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Short Report

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Tamoxifen retains cancer-preventive efficacy with concomitant hormonal contraceptive in a rat mammary tumorigenesis model

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Abstract

Whether hormonal contraceptives (HCs) impair tamoxifen efficacy in premenopausal women is unknown. Using a rat estrogen receptor-positive mammary tumorigenesis model, we found that combined HC (17α-ethinylestradiol + levonorgestrel) did not reduce the cancer-preventive tamoxifen efficacy. No cancers occurred with tamoxifen+HC treatment, 16.7% in tamoxifen-alone and 91.7% in controls ($p < 0.001$). Tamoxifen-associated reproductive adverse events were not increased by HC co-administration. HC use by premenopausal women taking tamoxifen merits clinical evaluation.

Main Text

Tamoxifen is a selective estrogen receptor modulator (SERM), and the only agent approved for breast cancer risk reduction in premenopausal women, reducing incidence by approximately 50%[1]. However, effective contraception is required during tamoxifen treatment due to its teratogenicity[2]; systematic reviews of inadvertent exposure report increased risk of fetal malformations in approximately 12.6% of pregnancies compared with 3.9% in the general population[2] although prospective data are non-existent. Copper IUDs do not increase breast cancer risk[3] but cause heavier menstrual bleeding and dysmenorrhea in approximately 40% of users, driving discontinuation at significantly higher rates than hormonal IUDs[4]. Hormonal contraceptives (HCs) offer greater tolerability and additional benefits including reduction of dysmenorrhea and ovarian and endometrial cancer risk. The levonorgestrel (LNG)-releasing IUD (up to 8 years) and etonogestrel subcutaneous implant (up to 5 years) both provide long-acting coverage during tamoxifen treatment. However, current HCs carry a modestly elevated breast cancer risk (OR 1.25, 95% CI 1.18–1.33)[5], but whether HCs reduce tamoxifen efficacy remains unknown. Prior chemoprevention trials either prohibited HC use (NSABP P-1) or did not analyze outcomes by contraceptive use (IBIS-I)[1,6] and a retrospective a retrospective cohort found no increase in HC-recurrence but did not analyze tamoxifen use.

Current guidelines recommend nonhormonal contraception (NCCN 2026 v1) based on absence of evidence[7] rather than proven harm; or permit hormonal IUDs with presumption of safety (ESMO 2025). To address this gap, we have evaluated the cancer-preventive efficacy of tamoxifen with concomitant HC use in a preclinical rat model. We hypothesized that the most widely used HC, 17α-ethinylestradiol plus levonorgestrel (EE+LNG) would not reduce the cancer-preventive efficacy of oral tamoxifen. We used a carcinogen-induced mammary tumor model in which *N*-Methyl *N*-Nitrosourea (MNU) treatment produces ER-positive cancers that are hormonally sensitive and are further promoted by exogenous hormones (estrogen or progestogens) in cycling female rats[8]. We used EE+LNG at a clinically relevant, human-equivalent dose (HED) of oral combination HC; this has not been previously studied in a rat mammary tumorigenesis model. In the MNU rat model, tamoxifen at an HED of 10 mg/day or more prevents mammary cancer completely[9,10]. To more closely simulate the human condition where tamoxifen is imperfect for cancer prevention, we chose a lower dose (HED 7 mg/day), which is also consistent with low-dose tamoxifen regimens in high-risk premenopausal women. The primary endpoint was histopathology-confirmed mammary cancer incidence; we also examined tamoxifen and HC effects on hormone levels, and on the uterus and ovary compared to untreated controls.

