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# Comparative Effects of Ginger and Ashwagandha Extracts, Alone and Combined, Versus Levothyroxine on Reproductive Endocrine Recovery in PTU-Induced Hypothyroid Female Rats Treated During Pregnancy

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**ABSTRACT:** **Introduction**: Hypothyroidism can disturb hypothalamic-pituitary-ovarian regulation, gonadotropin secretion and ovarian steroidogenesis, changes that are particularly relevant during pregnancy and the transition after parturition.

**Objective**: To compare the effects of 70% ethanolic extracts of Zingiber officinale (ginger) and Withania somnifera (ashwagandha), alone or combined, with levothyroxine on reproductive endocrine recovery in PTU-induced hypothyroid female rats treated during pregnancy.

**Methods**: Sixty adult female albino rats were allocated to six groups (10/group). G1 received water, whereas G2-G6 received propylthiouracil (PTU; 50 mg/kg/day) orally for 28 days. Hormonal analyses were based on n=3 animals/group in each phase. After confirmation of hypothyroidism and pregnancy, G1 remained the healthy control and G2 the untreated hypothyroid control. During the 21-day gestational treatment period, G3 received levothyroxine (20 µg/kg/day), G4 ginger (200 mg/kg/day), G5 ashwagandha (200 mg/kg/day), and G6 both extracts (200 mg/kg/day each). Serum LH, FSH, testosterone, estrogen, progesterone and prolactin were measured.

**Results**: Before treatment, PTU-exposed groups showed significantly lower LH, FSH and testosterone than G1, significantly higher estrogen, a general tendency toward lower progesterone, and no significant prolactin difference. One day after parturition, untreated G2 retained significantly lower LH, FSH, testosterone and progesterone than G1, whereas estrogen and prolactin did not differ significantly. With levothyroxine, FSH, testosterone and progesterone reached values statistically comparable with G1, while LH remained lower. Ginger improved LH and FSH and increased estrogen relative to G2, but testosterone and progesterone did not improve significantly relative to G2. With ashwagandha, LH, FSH and progesterone reached values statistically comparable with G1, whereas testosterone remained significantly lower than G1 and did not differ significantly from G2. The combined treatment improved LH, FSH and testosterone significantly versus G2, produced FSH and progesterone values statistically comparable with G1, and produced the highest numerical estrogen concentration. No

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significant group difference in prolactin was detected.

**Conclusions**: The treatments produced distinct reproductive endocrine recovery patterns. Ashwagandha showed the most complete plant-associated LH recovery, whereas the combination produced the broadest significant improvement versus the untreated hypothyroid group across LH, FSH and testosterone. The data did not demonstrate pharmacological synergy.

## 1. INTRODUCTION

Thyroid dysfunction can influence female reproductive physiology through interactions with the hypothalamic-pituitary-ovarian axis, ovarian steroidogenesis and reproductive hormone regulation. Experimental hypothyroidism in female rats has been associated with altered gonadotropins, ovarian steroid hormones and reproductive function [1-4]. These effects are important in pregnancy because maternal endocrine homeostasis must support both gestation and the transition to the postpartum reproductive state.

Propylthiouracil (PTU) is commonly used to induce experimental hypothyroidism. Reproductive responses to thyroid hormone deficiency are not uniform across studies: reductions in LH, FSH and testosterone have been reported in several models [3-5], whereas other PTU studies have shown different LH, testosterone, estrogen or progesterone responses depending on exposure conditions and reproductive stage [6,7]. Such variability emphasizes the importance of interpreting reproductive hormones in relation to the timing of sampling and the specific physiological state of the animals.

Thyroid hormones may also influence ovarian responsiveness directly. Thyroid hormone has been shown to amplify FSH-mediated functional differentiation and steroidogenic activity in granulosa cells [8], and thyroid-hormone-dependent stimulation of progesterone release has been demonstrated in rat granulosa cells [9]. Therefore, reproductive endocrine recovery after treatment of hypothyroidism may involve changes in both pituitary gonadotropins and ovarian steroid output.

Levothyroxine is the conventional replacement treatment for hypothyroidism and has been used experimentally to reverse reproductive endocrine disturbances associated with thyroid hormone deficiency [4,10]. Medicinal plant extracts have also been investigated for possible reproductive endocrine effects. Ginger (Zingiber officinale) has been reported to modify LH, FSH, estrogen and progesterone in female-rat models [11,12], while Withania somnifera (ashwagandha) has been associated with changes in gonadotropins and reproductive steroid hormones [13,14].

