation, and ovarian follicle development. Moreover, reduces oxidative stress in ovarian tissues(Begum & Mohan, 2023). These mechanisms facilitate the restoration of normal folliculogenesis, ovulation, and luteal function. **Lipid profile** modulation was evident, with HS1 and HS2 significantly improving HDL levels while reducing TC, LDL, and VLDL. Conversely, HS3 exhibited an increased trend in lipid metrics, indicating potential overactivation of lipid biosynthetic pathways. Cr Pic and Se modulate lipid metabolism by inhibiting SREBP-1c (Sterol Regulatory Element-Binding Protein-1c) and enhancing the activity of peroxisome proliferator-activated receptor alpha (PPARα), thereby reducing dyslipidemia(Han et al., 2024; Kazempour et al., 2023). This lipid correction attenuates ovarian inflammation and hyperandrogenism, central features of female reproductive health disorders. (Maktabi et al., 2017)observed reduced serum lipids in PMOS patients after six weeks of Zn supplementation. Improved lipid metabolism alleviates intra-ovarian inflammation and hyperandrogenism, thereby supporting ovulation and endometrial receptivity. The **abdominal circumference** showed a 6.36% and 2.65% reduction in HS2 and HS1, respectively, compared to H0, suggesting a decrease in visceral fat. Central adiposity is a recognised contributor to insulin resistance and elevated aromatase activity, resulting in altered estrogen-to-testosterone ratios (Maitra et al., 2024). Antioxidant-induced weight loss may, therefore, directly impact reproductive hormonal balance. (de Oliveira et al., 2024) confirmed that zinc and selenium supplementation reduced abdominal adiposity and oxidative stress in obese women. Nutrients such as chromium, manganese, and vitamin D reduce visceral fat mass by activating AMPK and suppressing proinflammatory cytokines in adipose tissue (Pal et al., 2025). These findings reinforce that managing visceral adiposity through targeted nutrition supports the normalisation of the ovarian axis. **Biomarkers of oxidative stress** confirmed the dose-dependent impact of supplementation. HS1 and HS2 showed significant increases in antioxidant enzymes—TAC, GSH, SOD, and CAT—while HS3 exhibited elevated malondialdehyde (MDA) and total oxidant status (TOS), markers of lipid peroxidation and redox imbalance. This U-shaped response suggests that moderate antioxidant intake enhances endogenous defence via NRF2 transcriptional activation, while excessive intake surpasses cellular buffering capacity, promoting paradoxical pro-oxidative states (Eddie-Amadi et al., 2023). Preserved redox balance protects mitochondrial function in granulosa cells, enhances oocyte competence, and improves embryo viability(Kobayashi et al., 2024).**Inflammation,** measured via C-reactive protein (CRP), was lowest in HS1 and HS2, affirming systemic anti-inflammatory effects. Chromium, via PPAR-γ (Peroxisome Proliferator-Activated Receptor Gamma) and GLUT-1(**Glucose Transporter Type 1)** activation, reduces IL-1β (Interleukin-1 Beta) expression, thereby mitigating inflammation-linked insulin resistance and hyperandrogenism (Amiri Siavashani et al., 2018). Vit D, with its immunomodulatory effects, further enhances these responses, supporting ovarian health (Ye et al., 2024). **HbA1c and insulin** results demonstrated an increase of 17.02% in HbA1c and 15.50% in insulin in HS3 from control (H0), whereas other groups showed a comparable optimal trend in HbA1c and insulin levels. Similar results were obtained by, (Farrokhian et al., 2020), who assessed 200 µg/day chromium for 12 weeks in 64 diabetic CHD patients, noting reductions in body weight, BMI, fasting glucose, insulin, HOMA-IR, hs-CRP, MDA, and diastolic blood pressure, with increased insulin sensitivity and total antioxidant capacity (Tatone et al.) ($p=0.001$ to $p=0.02$). Chromium's antioxidant properties decrease MDA, a lipid peroxidation marker, and increase TAC, neutralising reactive oxygen species that exacerbate reproductive imbalances. It also enhances insulin sensitivity by facilitating insulin receptor activation, improving glycemic control. (Portillo et al., 2020) highlighted the indirect role of folic acid in methionine and homocysteine metabolism through enzymes like MTHFR (5,10-Methylenetetrahydrofolate reductase), suggesting its potential to regulate epigenetics and redox balance relevant to female reproductive health issues. By supporting the folate cycle and SAM (S-adenosylmethionine) synthesis, folic acid may help modulate insulin signalling and ovarian steroidogenesis in females with reproductive health issues.**Liver enzymes ALT and AST** declined in HS1 and HS2, indicating an improvement in hepatic health. Antioxidant vitamins, particularly Se, Zn, Vit E, and D, protect hepatic tissue from lipid peroxidation and modulate hepatic estrogen metabolism, thereby influencing sex hormone-binding globulin (SHBG) levels and the free androgen index (Fathi et al., 2020; Yousefi-Nodeh et al., 2024). The regulation of hepatic pathways impacts the systemic hormonal milieu and supports overall reproductive axis stability. **Menstrual cyclicity** was preserved in HS1 and HS2 but prolonged in HS3, paralleling ovarian weight data. This suggests that moderate supplementation enhances folliculogenesis and maintains ovarian weight. While excess dose may disrupt endocrine rhythms and result in the accumulation of atretic follicles and cysts that can enlarge the ovary, thereby increasing its weight (Günalan et al., 2018). Vitamin E reduces granulosa cell apoptosis and mimics FSH signalling, thereby enhancing ovarian reserve and ovulatory function (Olaniyan et al., 2023). Vitamin D's influence on VDR (Vitamin D Receptor) expression in granulosa cells has also been shown to correlate with follicle maturation(Xu et al., 2021). Se nanoparticles were found to lower LH and testosterone levels while promoting corpus luteum formation in animal models, thereby validating their role in supporting ovulation(Abdallah et al., 2023). These findings validate the mechanistic underpinning of hormonal improvement observed in this study. **Uric acid** levels declined in HS1 and HS2, while HS3 showed a slight rise. **UA** serves as a biomarker of systemic inflammation and metabolic stress, both of which are implicated in female reproductive dysfunction. Elevated serum uric acid (UA) levels observed in female reproductive disorders like PCOS are

