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Hypothyroidism induces uterine hyperplasia and inflammation related to sex hormone receptors expression in virgin rabbits

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ABSTRACT

Aims: In women, uterine alterations have been associated with sex steroid hormones. Sex hormones regulate the expression of thyroid hormone receptors (TRs) in the uterus, but an inverse link is unknown. We analyzed the impact of hypothyroidism on histological characteristics, vascular endothelial growth factor (VEGF-A), progesterone receptors (PR), estrogen receptors (ER), thyroid hormone receptors (TRs), perilipin (PLIN-A), and lipid content in the uterus of virgin rabbits.

Main methods: Twelve Chinchilla-breed adult female rabbits were grouped into control (n = 6) and hypothyroid (n = 6; 0.02% of methimazole for 30 days). The thickness of endometrium and myometrium, number of uterine glands, and infiltration of immune cells were analyzed. The expression of VEGF-A, PR, ERα, and PLIN-A was determined by RT-PCR and western blot. The uterine content of triglycerides (TAG), total cholesterol (TC), and malondialdehyde (MDA) was quantified.

Key findings: Hypothyroidism promoted uterine hyperplasia and a high infiltration of immune cells into the endometrium, including macrophages CD163+. It also increased the expression of VEGF-A, TRA, and ERα-66 but reduced that of PR and ERα-46. The uterine content of PLIN-A, TAG, and TC was reduced, but that of MDA was augmented in hypothyroid rabbits.

Significance: Our results suggest that uterine hyperplasia and inflammation promoted by hypothyroidism should be related to changes in the VEGF-A, PR, ER, and TRs expression, as well as to modifications in the PLIN-A expression, lipid content, and oxidative status. These results suggest that hypothyroidism should affect the fertility of females.

1. Introduction

In women, endometriosis, uterine hyperplasia, dysfunctional uterine bleeding and myomas have been associated with alterations in serum levels of estradiol (E2), testosterone, and progesterone (P4) [1,2], as well as with changes in the uterine expression of progesterone (PRs) and estrogen (ERs) receptors [3,4]. Uterine alterations have also been related to a high expression of vascular endothelial growth factor (VEGF) [5], immune cells infiltration [5,6], and significant uterine lipid peroxidation [7]. Furthermore, uterine abnormalities are correlated with dyslipidemias and body weight gain [8], and recently with hypothyroidism [9–12].

Particularly, the impact of hypothyroidism on the uterus has been mostly studied in pregnant laboratory animals. In rats, this thyroid dysfunction increases gestational length, modifies myometrial contractions, and decreases litter size [13]. It also modifies the expression of thyroid hormones receptors (TRs) [14], promotes inflammation and alters the immune profile in the uterus affecting the trophoblast migration [15]. In rabbits, hypothyroidism reduces the thoracic size of embryos and increases the thickness of the endometrium, as well as

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reduces the content of total cholesterol (TC) and triacylglycerol (TAG), and the expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) in the uterine horn [16]. However, there are limited studies about the effect of hypothyroidism on the uterus from non-pregnant animal models. In young rats, hypothyroidism induces a low uterine anti-oxidative status [17]. A reduction in the uterus volume and luminal epithelium thickness have been found in hypothyroid non-pregnant rats [18]. It is necessary to perform more studies to analyze the impact of hypothyroidism on the uterus of non-pregnant animals.

The TRs and thyrotropin receptors (TSHR) are present in the endometrium and myometrium [19,20]. The expression or activity of these receptors can be modified by circulating E2 and P4 [16,21,22]. In contrast, the influence of thyroid hormones on the uterine expression of ERs and PRs is little known.

The present study aimed to analyze the impact of hypothyroidism on the histology, immune cells infiltration, vascularization, and expression of VEGF-A, PRs, ERα, TRs, and perilipin (PLIN-A), as well as the lipid content and lipid peroxidation in the uterus of adult virgin female rabbits.

2. Material and methods

Twelve chinchilla-breed nulliparous female rabbits (*Oryctolagus cuniculus*, 8–9 months of age) were housed under controlled temperature (20 ± 2 °C) and light: dark cycle of 16:8 h. This condition maintained most females in early proestrus [23]. They were daily provided with pellet food (120 g/day) and tap water *ad libitum*. Hypothyroidism was induced with a one-month administration of 0.02% methimazole (Sigma, MO, USA; approximate diary dosage of 10 mg/kg) in drinking water for 30 days. This treatment is useful to induce hypothyroidism in female rabbits [24]. At the end of this treatment, control (n = 6) and hypothyroid (n = 6) rabbits were anesthetized with sodium pentobarbital (90 mg/kg, i.p.), and subsequently euthanized with an overdose of the same anesthetic. Cardiac blood was obtained and serum concentrations of total triiodothyronine (T3), thyroxine (T4) and free T4 were quantified using chemiluminescence by the Diagnóstico Molecular y Servicio de Referencia S.A. de C.V. (Diagmo laboratory; México). Immediately after death, right and left uterine horns were excised. The Ethics Committee from the Universidad Autónoma de Tlaxcala approved this experimental design, according to the Guidelines of the Mexican Law for Production, Care and Use of Laboratory Animals.

A piece from the middle portion of the left uterine horn was collected and histologically processed to be cut in the cryostat at 5 μm. Another piece from the middle portion of the left uterine horn was embedded in paraplast X-tra (Sigma, MO, USA) and transversally cut at a thickness of 5 μm using a microtome (Thermo Scientific, Model Finesse 325, MA, USA). The middle portion of the right uterine horn was frozen at −80 °C for biochemical measures.

2.1. Morphometry of the uterine horns

One slide with six slices of the left uterine horn cut with microtome per animal was deparaffinized, rehydrated and stained with Masson's trichrome. Slides were observed and photographed. In the best-stained slice, pictures at 4× (Zeiss Axio Imager A1, Oberkochen, Germany) were taken. The area covered by endometrium and thickness of endometrium and myometrium (in 20 microscopic fields from one slice per animal) were measured using the software AxioVision 4.8 (Carl Zeiss Micro Imaging, Inc.). Histograms of the measurements for the thickness of endometrium and myometrium were obtained, and the percentage of the measurements > 1400 μm for endometrium and > 1000 μm for myometrium were calculated. In pictures at 400 magnifications, randomly pictures from 16 microscopic fields of the endometrium were taken per rabbit. The number, thickness and external cross-sectional area (CSA) of closed uterine glands were measured. The number of uterine fused glands was also counted. Moreover, the number of blood vessels and areas covered by them were quantified in 8 random fields per uterus. Additionally, other slides were stained with hematoxylin-eosin to detect the presence of immune cells inside epithelium in pictures at 1000 magnifications in 30 microscopic fields randomly selected.

Other slides were deparaffinized and processed for immunohistochemistry. Endogenous peroxidases were quenched, and endogenous binding was blocked with 10% of donkey serum (Jackson Immuno Research Inc. PA, USA). Independent sections were incubated with primary antibody to detect macrophages CD163 positives (1:20, goat polyclonal antibody Santa Cruz Biotechnology, TX, USA; sc-18794) for overnight at 4 °C. Subsequently, they were incubated with secondary antibodies (1:250; donkey anti-goat antibody Santa Cruz Biotechnology, TX, USA) and diluted in PBST for 2 h at 37 °C. Immunostaining was developed using the ABC method and sections were washed and counterstained with Mayer's hematoxylin. Macrophages CD163 positives secrete both pro- and anti-inflammatory cytokines and pro-angiogenic factors [25].

2.2. Expression of VEGF, ERα, PR, TRA, TRB, and PLIN in the uterine horns

A portion of the right uterine horn (~50 mg) was homogenized in trizol reagent (Invitrogen, CA, USA) according to manufacturer's protocol. The quantity and purity of RNA were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA). The RNA integrity was determined by electrophoresis. For this, 1 μg of each RNA sample in a 1.5% agarose gel with 0.5× tris-borate buffer (TB), and only total RNA samples with 260/280 and 230/260 relations close to 2.0, and integrity represented by two defined 28S and 18S bands without smear visualized on an agarose gel were used for RT-PCR. Then, 1 μg of RNA was used to synthesize the first-strand cDNA with the Moloney murine leukemia virus (M-MLV) reverse transcriptase (Thermo Scientific, MA, USA) following the manufacturer's instructions. Posteriorly, 2 μl of this reaction were subjected for PCR to amplify VEGF-A, PR, ERα and thyroid hormone receptors (alpha, TRA; and beta, TRB). Sense and antisense primers were purchased from Sigma-Aldrich (MO, USA). Table 1 contains all the information of the primers for PCR. Negative controls without cDNA were included in all experiments. The PCR reaction was performed as follows: an initial PCR activation step at 94 °C (5 min), 30 cycles of denaturation at 94 °C (20 s), annealing at 60 °C (30 s), and elongation at 72 °C (30 s). A final extension cycle was performed at 72 °C (3 min). The number of cycles performed was within the exponential phase of the amplification process. PCR products were separated by electrophoresis in a 1.5% agarose gel at 70 V for 120 min and stained with EpiQuik (Epigentek, NY, USA). The gel image was captured under a UV trans-illuminator and analyzed for band densitometry using the Image J software (National Institute of Health, USA). The expression of VEGF-A, PR, ERα, TRA, and TRB was

Table 1
Primers used for gene amplifications.