4–5 week-old female Sprague Dawley rats received a single intraperitoneal injection of MNU, to induce ER-positive mammary tumors resembling human luminal-type carcinomas[8,11]. At this single 50 mg/kg dose, MNU reliably induces mammary tumors without the reproductive-tract precancerous changes reported at higher, repeated intravenous doses (cumulative 60–150 mg/kg)[12], allowing us to attribute reproductive organ findings to tamoxifen and HC rather than the carcinogen itself. Three weeks later, 50 animals were randomized into four groups: untreated control, HC alone, tamoxifen alone and tamoxifen+HC (n=12-13 rats per group) **(Figure 1a)**. Tamoxifen was incorporated into the diet at 1.03 mg/kg/day (HED 7 mg/day), expecting 10–20% cancer incidence in the MNU rat model[9]. We implanted

subcutaneous pellets releasing a daily HED of EE 0.03 mg + LNG 0.15 mg, the standard oral dose in humans. Tumor development was monitored for six months. We found that no cancers occurred in the tamoxifen+HC group, cancer incidence was 16.7% in the tamoxifen alone group, 23.1% in the HC-alone group, and 91.7% in controls (p=0.0001) (**Figure 1b**). Compared to the control group, cancer incidence was significantly lower with HC-alone treatment (23.1% vs. 92%, p=0.0002) (**Figure 1c**). Benign fibroadenomas developed in 15.4% of rats in the tamoxifen-alone group and in 23.1% of the HC-alone group.

In women, EE+LNG works by suppressing gonadotropin releasing hormone (GnRH) which in turn controls luteinizing hormone (LH), follicle-stimulating hormone (FSH) and ovarian hormones (estradiol, and progesterone) by negative feedback on hypothalamic-pituitary-gonadal (HPG) axis[13] (**Figure 2a**). We assessed the effects of HC and tamoxifen on the HPG axis at the time of euthanasia. Median LH (mIU/mL) was similar across groups (control 3.11, HC 2.70, tamoxifen 2.25, tamoxifen+HC 1.99; p=ns). Median FSH (mIU/mL) was significantly lower than HC alone in the tamoxifen-treated groups: control 14.3, HC 11.8, tamoxifen 7.99, tamoxifen+HC 7.73; (p=0.005 for both tamoxifen-treated groups) (**Figure 2b**). Compared to controls, median estradiol (pg/mL) was lowered significantly by tamoxifen treatment, but not by HC treatment alone: control 14.9, HC 11.9, tamoxifen 8.82, tamoxifen+HC 9.47 (p =0.008 and p =0.018, respectively compared to control). Median progesterone (ng/mL) did not differ significantly from control in any group: control 161, HC 107, tamoxifen 41.8, tamoxifen+HC 35.5, However, compared to HC treatment alone, progesterone levels were significantly lower in both tamoxifen and tamoxifen+HC groups (p=0.016 and p=0.006, respectively) (**Figure 2c**). The estrous cycle phase was not controlled at euthanasia, as hormone assessment was a secondary endpoint; this may have limited sensitivity to detect group differences, particularly for LH.

Sex hormone-binding globulin (SHBG) is a marker of hepatic estrogenic effect; we observed no significant differences in median values among groups (**Supplementary Figure 1**). This contrasts with premenopausal women, in whom both combined HC and tamoxifen increase circulating SHBG. Rats and mice largely lack postnatal hepatic SHBG expression[14], which may explain the absence of a treatment-related signal in our experiment.

Next, we assessed effects of HC and tamoxifen on the uterus and ovaries. Normalized uterine weight (ratio of wet uterine weight to body weight) displayed medians of 2.21 in controls, 2.77 in HC, 1.51 in tamoxifen, 1.64 in tamoxifen+HC groups (**Figure 2d)**. Uterine weight was significantly lower in both tamoxifen-treated groups, compared to HC alone (p<0.0001, p=0.0045) and control (p=0.0005). Median endometrial thickness (μm) did not differ significantly with any treatment compared to controls; control 41.9, HC 38.5, tamoxifen 45.6, tamoxifen+HC 47.3 (**Figure 2e**), although it trended 8% lower with HC and 9–13% higher with tamoxifen and tamoxifen+HC than in controls. Endometrial thickness was significantly higher in tamoxifen+HC compared to HC alone (p<0.05), while tamoxifen alone did not differ significantly from HC (p=0.1745) (**Figure 2e**). Endometrial gland loss and non-keratinizing epithelial squamous metaplasia (ESM) occurred in 12/12 (100%) tamoxifen-alone and 11/13 (85%) tamoxifen+HC animals. Keratinizing ESM was infrequent in both tamoxifen-treated groups (2/12 and 1/13). Both keratinizing and non-keratinizing ESM are considered benign and ER-driven in estrogen-treated rats[15]. Long-standing clinical trial evidence indicates no increased endometrial cancer risk in premenopausal tamoxifen users, while in postmenopausal women tamoxifen increases risk of uterine adenocarcinomas, not squamous cell carcinomas. Absence of corpora lutea in all tamoxifen alone and tamoxifen+HC groups confirmed anovulatory status.