indicative of heightened oxidative stress, adiposity, and metabolic dysfunction. In the current study, supplementation with HS1 and HS2 was associated with a marked reduction in serum UA compared to the control, while HS3 showed only a marginal increase. Elevated UA has also been linked to insulin resistance and inflammation, both of which impair ovarian steroidogenesis and disrupt follicular development as observed by (Szczuko et al., 2019). This inflammatory milieu contributes to anovulation, irregular menstruation, and subfertility in females. Nutrients such as zinc and manganese play a crucial role in modulating UA metabolism and mitigating oxidative renal injury, thus contributing indirectly to improved reproductive function (Chen et al., 2024)(Nwadike et al., 2019). Furthermore, chromium picolinate has been shown to enhance renal urate clearance and lower serum UA levels, reflecting improved metabolic efficiency and potential endocrine restoration (Abdelfattah et al., 2024).Ovarian histological analysis revealed a significant improvement in follicle count, a reduction in atretic follicles, and a more regulated estrous cycle in the HS1 and HS2 groups. These structural improvements were paralleled by a decrease in ovarian weight, indicating better follicular recruitment and reduced cystic morphology. Mechanistically, Se nanoparticles and folic acid supplementation have been shown to suppress granulosa cell apoptosis, modulate inflammatory cytokines, and enhance oocyte quality and follicular maturation (El-Naby et al., 2020; Saberi et al., 2017).(Shaukat et al., 2025)reported that morin alleviated uterine oxidative injury via Nrf2 activation and NF-κB suppression, which aligns with our findings, where nutraceutical supplementation reduced oxidative stress and CRP levels, thereby improving ovarian function in healthy female rats. This highlights that regulating redox and inflammatory pathways is crucial for restoring reproductive health. This histological normalisation is a strong indicator of restored ovarian architecture and function, reinforcing the reproductive benefits of moderate-dose antioxidant nutraceuticals.

CONCLUSION

This study demonstrates that carefully dosed antioxidant-based nutraceutical supplementation, particularly as seen in HS1 and HS2, can significantly improve the metabolic, hormonal, and reproductive landscapes in a reproductive and endocrinal imbalancedfemale model. The findings highlight that nutraceuticals improve insulin sensitivity, modulate lipid and glucose metabolism, reduce oxidative stress, and enhance reproductive hormone profiles. These effects appear to be mediated through critical molecular pathways, including the activation of AKT, NRF2, and PPARγ, which collectively improve folliculogenesis, suppress inflammation, and support hormonal equilibrium. Conversely, excessive dosing, as seen in HS3, may lead to metabolic inefficiencies and counteract potential benefits.

Clinically, these findings underscore the importance of integrating nutraceuticals as a complementary therapeutic strategy in the management of female reproductive health issues. When used in conjunction with pharmacological agents and lifestyle interventions, optimised supplementation can play a crucial role in restoring reproductive health. However, limitations such as study duration, sample size, and species-specific physiology must be acknowledged. Future research should focus on long-term human and animal trials, personalised dose-response evaluations, and synergistic nutraceutical combinations to maximise therapeutic efficacy and ensure safety. A mechanistically informed, individualised supplementation strategy could mark a transformative step in reproductive health care for women affected by metabolic issues and PCOS.

Ethics statement

The Ethical Committee of GCUF reviewed this study, which did not raise anyethical issues.

Competing interests: The authors declare that they have no competinginterests.

Author's contribution: Kanwal Hafeez Khan conducted the experimental research, collected andprocessed the data, and drafted the manuscript. Mehr Un Nisa conceptualised and designed the study, supervised the research process, lab analysis, and guaranteed adherence to ethical standards. Nazir Ahmad analysed the data and corrected the text for intellectual content. Mehr Un Nisa and Huma Umbreen contributed to the study's methodology, data interpretation, and revision of the paper.

Acknowledgement

We are thankful to GCUF for providing access to the digital library and various departmental labs, especially the Nutritional Sciences department, for supplying us with Antioxidant enzymes kits. Additionally, the departments of Biochemistry, Botany, Pharmacology, and Zoology, along with APEX Feed Pvt Ltd, supplied basal feed for animals.

Funding: No Funding.

Results (graphs) and images:

Figure: 1 ; Nutrient Intake , Nutrient In Feces, Nutrient Digestibility,Feed Intake ,FCR, FER, And Body Weight Changes

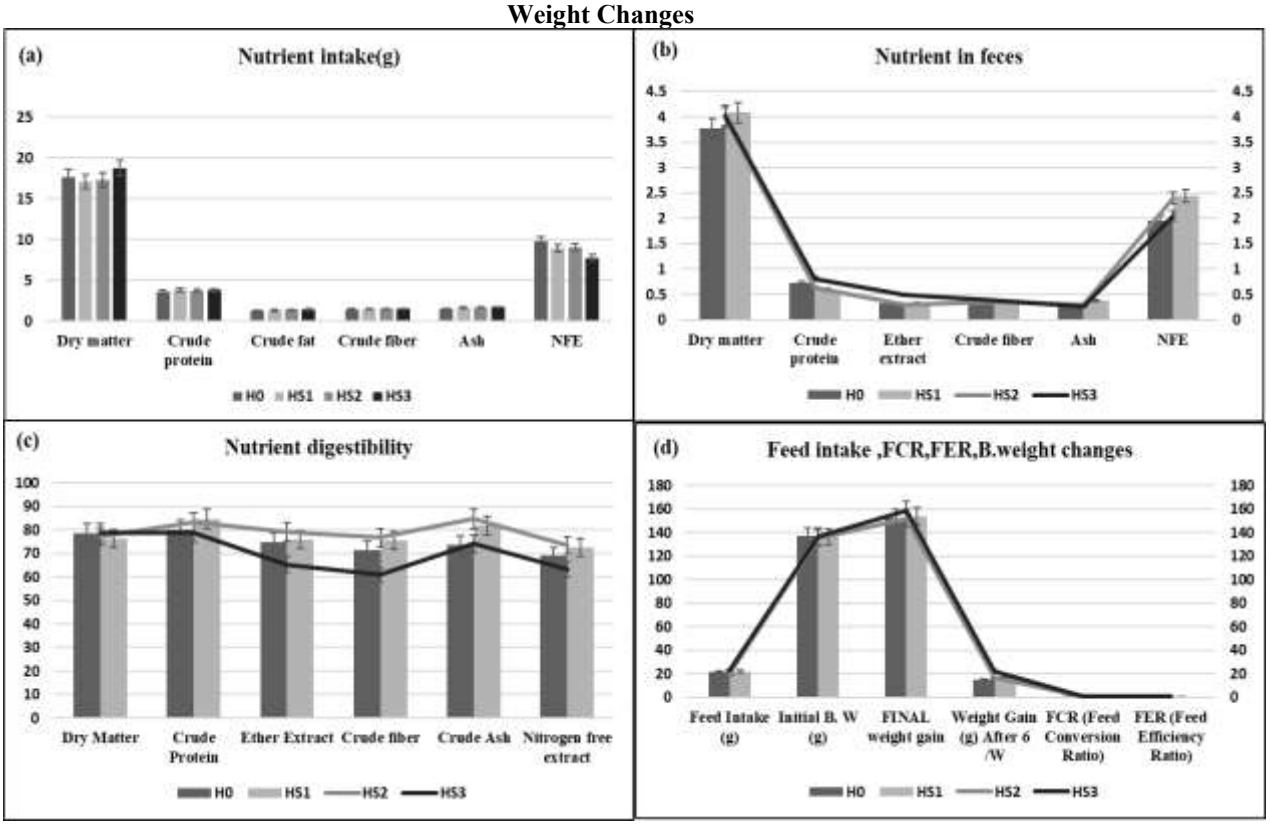


Figure:1; H0: (negative control; healthy), HS1(supplement level: 1 RDA based; rat), HS2(Supplement level: 2 (10 % extended from rat's RDA), HS3 (supplement level: 3 research tested doses). FCR: feed conversion ratio, FER: feed efficiency ratio.