Gene	Primer sequence	Amplified fragment
VEGF-A	FW: 5′-CCACACCGCCACCACCGACA-3′	149
VEGF-A	RV: 5′-CCAATTCCAAGAGGGCCCGT-3′	
PR	FW: 5′-GTCCTTGGAGGGCGAAAGTT-3′	163
PR	RV: 5′-ACAGGTTGATTAGAGGGGGA-3′	
ERα	FW: 5′-AGGGTTCCAGGCTTTGTGGA-3′	181
ERα	RV: 5′ CCACCATGCCCTCTACACATT-3′	
THRA	FW: 5′-ACAGTGCCAGGTCACCAGAT-3′	195
THRA	RV: 5′-GGATTGTGCGGCGAAAGAAG-3′	
THRB	FW: 5′-ACCTTGAACGGGGAAATGGC-3′	170
THRB	RV: 5′-CCTGGGCGATCTGAGGACAT-3′	
18S	FW: 5′-AGTGAAACTGCAATGGCTC-3′	167
18S	RV: 5′-CTGACCGGGTTGGTTTTGAT-3′	

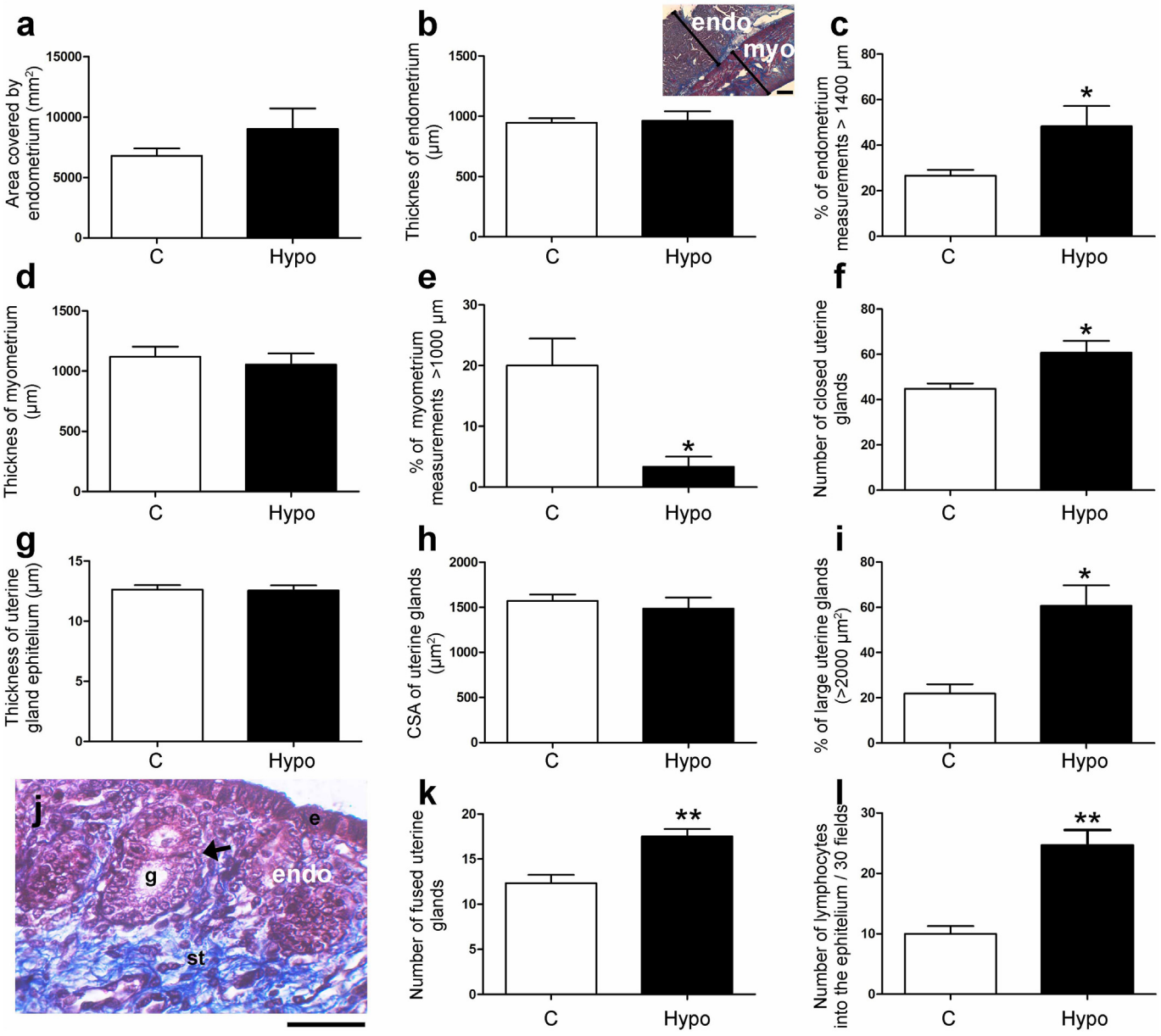


Fig. 1. Histological characteristics of the uterine horn from control (C, white bars) and hypothyroid (hypo, black bars) rabbits. Endometrium (endo; **a,** area covered; **b,** thickness; and **c,** percentage of measurements > 1400 µm). Myometrium (myo; **d,** thickness; **e**; percentage of measurements > 1000 µm). Uterine glands (**f**, number of closed glands; **g**, thickness of epithelium; **h**, CSA of glands; **i**, percentage of large glands; **j**, examples of fused glands; **k**, number of fused glands; and **l**, infiltration of lymphocytes). Results are expressed as mean ± S.E.M. $*p \leq 0.05$; $**p \leq 0.01$. Other abbreviations: g, uterine gland; st, stroma, e, epithelium. Scale: b = 400 µm and j = 50 µm. Arrow indicates a fused uterine gland.

normalized to that of the internal control 18S rRNA.

A portion of the right uterine horn (~50 mg) was disrupted in lysis buffer as reported elsewhere [26]. Total proteins were obtained by centrifugation at 15000 rpm, at 4 °C for 30 min and quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA). Protein extracts (VEGF-A, 20 µg; ERα, 30 µg; PR, 60 µg; and PLIN-A, 30 µg) were resolved onto SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Laboratories Headquarters, CA, USA). The PLIN-A regulates the lipid storage into droplets on tissues [27], and its expression is regulated by hypothyroidism in the ovary [26]. Membranes were stained with Ponceau's Red to confirm that protein content was equal in all lines. Membranes were soaked as indicated: 3.0% nonfat dry milk plus 2.0% bovine serum albumin (BSA) (PLIN-A, PR, and ERα) or 15% non-dry milk (VEGF-A), all diluted in PBS containing 0.2% Tween-20 (PBST). Then, they were incubated overnight at 4 °C with the following antibodies: rabbit polyclonal anti VEGF-A (0.5 µg/ml; Santa Cruz Biotechnology, sc-152); goat polyclonal anti PLIN-A (1 µg/ml, Abcam, MA, USA; ab60269), mouse monoclonal anti-PR (1 µg/ml, Abcam, ab58565) and mouse monoclonal anti-ERα (0.5 µg/ml; Santa Cruz Biotechnology, TX, USA; sc-8002). Following an incubation with a secondary antibody (VEGF-A: 1:20000 mouse anti-rabbit; Santa Cruz Biotechnology, sc-2357; PLIN-A: 1:2000 mouse anti-goat, Santa Cruz Biotechnology, sc-2354; PR and ERα: 1:5000 goat anti-mouse; Pierce, 1,858,413) conjugated with horseradish peroxidase at room temperature under constant agitation for 45 min. Chemiluminescent signals were detected exposing membranes to Kodak Biomax Light films (Sigma-Aldrich, MO, USA) using a chemiluminescence kit (West Pico Signal, Thermo Scientific, MA, USA). The band density for the antigen-antibody complex was calculated in a semi-quantitative way using a 14.1 megapixels digital Canon camera (SD1400IS, Canon, Mexico) and

