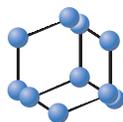
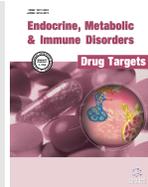


## RESEARCH ARTICLE

BENTHAM  
SCIENCE

## Hypothyroidism Alters the Uterine Lipid Levels in Pregnant Rabbits and Affects the Fetal Size



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**Abstract: Background:** Hypothyroidism has been related to low-weight births, abortion and prematurity, which have been associated with changes in the content of glycogen and vascularization of the placenta. Since hypothyroidism can cause dyslipidemia, it may affect the lipid content in the uterus affecting the development of fetuses.

**Objective:** To investigate the effect of hypothyroidism on the lipid levels in serum and uterus during pregnancy and their possible association with the size of fetuses.

**Method:** Adult female rabbits were grouped in control ( $n = 6$ ) and hypothyroid ( $n = 6$ ; treated with methimazole for 29 days before and 19 days after copulation). Food intake and body weight were daily registered. At gestational day 19 (GD19), dams were sacrificed under an overdose of anesthesia. Morphometric measures of fetuses were taken. Total cholesterol (TC), triglyceride (TAG), and glucose concentrations were quantified in blood, uterus and ovaries of dams. The expression of uterine  $\beta$ -hydroxysteroid dehydrogenase ( $\beta$ -HSD) was quantified by Western blot.

**Results:** Hypothyroidism reduced food intake and body weight of dams, as well as promoted low abdominal diameters of fetuses. It did not induce dyslipidemia and hyperglycemia at GD19 and did not modify the content of lipids in the ovary. However, it reduced the content of TAG and TC in the uterus, which was associated with uterine hyperplasia and an increased expression of  $\beta$ -HSD in the uterus.

**Conclusion:** Hypothyroidism alters the lipid content in the uterus that might subsequently affect the energy production and lipid signaling important to fetal development.

**Keywords:** thyroid hormones, total cholesterol, triglyceride,  $\beta$ -HSD, endometrium, methimazole

### 1. INTRODUCTION

During pregnancy, the elongation of the uterus requires a high content of lipids and glucose. Pregnancy involves a major steroidogenesis and energy production, an increase in the prostaglandins synthesis and an activation in the cell signaling [1]. Although the uterus receives nutrients from blood [2], it also accumulates glycogen and lipids [3, 4]. High serum concentrations of glucose and lipids during pregnancy have been associated with preterm birth and alterations in the development of the placenta [5-9].

Clinical and subclinical hypothyroidism have been associated with pregnancy loss, intrauterine growth restriction, fetal death, premature birth and low birth weight [10-12]. Actions of thyroid hormones on fertility, pregnancy and fetal development in animal models are scarce. It has

been reported that hypothyroid induces a low body weight at birth in rats, a high content of glycogen in the fetal and maternal placenta, and a low expression of vascular endothelial growth factor (VEGF) [13, 14]. However, the influence of hypothyroidism on the dams and fetuses is not yet fully understood. We have studied the effects of hypothyroidism on the fertility of rabbits, body weight of dams and fetuses, and its possible link to the content of lipids and glucose in serum, ovary and uterus of dams, as well as the histological characteristics of the uterus.

### 2. MATERIALS AND METHODS

Nine-to-eleven months old breed-chinchilla European virgin female rabbits (*Oryctolagus cuniculus*) were grouped in control (C;  $n = 6$ ) or hypothyroid (H;  $n = 6$ ). The number of dams per experimental group was determined according to previous studies [14] and recommendations of the Ethics Committee of our University. Rabbits were housed in individual cages of stainless-steel at  $20 \pm 2^\circ\text{C}$  room temperature under artificial lighting conditions (16:8 h;

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light:dark). Hypothyroidism was induced by oral administration of 0.02% methimazole (10 mg/kg) in the drinking water [14] from 30 days previous pregnancy to 19 days after conception (Fig. 1a). We previously confirmed the presence of hypothyroidism in virgin rabbits after 30 days of the dose, showing low triiodothyronine (T3) and thyroxine (T4), as well as high thyrotropin (TSH) levels [14]. Females copulated with sexual experienced males for 4 times to guarantee fertility [15] and the day of copulation was taken as the day 0 of gestation (GD0).

Female rabbits had continuous access to water and were fed with 180 g/day pellet chow for 1 month before copulation and with 250 g/day during pregnancy. The food intake per day was measured. At the end of the methimazole-treatment (GD19), rabbits were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and then, euthanized with an overdose. 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 Laboratory Animals.

### 2.1. Characteristics of Fetuses

Left and right horns of the uterus were longitudinally opened, and the number of fetuses and resorptions counted. Fetuses were removed for their yolk sacs and the body weight and length from head to rump, and abdomen and head diameters were measured (Fig. 1b).

### 2.2. Lipids Quantification

At day 20 of pregnancy, after 12 h fasting, blood samples of anesthetized female rabbits were obtained by cardiac puncture. Serum was stored at  $-80^{\circ}\text{C}$  until assayed. Total cholesterol (TC) and triglyceride (TAG) levels (mg/dL) were measured using standard enzymatic methods (Elitech, France; CHSL-0507 and TGML-0427) [16].

A middle portion of the right uterus (inter-implant) was frozen at  $-80^{\circ}\text{C}$  for biochemical analysis. Right ovaries were weighed and frozen at  $-80^{\circ}\text{C}$  for biochemical analysis. Both TAG and TC of the uterus and ovaries were extracted by the method of Folch and their concentrations measured as mg/g of tissue using standard kits (Elitech; see above) [17].

### 2.3. Glucose and Glycogen Quantification

The glucose concentration in serum was measured (mg/dL) by the glucose-oxidase method (Elitech; GPSL-0707). The content of glycogen in the uterus was extracted and determined by the acid-hydrolytic method [16], using the same commercial kit used for serum glucose. It was measured as  $\mu\text{mol}$  of glucosyl units/g of wet uterus weight.