Figure: 2;Metabolic Parameters

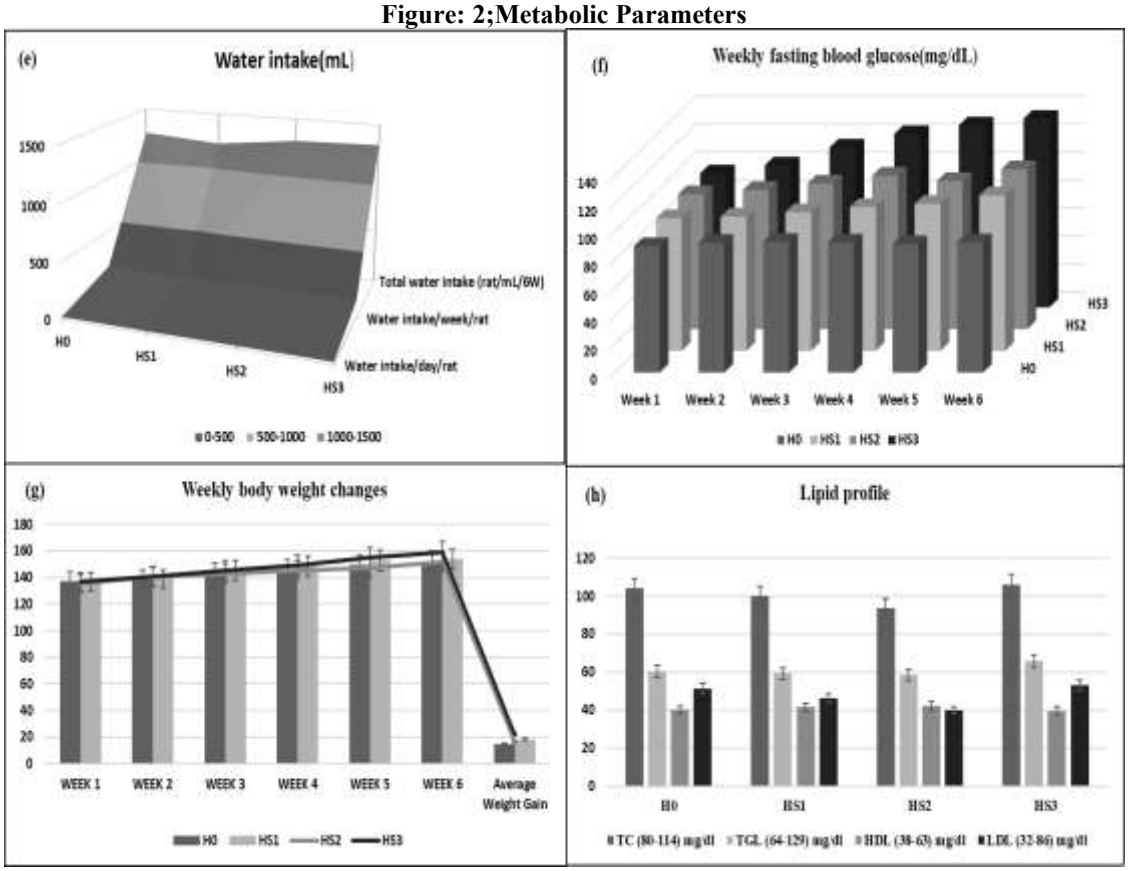


Figure: 2 (H0: (negative control; healthy), HS1(supplement level: 1 RDA based; rat), HS2(Supplement level: 2 (10 % extended from rat's RDA), HS3 (supplement level: 3 research tested doses) TC: Total Cholesterol, TGL: Triglycerides, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein.

Figure 3; Reproductive Hormones, Abd,Cir/W, Oxidative , Inflammatory Biomarkers, Liver Enzymes

