EFFECT OF TREATMENT WITH DEXAMETHASONE ON THYROID FUNCTION IN LACTATING FEMALE RATS

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ABSTRACT

The present study is designed to evaluate the effect of dexamethasone treatment on thyroid gland function in lactating female rats. Thirty six lactating female rats of (250±20gm) body weight were divided randomly into six equal groups (n=6).Three control groups (1-3) were injected subcutaneously with normal saline daily at lactation days 1-10,1-20 and 1-30 respectively. Three treatment groups (T1, T2 &T3) were injected subcutaneously 150 µg/kg/day dexamethasone (Dex) at lactation days 1-10, 1-20 and 1-30 respectively. At the end of each treatment period, the animals were sacrificed and blood samples were collected for the purpose of measuring the level of thyroid hormones and the thyroid glands of each animal were removed and maintained in 10% formalin for the study of histological changes. The results showed a significant ($P \leq 0,05$) decrease in serum levels of TSH in (1-30), T_4 in (1-20),(1-30)and in T_3 levels in all Dex treated groups compared with control groups.In addition histopathological study showed architectural changes including, microfollicles, other distended with colloid lined by low cuboidal thyrocytes ,vacuolation of colloid and some thyrocytes with areas of hyperplasia.

INTRODUCTION

Glucocorticoids (GCs) are a category of corticosteroids recognized as "stress hormones", because they participate in the initial hormonal response when exposed to acute and chronic stress (1; 2). Corticosteroids are one of the most useful and widely used drugs in veterinary medicine. Dexamethasone (Dex) is a commonly used synthetic drug that suppresses the immune response of an individual, which belongs to GCs and its effectiveness exceeds cortisol by 20-30 times. GC shifts from the cytosol to nucleus of the target cells, where it binds to receptors specific for GC within the regulatory DNA sequences and eventually, the expression of reactive genes is modified in GC (3; 4). Dexamethasone is powerful synthetic glucocorticoid that has long been used in the treatment of many metabolic diseases and inflammations resulting from infections in both veterinary and human medicine (5) . Clinically, dexamethasone is given to suppress inflammation (6).In addition to alleviating other diseases. It was used in veterinary medicine as a first line for animals treatment. Animal diseases in which dexamethasone is most effectively used in treatment include, but not limited to, inflammation, acetonemia / ketosis, nonspecific skin diseases, trauma, and stress (7 ; 8). Thyroid hormones regulate the metabolic processes necessary for normal growth and differentiation in the developing organism, as well as determining the metabolic rate in adults (8; 9), especially regarding regulating intracellular energy metabolism (10 ; 11). The thyroid tissue contains alpha and beta glucocorticoid receptors, which play an important role in the process of differentiation of thyroid cells, as it appeared that adenoma c of thyroid cells, have shown a decrease in the mRNA GC- alpha receptors and an increase in GC- beta receptors (12). The thyroid gland axis is particularly exposed to a large group of medicines and natural substances, the number of which increases every year. These substances influence all physiological and pharmacological aspect of thyroid gland (13). GCs have been shown to influence the thyroid metabolism by affecting the hypothalamus- pituitary-thyroid axis (14). Chronic administration (35 days days) of GCs at low doses as anti-inflammatory agent causes a significant changes in T3 and T4 peripheral metabolism by changing their binding to carrier proteins, changing their distribution, and reduction in the conversion of T_4 to T_3(14). The close relationship between pituitary-adrenal and pituitary-thyroid systems, is somewhat known. Moreover, the mechanisms of their interaction at various levels

have not been definitively established. This is of great importance because glucocorticoids and thyroid hormones play a major role in regulating the most important systems of vital activity and adaptation. The role of glucocorticosteroids in regulating thyroid cell function is interesting because of the noticeable growth of thyroid diseases in different regions of the world, as well as significantly improving prevention of iodine (15).Therefore, the present study was designed to determine the effect of dexamethasone treatment on hormonal levels and histological changes of thyroid gland in lactating female rats.

MATERIALS AND METHODS

Experimental Animals:

The experiment was carried out in the animal house of the college of Veterinary Medicine/ University of Basrah, Iraq. Thirty six well-experienced female adult rats, weighed (250± 30) g were used in this study. The animals were maintained under controlled condition in the animal house, three weeks without any treatment for acclimatization before the beginning of the experiment.