### 2.4. Uterine Histological Characteristics

A middle portion of the longitudinally opened left uterus (inter-implant) was histologically processed and cut at 7  $\mu\text{m}$  in a microtome. One section per rabbit was stained with Masson Trichrome method. Photomicrographs were taken using a microscope Zeiss Axio Imager A1 and the morphological variables were measured by the software Axiovision

4.8 (Carl Zeiss Micro Imaging, Inc. Germany). On each section, 1) the thickness of the endometrium and myometrium at 8-12 points per section using a 4x objective, 2) the external cross-sectional area (CSA) of 614 closed glands uterine for the C and 690 for the H group in 8 fields per section using a 10x objective, 3) the number of vessels (vascular and lymphatic), and 4) the projected area by these capillaries using a 40x objective were obtained.

### 2.5. Expression of 3 $\beta$ -hydroxysteroid Dehydrogenase (3 $\beta$ -HSD) in the Uterus

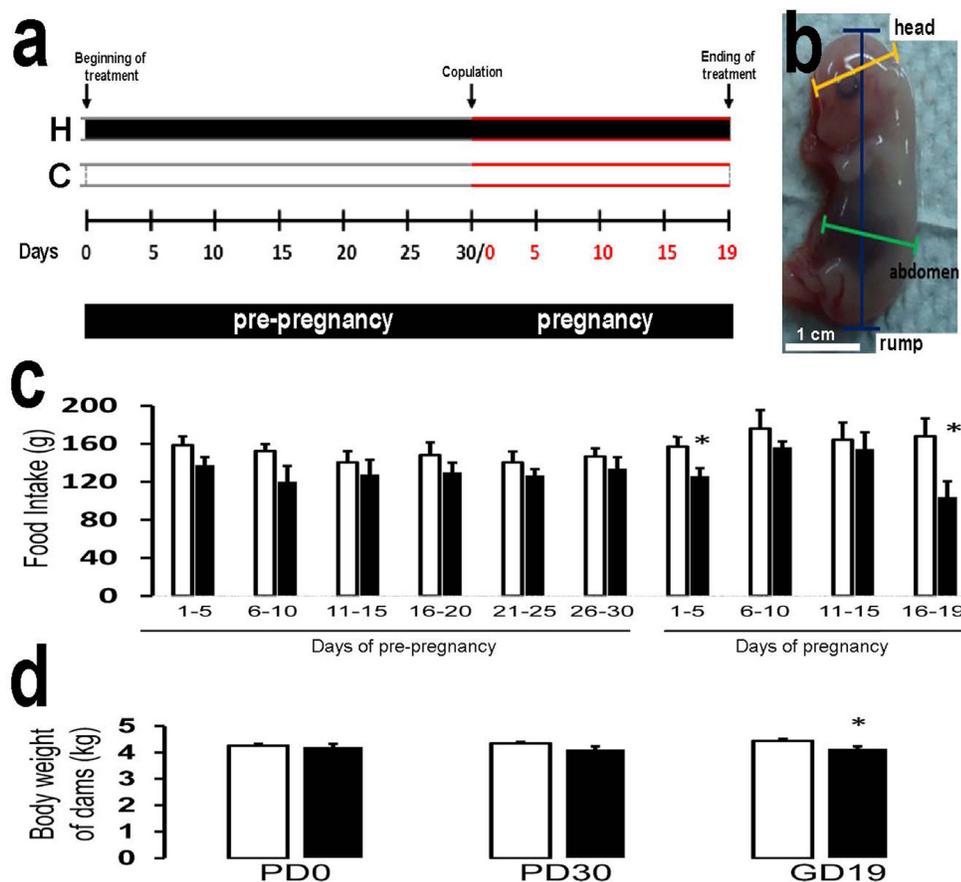
Tissue samples of the right uterus horns of C and H rabbits, weighing approx. 50 mg, were lysed as reported elsewhere [17]. The total protein per sample was obtained and 80  $\mu\text{g}$  of protein extracts was resolved using SDS-PAGE and blotted to nitrocellulose membranes (Enduro Labnet International, USA). Membranes were incubated overnight at  $4^{\circ}\text{C}$  with polyclonal anti-3 $\beta$ HSD antibody (1:50; sc-30820, Santa Cruz Biotechnology, USA), followed by incubation with a secondary goat anti-mouse horseradish peroxidase-conjugated antibody (1:2000; sc-2005, Santa Cruz Biotechnology) at room temperature for 45 min. Proteins were immunostained using a chemiluminescence kit (West Pico Signal, Thermo Fisher Scientific, USA) and analyzed with a chemiluminescent signal analyzer (MyECL Imager, Thermo Fisher Scientific). Two bands between 32 and 46 kDa were considered positive for 3 $\beta$ -HSD. The expression of 3 $\beta$ -HSD was measured by densitometry and normalized as the ratio between the 3 $\beta$ -HSD band density and the density of bands covering at least 90% of the length of each Ponceau's Red-stained lane [17, 18], using the ImageJ software (National Institutes of Health, USA).

### 2.6. Statistical Analysis

Data are mean  $\pm$  SE unless otherwise is stated. Student t-test or Mann-Whitney U-test was used to test variables differences between groups. Kolmogorov-Smirnov test was used to check the normality of distribution. Percentages were compared with Fischer tests. Statistical analyses to test significant differences ( $P \leq 0.05$ ) between variables and experimental groups were carried out with the program Prism v.5 for Windows (Graphpath Software, USA).

