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Original Article

An Abattoir Study of Ovine Maternal and Fetal Thyroid Lesions and the Respective Serum T3 andT4 Levels in an Endemic Goiter Region in Iran

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Abstract

The objectives of the present study were to investigate the prevalence of histologic thyroids lesions and the respective thyroid hormones changes of ewes in an endemic goiter region and to find out any impact of this condition on the fetal thyroid structures and serum thyroid hormones. In the present study a total number of 100 pregnant ewes and their fetuses slaughtered at slaughterhouse were selected for the study. The sera were prepared from the jugular vein blood of ewes and fetuses umbilical cords. The ewes and fetuses thyroids subjected to histopathological examination and radioimmunoassay procedure was used for sera T3 and T4 assay. The results showed a high prevalence of thyroid lesions in ewes and their fetuses. The different kinds of lesions including cysts, follicular hyperplasia, hemorrhage and inflammation were seen in ewes. In the fetuses thyroid structural changes were cysts resemble structures; hemorrhage and hyperemia. Mean T4 concentration was significantly higher in the ewes with pathologic thyroid compared to normal animals. However, there was not any significant difference between pathologic and normal ewes' thyroids on serum T3. Although, existence of lesions on fetal thyroids did not affect the serum concentrations of T4 and T3, there was a significant correlation between T4 and T3 serum concentrations of fetuses and their age. In conclusion, the significant prevalence of fetal and maternal thyroid pathological changes in the endemic goiter region raises a question about probable congenital source of these variations. The thyroid lesions significantly influenced the functions of mothers' gland but not fetuses.

Key words: Endemic goiter region, Fetus, Ewe, Thyroid lesion, Thyroxin

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Introduction

Thyroid glands have important role in growth and development of fetus during pregnancy and maintaining metabolic homeostasis in mammals.[1] Presence of an intact maternal gland during early pregnancy to produce thyroxin and support the development of central nervous system of the embryos is essential. In sheep, placental transfer of thyroid hormones is important during early gestation, but in contrast to man and rat, seems to be absent or at least strongly diminished in the second half of the gestation.[2] Maternal thyroid malfunction was associated with disorders in fetal development. It has been shown that maternal thyroidectomy before conception causes a reduction in fetal brain size and body growth at midgestation and from midgestation onward, fetal plasma thyroxin levels are not different from those of control fetuses, indicating that from midgestation onward, the fetus relays on own thyroxin hormone.[3]

Different fetal congenital disorders were reported in different species. Fetal growth retardation, still birth, abortion and prolonged gestation were the consequences of these problems. The fetal thyroidectomy after second half of gestation decreased fetal hormones causing organs damage, retarded fetal brain development and early postnatal death.[4-6] Thus, in sheep, maternal and fetal thyroid dysfunction during early and late pregnancy respectively can have some effects on newborn lamb performance. During fetal life the developing thyroid responses to the stimulant factors by undergoing structural changes such as hypertrophy, hyperplasia, cell death and fibrosis.[7] In the previous research we reported 41% of sheep slaughtered at slaughterhouse had different lesions in their thyroids [8] and in another abattoir study we showed various lesions in the thyroid of 59 % and 21 % of pregnant ewes and their fetuses, respectively.[9] These high incidences of thyroid pathologic changes among the sheep prompted us to measure thyroid hormones of pregnant ewes and their fetuses to see if these changes had any effect on the thyroid functions. The aim of the present study was to investigate the changes of fetal and maternal thyroid microstructure and serum concentration of T3 and T4 in an endemic goiter region.

Materials and Methods

Location of sampling. The research was carried out in Shahrekurd, center of Chaharmahal and Bakhtiari province, Iran (latitude 32°:20’ N, longitude 50°:51’ E and altitude 2061 m). Based on the previous report, this region was an endemic area for goiter in human and by adding iodine to the diets the problem could be solved.[10] There is no such a supportive program for domestic animals living in the area.