We report the first direct preclinical evidence that the cancer-preventive efficacy of tamoxifen at an HED of 7 mg/day is not reduced by HED of combined HC (EE+LNG) in an ER-positive mammary tumorigenesis model. Surprisingly, HC-alone treatment displayed significant cancer-preventive efficacy.

The reason for this is not clear; it may relate to overall decreased estrogen and progesterone exposure of the HC group over the 6 months of treatment, in a model that is exquisitely sensitive to hormonal effects. But this is speculative since we only measured hormones at a single timepoint at the end of the experiment. In humans, HC use is associated with a small but significant increase in breast cancer incidence[5,16]. TAM-associated reproductive adverse events were not exacerbated by HC co-administration.

Several limitations bear on the translational interpretation of our findings. Tamoxifen acts as a full central ER antagonist in rats, completely suppressing the LH/FSH surge and abolishing ovulation[17], whereas in premenopausal women, tamoxifen exerts only modest central antagonism, and ovulatory cycles are commonly maintained[18]. Additionally, we evaluated only one HC formulation, therefore the effects of the progestin-only IUDs and oral pills, or other combined oral formulations need further study. Recent large-scale registry data indicate that breast cancer risk varies substantially by progestin type, with desogestrel-containing formulations carrying markedly higher risk than LNG or drospirenone and combined oral drospirenone showing no statistically significant risk elevation[19]; whether these biological differences extend to differential effects on tamoxifen efficacy is unknown. Nevertheless, our findings are reassuring regarding the maintenance of tamoxifen efficacy in the presence of the most used HC world-wide and suggest that the current clinical caution against concomitant HC and tamoxifen use reflects an evidence gap that needs to be filled, rather than a demonstrated harm. Further prospective evaluation should be pursued in humans, using both laboratory and clinical research settings. Key questions for the field include whether specific HC formulations, particularly those with anti-androgenic progestins such as drospirenone can be safely paired with tamoxifen without compromising cancer prevention or uterine safety.

Methods

Materials

MNU (cat# HY-34758), 17α-ethinylestradiol (cat# HY-B0216), and levonorgestrel (cat# HY-B0257) were obtained from MedChem Express Inc (Monmouth Junction, NJ). 17a-ethynylestradiol 0.07 mg/pellet+levonorgestrel 0.3 mg/pellet, 90-day extended-release HC pellets (cat#NX-999) were custom-ordered from Innovative Research of America (Sarasota, FL). Custom formulations of global 14% protein diet (cat# 2014) with or without 30 ppm tamoxifen were obtained from Envigo.(Indianapolis, IN). Rat blood was collected in 1.3mL EDTA coated tubes (cat# 41.1395.105) obtained from Sarstedt (Nümbrecht, Germany). Tissues were fixed in 10% Neutral Buffered Formalin (cat#22-050-104) obtained from Fisher Scientific (Pittsburgh, PA). We obtained enzyme-linked immunosorbent assay (ELISA) kits to measure plasma hormones: estradiol (cat# ES180S-100) was obtained from Calbiotech (El Cajon, California), progesterone (cat# 80559) was obtained from Crystal Chem (Itasca, IL), follicle-stimulating hormone (cat# CSB-E06869r) and luteinizing hormone (cat# CSB-E12654r) were both obtained from CUSAbio (Houston, TX), and sex hormone binding globulin (cat# SEA396Ra) was obtained from Cloud-Clone Corp (Katy, Texas).