## 3. RESULTS

Food intake in the days previous to pregnancy was similar between C and H dams, but it significantly decreased in H dams at GD1-5 ( $t = 2.3$ ;  $df = 10$ ;  $P = 0.04$ ), and at GD16-19 ( $t = 2.5$ ;  $df = 10$ ;  $P = 0.02$ ; Fig. 1c). The body weight of dams at PD0 ( $4.2 \pm 0.06$  kg in C vs.  $4.2 \pm 0.1$  kg in H dams) and PD30 ( $4.3 \pm 0.05$  kg in C vs.  $4.1 \pm 0.1$  kg in H dams) was similar between groups. At GD19, it decreased in H ( $4.1 \pm 0.07$  kg) with respect to C dams ( $4.4 \pm 0.07$  kg;  $t = 2.7$ ;  $df = 10$ ;  $P = 0.02$ ; Fig. 1d). Both in the right and left uterine horns, no significant differences between the number of implants, resorptions and fetuses in C and H groups were found (Table 1). Morphometric characteristics of fetuses, such as body weight, body length (from head to rumps), and abdomen and head diameters were similar between both groups (Table 1). The averaged percentage of H fetuses with abdo-



**Fig. (1).** a. Protocol of untreated control (C) and methimazole-treated hypothyroid (H) dams. b. Picture shows a control rabbit fetus at 19 pregnancy day (GD19) and the locations where the head-rump length (blue line), and head (yellow line) and abdomen (green line) diameter measurements were done. c and d. Food intake and body weight of control (white bars; n=6) and hypothyroid (black bars; n=6) rabbits before (PD) and during pregnancy (GD). At all ages, food intake was less in hypothyroid dams, but it was significantly reduced at GD1-5 (20 %) and at GD16-19 (38 %). (\*)  $P \leq 0.05$ .

men diameter  $\geq 11.1$  mm from left and right horns was lower than in controls (Table 1).

Serum concentrations of glucose, TAG and TC were similar between C and H dams (Table 2). Also, the levels of TAG and TC in the ovary and glycogen in the uterus were similar between groups (Table 2). In contrast, TAG and TC uterine levels were lower in H dams than in controls (Table 2). The thickness of the endometrium was similar between C ( $797.5 \pm 62.0 \mu\text{m}$ ) and H ( $1065.2 \pm 144.2 \mu\text{m}$ ) dams Fig. 2a). Due to variability in the endometrium thickness, we obtained the percentage of endometrium segments  $> 900 \mu\text{m}$  being  $35.6 \pm 8.4$  for C and  $64.5 \pm 11.7$  for H dams (Fig. 2b) but it did not reach a statistical significance. However, the thickness of these wide segments in H ( $1259.2 \pm 102.1 \mu\text{m}$ ) was significantly greater than in control dams ( $1075.5 \pm 31.1 \mu\text{m}$ ;  $U = 5$ ;  $P = 0.04$ ; Fig. 2c). No differences between groups were found in the myometrium thickness (Fig. 2d), uterine gland CSA (Fig. 2e), capillaries number (Fig. 2f) and projected area (Fig. 2g) per field. In contrast, the uterine  $3\beta$ -HSD expression was significantly higher in H dams (220%) than in controls ( $U = 0.0$ ;  $P = 0.02$ ; Figs. 3a-c).

#### 4. DISCUSSION

Our results show a decreased body weight in H pregnant dams at GD16-19, possibly due to a low food intake. The

reduced food intake in H dams can be related to an increase in the perception of the bitter taste of methimazole, promoted during pregnancy [19]. Other studies have shown that hypothyroidism decreases the body weight and food intake in male rats associated with a regulation of the in the protein content of orexigenic neuropeptide Y (NPY) and anorexigenic proopiomelanocortin (POMC) in the arcuate nuclei, as well as a leptin resistance [20].

The minor body weight in H dams is probably one of the most important underlying causes of low TAG and TC content in the uterus and the decreased percentage of fetuses with large abdominal diameter. Additionally, H dams showed endometrium hyperplasia and an increased  $3\beta$ -HSD expression at the inter-implant sites. These effects may implicate direct actions of thyroid hormones because endometrium and placenta express type II and III deiodinases, thyroid hormone receptors, and monocarboxylate transporters (MCT) 8 and 10 [21-25].

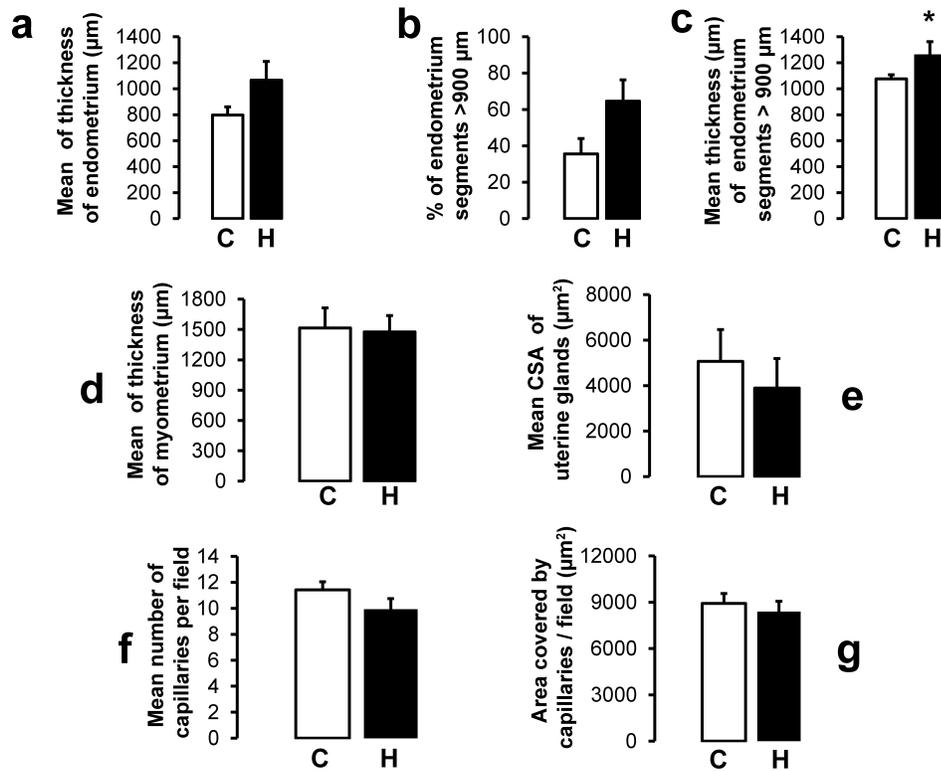
As previously reported, methimazole-treatment during 30 days reduces serum levels of thyroid hormones and increases thyrotropin levels in virgin rabbits [14]. This treatment increased the thickness of oviduct epithelium [14] and reduced the size of antral follicles in the ovary [16]. Considering that female rabbits were under methimazole treatment during 49 days in the present study, we can assume that they have

**Table 1.** Number of implants and resorptions in left, right and both uterine horns, as well as morphological characteristics of fetuses. The abdominal diameter was similar between control and hypothyroid fetuses, but the percentage of abdominal diameter  $\geq 11.1$  mm significantly decreased (42.8 % decrease) in hypothyroid fetuses. Non-significant, ns.