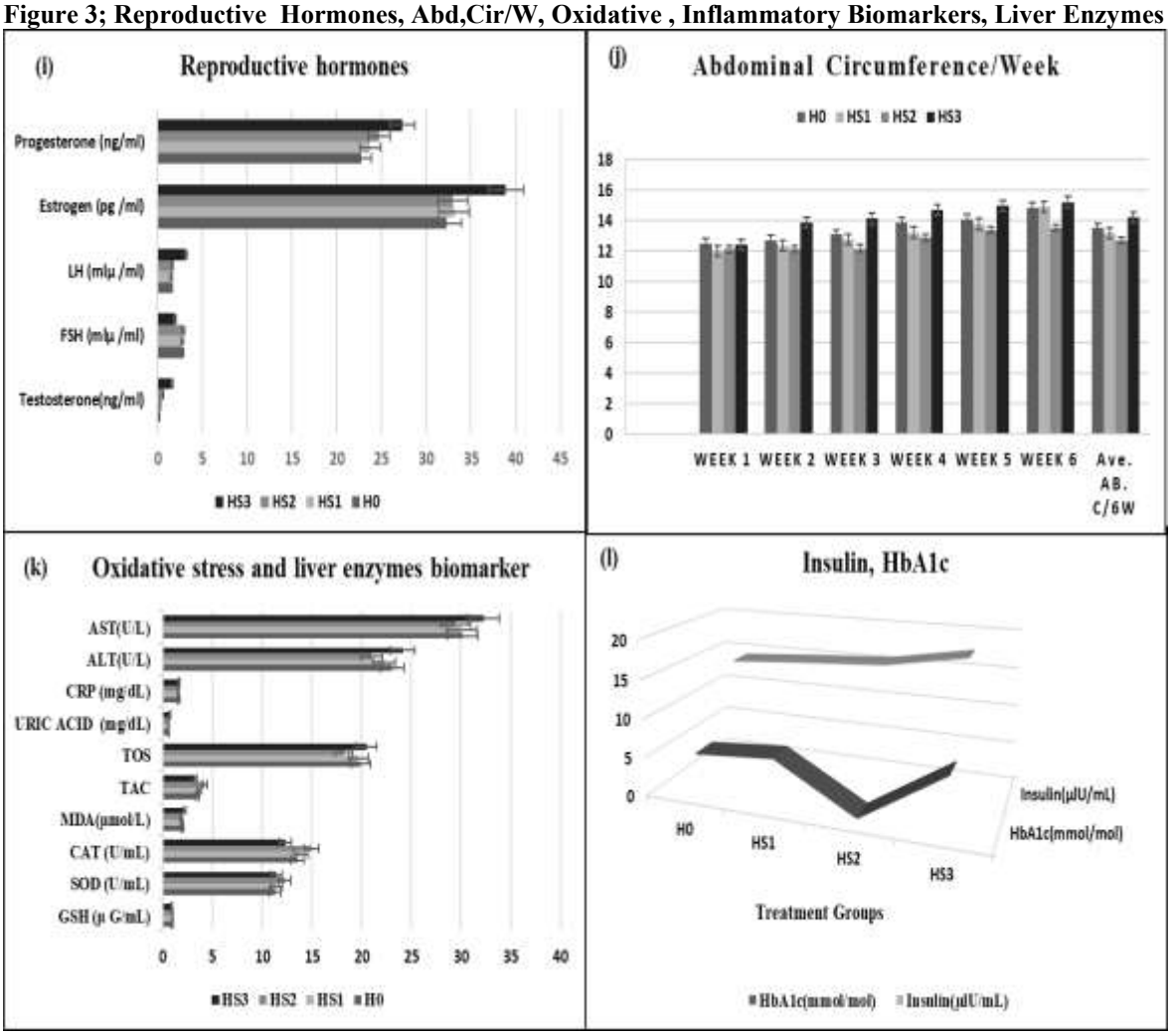


Fig:3 (H0: (negative control; healthy), HS1(supplement level: 1 RDA based; rat), HS2(Supplement level: 2 (10 % extended from rat's RDA), HS3 (supplement level: 3 research tested doses).LH: Luteinizing Hormone, FSH: Follicle-Stimulating Hormone, TC: Total Cholesterol, TGL: Triglycerides, LDL: Low-Density Lipoprotein, HDL: High-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, CRP: C-Reactive Protein, TAC: Total Antioxidant Capacity, TOS: Total Oxidant Status, CAT: Catalase, MDA: Malondialdehyde, SOD: Superoxide Dismutase, GSH: Glutathione, HbA1c: Glycated Hemoglobin.

Figure :4; estrous cycle and ovarian weight

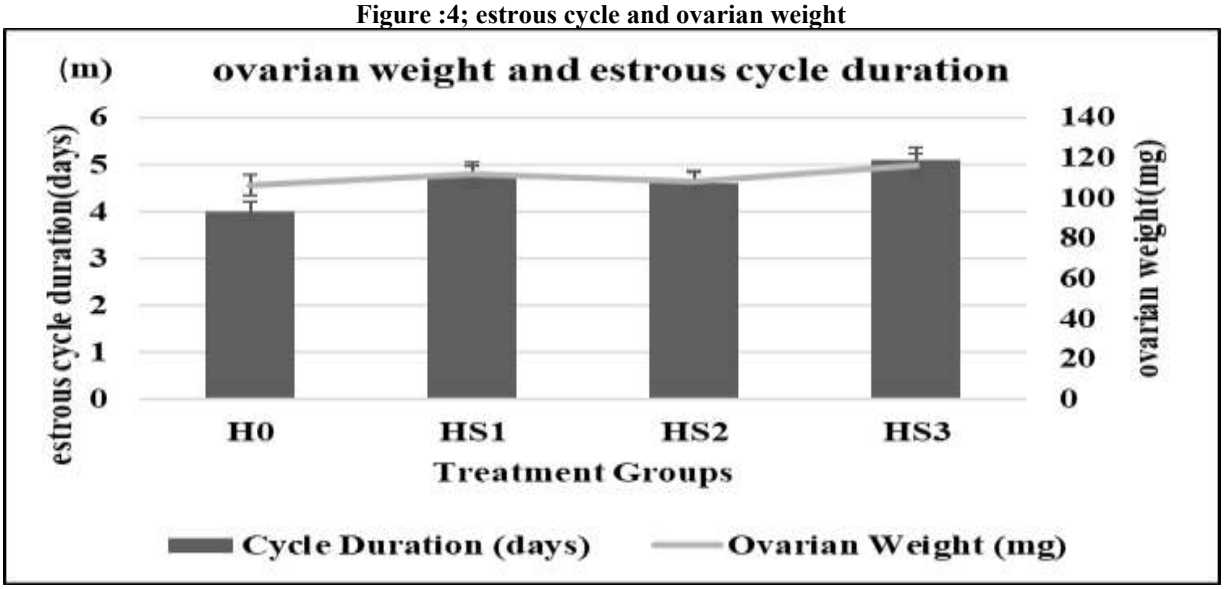
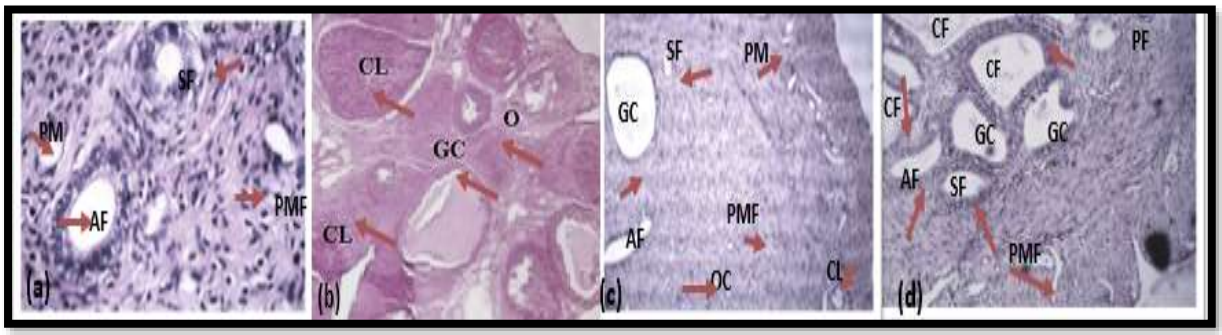


Fig:4 ((H0: (negative control; healthy), HS1(supplement level: 1 RDA based; rat), HS2(Supplement level: 2 (10 % extended from rat's RDA), HS3 (supplement level: 3 research tested doses).