Evidence directly comparing ginger, ashwagandha, their combination and levothyroxine within the same PTU-induced hypothyroid pregnancy model while assessing a broad panel of reproductive hormones remains limited. Therefore, this study aimed to compare the effects of these treatments on serum LH, FSH, testosterone, estrogen, progesterone and prolactin in female rats, with reproductive hormone profiles evaluated before treatment and one day after parturition following treatment throughout gestation.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of plant extracts

Ginger and ashwagandha were obtained from a local herbal market. Homogenized plant material (20 g) was defatted with 100 mL chloroform on an electric vibrator for 3 h. After removal of the chloroform layer and drying at 50 °C, 10 g of dried sample was extracted with ethanol/water (70/30, v/v) in an ultrasonic bath at room temperature for 1 h. After filtration, solvent was removed under vacuum using a rotary evaporator and the extract was dried at 40 °C to constant mass and stored at 4 °C.

### 2.2 Experimental animals and housing

Sixty adult female albino rats, 8-10 weeks old and weighing approximately 250-270 g, were obtained from the animal house of the Biotechnology Research Center, Al-Nahrain University. Animals were housed in plastic cages with metal mesh lids under identical laboratory conditions (22-25 °C; 40-60% relative humidity; continuous access to feed and water). The animals were

randomly divided into six groups, with 10 rats initially allocated to each group. The hormonal endpoints reported in this manuscript were based on n=3 animals per group in each experimental phase, and the two phases were analysed separately.

**2.3 Induction of hypothyroidism**

During the first experimental phase, G1 served as the negative control and received water only. Hypothyroidism was induced in G2-G6 by oral gavage of PTU at 50 mg/kg/day for 28 consecutive days. At the end of this period, hypothyroidism was confirmed before mating. Importantly, G2-G6 were induction groups only during this phase; no levothyroxine, ginger, ashwagandha or combined treatment had yet been administered. PTU administration was limited to the 28-day induction period.

**2.4 Mating and treatment during pregnancy**

After confirmation of hypothyroidism, females were mated with healthy males at a 1:1 ratio. Early pregnancy was confirmed by the presence of a vaginal plug and/or detection of sperm in a vaginal swab. Once pregnancy was confirmed, the second experimental phase began. G1 remained the healthy negative control and continued to receive water. G2 served as the untreated hypothyroid positive control. G3 received levothyroxine at 20 µg/kg/day, G4 received ginger extract at 200 mg/kg/day, G5 received ashwagandha extract at 200 mg/kg/day, and G6 received both ginger and ashwagandha extracts at 200 mg/kg/day each. Treatments were administered orally by gavage for 21 days throughout pregnancy. The second blood collection was performed one day after parturition; therefore, the second-phase hormone concentrations represent the one-day postpartum sampling point after treatment throughout gestation rather than hormone concentrations measured during pregnancy itself.

**2.5 Blood collection and reproductive hormone assays**

At each experimental phase, the animals selected for hormonal examination were anesthetized by chloroform inhalation until cessation of movement. Blood was then collected by cardiac puncture, transferred to serum-separator gel tubes and centrifuged at 2000 rpm for 10 min to obtain serum. Serum LH, FSH, testosterone, estrogen, progesterone and prolactin concentrations were measured using pre-prepared assay kits according to the manufacturer's instructions and the ELISA technique.

**2.6 Statistical analysis**

Statistical analysis was performed using SAS (Statistical Analysis System), and the least significant difference (LSD) procedure was used for comparison between group means. Results are presented as mean ± standard error (SE). Differences were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.01$. Hormonal analyses in each experimental phase were based on n=3 animals/group.

**3. RESULTS**

**3.1 Reproductive hormone profile after PTU induction**

After 28 days of PTU exposure and before mating or therapeutic intervention, all PTU-exposed groups (G2-G6) showed significantly lower LH, FSH and testosterone concentrations than G1. LH differed among groups at P=0.0021, while FSH and testosterone differed at P=0.0001. All PTU-exposed groups carried the same statistical letter for each of these hormones, indicating no significant differences among the induction groups before treatment.