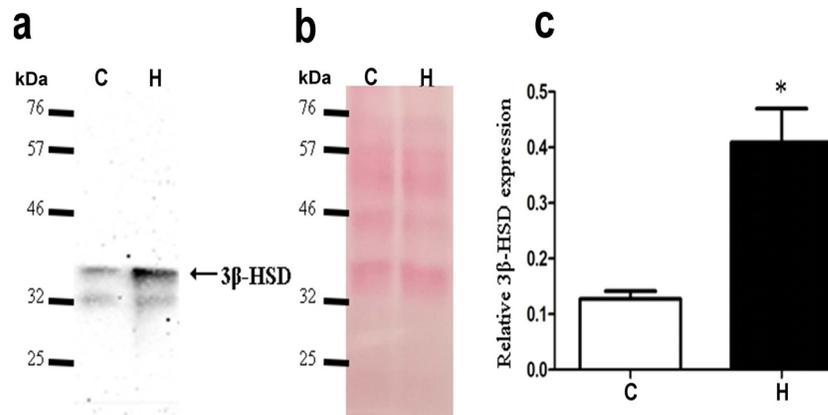
-	Control n=6	Hypothyroid n=6	Significance Between Groups
<b>Right horn of the uterus</b>			
Total of implants (resorptions and fetuses) per dam	4.6 $\pm$ 1.0	5.6 $\pm$ 0.6	ns
Number of fetal resorptions per dam	0.3 $\pm$ 0.2	0.6 $\pm$ 0.3	ns
Body-weight of fetuses (g)	2.3 $\pm$ 0.1	2.2 $\pm$ 0.05	ns
Head diameter of fetuses (mm)	10.0 $\pm$ 0.5	10.0 $\pm$ 0.1	ns
Abdominal diameter of fetuses (mm)	11.1 $\pm$ 0.1	10.7 $\pm$ 0.08	ns
Body length of fetuses (mm)	31.0 $\pm$ 0.7	30.4 $\pm$ 0.4	ns
<b>Left horn of the uterus</b>			
Total of implants (resorptions and fetuses) per dam	5.7 $\pm$ 0.7	3.6 $\pm$ 0.3	ns
Number of fetal resorptions per dam	0.1 $\pm$ 0.1	0.3 $\pm$ 0.2	ns
Body-weight of fetuses (g)	2.3 $\pm$ 0.1	2.3 $\pm$ 0.05	ns
Head diameter of fetuses (mm)	10.0 $\pm$ 0.2	10.0 $\pm$ 0.1	ns
Abdominal diameter of fetuses (mm)	11.0 $\pm$ 0.1	10.9 $\pm$ 0.1	ns
Body length of fetuses (mm)	31.0 $\pm$ 0.7	31.1 $\pm$ 0.3	ns
<b>Both uterine horns</b>			
Total of implants (resorptions and fetuses) per dam	9.8 $\pm$ 0.8	9.3 $\pm$ 0.7	ns
Number of fetal resorptions per dam	0.5 $\pm$ 0.2	1.0 $\pm$ 0.4	ns
Body-weight of fetuses	2.3 $\pm$ 0.1	2.3 $\pm$ 0.05	ns
Abdominal diameter of fetuses (mm)	11.1 $\pm$ 0.1	10.8 $\pm$ 0.1	ns
Body length of fetuses (mm)	31.0 $\pm$ 0.7	30.8 $\pm$ 0.3	ns
Percentage of fetuses with weight $\geq 2.2$ g	75.0 %	80.0 %	ns
Percentage of fetuses with abdominal diameter $\geq 11.1$ mm	60.7 %	26.0 %	0.0004

**Table 2.** Metabolic variables in serum, ovary and uterus of control and hypothyroid dams at 19 days of pregnancy. Total cholesterol, TC; triglycerides, TAG.

Variable	Control (n=6)	Hypothyroid (n=6)	Significance Between Groups
Serum glucose (mg/dL)	108.1 $\pm$ 10.2	108.3 $\pm$ 7.9	t=0.015; df=10; P=0.9
Serum TAG (mg/dL)	100.1 $\pm$ 3.3	122.5 $\pm$ 17.5	U=11.5; P= 0.3
Serum TC (mg/dL)	5.0 $\pm$ 0.4	6.9 $\pm$ 0.8	t=1.9; df=10; P=0.08
TAG in the ovary (mg/g of tissue)	23.7 $\pm$ 2.5	27.1 $\pm$ 4.1	t=0.7; df=10; P=0.4
TC in the ovary (mg/g of tissue)	18.8 $\pm$ 3.9	11.4 $\pm$ 2.0	t=1.654; df=10; P=0.1
Glycogen in the uterus ( $\mu$ mol glycosyl units/ g of tissue)	0.4 $\pm$ 0.08	0.5 $\pm$ 0.1	t=0.7; df=10; P=0.4
TAG in the uterus (mg/g of tissue)	22.6 $\pm$ 4.0	7.5 $\pm$ 1.3	t=3.5; df=10; P=0.005
TC in the uterus (mg/g of tissue)	9.4 $\pm$ 1.3	4.8 $\pm$ 0.9	t=2.7; df=10; P=0.01



**Fig. (2).** Morphometric characteristics of the uterus in control (C, n=6) and hypothyroid (H; n=6) rabbits. **a.** The mean thickness of the endometrium was similar between C and H rats, but the percentage and thickness of endometrium segments > 900 µm in H rats was greater than in controls (**b** and **c**, respectively). **d.** No differences between groups were found in the mean myometrium thickness. The mean cross-sectional area (CSA; **e**) of uterine glands, number of capillaries (**f**) and area covered by capillaries (**g**) were similar between groups. (\*) P = 0.04.