Image 1, Ovarian Histopathology



Description: Ovarian histological examination of healthy rats through Nikon Eclipse Ci-L, 40x/100x, stained histological sections with hematoxylin and eosin, **(a)** H0 showed normal folliculogenesis process with the growth of primary, secondary follicles**(b)** HS1 had increased primordial follicles (+6.3%; $p<0.05$) and vascularity but reduced corpus luteum (-6.3%; $p<0.05$). **(c)** HS2 was optimal: elevated primordial/antral follicles (+12.5%/+6.3%; $p<0.01$), healthy stroma. **(d)** The HS3 group was characterised by a prominent significant decline in antral follicles (-18.8%; $p < 0.001$), thinning of granulosa cells (100x), stromal fibrosis ($p < 0.001$), and a 3.2-fold higher risk of cysts ($p < 0.001$).CF: (Cystic follicles), CL: (Corpus luteum), GC: (Granulosa cells), O: (oocytes), PM (Primary follicle), SF (secondary follicle), PM (primordial follicle).

REFERENCES

1. Abdallah, A. B. E., El-Ghannam, M. A., Hasan, A. A., Mohammad, L. G., Mesalam, N. M., & Alsayed, R. M. (2023). Selenium Nanoparticles Modulate Steroidogenesis-Related Genes and Improve Ovarian Functions via Regulating Androgen Receptor Expression in Polycystic Ovary Syndrome Rat Model. *Biol Trace Elem Res*, *201*(12), 5721-5733. https://doi.org/10.1007/s12011-023-03616-0
2. Abdelfattah, D. S. E., Fouad, M. A., Elmeshad, A. N., El-Nabarawi, M. A., & Elhabal, S. F. (2024). Anti-Obesity Effect of Combining White Kidney Bean Extract, Propolis Ethanolic Extract and CrPi(3) on Sprague-Dawley Rats Fed a High-Fat Diet. *Nutrients*, *16*(2). https://doi.org/10.3390/nu16020310
3. Alsulami, M. N., & El-Saadony, M. T. (2023). Supplementing broiler diets with bacterial selenium nanoparticles enhancing performance, carcass traits, blood indices, antioxidant status, and caecal microbiota of Eimeria tenella-infected broiler chickens. *Poult Sci*, *102*(12), 103111. https://doi.org/10.1016/j.psj.2023.103111
4. Amiri Siavashani, M., Zadeh Modarres, S., Mirhosseini, N., Aghadavod, E., Salehpour, S., & Asemi, Z. (2018). The Effects of Chromium Supplementation on Gene Expression of Insulin, Lipid, and Inflammatory Markers in Infertile Women With Polycystic Ovary Syndrome Candidate for in vitro Fertilization: A Randomized, Double-Blinded, Placebo-Controlled Trial. *Front Endocrinol (Lausanne)*, *9*, 726. https://doi.org/10.3389/fendo.2018.00726
5. Amirkhizi, F., Taghizadeh, M., Khalese-Ranjbar, B., Hamedi-Shahraki, S., & Asghari, S. (2024). Association of Serum Selenium and Selenoprotein P with Oxidative Stress Biomarkers in Patients with Polycystic Ovary Syndrome. *Biol Trace Elem Res*, *202*(3), 947-954. https://doi.org/10.1007/s12011-023-03747-4
6. Basnet, J., Eissa, M. A., Cardozo, L. L. Y., Romero, D. G., & Rezq, S. (2024). Impact of Probiotics and Prebiotics on Gut Microbiome and Hormonal Regulation. *Gastrointest Disord (Basel)*, *6*(4), 801-815. https://doi.org/10.3390/gidisord6040056
7. Baykara, O. (2023). Effects of Natural Products on the Genetics of Gestational Diabetes Mellitus. *Natural Products and their Bioactives in Antidiabetic Drug Discovery*.
8. Begum, R. F., & Mohan, S. (2023). Pharmacological investigation of vitamin E with combined oral contraceptives on INHBA gene against PCOS that intricate through melatonin PKC pathway. *Syst Biol Reprod Med*, *69*(6), 450-464. https://doi.org/10.1080/19396368.2023.2276082
9. Chance, B., & Maehly, A. (1955). [136] Assay of catalases and peroxidases.
10. Chen, L., Li, T., Xu, J., Liao, S., Xu, H., Li, K., Ivanova, D., Si, Y., Zhang, X., Zhang, J., Li, F., Chen, X., Wang, Y., Xie, B., Shi, D., & Wang, M. (2025). Screening of Oxidative Stress-related Biomarkers and Antioxidant Drugs for Polycystic Ovary Syndrome Based on Bioinformatics and Molecular Docking. *Reprod Sci*, *32*(5), 1644-1660. https://doi.org/10.1007/s43032-025-01790-1
11. Chen, M. T., Sheu, J. Y., & Lin, T. H. (2000). Protective effects of manganese against lipid peroxidation. *J Toxicol Environ Health A*, *61*(7), 569-577. https://doi.org/10.1080/00984100050194090
12. Chen, X., Ge, J., Ma, S., Du, L., Chen, Z., Jiang, L., Liu, L., & Jia, Y. (2024). Long-term relatively high dietary manganese intake could decrease the risk of hyperuricemia: Twelve-year distinct dietary manganese consumption trajectories and the China Health and Nutrition Survey. *Nutr Metab Cardiovasc Dis*, *34*(8), 1984-1993. https://doi.org/10.1016/j.numecd.2024.05.008
13. Coughlan, K. A., Valentine, R. J., Ruderman, N. B., & Saha, A. K. (2013). Nutrient Excess in AMPK Downregulation and Insulin Resistance. *J Endocrinol Diabetes Obes*, *1*(1), 1008.
14. Council, N. R., Nutrition, C. o. A., & Nutrition, S. o. L. A. (1995). Nutrient requirements of laboratory animals: 1995.
15. de Oliveira, A. R. S., Cruz, K. J. C., Morais, J. B. S., Dos Santos, L. R., de Sousa Melo, S. R., Fontenelle, L. C., Severo, J. S., Beserra, J. B., de Sousa, T. G. V., de Freitas, S. T., de Oliveira, E. H. S., Maia, C. S. C., de Matos Neto, E. M., de Oliveira, F. E., Henriques, G. S., & Marreiro, D. D. N. (2024). Magnesium, selenium and zinc