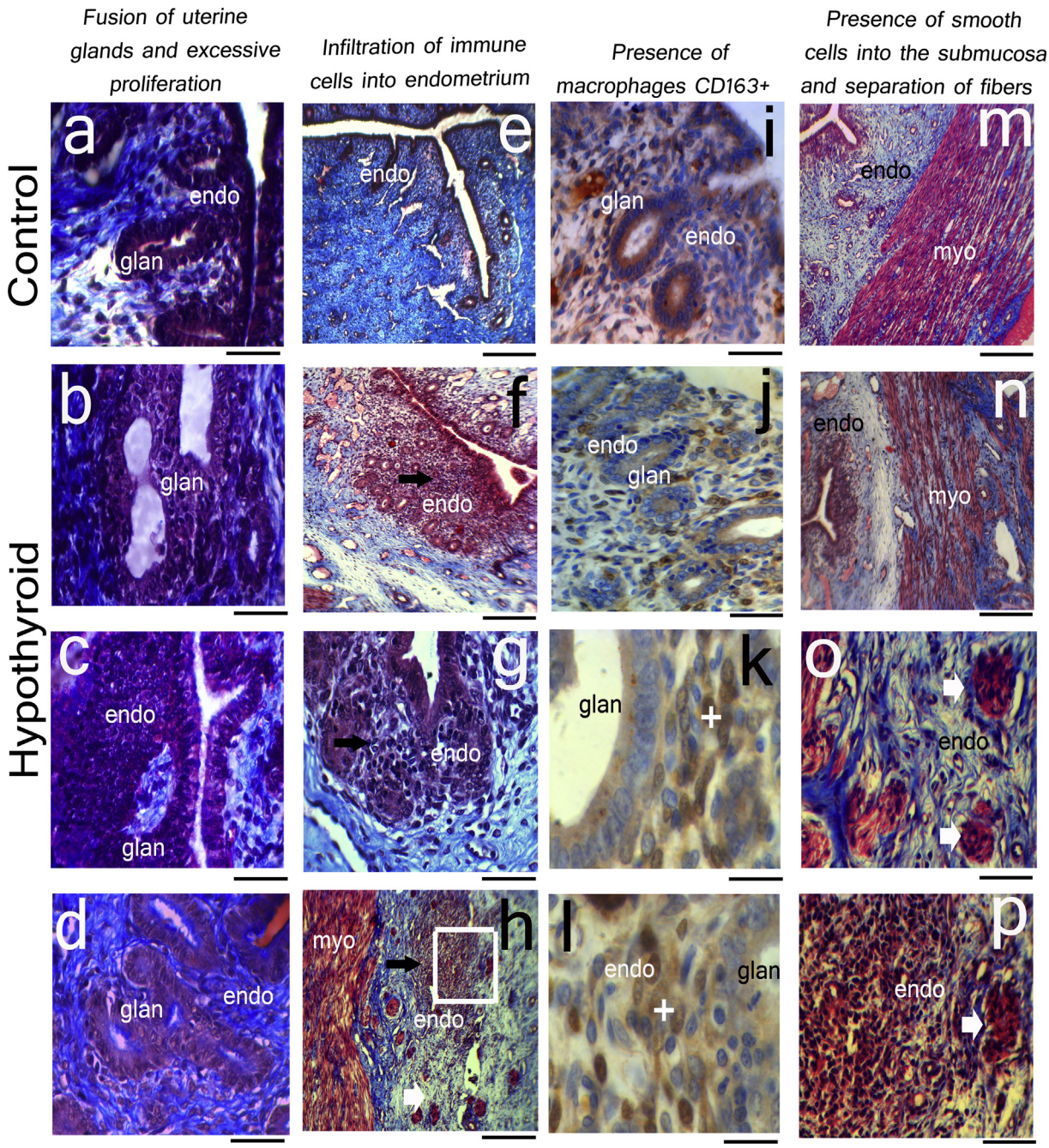


Fig. 2. Characteristics of the endometrium (endo) and myometrium (myo) of control and hypothyroid rabbits. Compared to control (**a**), hypothyroidism promoted the thickening of the glandular epithelium and the fusion of uterine glands (glan; **b–d**). A great infiltration of immune cells (black arrows) into the endometrium was observed in hypothyroid rabbits (**f–g**), even showing some points of inflammation (**h**) that were not observed in the uterus from control rabbits (**e**). Many of these immune cells were macrophages CD163+ (+) (control, **i**; hypothyroid, **j–l**). The organization of smooth muscle fibers of the myometrium was compacted in the control group (**m**) but separated in the hypothyroid group (**n**). Also, groups of smooth muscle cells were observed into the endometrium (white arrows; **o–p**) near inflammatory points. The picture **p** is the enlargement from the white square of the picture **h**. Scale a–d, g, i–j, and o–p = 50 μm; e–f, h, and m–n = 500 μm; k–i = 20 μm.

the Image J 14.45S software (National Institute of Health, USA). To correct differences in the total protein loaded in each lane VEGF-A, PR, ERα, and PLIN-A, protein content was normalized to that of α-tubulin. Therefore, blots were stripped with a 0.1 M glycine solution (pH 2.5, 0.5% SDS) for 2 h at 37 °C and incubated with mouse monoclonal anti-α-tubulin (0.7 μg/ml, Santa Cruz Biotechnology, sc-5286) at 4 °C overnight. Blots were then incubated with a goat-anti-mouse secondary antibody (1:5000; Pierce, 1,858,413) at room temperature for 45 min under constant agitation. Immunoblot chemiluminescent signals were detected as described.

2.3. Lipids in the uterine horns

The quantification of TC and TAG were done in samples of the right uterine horn (~100 mg) by using the method of Folch as reported elsewhere [16]. Enzymatic method-based kits of ELITech Clinical Systems (Sees, France) were used to measure TC (CHSL-0507) and TAG (TGML-0425). Uterine TC and TAG concentrations were expressed as mg of TC or TAG per gram of the uterus.

A portion of the right uterine horn (25 mg) was homogenated in ice-cold tris-buffer (20 mM, pH 7.4) and centrifuged at 3000 rpm for 10 min

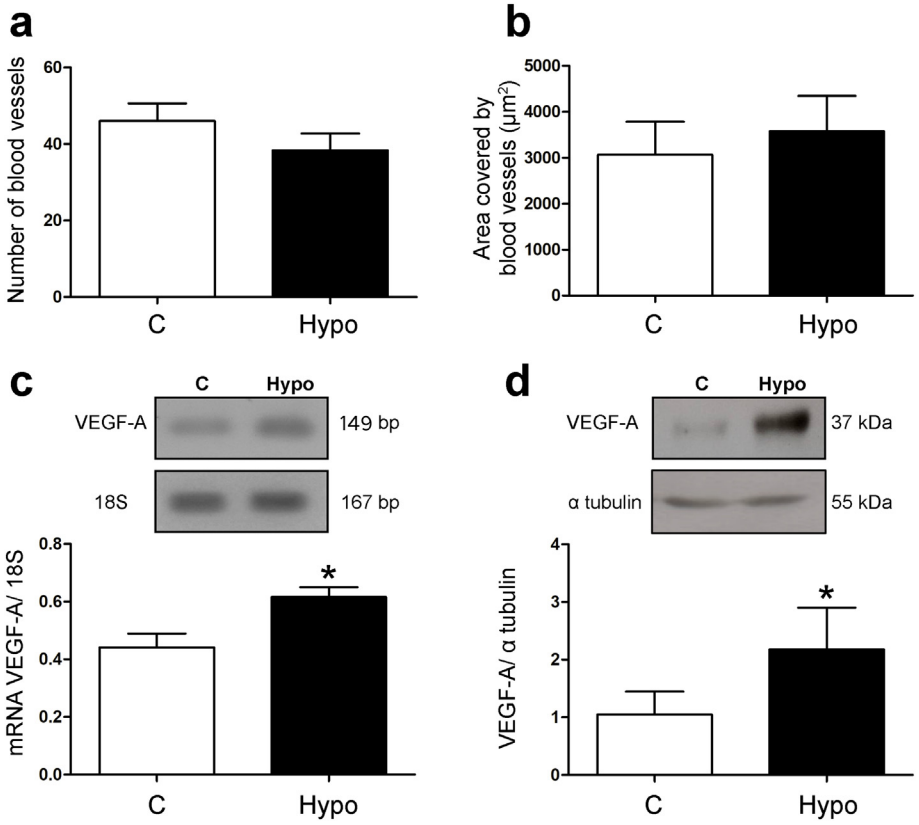


Fig. 3. (**a, b**) Blood vessel characteristics in the uterine horn from control (C, white bars) and hypothyroid (hypo, black bars) rabbits. Representative PCR or western blot (upper panel) and densitometry analysis showing the VEGF-A mRNA (**c**) and protein (**d**) expression in the uterine horn. Results are expressed as mean ± S.E.M. $^*p \leq 0.05$.

at 4 °C, and then the supernatant was collected and immediately tested for lipid peroxidation with ALDetect lipid peroxidation assay kit (BML-AK170-0001, Enzo Life Sciences, Ann Arbor, MI, USA). The kit uses a chromatographic reagent which reacts with malondialdehyde (MDA) at 70 °C, yielding a stable chromophore with maximum absorbance at 586 nm. Uterine MDA concentrations were expressed as μM de MDA/mg of the uterus.