**Fig. (3).** Hypothyroidism increases the expression of 3β-hydroxysteroid dehydrogenase (3β-HSD) in the pregnant uterus. **a.** Immunoblot showing the expression of 3β-HSD in the uterus of control (C) and hypothyroid (H) dams. **b.** Ponceau's Red stained membrane. **c.** Relative 3β-HSD expression in the uterus of C and H rabbits. Note that the 3β-HSD expression in H rabbits is 220% greater than in controls. Four animals per group were averaged. (\*) P = 0.02.

reduced serum concentrations of thyroid hormones and increased thyrotropin as it has been reported in shorter treatments (30 days) [14]. Despite hypothyroidism is a risk factor for pregnancy [26], the fertility of H dams was not modified after methimazole treatment for 49 days, possibly owing to the increased TC and glycogen contents in the ovary [17]. Interestingly, both the number of re-absorptions and the body weight of fetuses were unaffected in H dams [11, 13, 27]. This may be related to the physiology of rabbits. Since female rabbits, differently to rats, can simultaneously be pregnant and lactating without significant changes in the number, growth

and survival of fetuses and pups [28], suggesting that female rabbits might have a very active and specific placental metabolism to provide the requirement of nutrients to their embryos. Although methimazole-induced hypothyroidism did not affect the body weight of fetuses, it reduced their abdominal diameter. In agreement with this, hypothyroidism affects the organogenesis of ovine fetuses [29]. Moreover, modifications in the development of the placenta of hypothyroid rats have been reported [30].

The serum concentration of glucose was similar between H and C dams at GD19, but lower than those previously re-

ported for non-pregnant rabbits [31]. A possible explanation of this reduction is the use of glucose in organs or tissues, including the uterus, which might require a major intake of circulating glucose during pregnancy [32]. In humans, gestation is associated with dyslipidemia, particularly with increased TAG concentration at term [33]. In agreement with this, the TAG concentration in serum and ovaries at GD19 was similar between C and H dams, but these levels were higher than those reported in non-pregnant rabbits [16, 17]. This may result due to either an augmented intake of free fat acids or a decreased lipolysis in the ovary of H dams. In contrast, an increased lipolytic activity in the adipose tissue has been reported in pregnant rats [34]. The low content of TAG in the uterus of H dams suggests a major lipid peroxidation [35], probably as a compensatory effect against lipid accumulation in the placenta. This is common in the placenta of patients with preeclampsia [36], a condition associated with hypothyroidism [37]. Furthermore, low TAG uterine levels in H dams may alter the prostaglandin synthesis, affecting diverse functions of the uterus including the myometrium contractions [38]. In fact, hypothyroidism reduces the contraction amplitude of the uterus of rats [39]. Whilst, thyroxine treatment favors myometrial contraction in women [40].

Hypothyroidism can induce dyslipidemia in adult humans and animals [16-18, 41], which promotes the accumulation of lipids in the ovary [17]. Consequently, H dams had hypercholesterolemia at PD30 (prior matting). However, TC in serum decreases in both C and H dams after copulation, and no significant differences between groups were found at GD19. In contrast, the TC content in the ovary at GD19 increased in C but not in H dams, suggesting a possible disruption in the ovarian steroidogenesis [16]. The TC level in the uterus of nonpregnant rabbits is unknown. We have found that TC level at GD19 is low in H compared to C dams. The serum concentrations of progesterone and estradiol are unaffected in non-pregnant H rabbits [14], suggesting that a reduction of uterine TC in pregnant rabbits might affect the local steroidogenesis. Cholesterol is the precursor of steroid hormones progestogens, estrogens, and glucocorticoids in both uterus and placenta [42]. Accordingly, an increased 3 $\beta$ -HSD expression in the uterus of H dams at GD19 was observed, which participates in the synthesis of progesterone and androstenedione.

Progesterone participates in the endometrial proliferation [43] and the uterine hyperplasia is accompanied by a high expression of 3 $\beta$ -HSD [44]. Our data suggest that the increased endometrial thickness observed in the uterus of H dams might have a causal link to increased 3 $\beta$ -HSD levels. In agreement, hypothyroidism is a risk factor for endometrial hyperplasia [45] and enlargement of the uterus [46]. However, further studies should be done to analyze the role of thyroid hormones on the progesterone content in the uterus and the expression of their receptors.

## CONCLUSION

Hypothyroidism affects the lipid content in the interimplant zones of the uterus. This can be related to steroidogenesis and hyperplasia of the uterus, as well as the reduced size of fetuses. Present results may help understand the association between hypothyroidism and low-weight at birth observed in humans.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Ethics Committee of the Autonomous University of Tlaxcala, Mexico approved this study with reference/protocol number 038-2010-2014.

## HUMAN AND ANIMAL RIGHTS

No humans were involved in the study. All animal procedures were followed according to the Guidelines of the Mexican Law for Production, Care and Use of Laboratory Animals.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analysed during the current study are not publicly available as our University does not have a repository site but are available from the corresponding author on reasonable request.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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## REFERENCES