deficiency compromises antioxidant defense in women with obesity. *Biometals*, *37*(6), 1551-1563. https://doi.org/10.1007/s10534-024-00625-x
16. Eddie-Amadi, B. F., Ezejiofor, A. N., Orish, C. N., & Orisakwe, O. E. (2023). Zn and Se abrogate heavy metal mixture induced ovarian and thyroid oxido-inflammatory effects mediated by activation of NRF2-HMOX-1 in female albino rats. *Curr Res Toxicol*, *4*, 100098. https://doi.org/10.1016/j.crtox.2022.100098
17. El-Naby, A. A. H., Ibrahim, S., Hozyen, H. F., Sosa, A. S. A., Mahmoud, K. G. M., & Farghali, A. A. (2020). Impact of nano-selenium on nuclear maturation and genes expression profile of buffalo oocytes matured in vitro. *Mol Biol Rep*, *47*(11), 8593-8603. https://doi.org/10.1007/s11033-020-05902-9
18. Elmalkey, N. (2018). Effect of Selinum on Ovarian Structure, Function and Serum Osteopontin Level in Rat Model of Polycystic Ovary Syndrome. *The Medical journal of Cairo University*, *86*, 2435-2445.
19. Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin Biochem*, *37*(4), 277-285. https://doi.org/10.1016/j.clinbiochem.2003.11.015
20. Evans, H. M., & Emerson, G. A. (1943). The Prophylactic Requirement of the Rat for Alpha Tocopherol: Two Text Figures and One Plate (Four Figures). *The Journal of Nutrition*, *26*(6), 555-568.
21. Farahavar, A., Rostami, Z., Alipour, D., & Ahmadi, A. (2020). The effect of pre-breeding vitamin E and selenium injection on reproductive performance, antioxidant status, and progesterone concentration in estrus-synchronized Mehraban ewes. *Trop Anim Health Prod*, *52*(4), 1779-1786. https://doi.org/10.1007/s11250-019-02183-8
22. Farrokhian, A., Mahmoodian, M., Bahmani, F., Amirani, E., Shafabakhsh, R., & Asemi, Z. (2020). The Influences of Chromium Supplementation on Metabolic Status in Patients with Type 2 Diabetes Mellitus and Coronary Heart Disease. *Biol Trace Elem Res*, *194*(2), 313-320. https://doi.org/10.1007/s12011-019-01783-7
23. Fathi, M., Alavinejad, P., Haidari, Z., & Amani, R. (2020). The Effect of Zinc Supplementation on Steatosis Severity and Liver Function Enzymes in Overweight/Obese Patients with Mild to Moderate Non-alcoholic Fatty Liver Following Calorie-Restricted Diet: a Double-Blind, Randomized Placebo-Controlled Trial. *Biol Trace Elem Res*, *197*(2), 394-404. https://doi.org/10.1007/s12011-019-02015-8
24. Fazel Torshizi, F., Chamani, M., Khodaei, H. R., Sadeghi, A. A., Hejazi, S. H., & Majidzadeh Heravi, R. (2020). Therapeutic effects of organic zinc on reproductive hormones, insulin resistance and mTOR expression, as a novel component, in a rat model of Polycystic ovary syndrome. *Iran J Basic Med Sci*, *23*(1), 36-45. https://doi.org/10.22038/ijbms.2019.36004.8586
25. Galhardi, C. M., Diniz, Y. S., Rodrigues, H. G., Faine, L. A., Burneiko, R. C., Ribas, B. O., & Novelli, E. L. (2005). Beneficial effects of dietary copper supplementation on serum lipids and antioxidant defenses in rats. *Ann Nutr Metab*, *49*(5), 283-288. https://doi.org/10.1159/000087294
26. Günalan, E., Yaba, A., & Yılmaz, B. (2018). The effect of nutrient supplementation in the management of polycystic ovary syndrome-associated metabolic dysfunctions: A critical review. *J Turk Ger Gynecol Assoc*, *19*(4), 220-232. https://doi.org/10.4274/jtgga.2018.0077
27. H.Al-Mosawi, R., & Hamood, N. J. (2020). The effect of folic acid on lipid peroxidation in women with polycystic ovary syndrome. *International Journal of Psychosocial Rehabilitation*, *24*(9), 4346-4355. https://doi.org/10.61841/bsck6t25
28. Han, Y., Hou, Y., Han, Q., Yuan, X., & Chen, L. (2024). Dietary supplements in polycystic ovary syndrome–current evidence [Mini Review]. *Frontiers in Endocrinology*, *Volume 15 - 2024*. https://doi.org/10.3389/fendo.2024.1456571
29. Helvaci, N., & Yildiz, B. O. (2025). Polycystic ovary syndrome as a metabolic disease. *Nat Rev Endocrinol*, *21*(4), 230-244. https://doi.org/10.1038/s41574-024-01057-w
30. Hrelia, S., & Angeloni, C. (2021). New Mechanisms of Action of Natural Antioxidants in Health and Disease II. *Antioxidants (Basel)*, *10*(8). https://doi.org/10.3390/antiox10081200
31. Karami, M., Almaghrabi, S., Banasr, O., & Hussein, K. (2025). Serum Selenium Levels and Their Relationship with Insulin Resistance in Individuals with Polycystic Ovary Syndrome. *Biol Trace Elem Res*, *203*(5), 2587-2594. https://doi.org/10.1007/s12011-024-04435-7
32. Kazeminia, M., Rajati, F., Rasulehvandi, R., & Rajati, M. (2024). The effect of vitamin D on the hormonal profile of women with polycystic ovarian syndrome: a systematic review and meta-analysis. *Middle East Fertility Society Journal*, *29*(1), 45.
33. Kazempour, R., Abedi, P., Siahkal, S. F., Sharifipour, F., & Zahedian, M. (2023). Effect of Selenium Supplementation on Biochemical Markers of Women with Polycystic Ovarian Syndrome: A Systematic Review. *Prev Nutr Food Sci*, *28*(2), 121-133. https://doi.org/10.3746/pnf.2023.28.2.121
34. Kisley, L. R., Sakai, R. R., Ma, L. Y., & Fluharty, S. J. (1999). Ovarian steroid regulation of angiotensin II-induced water intake in the rat. *Am J Physiol*, *276*(1), R90-96. https://doi.org/10.1152/ajpregu.1999.276.1.R90
35. Kobayashi, H., Yoshimoto, C., Matsubara, S., Shigetomi, H., & Imanaka, S. (2024). Altered Energy Metabolism, Mitochondrial Dysfunction, and Redox Imbalance Influencing Reproductive Performance in Granulosa Cells and Oocyte During Aging. *Reprod Sci*, *31*(4), 906-916. https://doi.org/10.1007/s43032-023-01394-7
36. Kuryłowicz, A. (2023). Estrogens in Adipose Tissue Physiology and Obesity-Related Dysfunction. *Biomedicines*, *11*(3). https://doi.org/10.3390/biomedicines11030690
37. Li, L., & Yang, X. (2018). The Essential Element Manganese, Oxidative Stress, and Metabolic Diseases: Links and Interactions. *Oxid Med Cell Longev*, *2018*, 7580707. https://doi.org/10.1155/2018/7580707