**Table 1. Serum LH, FSH and testosterone after 28 days of PTU induction.**

| Group | LH (mIU/mL) | FSH (mIU/mL) | Testosterone (ng/dL) |
|---|---|---|---|
| **G1** | 1.83 ± 0.06 a | 42.30 ± 2.77 a | 16.83 ± 1.35 a |
| **G2** | 0.800 ± 0.06 b | 16.23 ± 1.84 b | 5.70 ± 0.43 b |
| **G3** | 1.00 ± 0.26 b | 18.23 ± 3.32 b | 7.06 ± 0.46 b |
| **G4** | 0.766 ± 0.23 b | 17.50 ± 0.91 b | 5.80 ± 0.47 b |

| | | | |
|---|---|---|---|
| **G5** | 0.667 ± 0.12 b | 14.83 ± 2.61 b | 6.46 ± 0.91 b |
| **G6** | 0.833 ± 0.03 b | 18.76 ± 1.07 b | 6.20 ± 0.40 b |
| **LSD** | 0.483 ** | 6.996 ** | 2.341 ** |
| **P value** | 0.0021 | 0.0001 | 0.0001 |

Values are mean ± SE (n=3/group). Means with different letters within the same column differed significantly. **P ≤ 0.01. During this phase G2-G6 were PTU induction groups only; therapeutic treatments had not yet begun.

Estrogen also differed significantly among groups (P=0.0466). G1 recorded 97.67 ± 6.48 pg/mL, whereas all PTU-exposed groups carried the statistical letter (a) and had significantly higher concentrations than G1. Progesterone differed at P=0.0497; although all PTU-exposed groups were numerically lower than G1, only G4 differed significantly from G1 because G2, G3, G5 and G6 shared overlapping statistical letters with the control. Prolactin did not differ significantly among groups (P=0.827).

**Table 2.** Serum estrogen, progesterone and prolactin after 28 days of PTU induction.

| Group | Estrogen (pg/mL) | Progesterone (ng/mL) | Prolactin (ng/mL) |
|---|---|---|---|
| **G1** | 97.67 ± 6.48 b | 19.67 ± 1.66 a | 18.00 ± 1.15 |
| **G2** | 111.33 ± 4.05 a | 16.00 ± 1.53 ab | 15.00 ± 1.15 |
| **G3** | 116.33 ± 3.18 a | 15.67 ± 3.48 ab | 15.33 ± 2.40 |
| **G4** | 117.33 ± 1.85 a | 10.33 ± 1.33 b | 15.00 ± 2.88 |
| **G5** | 113.67 ± 4.17 a | 15.00 ± 3.51 ab | 15.33 ± 2.18 |
| **G6** | 116.00 ± 3.60 a | 15.00 ± 1.15 ab | 14.00 ± 2.08 |
| **LSD** | 12.73 * | 7.189 * | 6.40 NS |
| **P value** | 0.0466 | 0.0497 | 0.827 |

Values are mean ± SE (n=3/group). Means with different letters within the same column differed significantly. *P ≤ 0.05; NS, non-significant. No statistical letters are shown for prolactin because the overall group effect was non-significant.

### 3.2 Reproductive hormone profile after gestational treatment

Following the 21-day gestational treatment period, blood was collected one day after parturition. Group differences were highly significant for LH (P<0.0001), FSH (P=0.0001) and testosterone (P=0.0002), with LSD values of 0.475, 8.646 and 3.157, respectively. G2 retained significantly lower LH, FSH and testosterone than G1.

Levothyroxine-treated G3 showed FSH and testosterone concentrations statistically comparable with G1, while LH improved significantly versus G2 but remained below G1. Ginger-treated G4 significantly increased LH and FSH versus G2, but both remained below G1; testosterone did not differ significantly from G2. In Ashwagandha-treated G5, LH and FSH reached values statistically comparable with G1, whereas testosterone increased numerically but did not differ significantly from G2. The combined treatment G6 significantly increased LH, FSH and testosterone versus G2. FSH was statistically comparable with G1 and G3 and significantly higher than G4 and G5; testosterone was higher than G2 and G4 but lower than G1 and G3 and statistically comparable with G5.