Estimating fetal age and weight. Fetuses were weighed with a digital scales and crown rump length (CRL) of fetuses were measured with a regular tape measure. Fetal age was estimated according to previous equation based on previous reported formula: X=2.1(Y+17), where X is the estimated age of fetus in day and Y is the length of CRL in cm.[11]

Blood and thyroid sample. Maternal thyroid and blood samples were taken from 100 pregnant ewes slaughtered at local abattoir in a period of 6 months from January to June 2008. The uterus was excised and after removal of the fetus, their weight and CRL were recorded. The maternal and fetal thyroid dissected and their small pieces were fixed for examination. Fetal blood samples were taken from umbilical cord and their respective mother’s blood were collected from jugular vein. After collection, the blood samples were allowed to stand for 20 - 30 minutes and then they were centrifuged at 2000 to 3000 rpm for 15

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minutes. The sera was separated and stored at -22°C till assayed.

Hormone assays. Serum T3 was measured with radioimmunoassay kit (IM1699-IM3287, Immunotech, Czech Republic). Inter- and intra-assay coefficient of variation for 10 assays were 7.7 and 6.3 %, respectively. Serum T4 was measured with a radioimmunoassay kit (IM1447-IM3286, Immunotech, Czech Republic). Inter- and intra-assay coefficient of variation for 10 assays were 8.6 and 6.2 %, respectively. The antibodies, which use in these immunoassays, were highly specific for T3 and T4. Extremely low cross-reaction were obtained with several related molecules.

Thyroid histopathology examination. The thyroid glands were dissected free of fat, fixed in 10 % buffered formalin for 24-48 h, dehydrated in ethyl alcohol series (70, 80, 90, 100, 100-I 100-II %) and embedded in paraffin wax. Tissue sections were cut at 5 μm thickness, stained with hematoxylin and eosin (H&E) for histopathological examination.

Statistical analysis. The ewes and fetuses thyroid histological finding were classified as normal or pathologic and the pathologic thyroids were defined as the thyroids with single or multiple lesions. The percentage of pathologic thyroids was compared with the normal using chi-square test. In this model the serum T4 and T3 concentrations were analyzed between different groups using general linear model (GLM) procedure of SAS (Statistical Analysis System, 8th ed., 1996). The Pearson correlation test was used to find any correlation between the ewes T4 and T3 and that of the fetus. This test also applied to find any correlation between concentrations of fetuses' hormones with fetal age and weight. The Spearman correlation test was used to find any correlation between existence of the pathology and the serum hormones concentration from the mother and the fetus. The correlation between the fetal pathology with fetal age, sex and ewes' pathology was also analyzed using Spearman correlation test. Regression analysis was used to find any association between fetal age and weight with different hormone concentrations. Data have been shown as least square means (LS Mean) and standard error of mean (*S.E.*).

Results

Pathology. The results of this study revealed very high prevalence of pathologic thyroids lesions in the ewes 65.9 % (58/88) (Table 1) and fetuses 65 % (65/100) (Table 2). Two types of goiter were seen in 36 thyroid samples: follicular hyperplasia and hyperplasic goiter with different degree which was noticed in 32 out of 36 (Fig 1A and B) and colloid goiter in 4 out of 36 (Fig 1C), Ultimobranchial cyst in 8 out of 88 with stratified squamous epithelial cells and keratinized material in lumen (Fig 1D), thyroiditis (lymphocytic and suppurative) in 4 out of 88. In 10 thyroids more than one lesion was observed.

The thyroid samples from 35 fetuses were normal (Fig 2A). Histopathologic results of fetal thyroids revealed different degree of hyperemia with accumulation of red blood cells in follicle walls and hemorrhage of thyroid (Fig 2B). Cyst with eosinophilic materials was detected in 9 fetal thyroids (Fig 2 C and D). Hemorrhage with follicular cell necrosis was seen in one case.

Hormones. The mean serum concentration of T4 (nmol L^{-1}) was significantly higher ($p = 0.03$) in the thyroids with pathologies (118.7 ± 5.5) compared to the normal (95.4 ± 9.04) glands. There was no difference ($P > 0.05$) between pathologic and non-pathologic thyroids on serum concentration of T3 [2.1 ± 0.11 vs. 2.07 ± 0.19 (nmol L^{-1})], respectively. Table 1 shows the mean serum concentrations of assayed hormones in the ewes with different thyroid microstructures. The highest serum T4 was

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noticed in ewes with ultimo bronchial lesions which was significantly higher than that of ewes with normal thyroids. (Table1).)