Additionally, a slide with left uterine horn slices cut in cryostat was stained with Sudan black to detect oxidative lipids, counterstained with Harris hematoxylin, and mounted in glycerol. Sections were observed in a light microscope (Zeiss Axio Imager A1, Oberkochen, Germany) and pictures were made with a digital camera (ProgRES, Jenoptik, Jena Germany) at 400 and 1000 magnifications. The presence of granules of oxidative lipids in the endometrium (luminal epithelium, uterine glands, and stroma) was evaluated semi-qualitatively as previously described [26]. Categories were established: (+++) for a high proportion of stained granules; (++) for a moderate proportion of stained granules; and (+) for a low proportion of stained granules.

2.4. Statistical analysis

Statistical analyses were performed with the GraphPad Prism v 5.01 software (GraphPad Sofware, Inc., CA, USA). Results were expressed as mean ± S.E.M. Student *t*-tests were performed to determine significant differences between control and hypothyroid rabbits. The values of $p \leq 0.05$ were considered statistically significant.

3. Results

The area covered by the endometrium and their media thickness was similar between control and hypothyroid groups (Fig. 1a–b; see in the picture the measurements for the thickness of endometrium and myometrium). However, the percentage of the measurements of the thickness of the endometrium (> 1400 μm) was significantly high in the hypothyroid group (Fig. 1c). No differences were found in the thickness of myometrium between groups (Fig. 1d), but a low percentage of measurements > 1000 μm were found in the hypothyroid group (Fig. 1e). The number of closed uterine glands was higher in hypothyroid dams (Fig. 1f), but the thickness and the CSA of closed uterine glands were not modified (Fig. 1g–h). The percentage of large uterine glands (> 2000 μm^2) was higher in the hypothyroid group (Fig. 1i). Hypothyroidism also increased the number of fused uterine glands, which were commonly found near the luminal epithelium (Fig. 1j–k). The number of lymphocytes into the luminal epithelium of the uterine horn from hypothyroid rabbits was higher than in control ones (Fig. 1l).

Tissue characteristics for endometrium and myometrium from control and hypothyroid rabbits are shown in Fig. 2. Compared to controls (Fig. 2a), hypothyroidism promoted the thickening of the glandular epithelium and the fusion of uterine glands (Fig. 2b–d). Although the infiltration of immune cells in the endometrium from control rabbits is common (Fig. 2e), a great infiltration was observed in hypothyroid rabbits (Fig. 2f–g). Even it was possible to observe some points of inflammation (Fig. 2h). Many of these immune cells infiltrated into the endometrium were macrophages CD163+ (Fig. 2i; for controls; and Fig. 2j–l for hypothyroid). For the control group, myometrium was observed uniform and the smooth muscle fibers were together (Fig. 2m). However, the hypothyroid group showed myometrium with separated muscle fibers (Fig. 2n). Even some groups of smooth muscle cells were observed into the endometrium near of inflammatory points forming circle or oval forms (Fig. 2o–p). The number and area covered by capillaries were similar between females from the control and hypothyroid groups (Fig. 3a–b). However, the expression of VEGF-A mRNA and protein levels was higher in the hypothyroid group (Fig. 3c–d).

Hypothyroidism decreased PR mRNA expression in the uterine horn (Fig. 4a) as well as the content of its two isoforms (PR-A and PR-B) (Fig. 4b). The expression of ERα mRNA was unmodified by

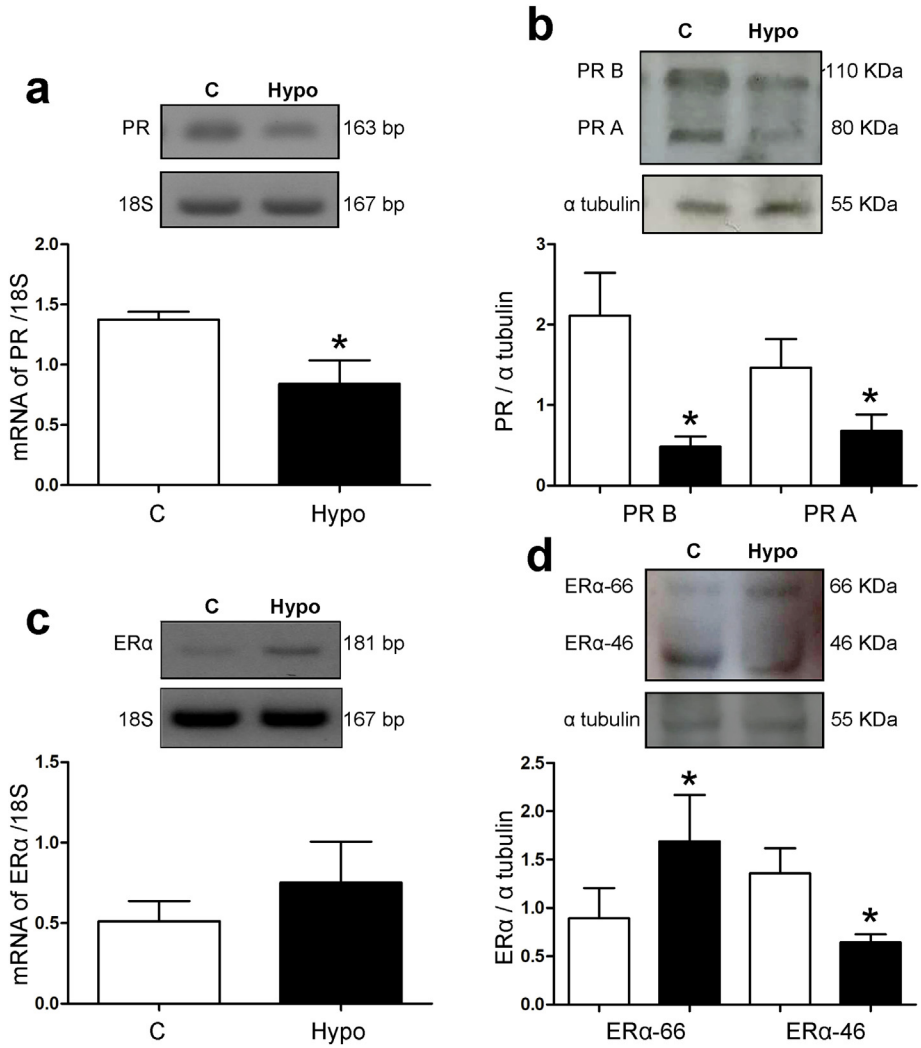


Fig. 4. Hypothyroidism affects the PR and ERα expression in the uterine horn of rabbits. Representative PCR (upper panel) and densitometry analysis showing PR (**a**) or ERα (**c**) mRNA expression of control (C, white bars, n = 6) and hypothyroid (Hypo, black bars, n = 6). Representative western blot detection of PR (**b**) and ERα (**d**) in control (C, white bars, n = 6) and hypothyroid (Hypo, black bars, n = 6). Results are expressed as mean ± S.E.M. $^{*}p \leq 0.05$.

hypothyroidism (Fig. 4c). However, uterine expression of the large isoform (66 kDa) of ERα in hypothyroid female rabbits was higher than that in controls, the opposite was found for the truncated isoform (46 kDa, Fig. 4d).

In addition, hypothyroid rabbits showed a tendency to have a lower concentration of T4 and free T4 index ($p = 0.06$) and presented a significant reduction in the concentration of T3 as compared to control animals (Fig. 5a–c). Hypothyroid rabbits showed an increased expression of TRA (Fig. 5d) and a tendency to present a high expression of TRB (Fig. 5e; $p = 0.06$).

The expression of PLIN-A in the uterine horn in the hypothyroid group was lower compared to controls (Fig. 6a). The content of TAG and TC was also lower in hypothyroid rabbits (Fig. 6b–c). In contrast, a high concentration of MDA was found in the hypothyroid group (Fig. 6d). The presence of oxidative lipids identified by Sudan black stain in the epithelium, uterine glands, and stroma in the hypothyroid group was lower than in controls (Fig. 6e–g). However, the gland secretion showed higher staining to Sudan black in the hypothyroid group.