**Table 3.** Serum LH, FSH and testosterone one day after parturition following treatment throughout the 21-day gestational period.

| Group | LH (mIU/mL) | FSH (mIU/mL) | Testosterone (ng/dL) |
|---|---|---|---|
| **G1** | 3.75 ± 0.05 a | 45.32 ± 2.03 ab | 15.09 ± 1.30 a |
| **G2** | 1.73 ± 0.18 d | 23.40 ± 1.83 d | 6.86 ± 0.26 c |
| **G3** | 3.04 ± 0.09 bc | 50.13 ± 3.94 a | 15.16 ± 1.46 a |

| | | | |
|---|---|---|---|
| **G4** | 2.88 ± 0.10 c | 34.37 ± 2.14 c | 7.00 ± 0.10 c |
| **G5** | 3.36 ± 0.13 ab | 37.16 ± 2.13 bc | 9.43 ± 0.49 bc |
| **G6** | 3.15 ± 0.27 bc | 50.20 ± 3.89 a | 11.36 ± 1.47 b |
| **LSD** | 0.475 ** | 8.646 ** | 3.157 ** |
| **P value** | <0.0001 | 0.0001 | 0.0002 |

Values are mean ± SE (n=3/group). Means with different letters within the same column differed significantly. **P ≤ 0.01. G1, negative control; G2, untreated hypothyroid control; G3, levothyroxine 20 µg/kg/day; G4, ginger 200 mg/kg/day; G5, ashwagandha 200 mg/kg/day; G6, ginger 200 mg/kg/day + ashwagandha 200 mg/kg/day.

Estrogen differed significantly among groups at the one-day postpartum sampling point (P=0.0303), progesterone differed highly significantly (P=0.0093), and prolactin remained non-significant (P=0.4032). G2 showed progesterone significantly lower than G1, while estrogen and prolactin did not differ significantly from G1. In G3, progesterone increased significantly versus G2 and became statistically comparable with G1; estrogen and prolactin remained comparable with both controls. G4 showed estrogen significantly higher than G2 but comparable with G1, while progesterone remained comparable with G2 and lower than G1. In G5, progesterone increased significantly versus G2 and reached a value statistically comparable with G1, while estrogen and prolactin did not differ significantly from either control. In G6, progesterone also increased significantly versus G2 and reached a value statistically comparable with G1; G6 produced the highest estrogen concentration, which was significantly higher than G1, G2 and G3 but statistically comparable with G4 and G5. No significant group difference in prolactin was detected.

**Table 4.** Serum estrogen, progesterone and prolactin one day after parturition following treatment throughout the 21-day gestational period.

| Group | Estrogen (pg/mL) | Progesterone (ng/mL) | Prolactin (ng/mL) |
|---|---|---|---|
| **G1** | 125.56 ± 3.48 bc | 23.35 ± 1.55 a | 17.00 ± 1.09 |
| **G2** | 119.00 ± 4.58 c | 14.00 ± 1.00 c | 13.33 ± 1.45 |
| **G3** | 128.00 ± 1.52 bc | 22.37 ± 2.03 a | 14.33 ± 0.33 |
| **G4** | 135.00 ± 5.19 ab | 17.33 ± 1.85 bc | 14.25 ± 1.20 |
| **G5** | 130.33 ± 5.24 ac | 22.00 ± 1.73 ab | 16.00 ± 1.15 |
| **G6** | 142.00 ± 3.60 a | 22.31 ± 1.45 ab | 15.33 ± 1.85 |
| **LSD** | 12.751 * | 5.038 ** | 3.894 NS |
| **P value** | 0.0303 | 0.0093 | 0.4032 |

Values are mean ± SE (n=3/group). Means with different letters within the same column differed significantly. *P ≤ 0.05; **P ≤ 0.01; NS, non-significant. No statistical letters are shown for prolactin because the overall group effect was non-significant.

## 4. DISCUSSION

The present study demonstrated two major reproductive endocrine findings. First, the 28-day PTU induction phase produced a broad disturbance before mating, characterized by significant suppression of LH, FSH and testosterone, significant elevation of estrogen, a general tendency toward lower progesterone, and no significant change in prolactin. Second, treatment throughout pregnancy with levothyroxine, ginger, ashwagandha or their combination produced distinct recovery patterns when hormones were measured one day after parturition.

The induction-phase reductions in LH and FSH agree with Bruni et al. [5], who showed reduced gonadotropins during experimental hypothyroidism, and with Tohei et al. [3] and Hatsuta et al. [4], who reported reduced LH and low testosterone

in hypothyroid female rats. The uniform statistical grouping of G2-G6 before treatment is methodologically important because it indicates that the reproductive endocrine disturbance was comparable among the future treatment groups before any therapeutic intervention.