- [1] Ribeiro, E.S.; Santos, J.E.P.; Thatcher, W.W. Role of lipids on elongation of the preimplantation conceptus in ruminants. *Reproduction*, **2016**, *152*(4), R115-R126. [<http://dx.doi.org/10.1530/REP-16-0104>] [PMID: 27335133]
- [2] Pèrè, M.-C.; Etienne, M. Nutrient uptake of the uterus during the last third of pregnancy in sows: Effects of litter size, gestation stage and maternal glycemia. *Anim. Reprod. Sci.*, **2018**, *188*, 101-113. [<http://dx.doi.org/10.1016/j.anireprosci.2017.11.014>] [PMID: 29187294]
- [3] Wathes, D.C.; Clempson, A.M.; Pollott, G.E. Associations between lipid metabolism and fertility in the dairy cow. *Reprod. Fertil. Dev.*, **2012**, *25*(1), 48-61. [<http://dx.doi.org/10.1071/RD12272>] [PMID: 23244828]
- [4] Dean, M.; Hunt, J.; McDougall, L.; Rose, J. Uterine glycogen metabolism in mink during estrus, embryonic diapause and pregnancy. *J. Reprod. Dev.*, **2014**, *60*(6), 438-446. [<http://dx.doi.org/10.1262/jrd.2014-013>] [PMID: 25225159]
- [5] Catov, J.M.; Bodnar, L.M.; Kip, K.E.; Hubel, C.; Ness, R.B.; Harger, G.; Roberts, J.M. Early pregnancy lipid concentrations and spontaneous preterm birth. *Am. J. Obstet. Gynecol.*, **2007**, *197*(6), 610.e1-610.e7.