4. Discussion

Our results show that in the uterine horn of virgin rabbits, hypothyroidism promoted several histological alterations such as endometrial hyperplasia, with high infiltration of immune cells that in some cases can be observed as points of inflammation. This inflammation is associated with high epithelial proliferation and fusion of glands. Even a detachment of epithelial tissue can be found. Our results are essential considering the involving of thyroid hormones in the development of possible myomas and uterine adherent tissues affecting the fertility of hypothyroid females [9,10,12]. In general, endometrial fibrosis has been associated with intrauterine adhesions, in which there is a differentiation between epithelium, fibroblasts, and myoblasts promoted by transforming growth factor (TGF)-β, and remodeling of the extracellular matrix [28]. In this regard, hypothyroidism stimulates the proliferation of epithelium from the intestine, oviduct, lung, and thyroid [24,29], now we extended this finding in the uterus of virgin females. In concordance to our results, endometrial hyperplasia caused by prenatal hypothyroidism was confirmed by the vast number of fused uterine glands [16]. This could be considered as mild cystic hyperplasia of superficial glands [30]. Additionally, a low thickness in the myometrium layer was observed. This could be related to the abnormal uterine contraction patterns reported in non-pregnant hypothyroid rats [31]. In this regard, T3 regulates the expression of alpha-smooth muscle actin in cardiomyocytes [32] and aorta [33]. In addition, thyroid hormones can modulate chemotaxis, lipoperoxidation, and cytokines production in several immune cells [34]. A great infiltration of CD163+ macrophages was observed in hypothyroid uterine horn, suggesting

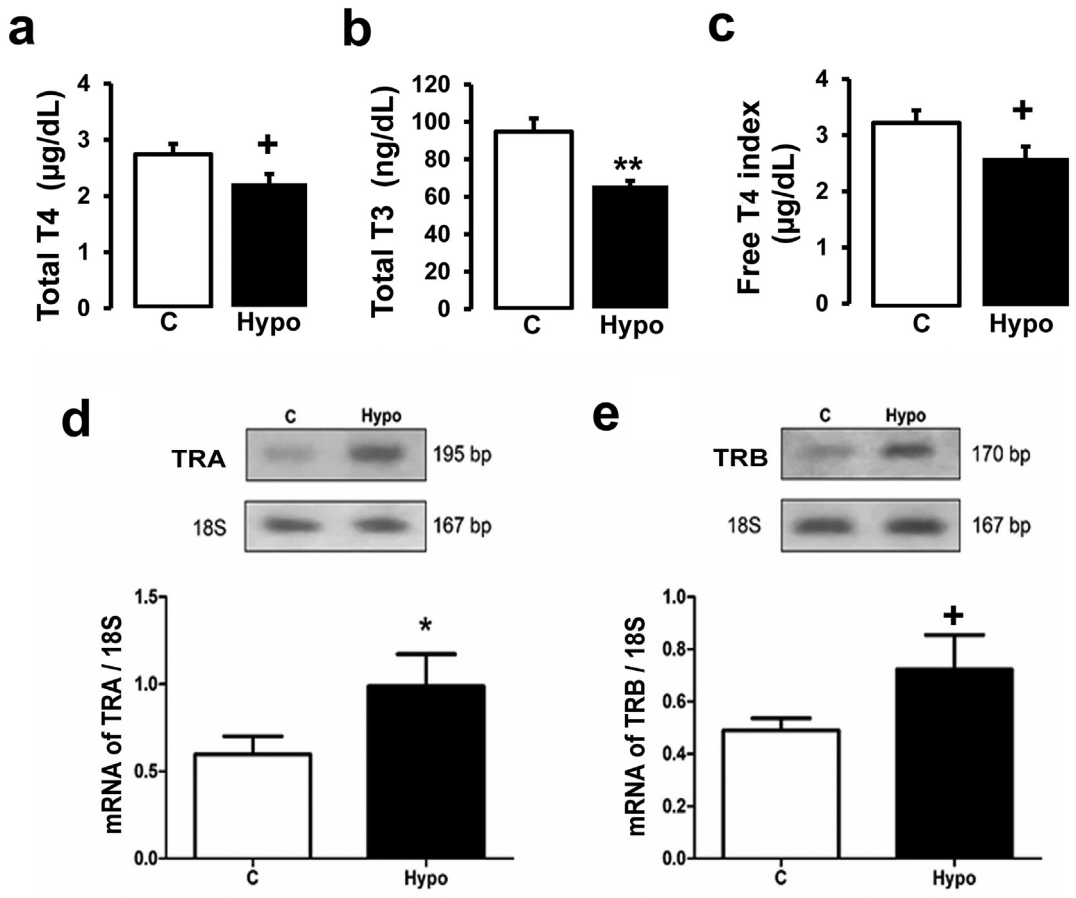


Fig. 5. Concentrations of total T4 (**a**) and T3 (**b**) in the serum of control (C; white bars, n = 6) and hypothyroid (Hypo; black bars, n = 6) female rabbits and free T4 index (**c**). Representative RT-PCR and densitometry analysis showing the TRA (**d**) and TRB (**e**) mRNA expression. Results are expressed as mean ± S.E.M. $^{*}p \leq 0.05$; $^{*}p \leq 0.01$; $+p = 0.06$.

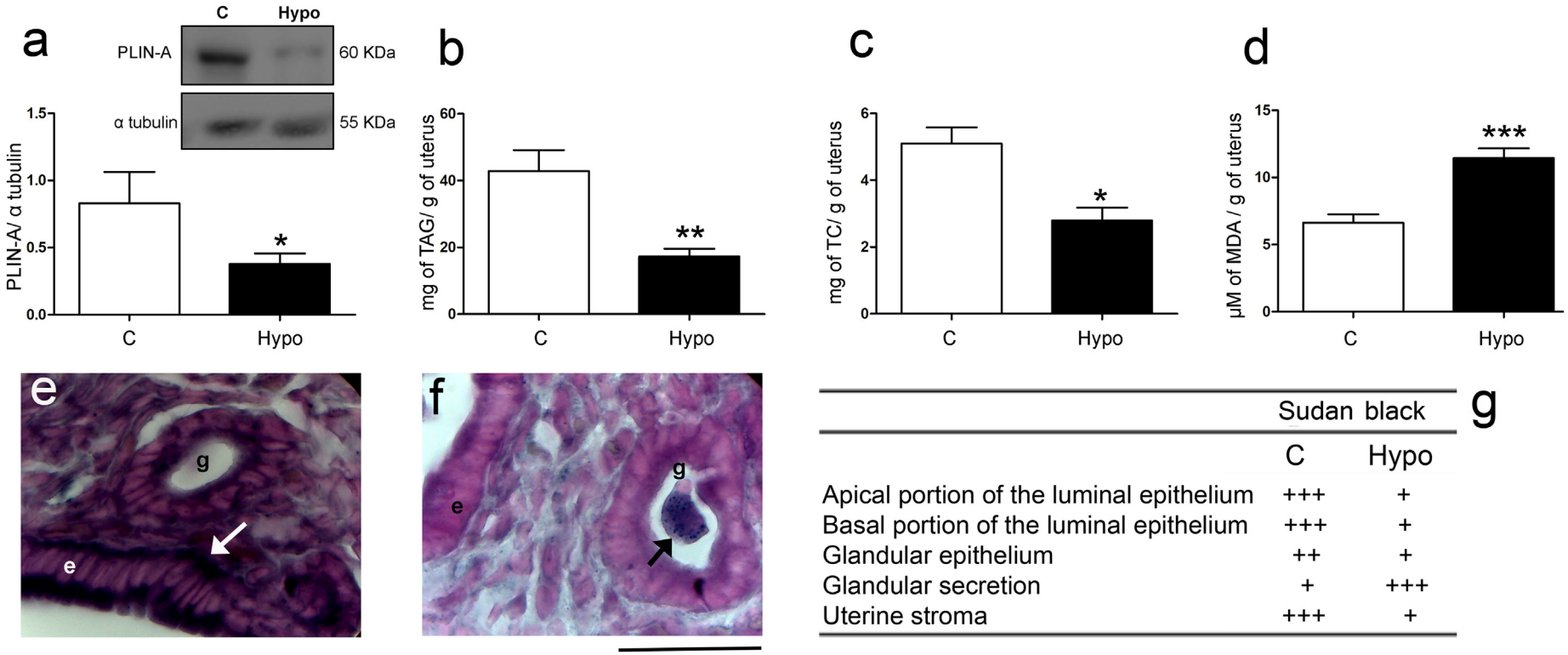
endometritis [6]. Macrophages CD163+ secrete TNF-α, IL-6, MCP-1, and VEGF-A [25], supporting the presence of uterine inflammation.