38. Li, M., Hu, S., Sun, J., & Zhang, Y. (2024). The role of vitamin D3 in follicle development. *J Ovarian Res*, *17*(1), 148. https://doi.org/10.1186/s13048-024-01454-9
39. Maitra, C., Raj, R., Maitra, A., & Gupta, R. (2024). Variation of Adipokines in Obese versus Non Obese Patients with Polycystic Ovary Syndrome and its Association to Visceral Adiposity Index: A Cross-sectional Study. *JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH*.
40. https://doi.org/10.7860/JCDR/2024/67554.20077
41. Maktabi, M., Jamilian, M., & Asemi, Z. (2017). RETRACTED ARTICLE: Magnesium-Zinc-Calcium-Vitamin D Co-supplementation Improves Hormonal Profiles, Biomarkers of Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome: a Randomized, Double-Blind, Placebo-Controlled Trial. *Biological Trace Element Research*, *182*, 21-28. https://doi.org/10.1007/s12011-017-1085-0
42. Manzoor, F., Nisa, M. U., Hussain, H. A., Ahmad, N., & Umbreen, H. (2020). Effect of different levels of hydrolysable tannin intake on the reproductive hormones and serum biochemical indices in healthy female rats. *Sci Rep*, *10*(1), 20600. https://doi.org/10.1038/s41598-020-77672-0
43. Mohamed, A. S. A., Abd El Latif, M. A., Hussein, E. A. M., Toson, E. M. A., Saleh, M., Kokoszynski, D., Elnesr, S. S., Mohany, M., Al-Rejaie, S. S., & Elwan, H. (2023). Efficacy of Dietary Supplementation with Zinc-Chromium Mixture, Organic Selenium, or Their Combinations on Growth Performance, Carcass Traits, and Blood Profiles of Broilers under Heat Stress Conditions. *Animals (Basel)*, *13*(15). https://doi.org/10.3390/ani13152539
44. Mohmmed, A., Awad, N., & Al-Fartosy, A. (2019). Study of Trace Elements Selenium, Copper, Zinc and Manganese Level in Polycystic Ovary Syndrome (PCOS). *International Journal For Research in Applied Sciences and Biotechnology*, *06*, 16-22. https://doi.org/10.31033/ijrasb.6.6.4
45. Nisa, M. U., Khan, A., Sarwar, M., Lee, W., Lee, H., Ki, K., Ahn, B., & Kim, H. (2006). Influence of Corn Steep Liquor on Feeding Value of Urea Treated Wheat Straw in Buffaloes Fed at Restricted Diets. *Asian-Australasian Journal of Animal Sciences*, *19*, 1610-1616. https://doi.org/10.5713/ajas.2006.1610
46. Nwadike, C., Adindu, E., Nosiri, C., Ezekwe, S., Anyagwa, M., Aloy-Amadi, O., & Onyeabo, C. (2019). Trace Elements, Antioxidants, Uric Acid, C-Reactive Proteins and Estrogen Levels of Preeclamptic Pregnant Women in Owerri, Nigeria. *Asian Journal of Medicine and Health*, 1-10.
47. https://doi.org/10.9734/ajmah/2019/v16i330146
48. Olaniyan, O., Ayobami, D., Adetunji, C., Okotie, G., Dare, J., Adigun, B., & Adebayo, F. (2023). Vitamin E modulates androgen receptor gene expression to attenuate ovarian dysfunctions in a rat model of dehydroepiandrosterone-induced polycystic ovary. *Asian Pacific Journal of Reproduction*, *12*, 81-89. https://doi.org/10.4103/2305-0500.372379
49. Pal, R. S., Punniyakotti, S., Kaur, M., & Pal, Y. (2025). Role of Micronutrients in Obesity and Weight Management. In *Nutraceuticals in Obesity Management and Control* (pp. 287-320). Apple Academic Press.
50. Panti, A. A., Shehu, C. E., Saidu, Y., Tunau, K. A., Nwobodo, E. I., Jimoh, A., Bilbis, L. S., Umar, A. B., & Hassan, M. (2018). Oxidative stress and outcome of antioxidant supplementation in patients with polycystic ovarian syndrome (PCOS). *Int J Reprod Contracept Obstet Gynecol*, *7*(5), 1667.
51. Papalou, O., Victor, V. M., & Diamanti-Kandarakis, E. (2016). Oxidative Stress in Polycystic Ovary Syndrome. *Curr Pharm Des*, *22*(18), 2709-2722. https://doi.org/10.2174/1381612822666160216151852
52. Pham, V. T., Calatayud, M., Rotsaert, C., Seifert, N., Richard, N., Van den Abbeele, P., Marzorati, M., & Steinert, R. E. (2021). Antioxidant Vitamins and Prebiotic FOS and XOS Differentially Shift Microbiota Composition and Function and Improve Intestinal Epithelial Barrier In Vitro. *Nutrients*, *13*(4). https://doi.org/10.3390/nu13041125
53. Portillo, F., Vázquez, J., & Pajares, M. A. (2020). Protein-protein interactions involving enzymes of the mammalian methionine and homocysteine metabolism. *Biochimie*, *173*, 33-47.
54. Refaat, B., & El-Boshy, M. (2021). Effects of supraphysiological vitamin D(3) (cholecalciferol) supplement on normal adult rat ovarian functions. *Histochem Cell Biol*, *155*(6), 655-668. https://doi.org/10.1007/s00418-021-01975-0
55. Saberi, A., Salarkia, E., Safi, Z., & Sepehri, G. (2017). Effects of Subacute Administration of Co-Trimoxazole and Folic Acid on Ovarian Tissue in Adult Female Rats. *Iran J Med Sci*, *42*(6), 561-568.
56. Shaukat, A., Rajput, S. A., Hanif, S., Shukat, R., Ali, M., Shaukat, I., Hassan, M., Aleem, M. T., & Mohsin, M. (2025). Morin Inhibits Inflammation, Oxidative Stress and Ferroptosis in Lipopolysaccharide-Induced Endometritis. *Pakistan Veterinary Journal*, *45*(2).
57. Silvestris, E., Lovero, D., & Palmirotta, R. (2019). Nutrition and Female Fertility: An Interdependent Correlation. *Front Endocrinol (Lausanne)*, *10*, 346. https://doi.org/10.3389/fendo.2019.00346
58. Sonawdekar, S., Parab, M. M., Shrivastava, A. K., & Gupta, P. P. (2024). Nutraceuticals for hormonal imbalance management. In *Nutraceuticals Inspiring the Contemporary Therapy for Lifestyle Diseases* (pp. 65-79). CRC Press.
59. Szczuko, M., Zapalowska-Chwyć, M., & Drozd, R. (2019). A Low Glycemic Index Decreases Inflammation by Increasing the Concentration of Uric Acid and the Activity of Glutathione Peroxidase (GPx3) in Patients with Polycystic Ovary Syndrome (PCOS). *Molecules*, *24*(8). https://doi.org/10.3390/molecules24081508
60. Tatone, C., Di Emidio, G., Placidi, M., Rossi, G., Ruggieri, S., Taccaliti, C., D'Alfonso, A., Amicarelli, F., & Guido, M. (2021). AGEs-related dysfunctions in PCOS: evidence from animal and clinical research. *J Endocrinol*, *251*(2), R1-r9. https://doi.org/10.1530/joe-21-0143
61. Trummer, C., Schwetz, V., Kollmann, M., Wölfler, M., Münzker, J., Pieber, T. R., Pilz, S., Heijboer, A. C., Obermayer-Pietsch, B., & Lerchbaum, E. (2019). Effects of vitamin D supplementation on metabolic and