A partially different response was reported by Liu et al. [6], who observed decreased FSH in PTU-induced hypothyroid female rats but no significant LH reduction and an increase rather than a decrease in testosterone. Differences in PTU exposure, severity of hypothyroidism, animal age, ovarian status and estrous-cycle stage at sampling may contribute to the contrasting responses. These factors are especially relevant because gonadotropins and ovarian steroids fluctuate across the reproductive cycle.

The significant estrogen elevation during induction agrees with Hapon et al. [7], who reported increased circulating estradiol during short-term PTU-induced hypothyroidism and increased ovarian aromatase expression. Li et al. [15] also reported an increasing tendency in estradiol and the estradiol-to-progesterone relationship in a different hypothyroid model. In contrast, Liu et al. [6] and Tohei et al. [3] reported reduced estradiol. The different directions across studies reinforce the influence of model severity, reproductive stage and sampling time on ovarian steroid profiles.

Progesterone showed a more heterogeneous induction-phase response. All PTU-exposed groups were numerically lower than G1, but statistical significance was confined to G4, which had not yet received ginger and therefore cannot be interpreted as a treatment effect. The lower progesterone tendency is mechanistically supported by Chen et al. [16], who demonstrated that PTU can reduce progesterone release from rat granulosa cells and interfere with StAR-associated steroidogenesis, P450scc and 3β-HSD. Thyroid hormone also supports FSH-mediated granulosa-cell differentiation [8] and progesterone release [9], providing a physiological basis for disruption of ovarian steroidogenesis during thyroid hormone deficiency.

At the one-day postpartum sampling point, the untreated hypothyroid group G2 retained significantly lower LH, FSH, testosterone and progesterone than G1, while estrogen and prolactin were not significantly different. This pattern indicates persistent reproductive endocrine dysfunction despite PTU withdrawal after the induction phase. The progesterone decrease is consistent with Kong et al. [17], who reported markedly lower progesterone in hypothyroid female rats. The absence of a significant estrogen difference is compatible with Armada-Dias et al. [18], who found no significant serum estradiol difference between control and hypothyroid female rats.

The timing of the second sample is central to interpretation. Connor and Davis [19] demonstrated marked changes in estradiol and progesterone during the first 24 h after parturition, while Fox and Smith [20] described a postpartum preovulatory gonadotropin surge associated with the labor process. The exact stage of postpartum estrus was not determined in the present study; therefore, these physiological events provide context for the one-day postpartum values but should not be interpreted as a directly measured cause of the observed concentrations.

Levothyroxine produced substantial reproductive endocrine recovery. FSH and testosterone became statistically comparable with G1, LH increased significantly relative to G2 but remained lower than G1, and progesterone recovered to a G1-comparable concentration. Estrogen and prolactin did not differ significantly from either control group. These findings are consistent with Erbas and Gedikli [10], who reported significant amelioration of FSH, LH and other measured reproductive hormones after L-thyroxine treatment in PTU-induced hypothyroid female rats, and with Hatsuta et al. [4], who showed reversal of several reproductive endocrine abnormalities after T4 treatment. The incomplete LH recovery in the present study indicates that different components of the hypothalamic-pituitary-ovarian axis did not recover to the same extent at the one-day postpartum sampling point.

Ginger produced a more selective response. LH and FSH improved significantly versus G2 but remained below G1, whereas testosterone did not differ significantly from G2. Estrogen increased significantly versus G2 and remained statistically comparable with G1, while progesterone did not improve significantly versus G2 and remained below G1. This pattern is partly consistent with Safavi Neini and Fardaei [11], who reported significant increases in LH at 180 mg/kg and estrogen at 120 and 180 mg/kg after ginger administration in female rats. Dizay et al. [21] reported no significant progesterone response to ginger in a hypothyroid female-rat protocol. Atashpour et al. [12], however, reported increased progesterone and reduced pathologically elevated estrogen in a PCOS model. The contrasting direction is plausibly related to the different endocrine background, treatment conditions and reproductive state.