Although hypothyroidism did not affect the number nor the area covered by blood vessels, a high expression of VEGF-A in the uterine horn was found. Uterine hyperplasia and pyometra are associated with a major expression of VEGF-A, improving the blood flow and reducing the vascular resistance [35]. Moreover, VEGF is regulated by P4 [36] and E2 [37]. Uterine hyperplasia and inflammation are related to an increase in ER and a decrease in PR expression [2–4,9,38]. In contrast, P4 has opposite effects on the hyperplasia induced by E2 [39]. In addition, we reported that hypothyroidism causes endometrial hyperplasia in pregnant rabbits, associated with an increase in the 3β-hydroxysteroid dehydrogenase (3β-HSD) [16], this is hypothyroidism may affect the local synthesis of sex hormones in the uterus.

As we confirmed in the present study, the methimazole dose (10 mg/kg) used is effective to reduce thyroid hormones concentrations in serum [24]. Besides, the expression of TRs was higher in hypothyroid animals. In contrast, hypothyroidism down-regulates the expression of TRs and TSHR in the uterine horn at the implantation period in rats [41]. In this regard, it is known that the expression of TRs in the rat uterus varies with the phase of the estrous cycle, being lower at the proestrus and diestrus [40]. This suggests differential regulation of TRs depending on the sex hormones status. According to other studies [23], our rabbits were at proestrus, and we have previously shown that hypothyroidism does not modify the concentration of estradiol and progesterone in non-pregnant rabbits [24]. This suggests that the increment in the expression of uterine TRs should depend on the reduced concentrations of thyroid hormones in serum and on the modifications in the expression of sex hormones receptors in the uterus.

Thus, the direct effects of thyroid hormones on TRs to regulate the expression of mRNA and protein of PR and ER in the uterus [19–21] may be proposed. In this regard, a decrease in the PR-A is necessary for implantation [42], an excessive reduction in the expression and activity of PR promotes a shortened luteal phase affecting the endometrial development [42]. A decrease in the synthesis of P4 and expression of PR has been associated with a higher activity of pro-inflammatory cytokines, metalloproteases and chemokines [42] Also, a deficiency in P4 and PR-aberrant is related to miscarriage and myomas [43]. Two isoforms of ERα (one large of 66 kDa, and other short of 46 kDa) were found in the uterus of rabbits, as previously reported in women [44]. Hypothyroidism increased the long form of ERα and diminished the short one. In concordance, it has been reported that ERα-46 modulates the expression of ERα-66 in the mammary gland and endometrium [44,45]. In the mammary gland, hypothyroidism affects the expression of PR (A and B) and ERα involving the regulation of nuclear receptor co-activator (NCOA) [46].

Hypothyroidism in female rabbits induces hypercholesterolemia [47]. However, it reduces the content of TAG and TC, the expression of PLIN-A, and the presence of oxidized lipid granules in virgin rabbits. The importance of these results lies in the role of cholesterol as a cell membrane component and precursor of sex hormones. The uterus has a *novo* synthesis of cholesterol since express lanosterol 14alpha-demethylase, which can be regulated by estrogens [48]. The triglycerides reduction in hypothyroid rabbits could affect prostaglandin synthesis [49]. The content of oxidized lipids may have an important role in the implantation [50]. In contrast, a great lipoperoxidation was observed in



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	Sudan black	
	C	Hypo
Apical portion of the luminal epithelium	+++	+
Basal portion of the luminal epithelium	+++	+
Glandular epithelium	++	+
Glandular secretion	+	+++
Uterine stroma	+++	+

Fig. 6. Lipids in the uterine horn of control (C, white bars) and hypothyroid (hypo, black bars) rabbits. Expression of PLIN-A (**a**), as well as the content of triglycerides (TAG; **b**), total cholesterol (TC; **c**), and malondialdehyde (MDA; **f**). Results are expressed as mean ± S.E.M. $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$. The uterus of control (**d**) and hypothyroid (**e**) was stained with Sudan black. Categories were established: (+ + +) for a high proportion of stained granules; (+ +) for a moderate proportion of stained granules; and (+) for a low proportion of stained granules. Scale = 50 µm. Arrows indicate oxidized lipids droplets in the uterine gland, g, and epithelium, e.

the hypothyroid uterus. In agreement, the high content of MDA with low activity of antioxidant enzymes has been reported in women with hyperplasia [7]. Changes in the uterine lipoperoxidation have been observed in young hypothyroid rats [17]. The content of uterine lipids and oxidative status of the endometrium could be affected by local synthesis of E2 and P4 or their respective receptors [51].

These variations in the lipid content of the uterus from the hypothyroid group could be related to modifications in the steroidogenesis, considering that hypothyroidism modifies the expression of P450scc [52], 3β-HSD [16] and 17β-HSD [53], and aromatase [47] in the ovaries and uterine horn of virgin or pregnant females. Both steroidogenesis and sexual hormones signaling may have an important role in the uterine hyperplasia and inflammation observed in hypothyroid rabbits, possibly mediating the expression of steroidogenic factor 1 (SF1), which has been related to abnormal uterine gland morphogenesis, an inhibition of steroid hormone signaling, and activation of an immune response [54].

In contrast to rats, rabbits reflex ovulatory and can stay in continuous early proestrus by light/dark cycle regulation [23]. This permits to manipulate the levels of thyroid hormones, with a stable concentration of sex hormones [24]. Moreover, rabbits have been chosen as an animal model for uterine adenocarcinoma, knowing the normal and pathology histology of their uterus [55]. Our study suggests that hypothyroidism is another cause of uterine hyperplasia.

5. Conclusion

Findings of the present study confirm that the hypothyroidism induces uterine hyperplasia and inflammation in virgin females, which is associated with an increase in the VEGF-A, a decrease in the expression of PR (A and B), and modifications in that of ERα. Also, hyperplasia involves changes in the uterine content of lipids and lipoperoxidation. Therefore, our findings indicate a direct effect of thyroid hormones on the uterine horn, regulating actions of sex hormones. Present results might be helpful to clarify the relationship between hypothyroidism and abnormal bleeding and miscarriage in females. Even female secondary infertility may be linked to hypothyroidism.

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Declaration of Competing Interest

The authors disclose any financial or personal relationships with other people or organizations that could inappropriately bias or influence in the work.

References

[1] J.Y. Wong, E.B. Gold, W.O. Johnson, J.S. Lee, Circulating sex hormones and risk of uterine fibroids: Study of Women's Health across the Nation (SWAN), J. Clin. Endocrinol. Metab. 101 (2015) 123–130, https://doi.org/10.1210/jc.2015-2935.

[2] K. Upson, K.H. Allison, S.D. Reed, C.D. Jordan, K.M. Newton, E.M. Swisher, J.A. Doherty, R.L. Garcia, Biomarkers of progestin therapy resistance and endometrial hyperplasia progression, Am. J. Obstet. Gynecol. 207 (2012) e1–e8, https://doi.org/10.1016/j.ajog.2012.05.012.

[3] B. Pieczyńska, S. Wojtylak, A. Zawrocki, W. Biernat, Analysis of PTEN, estrogen receptor α and progesterone receptor expression in endometrial hyperplasia using tissue microarray, Pol. J. Pathol. 62 (2011) 133–138.

[4] E. Laas, M. Ballester, A. Cortez, J. Gonin, G. Canlorbe, E. Daraï, O. Graesslin, Supervised clustering of immunohistochemical markers to distinguish atypical and non-atypical endometrial hyperplasia, Gynecol. Endocrinol. 31 (2015) 282–285, https://doi.org/10.3109/09513590.2014.989981.

[5] A.K. Elfayomy, S.M. Almasry, G.M. Attia, F.A. Habib, Enhanced expression of vascular endothelial growth factor and increased microvascular density in women with endometrial hyperplasia: a possible relationship with uterine natural killer cells, Romanian J. Morphol. Embryol. 56 (2015) 725–734.

[6] A. Cominelli, H.P. Gaide Chevronnay, P. Lemoine, P.J. Courtoy, E. Marbaix, P. Henriet, Matrix metalloproteinase-27 is expressed in CD163+/CD206+ M2 macrophages in the cycling human endometrium and in superficial endometriotic lesions, Mol. Hum. Reprod. 20 (2014) 767–775, https://doi.org/10.1093/molehr/gau034.