- [6] [http://dx.doi.org/10.1016/j.ajog.2007.04.024] [PMID: 18060950]  
Chen, X.; Scholl, T.O.; Stein, T.P.; Steer, R.A.; Williams, K.P. Maternal Circulating Lipid Profile during Early Pregnancy: Racial/Ethnic Differences and Association with Spontaneous Preterm Delivery. *Nutrients*, **2017**, *9*(1), 9.
- [7] [http://dx.doi.org/10.3390/nu9010019] [PMID: 28045435]  
Jiang, S.; Jiang, J.; Xu, H.; Wang, S.; Liu, Z.; Li, M.; Liu, H.; Zheng, S.; Wang, L.; Fei, Y.; Li, X.; Ding, Y.; Wang, Z.; Yu, Y. Maternal dyslipidemia during pregnancy may increase the risk of preterm birth: A meta-analysis. *Taiwan. J. Obstet. Gynecol.*, **2017**, *56*(1), 9-15.
- [8] [http://dx.doi.org/10.1016/j.tjog.2016.07.012] [PMID: 28254234]  
Ornoy, A.; Reece, E.A.; Pavlinkova, G.; Kappen, C.; Miller, R.K. Effect of maternal diabetes on the embryo, fetus, and children: congenital anomalies, genetic and epigenetic changes and developmental outcomes. *Birth Defects Res. C Embryo Today*, **2015**, *105*(1), 53-72.
- [9] [http://dx.doi.org/10.1002/bdrc.21090] [PMID: 25783684]  
Weiner, E.; Barber, E.; Feldstein, O.; Schreiber, L.; Dekalo, A.; Mizrahi, Y.; Bar, J.; Kovo, M. The placental component and neonatal outcome in singleton vs. twin pregnancies complicated by gestational diabetes mellitus. *Placenta*, **2018**, *63*, 39-44.
- [10] [http://dx.doi.org/10.1016/j.placenta.2018.01.010] [PMID: 29486855]  
Arbib, N.; Hadar, E.; Sneh-Arbib, O.; Chen, R.; Wiznitzer, A.; Gabbay-Benziv, R. First trimester thyroid stimulating hormone as an independent risk factor for adverse pregnancy outcome. *J. Matern. Fetal Neonatal Med.*, **2017**, *30*(18), 2174-2178.
- [11] [http://dx.doi.org/10.1080/14767058.2016.1242123] [PMID: 27677438]  
Hou, J.; Yu, P.; Zhu, H.; Pan, H.; Li, N.; Yang, H.; Jiang, Y.; Wang, L.; Wang, B.; Wang, Y.; You, L.; Chen, S. The impact of maternal hypothyroidism during pregnancy on neonatal outcomes: a systematic review and meta-analysis. *Gynecol. Endocrinol.*, **2016**, *32*(1), 9-13.
- [12] [http://dx.doi.org/10.3109/09513590.2015.1104296] [PMID: 26527131]  
Maraka, S.; Ospina, N.M.S.; O'Keeffe, D.T.; Espinosa De Ycaza, A.E.; Gionfriddo, M.R.; Erwin, P.J.; Coddington, C.C., III; Stan, M.N.; Murad, M.H.; Montori, V.M. Subclinical Hypothyroidism in Pregnancy: A Systematic Review and Meta-Analysis. *Thyroid*, **2016**, *26*(4), 580-590.
- [13] [http://dx.doi.org/10.1089/thy.2015.0418] [PMID: 26837268]  
Silva, J.F.; Vidigal, P.N.; Galvão, D.D.; Boeloni, J.N.; Nunes, P.P.; Ocarino, N.M.; Nascimento, E.F.; Serakides, R. Fetal growth restriction in hypothyroidism is associated with changes in proliferative activity, apoptosis and vascularisation of the placenta. *Reprod. Fertil. Dev.*, **2012**, *24*(7), 923-931.
- [14] [http://dx.doi.org/10.1071/RD11219] [PMID: 22935153]  
Anaya-Hernández, A.; Rodríguez-Castelán, J.; Nicolás, L.; Martínez-Gómez, M.; Jiménez-Estrada, I.; Castelán, F.; Cuevas, E. Hypothyroidism affects differentially the cell size of epithelial cells among oviductal regions of rabbits. *Reprod. Domest. Anim.*, **2015**, *50*(1), 104-111.
- [15] [http://dx.doi.org/10.1111/rda.12455] [PMID: 25405800]  
Dukelow, W.R.; Williams, W.L. Survival of capacitated spermatozoa in the oviduct of the rabbit. *J. Reprod. Fertil.*, **1967**, *14*(3), 477-479.
- [16] [http://dx.doi.org/10.1530/jrf.0.0140477] [PMID: 6071013]  
Rodríguez-Castelán, J.; Méndez-Tepepa, M.; Carrillo-Portillo, Y.; Anaya-Hernández, A.; Rodríguez-Antolín, J.; Zambrano, E.; Castelán, F.; Cuevas-Romero, E. Hypothyroidism Reduces the Size of Ovarian Follicles and Promotes Hypertrophy of Periovarian Fat with Infiltration of Macrophages in Adult Rabbits. *BioMed Res. Int.*, **2017**, *20173795950*
- [17] [http://dx.doi.org/10.1155/2017/3795950] [PMID: 28133606]  
Rodríguez-Castelán, J.; Méndez-Tepepa, M.; Rodríguez-Antolín, J.; Castelán, F.; Cuevas-Romero, E. Hypothyroidism affects lipid and glycogen content and peroxisome proliferator-activated receptor  $\delta$  expression in the ovary of the rabbit. *Reprod. Fertil. Dev.*, **2018**, *30*(10), 1380-1387.
- [18] [http://dx.doi.org/10.1071/RD17502] [PMID: 29720336]  
Frank, N.; Sojka, J.E.; Latour, M.A.; McClure, S.R.; Polazzi, L. Effect of hypothyroidism on blood lipid concentrations in horses. *Am. J. Vet. Res.*, **1999**, *60*(6), 730-733.
- [19] [http://dx.doi.org/10.1093/chemse/bjx005] [PMID: 28334158]  
Choo, E.; Dando, R. The Impact of Pregnancy on Taste Function. *Chem. Senses*, **2017**, *42*(4), 279-286.
- [20] [http://dx.doi.org/10.1089/thy.2015.0384] [PMID: 26538454]  
Calvino, C.; Império, G.E.; Wilieman, M.; Costa-E-Sousa, R.H.; Souza, L.L.; Trevenzoli, I.H.; Pazos-Moura, C.C. Hypothyroidism Induces Hypophagia Associated with Alterations in Protein Expression of Neuropeptide Y and Proopiomelanocortin in the Arcuate Nucleus, Independently of Hypothalamic Nuclei-Specific Changes in Leptin Signaling. *Thyroid*, **2016**, *26*(1), 134-143.
- [21] [http://dx.doi.org/10.1080/10.1089/thy.2015.0384] [PMID: 26538454]  
Chan, S.Y.; Franklyn, J.A.; Pemberton, H.N.; Bulmer, J.N.; Visser, T.J.; McCabe, C.J.; Kilby, M.D. Monocarboxylate transporter 8 expression in the human placenta: the effects of severe intrauterine growth restriction. *J. Endocrinol.*, **2006**, *189*(3), 465-471.
- [22] [http://dx.doi.org/10.1677/joe.1.06582] [PMID: 16731778]  
Rodríguez-Castelán, J.; Anaya-Hernández, A.; Méndez-Tepepa, M.; Martínez-Gómez, M.; Castelán, F.; Cuevas-Romero, E. Distribution of thyroid hormone and thyrotropin receptors in reproductive tissues of adult female rabbits. *Endocr. Res.*, **2017**, *42*(1), 59-70.
- [23] [http://dx.doi.org/10.1080/07435800.2016.1182185] [PMID: 27268091]  
Galton, V.A.; Martinez, E.; Hernandez, A.; St Germain, E.A.; Bates, J.M.; St Germain, D.L. The type 2 iodothyronine deiodinase is expressed in the rat uterus and induced during pregnancy. *Endocrinology*, **2001**, *142*(5), 2123-2128.
- [24] [http://dx.doi.org/10.1210/endo.142.5.8169] [PMID: 11316780]  
Galton, V.A.; Martinez, E.; Hernandez, A.; St Germain, E.A.; Bates, J.M.; St Germain, D.L. Pregnant rat uterus expresses high levels of the type 3 iodothyronine deiodinase. *J. Clin. Invest.*, **1999**, *103*(7), 979-987.
- [25] [http://dx.doi.org/10.1172/JCI6073] [PMID: 10194470]  
Huang, S.A.; Dorfman, D.M.; Genest, D.R.; Salvatore, D.; Larsen, P.R. Type 3 iodothyronine deiodinase is highly expressed in the human uteroplacental unit and in fetal epithelium. *J. Clin. Endocrinol. Metab.*, **2003**, *88*(3), 1384-1388.
- [26] [http://dx.doi.org/10.1210/jc.2002-021291] [PMID: 12629133]  
Teng, W.; Shan, Z.; Patil-Sisodia, K.; Cooper, D.S. Hypothyroidism in pregnancy. *Lancet Diabetes Endocrinol.*, **2013**, *1*(3), 228-237.
- [27] [http://dx.doi.org/10.1016/S2213-8587(13)70109-8] [PMID: 24622371]  
Bolarinwa, A.F.; Olaleye, S.B. Blastocyst implantation: effect of thyroidectomy and thyroxine replacement in the rat. *Afr. J. Med. Med. Sci.*, **1997**, *26*(3-4), 135-137.
- [28] [PMID: 10456155]  
Martínez-Gómez, M.; Juárez, M.; Distel, H.; Hudson, R. Overlapping litters and reproductive performance in the domestic rabbit. *Physiol. Behav.*, **2004**, *82*(4), 629-636.
- [29] [http://dx.doi.org/10.1016/j.physbeh.2004.05.011] [PMID: 15327910]  
Erenberg, A.; Omori, K.; Menkes, J.H.; O, W.; Fisher, D.A. Growth and development of the thyroidectomized ovine fetus. *Pediatr. Res.*, **1974**, *8*(9), 783-789.
- [30] [http://dx.doi.org/10.1203/00006450-197409000-00001] [PMID: 4413605]  
Silva, J.F.; Ocarino, N.M.; Serakides, R. Maternal thyroid dysfunction affects placental profile of inflammatory mediators and the intrauterine trophoblast migration kinetics. *Reproduction*, **2014**, *147*(6), 803-816.
- [31] [http://dx.doi.org/10.1530/REP-13-0374] [PMID: 24534949]  
Rodríguez-Castelán, J.; Nicolás, L.; Morimoto, S.; Cuevas, E. The Langerhans islet cells of female rabbits are differentially affected by hypothyroidism depending on the islet size. *Endocrine*, **2015**, *48*(3), 811-817.
- [32] [http://dx.doi.org/10.1007/s12020-014-0418-4] [PMID: 25213470]  
Crouse, M.S.; McLean, K.J.; Crosswhite, M.R.; Reynolds, L.P.; Dahlen, C.R.; Neville, B.W.; Borowicz, P.P.; Caton, J.S. Nutrient transporters in bovine uteroplacental tissues on days sixteen to fifty of gestation. *J. Anim. Sci.*, **2016**, *94*(11), 4738-4747.
- [33] [http://dx.doi.org/10.2527/jas.2016-0857] [PMID: 27898936]  
Montelongo, A.; Lasunción, M.A.; Pallardo, L.F.; Herrera, E. Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. *Diabetes*, **1992**, *41*(12), 1651-1659.
- [http://dx.doi.org/10.2337/diab.41.12.1651] [PMID: 1446807]