The mean serum concentration of T4 [68.6 ± 5.8 vs. 57.5 ± 8.2 (nmol L^{-1}); p = 0.27] and T3 [1.95 ± 0.22 vs. 1.24 ± 0.31 (nmol L^{-1}); p = 0.066] were not significantly different between the fetuses with the thyroid pathology and healthy fetuses. The details of hormones in the fetuses with thyroid lesions are shown in Table 2. Only presence of cysts caused significantly increase of serum T4 and T3. No association between fetus and ewes T4 (r = -0.1, p = 0.28) and T3 (r = 0.04, p = 0.7) were observed. A significant correlation between fetal age with serum T4 (r = 0.776, p = 0.0001) and T3 (r = 0.586, p = 0.0001) concentration was observed. Table 3 shows the regression analysis between fetal serum hormones with age and weight.

The mean serum concentrations of thyroid hormones in fetuses relative to gestational age are shown in Table 4.

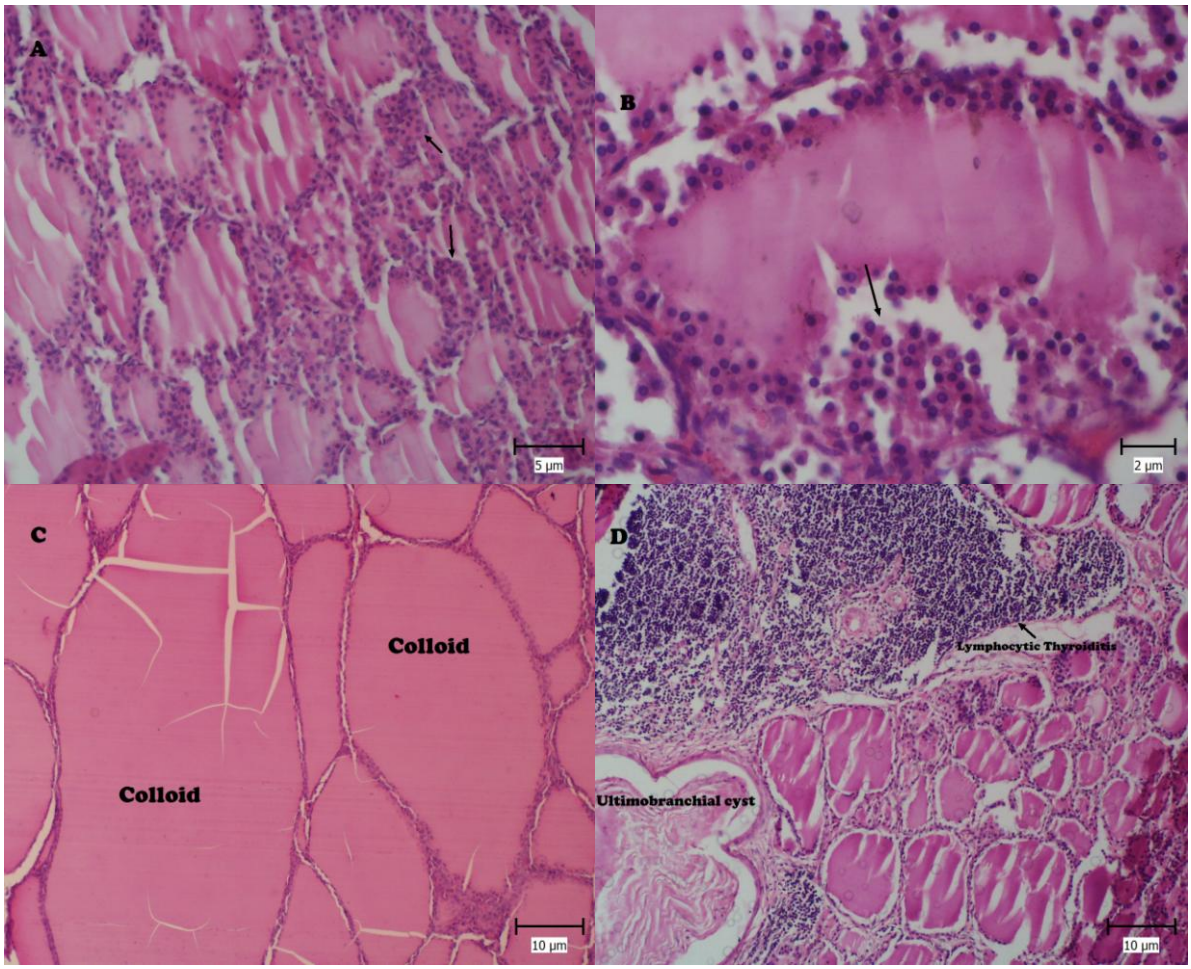

Fig 1. A: Hyperplastic Goiter: The characteristic of this lesion is proliferation of follicular cells (arrows). (H&E). **B**: Part of Fig 1A. Note different epithelial cells which locate in more than one layer and projected to the lumen. (H&E). **C**: Colloid Goiter: very big follicles with colloid accumulation (H&E, Bar = 10 µm).**D**: Ultimobranchial cyst and Lymphocytic thyroiditis (H&E).

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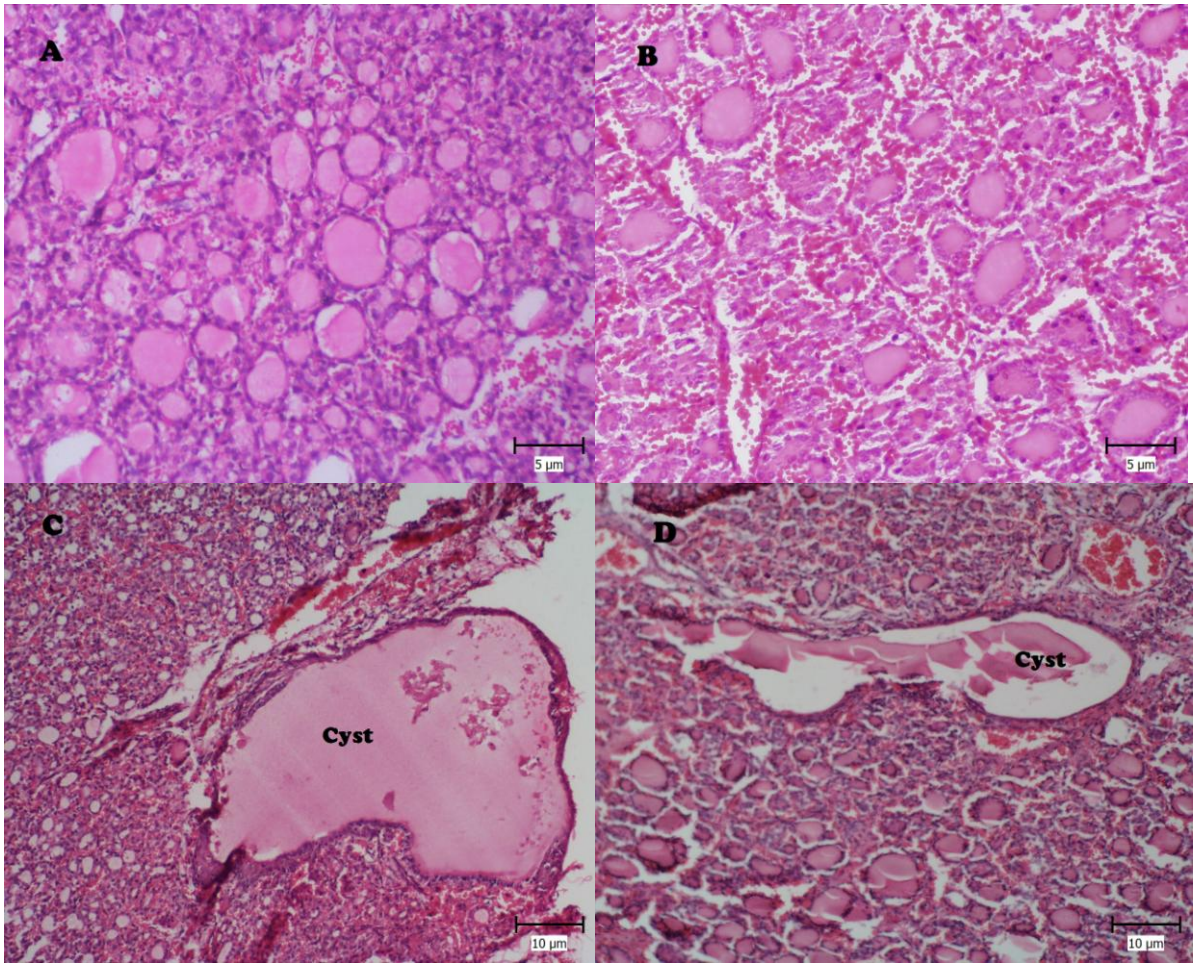