[7] S. Pejić, A. Todorović, V. Stojiljković, J. Kasapović, S.B. Pajović, Antioxidant enzymes and lipid peroxidation in endometrium of patients with polyps, myoma, hyperplasia and adenocarcinoma, Reprod. Biol. Endocrinol. 7 (2009) 149, , https://doi.org/10.1186/1477-7827-7-149.

[8] S. Özdemir, G. Batmaz, S. Ates, C. Celik, F. Incesu, C. Peru, Relation of metabolic syndrome with endometrial pathologies in patients with abnormal uterine bleeding, Gynecol. Endocrinol. 31 (2015) 725–729, https://doi.org/10.3109/09513590.2015.1058355.

[9] E. Soleymani, K. Ziari, O. Rahmani, M. Dadpay, M. Taheri-Dolatabadi, K. Alizadeh, N. Ghanbarzadeh, Histopathological findings of endometrial specimens in abnormal uterine bleeding, Arch. Gynecol. Obstet. 289 (2014) 845–849, https://doi.org/10.1007/s00404-013-3043-1.

[10] Y. Hu, Q. Wang, G. Li, X. Sun, C. Liu, Ultrasonic morphology of uterus and ovaries in girls with pituitary hyperplasia secondary to primary hypothyroidism, Horm. Metab. Res. 45 (2013) 669–674, https://doi.org/10.1055/s-0033-1345141.

[11] N.S. Ajmani, V. Sarbhai, N. Yadav, M. Paul, A. Ahmad, A.K. Ajmani, Role of thyroid dysfunction in patients with menstrual disorders in tertiary care center of Walled city of Delhi, J. Obstet. Gynaecol. India. 66 (2016) 115–119, https://doi.org/10.1007/s13224-014-0650-0.

[12] J. Ott, C. Kurz, R. Braun, R. Promberger, R. Seemann, E. Vytiska-Binstorfer, K. Walch, Overt hypothyroidism is associated with the presence of uterine leiomyoma: a retrospective analysis, Eur. J. Obstet. Gynecol. Reprod. Biol. 177 (2014) 19–22, https://doi.org/10.1016/j.ejogrb.2014.03.003.

[13] F. Bagheripuor, M. Ghanbari, A. Piryaei, A. Ghasemi, Effects of fetal hypothyroidism on uterine smooth muscle contraction and structure of offspring rats, Exp. Physiol. 103 (2018) 683–692, https://doi.org/10.1113/EP086564.

[14] A.S.M. Sayem, N. Giribabu, S. Muniandy, N. Salleh, Effects of thyroxine on expression of proteins related to thyroid hormone functions (TR-α, TR-β, RXR and ERK1/2) in uterus during peri-implantation period, Biomed. Pharmacother. 96 (2017) 1016–1021, https://doi.org/10.1016/j.biopha.2017.11.128.

[15] J.F. Silva, N.M. Ocarino, R. Serakides, Maternal thyroid dysfunction affects placental profile of inflammatory mediators and the intrauterine trophoblast migration kinetics, Reproduction 147 (2014) 803–816, https://doi.org/10.1530/REP-13-0374.

[16] J. Rodríguez-Castelán, D. Zepeda-Pérez, M. Méndez-Tepepa, M. Castillo-Romano, M. Espindola-Lozano, A. Anaya-Hernández, P. Berbel, E. Cuevas-Romero, Hypothyroidism modifies the uterine lipid levels in pregnant rabbits and affects the fetal size, Endocr Metab Immune Disord Drug Targets (2018), https://doi.org/10.2174/1871530318666181102093621 Epub ahead of Print.

[17] L. Kong, Q. Wei, J.S. Fedail, F. Shi, K. Nagaoka, G. Watanabe, Effects of thyroid hormones on the antioxidative status in the uterus of young adult rats, J. Reprod. Dev. 61 (2015) 219–227, https://doi.org/10.1262/jrd.2014-129.

[18] I.M. Inuwa, M.A. Williams, A morphometric study on the endometrium of rat uterus in hypothyroid and thyroxine treated hypothyroid rats, Ups. J. Med. Sci. 111 (2006) 215–225.

[19] M. Hulchiy, H. Zhang, J.M. Cline, A.L. Hirschberg, L. Sahlin, Receptors for thyrotropin-releasing hormone, thyroid-stimulating hormone, and thyroid hormones in the macaque uterus: effects of long-term sex hormone treatment, Menopause 19 (2012) 1253–1259, https://doi.org/10.1097/gme.0b013e318252e450.

[20] J. Rodríguez-Castelán, A. Anaya-Hernández, M. Méndez-Tepepa, M. Martínez-Gómez, F. Castelán, E. Cuevas-Romero, Distribution of thyroid hormone and thyrotropin receptors in reproductive tissues of adult female rabbits, Endocr. Res. 42 (2017) 59–70, https://doi.org/10.1080/07435800.2016.1182185.

[21] A.S.M. Sayem, N. Giribabu, K. Karim, L.K. Si, S. Muniandy, N. Salleh, Differential expression of the receptors for thyroid hormone, thyroid stimulating hormone, vitamin D and retinoic acid and extracellular signal-regulated kinase in uterus of rats under influence of sex-steroids, Biomed. Pharmacother. 100 (2018) 132–141, https://doi.org/10.1016/j.biopha.2018.02.008.

[22] H.S. Keeping, A.M. Newcombe, P.H. Jellinck, Modulation of estrogen-induced peroxidase activity in the rat uterus by thyroid hormones, J. Steroid. Biochem. 16 (1982) 45–49.

[23] T.M. Mousa-Balabel, R.A. Mohamed, Effect of different photoperiods and melatonin treatment on rabbit reproductive performance, Vet. Q. 31 (2011) 165–171, https://doi.org/10.1080/01652176.2011.642533.

[24] A. Anaya-Hernández, J. Rodríguez-Castelán, L. Nicolás, M. Martínez-Gómez, I. Jiménez-Estrada, F. Castelán, E. Cuevas, Hypothyroidism affects differentially the cell size of epithelial cells among oviductal regions of rabbits, Reprod. Domest. Anim. 50 (2015) 104–111, https://doi.org/10.1111/rda.12455.

[25] A.L. Jensen, J. Collins, E.P. Shipman, C.R. Wira, P.M. Guyre, P.A. Pioli, A subset of human uterine endometrial macrophages is alternatively activated, Am. J. Reprod. Immunol. 68 (2012) 374–386, https://doi.org/10.1111/j.1600-0897.2012.01181.x.

[26] J. Rodríguez-Castelán, M. Méndez-Tepepa, J. Rodríguez-Antolín, F. Castelán, E. Cuevas-Romero, Hypothyroidism affects lipid and glycogen content and peroxisome proliferator-activated receptor δ expression in the ovary of the rabbit, Reprod. Fertil. Dev. 30 (2018) 1380–1387, https://doi.org/10.1071/RD17502.

[27] H. Itabe, T. Yamaguchi, S. Nimura, N. Sasabe, Perilipins: a diversity of intracellular lipid droplet proteins, Lipids Health Dis. 16 (2017) 83, , https://doi.org/10.1186/s12944-017-0473-y.