Experimental design:

Animal were divided randomly into six groups; three control groups were injected subcutaneously with normal saline from day 1-10, 1- 20 and 1 - 30 of lactation respectively and three treatment groups (T1, T2 and T3)were injected subcutaneously with 150 µg/kg/day dexamethasone from day 1-10,1 -20 days , 1- 3o of lactation respectively.

The animals were euthanized via chloroform at the end of each experimental period,. Blood samples were obtained directly from the heart using a disposable 5 ml syringes from anaesthetized rats between 9.00 to 11.00 A.M in order to minimize the diurnal variation of hormones levels. The collected blood samples were poured into serum separator tubes and centrifuged at 3000 rpm for 15 minutes to obtain the serum samples, and kept in Eppendorf tubes and stored at -20 ºC until used for hormonal assay, after that the animals were sacrificed and thyroid glands were rapidly removed and maintained in 10% formalin for histological study.

Hormonal assay:Enzyme-Linked Immunosorbent Assay(ELISA):

T3 concentration was assayed by using ELISA kits from Monobind Inc.lake forest CA 92630; USA, Product code : l25-300(16). Similarly T4 concentration was assayed by ELISAKit (Mononobind Inc. lake forest CA 92630, USA).Product Code: 225-300 (17).TSH also was measured by using ELISA kit from (Calbiotech Inc. a life science company, USA), Product Code: TS227T(18).

Histological study:

Thyroid glands, were dehydrated a series of alcohol and cleaned in xylol, then tissue were embedded in paraffin wax. Sections were stained with haematoxylin followed by eosin and examined under light microscope (19).

Statistical analysis:

The data are expressed as mean values ± SD. Statistical analysis was performed using one-wayanalysis of variance (ANOVA) to assess significant differences between treatment groups. The criterion for statistical significance was set at $P<0.05$. All statistical analyzes were performed using SPSS statistical version 8 software package (SPSS Inc., USA).

RESULTS

Table (1) revealed that TSH levels decreased significantly ($P\leq 0, 05$) in rats group treated with Dex 150µg/kg. Bw/day from day 1 of lactation for 30 days compared with control and other treatment groups. Moreover a significant reduction ($P\leq0.05$)in serum T4 levelswere recorded in T1 & T2 (rats dams treated with Dex 150µg/kgbw/day from day 1 of lactation for 20 & 30 days) respectively in comparison with control groups and other treatment group. Finally a significant decrease ($P\leq0.05$) in T3 levels were recorded in all treatment groups compared with control groups.

Table 1: Effect of DEX treatment during lactation on thyroid function in female rats(Mean±SD) (n=6)

Parameters / Groups	TSH(µlU/ml)	T_4 (µg/dl)	T_3 (ng/ml)
Control (1)	0.13±0.002**a**	2.53±0.05 **a**	1.90±0.30 **ab**
Control (2)	0.13±0.003 **a**	2.52±0.04**a**	1.94±0.36**a**
Control (3)	0.13±0.002 **a**	2.53±0.05 **a**	1.92±0.29 **a**
T1	0.13±0.00 **a**	2.54±0.02**a**	1.13±0.06 **b**
T2	0.13±0.00 **a**	1.84±0.55 **b**	1.09±0.05 **b**
T3	0.02±0.03**b**	1.81±0.39 **b**	0.78±0.34 **b**
LSD	0.11	0.67	0.79

The different letters refer to significant differences among groups at ($p \leq 0.05$) level.

n=number of animals in each group Control groups (1,2,3): female rats were injected (s/c) with normal saline from first day of lactation for 10, 20 and 30 days respectively.

T 1,2,3 :Female rats injected (s/c) with Dex 150 µg/kg bw/day from first day of lactation for 10, 20 and 30 days respectively.

Histological Study

Microscopic picture of control rats thyroid gland showed thyroid follicles of different sizes filled with homogenous colloid and lined by cuboidal thyrocytes, surrounded by normal parafollicular cells(C cells), as shown in Figure(1). While thyroid section in female group treated with Dex for 10 days shows,central microfollicles with vacuolated colloid( ), some follicles lined by vacuolated thyrocytes, perpheral distend thyroid follicles, filled with colloid

and lined by low cuboidal or squamous thyrocytesas shown in Figure(2). The thyroid glands of female group treated with Dex for 20 daysshowing architectural changes, micro-thyroid follicles filled with small amount of colloid and lined by cuboidal cells, some area of hyperplasia of follicular cells , vacuolation of some thyroid follicles , other distended thyroid follicles, filled with colloid and lined by low cuboidal or squamous thyrocytes figure(3). Thyroid gland of lactating female rats treated with Dex for 30 days, showing , some thyroid follicles are distended, filled with colloid and lined by low cuboidal or appear squamous thyrocytes, most other follicles lost its normal architecture figure(4).