Fig 2. A: Normal fetal thyroid. Note to different size of follicles with colloid. (H&E). **B**: Hyperemia in fetal thyroid. Note to the accumulation of red blood cells between follicles (H&E). **C** and D: Branchial Cyst in fetal thyroid. Big cavity containing eosinophilic materials with cuboidal epithelial is obvious (H&E).

Table 1. Distribution of different pathologic lesions ewes' thyroids and related concentration of serum T4 and T3 (Least square mean ± SE)

Type of Lesions	No. (%)	T3	T4
Follicular hyperplasia/Goiter	32	1.9 ± 0.49	119.3 ± 19.8
Colloid Goiter	4	2.4 ± 0.03	95 ± 60.5
Ultimobrnachial Cyst	8	0.25 ± 0.05	212 ± 37.1
Lymphocytic Thyroiditis	3	2.9 ± 0.49	132.3 ± 60.5
Suppurative Thyroiditis	1	2.3 ± 0.85	119 ± 104.9
Normal	30	1.96 ± 0.16	98.3 ± 20.2
More than one lesion	10	2.25 ± 0.26	124.5 ± 33.2

Table 2. Distribution of different pathologic lesions in the fetuses' thyroids and related concentration of cord serum T4 and T3 (Ls mean ± SE)

Type of Lesions	No.	T3	T4
Hyperemia and hemorrhage	56	1.6 ± 1.4	62.8 ± 42.2
Branchial Cyst	9	4.2 ± 2.5*	104.33 ± 35.5*
Normal	35	1.2 ± 1.7	54.8 ± 53.3

The values with * sign within rows are significantly different with normal thyroids ($P < 0.05$)

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Table 3. The regression analyses between fetal analyzed hormones and fetal ages and weights.

Regression analysis	
T4 = 1.64 × age - 80.4	P = 0.0000
T3 = 0.057 × age - 3.26	P = 0.0001
T4 = 0.0001 × weight + 0.691	P = 0.0001
T3 = 0.042 × weight + 22.2	P = 0.0001

Table 4. The fetal cord serum T4 and T3 concentrations (Ls Mean ± SE) according to the gestational age of the ovine fetuses

Age (days)	No.	T4 (nmol L^{-1})	T3 (nmol L^{-1})
40 - 50	3	22.5 ± 9.25^{cd}	0.1 ± 0.03^{ab}
51 - 60	10	2.9 ± 0.69^{d}	0.04 ± 0.02^{b}
61 - 70	21	19.8 ± 4.14^{cd}	0.16 ± 0.11^{b}
71 - 80	13	35.5 ± 4.91^{c}	0.32 ± 0.11^{b}
81 - 90	16	70.5 ± 9.68^{b}	1.72 ± 0.29^{ac}
91 - 100	19	81.8 ± 8.3^{b}	2.8 ± 0.36^{cd}
101 - 110	16	111.7 ± 8.61^{a}	3.2 ± 0.32^{d}
111 - 120	11	85.5 ± 13.79^{b}	4.8 ± 1.58^{e}
>121	8	126.4 ± 11.07^{a}	2.2 ± 0.67^{acd}

^{abcd} The values with different letter significantly differ within column ($P < 0.05$)