Animal Study

All procedures followed the Animal Care and Use Committee guidelines of Northwestern University. We obtained 50 female Sprague Dawley rats (Strain #202F, 3-4 weeks old) from the Inotiv Inc (Indianapolis, IN). After 1-week acclimation, the rats received a single intraperitoneal injection of MNU and fed with base 14% protein diet to minimize phytoestrogen. MNU was dissolved in physiological saline, pH 5.0 and given at 50 mg/kg as described in our previous study[8]. Three weeks later, they were randomized to four intervention groups: control, HC alone, tamoxifen alone, and tamoxifen+HC (n=12–13 per group). Subcutaneous 90-day extended-release pellets containing EE (0.07 mg) + LNG (0.3 mg) were implanted and replaced at 90 days. Tamoxifen was incorporated into the diet at 1.03 mg/kg/day (HED 7 mg/day), a dose selected to allow 10–20% cancer incidence. HC pellets were subcutaneously implanted in dorsal

neck area and replaced around 90 days. Tamoxifen diets started one day after HC implantation. We monitored diet consumption, body weight and mammary tumor formation twice weekly. Animals underwent euthanasia and necropsy when tumor burden reached 4cm3 or at the 6-month study completion. Mammary tumor latency, weight and volumes were recorded. Wet uterine and ovaries were weighed.

Tissue Processing and Histology Evaluation

Whole mammary glands, and mammary tumors were collected. Uterine specimens were collected with ovaries. One horn with ovary was for longitudinal sections. The other horn was cut into 6 transverse pieces. Tissue specimens were fixed in 10% neutral-buffered formalin buffer (72 h) and processed to paraffin embedded blocks, sectioned at 4μm, Hematoxylin and Eosin (H&E) stained, and digitally scanned at the Pathology Core facility of Northwestern University. Uterine tissues were positioned such that one horn yielded longitudinal slices, and the 6 pieces yielded transverse slices.

Histology of mammary glands and mammary tumors and ovarian tissues were histopathologically evaluated by a single blinded laboratory pathologist (M.C.B.) and tumors were classified according to Russo and Russo [20].

Luminal epithelial thickness was measured from complete uterus transverse sections[21,22] by averaging five replicate measurements taken at thickest region around luminal epithelium for at least three complete transverse H&E pieces of uterus using NDP.view2Plus software (Hamamatsu Photonics Inc.). For a sample to pass quality check, at least three of 6 transverse samples were required to have intact, inner myometrium, outer myometrium, endometrium, and no missing endometrial luminal epithelium. Uterine transverse sections from animals 15 (control group), 33, and 36 (both HC+TAM group) failed to meet criteria and omitted. Median and mean number of transverse samples passing quality check = 4.0. Uterine and ovarian histopathology were classified by a single blinded laboratory pathology (M.C.B.) for ovarian cysts, ovarian corpora lutea, ovarian follicles, epithelial ESM, and presence of epithelial glands[15,23].

Hormone Assay

Rat blood plasma samples were quantified in singlet with Enzyme-Linked Immunosorbent Assay (ELISA) and a Spectra Max Plus 384 microplate reader from Molecular Devices (San Jose, CA) at the Northwestern University Comprehensive Metabolic Core.

Statistical Analysis

Statistical analysis was done in GraphPad Prism. Invasive cancer free survival curve and cancer incidence was analyzed with the log-rank (Mantel-Cox) test. Kruskal–Wallis test with Dunn's correction for multiple comparisons was used to analyze circulating hormone concentration, ratio of uterine weight to body weight, and endometrial luminal epithelial thickness.

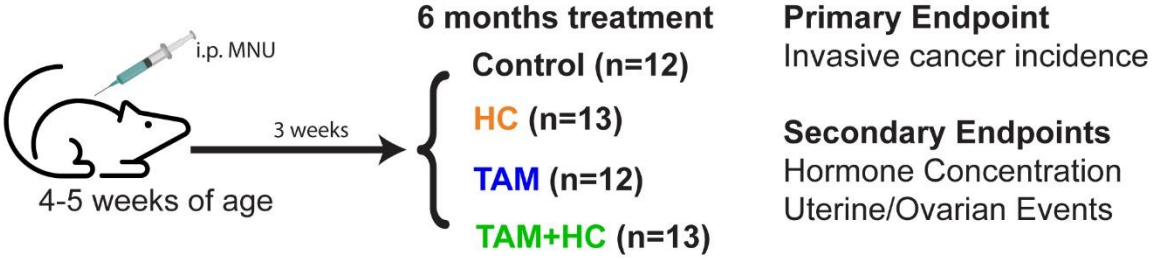
Data availability: all data will be made available on manuscript acceptance