Ashwagandha produced a different pattern. LH and FSH were significantly higher than G2 and statistically comparable with G1, whereas testosterone increased numerically but did not differ significantly from G2 and remained lower than G1. Progesterone also recovered to a value statistically comparable with G1, while estrogen and prolactin remained statistically comparable with both controls. The gonadotropin and progesterone responses agree with Megahd and Gabal [13], who reported improvement of reproductive hormones in female rats after ashwagandha in an experimental utero-ovarian injury model. Al-Qarawi et al. [14] also demonstrated that Withania somnifera can influence gonadotropin concentrations in female rats.

The combined ginger-ashwagandha treatment produced the broadest statistically significant improvement versus G2 among the plant-treated groups for LH, FSH and testosterone, because all three increased significantly. FSH became statistically comparable with G1 and levothyroxine, while testosterone improved versus G2 and ginger but remained below G1 and did not differ significantly from ashwagandha. LH improved versus G2 but remained below G1; notably, ashwagandha alone was the only plant treatment with LH statistically comparable with G1. Thus, the combination should not be interpreted as uniformly superior to ashwagandha.

For ovarian steroids, progesterone in the combined group increased significantly versus G2 and reached a concentration statistically comparable with G1, while G6 produced the highest estrogen value. Estrogen in G6 was significantly higher than G1, G2 and G3 but remained statistically comparable with G4 and G5. This represents a treatment-associated elevation rather than normalization toward the healthy control. No significant group difference in prolactin was detected. No directly comparable study was identified in the literature reviewed in which ginger and ashwagandha were administered together during pregnancy to PTU-induced hypothyroid female rats with simultaneous assessment of this reproductive hormone panel.

No statistically significant group difference in prolactin was detected during either experimental phase. Hapon et al. [2] demonstrated that prolactin responses to hypothyroidism can vary with reproductive stage and reported normal prolactin during lactation despite other stage-dependent alterations. In contrast, Hapon et al. [7] and Tohei et al. [3] reported increased prolactin in hypothyroid female rats under different experimental and reproductive conditions. Differences in exposure duration, severity of thyroid dysfunction, pituitary responsiveness and reproductive stage may contribute to these discrepant prolactin responses.

Overall comparison of the treatment groups showed distinct rather than uniformly superior response patterns. Levothyroxine produced the most complete testosterone recovery and produced FSH and progesterone concentrations statistically comparable with G1. Ashwagandha was the only plant treatment in which LH became statistically comparable with G1, and its FSH and progesterone concentrations were also statistically comparable with G1. The combined treatment produced the broadest statistically significant improvement versus G2 across LH, FSH and testosterone, but its testosterone concentration did not differ significantly from Ashwagandha and its LH remained below G1. For progesterone, G3 was significantly higher than G4, although the difference was marginal relative to the LSD threshold; G3 did not differ significantly from G5 or G6, and G4 did not differ significantly from G5 or G6. The estrogen elevation in G6 was treatment-associated rather than a normalization response because it exceeded G1, G2 and G3 while remaining statistically comparable with G4 and G5. These findings support treatment-specific reproductive endocrine effects without demonstrating pharmacological synergy.

## 5. STUDY LIMITATIONS

The exact estrous or postpartum-estrus stage at the second blood collection was not determined. Blood was collected one day after parturition, so the second-phase values do not represent hormone concentrations during pregnancy itself. The study did not directly measure ovarian aromatase activity, steroidogenic enzymes or GnRH activity. Therefore, mechanistic interpretations remain physiologically plausible rather than directly demonstrated.

## 6. CONCLUSION

PTU-induced hypothyroidism was accompanied by broad reproductive endocrine disruption before treatment, including suppression of LH, FSH and testosterone, elevation of estrogen and a tendency toward lower progesterone, while prolactin showed no significant group difference. One day after parturition, the untreated hypothyroid group retained significant reductions in LH, FSH, testosterone and progesterone. Levothyroxine produced the most complete testosterone recovery, while FSH and progesterone reached concentrations statistically comparable with the healthy control. Ginger improved LH

and FSH and increased estrogen but produced incomplete testosterone and progesterone recovery. With Ashwagandha, LH, FSH and progesterone reached concentrations statistically comparable with the healthy control, while the combined treatment produced the broadest significant improvement versus the untreated hypothyroid group across LH, FSH and testosterone, with progesterone also reaching a concentration statistically comparable with the healthy control. However, the combination did not demonstrate uniform superiority over Ashwagandha alone and the results do not confirm pharmacological synergy. No statistically significant treatment-associated difference in prolactin was detected.

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