Discussion

The results of maternal thyroid structure and function showed a high prevalence of morphological and functional goiter in ewes. In the present study the thyroids of more than 65% of ewes showed different types of pathologic lesions. Thirty four percent of the thyroids had a single lesion composed of colloid goiter, follicular hyperplasia goiter or ultimo bronchial cysts. More than 10% of the thyroids showed multiple lesions. The previous studies also in sheep at the other regions of Iran showed 41 [8] and 59% [9] thyroid lesions. An abattoir study in cattle in the north part of the country revealed an incidence of 15% thyroid pathologies.[12] In the present study it was shown that the serum T4 concentration in the ewes with the thyroid lesions was higher than healthy subjects. However, the mean serum T4 concentration in the healthy ewes was higher than the normal values (58.5 ± 14.5; 38-79.2 nmol L^{-1}) for sheep, [13] this could be due to the pregnancy in all the ewes in the present study. It has been shown that serum total T4 and T3 steadily increase during pregnancy to approximately 1.5 times the non-pregnant level.[14-16] Report from other part of Iran showed that serum T4 (6.3 ± 0.17 μg dL^{-1}) in healthy sheep was lower than that of finding at the current study.[8] On the other hand, it was also shown that the normal value for total T4 was101.29 ± 42.86 nmol L^{-1} which is almost in line with the finding of the present study.[17] The results of this study showed that presence of lesions in the thyroid was associated with high levels of serum T4 and the thyroids with inflammation and multiple lesions, significantly secreted T4.

The serum T3 concentration (2.09 ± 0.15 nmol L^{-1}) in the present study was not affected by the thyroid structural changes and it was within the normal range (1.53 ± 0.43 nmol L^{-1})[13] and was also in agreement with the previous research which reported the value of 1.31 ± 0.26 (nmol L^{-1}) for T3 in ewes.[17] The more analysis of our data showed that the inflammation or presence of multiple lesions may increase the serum levels of T3 in sheep.

The high prevalence of the thyroid dysfunction and structural abnormalities in the ewes and their fetuses raised the

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likelihood of hereditary nature of these lesions in sheep in current study.

As previously described the present study was conducted in an endemic goiter region of Iran that when human diets were supplemented with iodine the rate of goiter in human population showed a significant decline.[10]

The results of the present study showed a high prevalence of thyroid lesions in the ovine fetuses. The importance of these lesions and their impacts on animal performance are not clear at the present time and remained to be investigated. However, there are many reports of individual cases of fetal thyroid abnormalities with different pregnancy outcome. Congenital colloid goiter in camel[18] and thyroid hyperplasia in horse[19] fetuses were associated with abortion and other structural organ abnormalities. Other cases of congenital abnormalities like adenoma in the bovine fetal thyroid[20] and congenital cervical immature teratoma in the left lobe of the human fetal thyroid gland[21] also were reported. In cow, congenital hypoplasia of fetal adrenal and thyroid glands were associated with prolonged gestation.[22]. In the present study to reveal the effect of thyroid lesions on its function, the T4 and T3 of umbilical cord blood of the fetuses were measured. There was no significant different between normal thyroids hormones compared with the pathologic glands. However, the thyroids with cyst like structures significantly produced T4 and T3 concentrations compare with the normal and other types of lesions.

The interaction of maternal and fetal thyroid function has been investigated in human. While some studies reported that maternal hyperthyroidism resulted in fetal congenital hypothyroidism, [5, 23-25] the others indicating fetal congenital hyperthyroidism in hyperthyroid ewes.[4,6]

Feeding of female goats at different stages of gestation with subabul (Leucaena Leucocephala) leaves was associated with 44.5 % of abortion that all of aborted fetuses and their mothers had enlarged thyroid gland and showed parenchymatous and colloidal goiter indicating that subabul feeding not only caused hypothyroidism and reproductive failures in the female goats but also caused congenital goiter in the progeny.[26] Cyanide toxicity in goats also induced histological lesions like vacuolation of thyroid follicular cells, increased number of vacuoles on thyroid follicular colloid in fetuses and their mothers.[27]

The results of this research revealed that thyroid lesions are very high among sheeps in Shahrekurd and they may have them from their fetal life. It was also shown that these lesions could interfere with the thyroid functions. It is not clear to what extent, these changes of thyroid structures and functions could influence the animal growth and fertility and more research is needed to be carried out to clarify these ambiguities.

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