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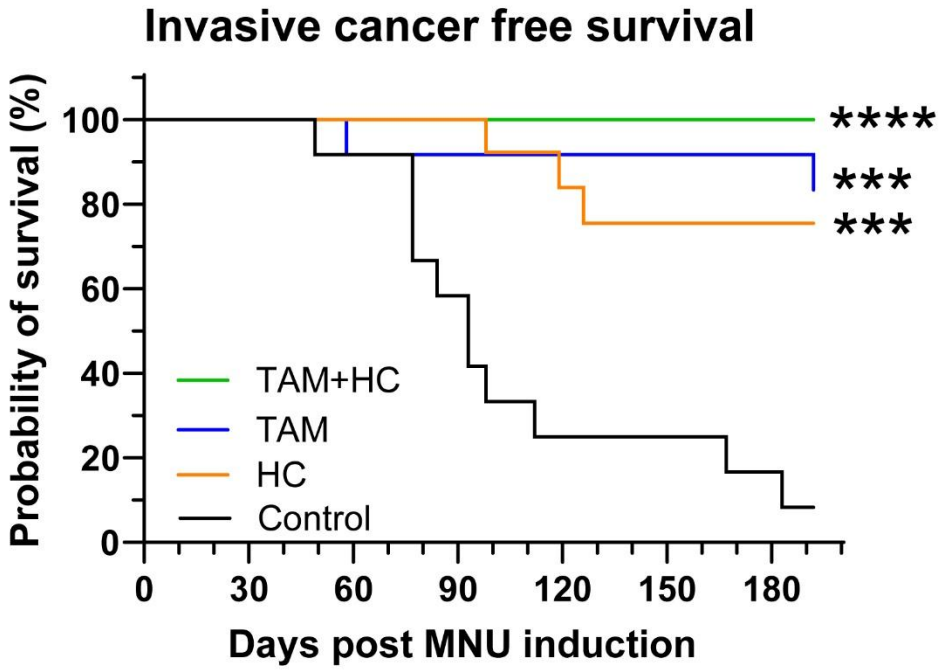
Author contributions: O.L. and S.A.K. contributed to conceptualization and manuscript review and editing. O.L. designed the experiments. O.L. and A.D.E. conducted experiments, acquired data, analyzed the data, and drafted the manuscript. M.C.B. performed pathology review and contributed to manuscript review and editing.

Competing interests: No authors have competing interests to declare.

a.



b.



c.

Treatment Groups	Invasive Cancer	Fibroadenoma	Hyperplasia
Control (n=12)	11 (91.7%)	0 (0%)	0 (0%)
HC (n=13)	3 (23.1%)	3 (23.1%)	0 (0%)
TAM (n=12)	2 (16.7%)	0 (0%)	0 (0%)
TAM + HC (n=13)	0 (0%)	2 (15.4%)	1 (7.7%)

Figure 1. Cancer-preventive efficacy of tamoxifen (TAM) is preserved with HC co-administration. **a)** Study schema. Female SD rats (n=50) received a single injection of MNU (50 mg/kg i.p.) at 4–5 weeks of age. Three weeks later the animals were randomized to four intervention groups and followed for six months. HC pellets (EE 0.06 mg + LNG 0.28 mg, 90-day release) were implanted subcutaneously and replaced at day 90. TAM was delivered in the diet at 1.03 mg/kg/day. **b)** Invasive cancer free survival curves. Comparison between all 4 groups $p < 0.0001$ and compared to control was done with the log-rank (Mantel-Cox) test $p < 0.001$ (***), $p < 0.0001$ (****). **c)** Summary of histology of tumors and mammary gland table.