[28] H.Y. Zhu, T.X. Ge, Y.B. Pan, S.Y. Zhang, Advanced role of Hippo signaling in endometrial fibrosis: implications for intrauterine adhesion, Chin. Med. J. 130 (2017) 2732–2737, https://doi.org/10.4103/0366-6999.218013.
[29] C. Frau, M. Godart, M. Plateroti, Thyroid hormone regulation of intestinal epithelial stem cell biology, Mol. Cell. Endocrinol. 459 (2017) 90–97, https://doi.org/10.1016/j.mce.2017.03.002.
[30] N. Reusche, A. Beineke, C. Urhausen, M. Beyerbach, M. Schmicke, S. Kramer, A.R. Günzel-Apel, Proliferative and apoptotic changes in the healthy canine endometrium and in cystic endometrial hyperplasia, Theriogenology. 114 (2018) 14–24, https://doi.org/10.1016/j.theriogenology.2018.03.018.
[31] S. Corriveau, S. Blouin, É. Raiche, M.A. Nolin, É. Rousseau, J.C. Pasquier, Levothyroxine treatment generates an abnormal uterine contractility patterns in an *in vitro* animal model, J. Clin. Transl. Endocrinol. 2 (2015) 144–149, https://doi.org/10.1016/j.jcte.2014.09.005.
[32] M.A. Gosteli-Peter, B.A. Harder, H.M. Eppenberger, J. Zapf, M.C. Schaub, Triiodothyronine induces over-expression of alpha-smooth muscle actin, restricts myofibrillar expansion and is permissive for the action of basic fibroblast growth factor and insulin-like growth factor I in adult rat cardiomyocytes, J. Clin. Invest. 98 (1996) 1737–1744.
[33] X. Wang, Z. Sun, Thyroid hormone induces artery smooth muscle cell proliferation: discovery of a new TRalpha1-Nox1 pathway, J. Cell. Mol. Med. 14 (2010) 368–380.
[34] E.L. Jara, N. Muñoz-Durango, C. Llanos, C. Fardella, P.A. González, S.M. Bueno, A.M. Kalergis, C.A. Riedel, Modulating the function of the immune system by thyroid hormones and thyrotropin, Immunol. Lett. 184 (2017) 76–83, https://doi.org/10.1016/j.imlet.2017.02.010.
[35] G.A. Veiga, R.H. Miziara, D.S. Angrimani, P.C. Papa, B. Cogliati, C.I. Vannucchi, Cystic endometrial hyperplasia-pyometra syndrome in bitches: identification of hemodynamic, inflammatory, and cell proliferation changes, Biol. Reprod. 96 (2017) 58–69, https://doi.org/10.1095/biolreprod.116.140780.
[36] M. Kim, H.J. Park, J.W. Seol, J.Y. Jang, Y. Cho, J.P. Lydon, F.J. Demayo, M. Shibuya, N. Ferrara, H.K. Sung, A. Nagy, K. Alitalo, G.Y. Koh, VEGF-A regulated by progesterone governs uterine angiogenesis and vascular remodeling during pregnancy, EMBO Mol. Med. 5 (2013) 1415–1430, https://doi.org/10.1002/emmm.201302618.
[37] J. Zhang, H. Song, Y. Lu, H. Chen, S. Jiang, L. Li, Effects of estradiol on VEGF and bFGF by Akt in endometrial cancer cells are mediated through the NF-κB pathway, Oncol. Rep. 36 (2016) 705–714, https://doi.org/10.3892/or.2016.4888.
[38] A.V. Kubyshkin, L.L. Aliev, I.I. Fomochkina, Y.P. Kovalenko, S.V. Litvinova, T.G. Filonenko, N.V. Lomakin, V.A. Kubyshkin, O.V. Karapetian, Endometrial hyperplasia-related inflammation: its role in the development and progression of endometrial hyperplasia, Inflamm. Res. 65 (2016) 785–794, https://doi.org/10.1007/s00011-016-0960-z.
[39] A. Gompel, Progesterone, progestins and the endometrium in perimenopause and in menopausal hormone therapy, Climacteric 21 (2018) 321–325, https://doi.org/10.1080/13697137.2018.1446932.
[40] A.S.M. Sayem, N. Giribabu, K. Karim, L.K. Si, S. Muniandy, N. Salleh, Differential expression of the receptors for thyroid hormone, thyroid stimulating hormone, vitamin D and retinoic acid and extracellular signal-regulated kinase in uterus of rats under influence of sex-steroids, Biomed. Pharmacother. 100 (2018) 132–141, https://doi.org/10.1016/j.biopha.2018.02.008.
[41] N. Salleh, A.S.M. Sayem, N. Giribabu, S.L. Khaing, Expression of proteins related to thyroid hormone function in the uterus is down-regulated at the day of implantation in hypothyroid pregnant rats, Cell Biol. Int. 43 (2019) 486–494, https://doi.org/10.1002/cbin.11114.
[42] M. Wetendorf, S.P. Wu, X. Wang, C.J. Creighton, T. Wang, R.B. Lanz, L. Blok, S.Y. Tsai, M.J. Tsai, J.P. Lydon, F.J. DeMayo, Decreased epithelial progesterone receptor A at the window of receptivity is required for preparation of the endometrium for embryo attachment, Biol. Reprod. 96 (2017) 313–326, https://doi.org/10.1095/biolreprod.116.144410.
[43] B. Patel, S. Elguero, S. Thakore, W. Dahoud, M. Bedaiwy, S. Mesiano, Role of nuclear progesterone receptor isoforms in uterine pathophysiology, Hum. Reprod. Update 21 (2015) 155–173, https://doi.org/10.1093/humupd/dmu056.
[44] G. Flouriot, H. Brand, S. Denger, R. Metivier, M. Kos, G. Reid, V. Sonntag-Buck, F. Gannon, Identification of a new isoform of the human estrogen receptor-alpha (hER-alpha) that is encoded by distinct transcripts and that is able to repress hER-alpha activation function 1, EMBO J. 19 (2000) 4688–4700, https://doi.org/10.1093/emboj/19.17.4688.
[45] C.M. Klinge, K.A. Riggs, N.S. Wickramasinghe, C.G. Emberts, D.B. McConda, P.N. Barry, J.E. Magnusen, Estrogen receptor alpha 46 is reduced in tamoxifen resistant breast cancer cells and re-expression inhibits cell proliferation and estrogen receptor alpha 66-regulated target gene transcription, Mol. Cell. Endocrinol. 323 (2010) 268–276, https://doi.org/10.1016/j.mce.2010.03.013.
[46] F. Campo-VerdeArboccó, C.V. Sasso, D.L. Nasif, M.B. Hapon, G.A. Jahn, Effect of hypothyroidism on the expression of nuclear receptors and their co-regulators in mammary gland during lactation in the rat, Mol. Cell. Endocrinol. 412 (2015) 26–35, https://doi.org/10.1016/j.mce.2015.05.026.
[47] J. Rodríguez-Castelán, M. Méndez-Tepepa, Y. Carrillo-Portillo, A. Anaya-Hernández, J. Rodríguez-Antolín, E. Zambrano, F. Castelán, E. Cuevas-Romero, Hypothyroidism reduces the size of ovarian follicles and promotes hypertrophy of periovarian fat with infiltration of macrophages in adult rabbits, Biomed. Res. Int. 2017 (2017) 3795950, , https://doi.org/10.1155/2017/3795950.
[48] X. Song, P. Tai, J. Yan, B. Xu, X. Chen, H. Ouyang, M. Zhang, G. Xia, Expression and regulation of lanosterol 14alpha-demethylase in mouse embryo and uterus during the peri-implantation period, Reprod. Fertil. Dev. 20 (2008) 964–972.
[49] M.A. Chaud, A.M. Franchi, M. Viggiano, A.L. Gimeno, M.A. Gimeno, Effect of exogenous phospholipase A2 and triacylglycerol lipase on the synthesis and release of monoenoic and bisenoic prostaglandins from isolated rat uterus, Prostaglandins Leukot. Essent. Fatty Acids. 44 (1991) 211–215.
[50] C. Alberto-Rincon Mdo, C.S. de Paiva, P.A. Abrahamsohn, T.M. Zorn, Histochemical demonstration of phospholipid containing choline in the cytoplasm of murine decidual cells, Acta Anat. 150 (1994) 119–126.
[51] M.M. Singh, R.N. Trivedi, S.C. Chauhan, V.M. Srivastava, A. Makker, S.R. Chowdhury, V.P. Kamboj, Uterine estradiol and progesterone receptor concentration, activities of certain antioxidant enzymes and dehydrogenases and histoarchitecture in relation to time of secretion of nidatory estrogen and high endometrial sensitivity in rat, J. Steroid Biochem. Mol. Biol. 59 (1996) 215–224.
[52] J.J. Chen, S.W. Wang, E.J. Chien, P.S. Wang, Direct effect of propylthiouracil on progesterone release in rat granulosa cells, Br. J. Pharmacol. 139 (2003) 1564–1570, https://doi.org/10.1038/sj.bjp.0705392.
[53] D. Sarkar, A. Chakraborty, D. Mahapatra, A.K. Changra, Morphological and functional alterations of female reproduction after exposure of bamboo shoots of North East India, Asian Pac, J. Reprod. 6 (2017) 151, https://doi.org/10.12980/apjr.6.20170402.
[54] Y.M. Vasquez, S.P. Wu, M.L. Anderson, S.M. Hawkins, C.J. Creighton, M. Ray, S.Y. Tsai, M.J. Tsai, J.P. Lydon, F.J. DeMayo, Endometrial expression of steroidogenic factor 1 promotes cystic glandular morphogenesis, Mol. Endocrinol. 30 (2016) 518–532, https://doi.org/10.1210/me.2015-1215.
[55] C.A. Bertram, K. Müller, R. Klopfleisch, Genital tract pathology in female pet rabbits (Oryctolagus cuniculus): a retrospective study of 854 necropsy examinations and 152 biopsy samples, J. Comp. Pathol. 164 (2018) 17–26, https://doi.org/10.1016/j.jcpa.2018.08.003.