endocrine parameters in PCOS: a randomized-controlled trial. *Eur J Nutr*, *58*(5), 2019-2028. https://doi.org/10.1007/s00394-018-1760-8
62. Umer, M., Nisa, M. U., Ahmad, N., Rahim, M. A., & Al-Asmari, F. (2023). Effects of different levels of dried onion powder on nutrient digestibility, biochemical parameters, and nitrogen balance in Wistar albino rats with induced hyperuricemia. *Front Physiol*, *14*, 1273286. https://doi.org/10.3389/fphys.2023.1273286
63. Vašková, J., Klepcová, Z., Špaková, I., Urdzík, P., Štofilová, J., Bertková, I., Kľoc, M., & Rabajdová, M. (2023). The Importance of Natural Antioxidants in Female Reproduction. *Antioxidants (Basel)*, *12*(4). https://doi.org/10.3390/antiox12040907
64. Villa, P., Amar, I. D., Bottoni, C., Cipolla, C., Dinoi, G., Moruzzi, M. C., Scambia, G., & Lanzone, A. (2017). The impact of combined nutraceutical supplementation on quality of life and metabolic changes during the menopausal transition: a pilot randomized trial. *Arch Gynecol Obstet*, *296*(4), 791-801. https://doi.org/10.1007/s00404-017-4491-9
65. Wahid, S., Ramli, M. D., Fazleen, N., Rosli, M. N., & Mokhtar, M. H. (2024). Exploring the Therapeutic Potential of Natural Products in Polycystic Ovarian Syndrome (PCOS): A Mini-Review of Lipid Profile, Blood Glucose, and Ovarian Histological Improvements. *Life*, *14*, 150. https://doi.org/10.3390/life14010150
66. WHO, W. H. O. (2026). *Polycystic ovary syndrome fact sheet* WHO. https://www.who.int/news-room/fact-sheets/detail/polycystic-ovary-syndrome
67. Xu, F., Wolf, S., Green, O., & Xu, J. (2021). Vitamin D in follicular development and oocyte maturation. *Reproduction*, *161*(6), R129-r137. https://doi.org/10.1530/rep-20-0608
68. Yao, X., Liu, R., Li, X., Li, Y., Zhang, Z., Huang, S., Ge, Y., Chen, X., & Yang, X. (2021). Zinc, selenium and chromium co-supplementation improves insulin resistance by preventing hepatic endoplasmic reticulum stress in diet-induced gestational diabetes rats. *J Nutr Biochem*, *96*, 108810.
69. https://doi.org/10.1016/j.jnutbio.2021.108810
70. Ye, Y. M., Wei, M. H., Lv, K. N., Xue, X. H., Shen, R., & Liu, J. H. (2024). Effects of an anti-inflammatory diet (AID) on maternal and neonatal health outcomes in pregnant Chinese patients with inflammatory bowel disease treated with infliximab (IFX). *Scand J Gastroenterol*, *59*(12), 1297-1305.
71. https://doi.org/10.1080/00365521.2024.2423828
72. Yousefi-Nodeh, H., Farshbaf-Khalili, A., Sadeghzadeh Oskouei, B., Jafarilar-Aghdam, N., Kazemi-Zanjani, N., & Pourzeinali, S. (2024). Curcumin and vitamin E improve hot flashes, lipid profile, and fasting blood glucose without any detrimental effect on the liver and renal function in postmenopausal women: A triple-blind placebo-controlled clinical trial. *Health Care Women Int*, *45*(11), 1184-1206.
73. https://doi.org/10.1080/07399332.2022.2117815
74. Zeng, S., Xu, J., Xie, C., & Jiang, W. (2024). The clinical significance of monitoring serum levels of glutathione peroxidase and selenium in patients with polycystic ovary syndrome. *Medical Research*, *6*, 35-41. https://doi.org/10.6913/mrhk.060106

75. Abbreviations

Abbreviation	Full Term
8-OHdG	8-hydroxy-2′-deoxyguanosine
AIN-93G	American Institute of Nutrition-93 Growth
AKT	Protein Kinase B
ALT	Alanine aminotransferase
AMPK	AMP-activated protein kinase
AST	Aspartate aminotransferase
BD	Basal diet
CAT	Catalase
CHOD-PAP	Cholesterol oxidase phenol + aminophenazone
CRD	Completely randomised design
CrPic	Chromium picolinate
CRP	C-reactive protein
DM	Dry matter
EE	Ether extract
ER	Endoplasmic reticulum
FCR	Feed conversion ratio
FER	Feed efficiency ratio
FSH	Follicle-stimulating hormone
GLUT-1	Glucose transporter 1
GSH	Reduced glutathione
HbA1c	Glycated haemoglobin A1c
IL-1β	Interleukin-1 beta
INHBA	Inhibin subunit beta A
IRS-1	Insulin receptor substrate-1

LDL	Low-density lipoprotein
LH	Luteinising hormone
MDA	Malondialdehyde
MTHFR	Methylenetetrahydrofolate reductase
NFE	Nitrogen-free extract
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NRF2	Nuclear factor erythroid 2-related factor 2
PCOS	Polycystic ovary syndrome
PI3K	Phosphoinositide 3-kinase
PMOS	Polyendocrine metabolic ovarian syndrome
PPAR-α	Peroxisome proliferator-activated receptor alpha
PPAR-γ	Peroxisome proliferator-activated receptor gamma
RDA	Recommended dietary allowance
RIA	Radioimmunoassay
ROS	Reactive oxygen species
SAM	S-adenosylmethionine
SHBG	Sex hormone-binding globulin
SOD	Superoxide dismutase
SREBP-1c	Sterol regulatory element-binding protein 1c
TAC	Total antioxidant capacity
TC	Total cholesterol
TGF-β	Transforming growth factor beta
TOS	Total oxidant status
UA	Uric acid
VDR	Vitamin D receptor
VLDL	Very low-density lipoprotein