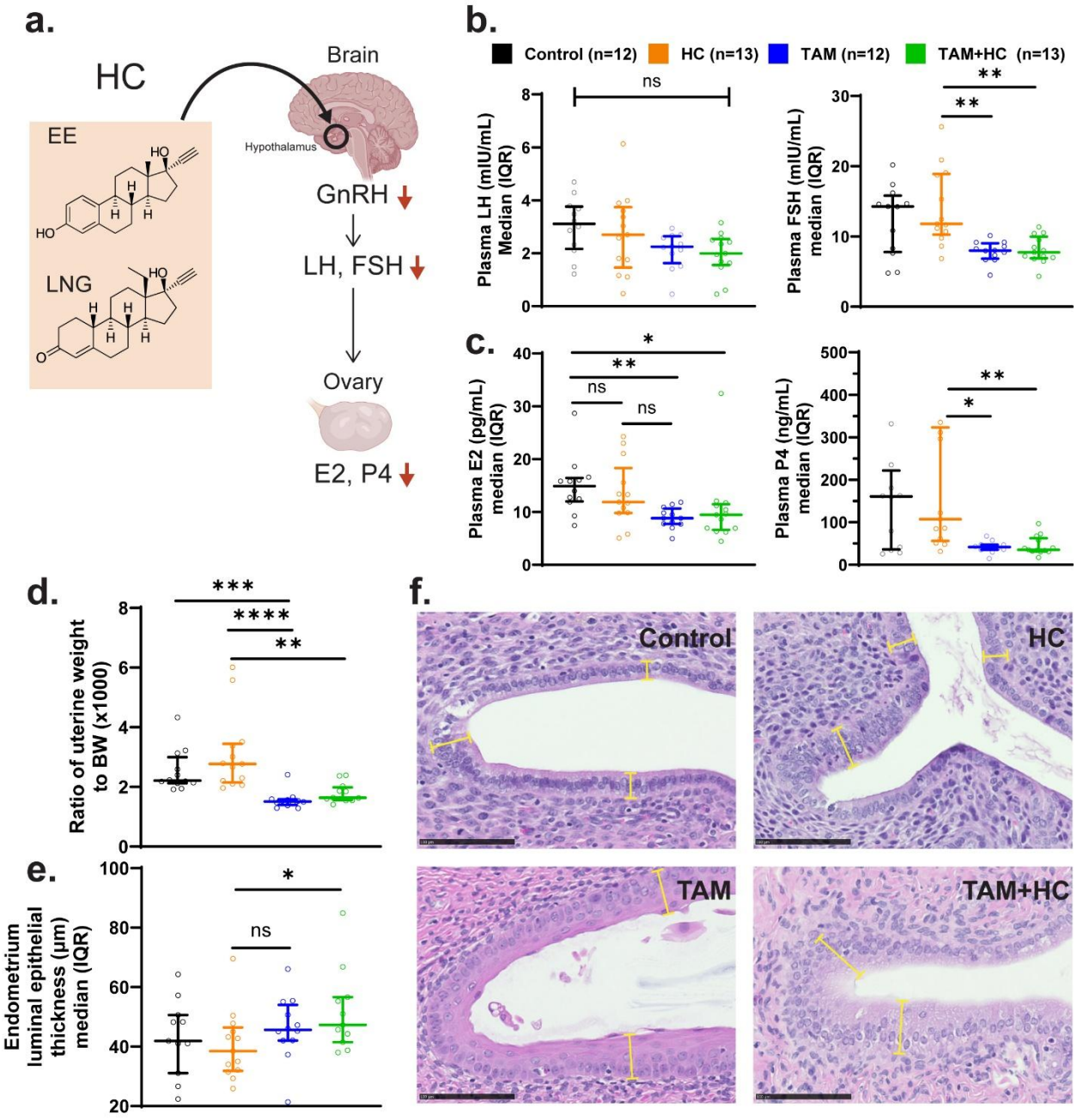


Figure 2. HC co-administration does not exacerbate tamoxifen (TAM)-associated reproductive effects. **a)** Schematic of the expected HC (EE+LNG) repression on human HPG and ovarian axis. HPG axis organ representations were sourced from BioRender. **b)** Circulating gonadotropins (LH, FSH) concentration at study completion. **c)** Circulating ovarian hormones (E2, P4) concentration at study completion. **d)** Uterine wet weight normalized to body weight at study completion. **e)** Transverse section endometrial luminal epithelial thickness. **f)** Representative H&E transverse sections with representative endometrial luminal epithelial measurements (yellow); scale bar, 100 µm. Statistical significance was calculated using Kruskal–Wallis test with Dunn's multiple comparison: adjusted $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***), $p<0.0001$ (****); ns, not significant.