- [34] López-Soldado, I.; Ortega-Senovilla, H.; Herrera, E. Maternal adipose tissue becomes a source of fatty acids for the fetus in fasted pregnant rats given diets with different fatty acid compositions. *Eur. J. Nutr.*, **2018**, *57*(8), 2963-2974. [http://dx.doi.org/10.1007/s00394-017-1570-4] [PMID: 29127477]
- [35] Kong, L.; Wei, Q.; Fedail, J.S.; Shi, F.; Nagaoka, K.; Watanabe, G. Effects of thyroid hormones on the antioxidative status in the uterus of young adult rats. *J. Reprod. Dev.*, **2015**, *61*(3), 219-227. [http://dx.doi.org/10.1262/jrd.2014-129] [PMID: 25797533]
- [36] Brown, S.H.J.; Eather, S.R.; Freeman, D.J.; Meyer, B.J.; Mitchell, T.W. A Lipidomic Analysis of Placenta in Preeclampsia: Evidence for Lipid Storage. *PLoS One*, **2016**, *11*(9)e0163972. [http://dx.doi.org/10.1371/journal.pone.0163972] [PMID: 27685997]
- [37] Männistö, T.; Karumanchi, S.A.; Pouta, A.; Väärämäki, M.; Mendola, P.; Miettola, S.; Surcel, H.-M.; Bloigu, A.; Ruokonen, A.; Järvelin, M.-R.; Hartikainen, A.-L.; Suvanto, E. Preeclampsia, gestational hypertension and subsequent hypothyroidism. *Pregnancy Hypertens.*, **2013**, *3*(1), 21-27. [http://dx.doi.org/10.1016/j.preghy.2012.09.001] [PMID: 23439671]
- [38] Herington, J.L.; O'Brien, C.; Robuck, M.F.; Lei, W.; Brown, N.; Slaughter, J.C.; Paria, B.C.; Mahadevan-Jansen, A.; Reese, J. Prostaglandin-Endoperoxide Synthase 1 Mediates the Timing of Parturition in Mice Despite Unhindered Uterine Contractility. *Endocrinology*, **2018**, *159*(1), 490-505. [http://dx.doi.org/10.1210/en.2017-00647] [PMID: 29029054]
- [39] Parija, S.C.; Mishra, S.K.; Raviprakash, V. Hypothyroid state reduces calcium channel function in 18-day pregnant rat uterus. *Indian J. Exp. Biol.*, **2006**, *44*(1), 19-27. [PMID: 16430086]
- [40] Corriveau, S.; Pasquier, J.C.; Blouin, S.; Bellabarba, D.; Rousseau, É. Chronic levothyroxine and acute T3 treatments enhance the amplitude and time course of uterine contractions in human. *Am. J. Physiol. Endocrinol. Metab.*, **2013**, *304*(5), E478-E485. [http://dx.doi.org/10.1152/ajpendo.00346.2012] [PMID: 23249699]
- [41] Jayasingh, I.A.; Puthuran, P. Subclinical hypothyroidism and the risk of hypercholesterolemia. *J. Family Med. Prim. Care*, **2016**, *5*(4), 809-816. [http://dx.doi.org/10.4103/2249-4863.2011177] [PMID: 28348996]
- [42] Chatuphonprasert, W.; Jarukamjorn, K.; Ellinger, I. Physiology and Pathophysiology of Steroid Biosynthesis, Transport and Metabolism in the Human Placenta. *Front. Pharmacol.*, **2018**, *9*, 1027. [http://dx.doi.org/10.3389/fphar.2018.01027] [PMID: 30258364]
- [43] Pan, J.L.; Yuan, D.Z.; Zhao, Y.B.; Nie, L.; Lei, Y.; Liu, M.; Long, Y.; Zhang, J.H.; Blok, L.J.; Burger, C.W.; Yue, L.M. Progesterone-induced miR-133a inhibits the proliferation of endometrial epithelial cells. *Acta Physiol. (Oxf.)*, **2017**, *219*(3), 683-692. [http://dx.doi.org/10.1111/apha.12762] [PMID: 27458709]
- [44] Gultiken, N.; Yarim, M.; Yarim, G.F.; Gacar, A.; Mason, J.I. Expression of 3 $\beta$ -hydroxysteroid dehydrogenase in ovarian and uterine tissue during diestrus and open cervix cystic endometrial hyperplasia-pyometra in the bitch. *Theriogenology*, **2016**, *86*(2), 572-578. [http://dx.doi.org/10.1016/j.theriogenology.2016.02.006] [PMID: 27020880]
- [45] Soleymani, E.; Ziari, K.; Rahmani, O.; Dadpay, M.; Taheri-Dolatabadi, M.; Alizadeh, K.; Ghanbarzadeh, N. Histopathological findings of endometrial specimens in abnormal uterine bleeding. *Arch. Gynecol. Obstet.*, **2014**, *289*(4), 845-849. [http://dx.doi.org/10.1007/s00404-013-3043-1] [PMID: 24121689]
- [46] Hu, Y.; Wang, Q.; Li, G.; Sun, X.; Liu, C. Ultrasonic morphology of uterus and ovaries in girls with pituitary hyperplasia secondary to primary hypothyroidism. *Horm. Metab. Res.*, **2013**, *45*(9), 669-674. [http://dx.doi.org/10.1055/s-0033-1345141] [PMID: 23670347]