Supplemental Figure

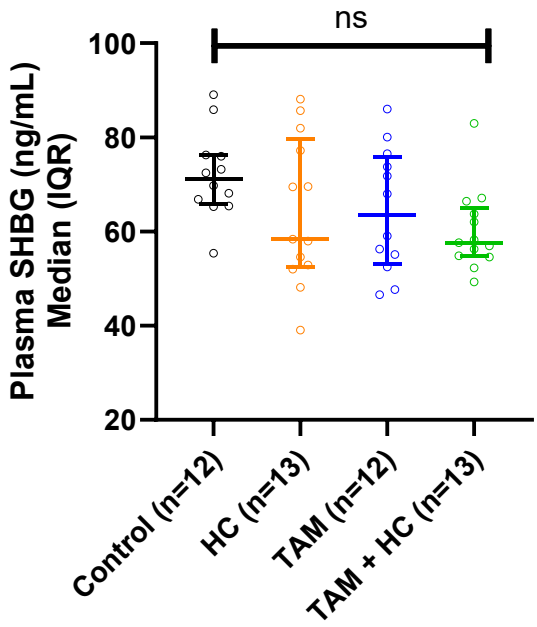


Figure S1. Circulating SHBG concentration at study completion. Statistical significance was calculated using Kruskal–Wallis test with Dunn's multiple comparison: ns, not significant.

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	Item	Question(s)	Answers
1	Study Design	Are all experimental and control groups clearly identified?	☒ Yes, for at least one experiment ☐ No
		Is the experimental unit (e.g. an animal, litter or cage of animals) clearly identified?	☒ Yes, for at least one experiment ☐ No
2	Sample Size	Is the exact number of experimental units in each group at the start of the study provided (e.g. in the format '*n*='')?	☒ Yes, for at least one experiment ☐ No
		Is the method by which the sample size was chosen explained?	☒ Yes, for at least one experiment ☐ No
3	Inclusion & Exclusion Criteria	Are the criteria used for including and excluding animals, experimental units, or data points provided?	☒ Yes, for at least one experiment ☐ No
		Are any exclusions of animals, experimental units, or data points reported, or is there a statement indicating that there were no exclusions?	☒ Yes, for at least one analysis ☐ No
4	Randomisation	Is the method by which experimental units were allocated to control and treatment groups described?	☒ Yes, for at least one experiment ☐ No
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6	Outcome Measures	For all experimental outcomes presented, are details provided of exactly what parameter was measured?	☒ Yes, for at least one experiment ☐ No
7	Statistical Methods	Is the statistical approach used to analyse each outcome detailed?	☒ Yes, for at least one analysis ☐ No
		Is there a description of any methods used to assess whether data met statistical assumptions?	☒ Yes, for at least one analysis ☐ No ☐ Not applicable
8	Experimental Animals	Are all species of animal used specified?	☒ Yes, for at least one experiment ☐ No
		Is the sex of the animals specified?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to species
		Is at least one of age, weight or developmental stage of the animals specified?	☒ Yes, for at least one experiment ☐ No
9	Experimental Procedures	Are both the timing and frequency with which procedures took place specified?	☒ Yes, for at least one experiment ☐ No
		Are details of acclimatisation periods to experimental locations provided?	☐ Yes, for at least one experiment ☐ No
10	Results	Are descriptive statistics for each experimental group provided, with a measure of variability (e.g. mean and SD, or median and range)?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to the type of data collected
		Is the effect size and confidence interval provided?	☒ Yes, for at least one experiment ☐ No ☐ Not applicable to the type of analysis used

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