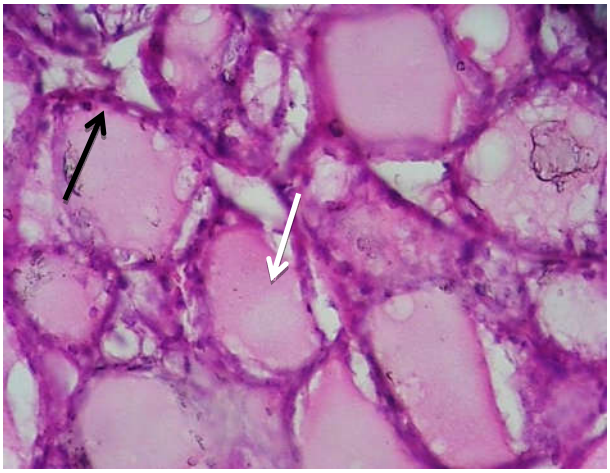

Fig.1: Thyroid gland of female control rats , shows normal architecture, thyroid follicles, filled with colloid(white arrow) and lined by cuboidal thyrocytes(black arrow) (H&E) 400X

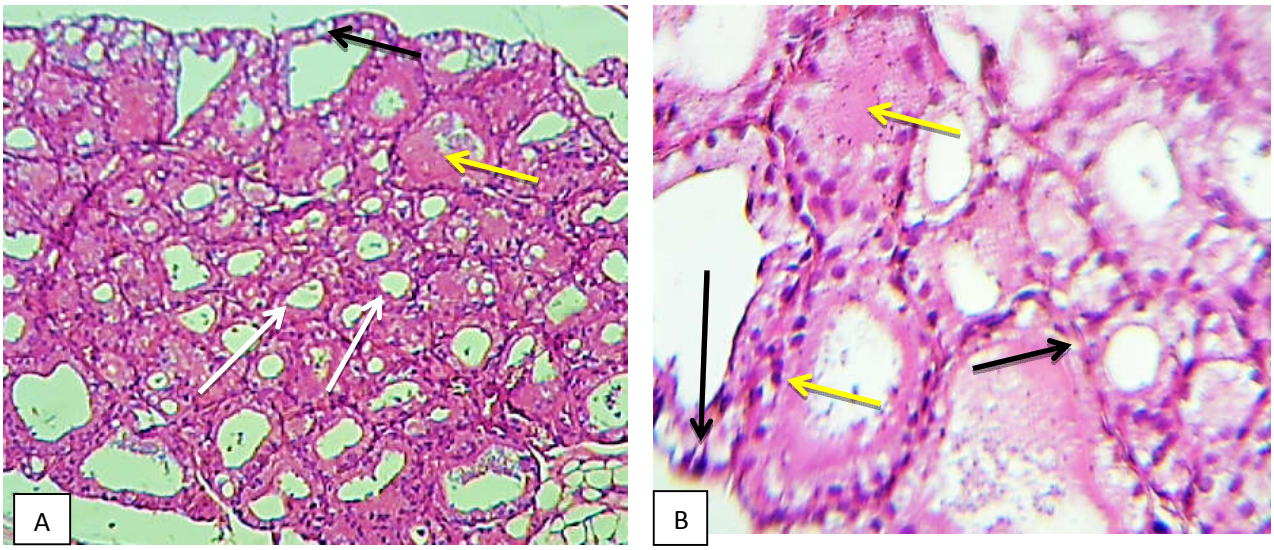

Fig.2: Thyroid gland of lactatingfemale rats group treated with Dex for 10 days, showingMicrofollicleswith vacuolated colloid(white arrow), some follicles lined by vacuolated thyrocytes(black arrow), other distend thyroid follicles filled with colloidand lined by low cuboidal or squamous thyrocytes(yellow arrow),(A&B).Stain (H&E) (A)100X, (B)400X

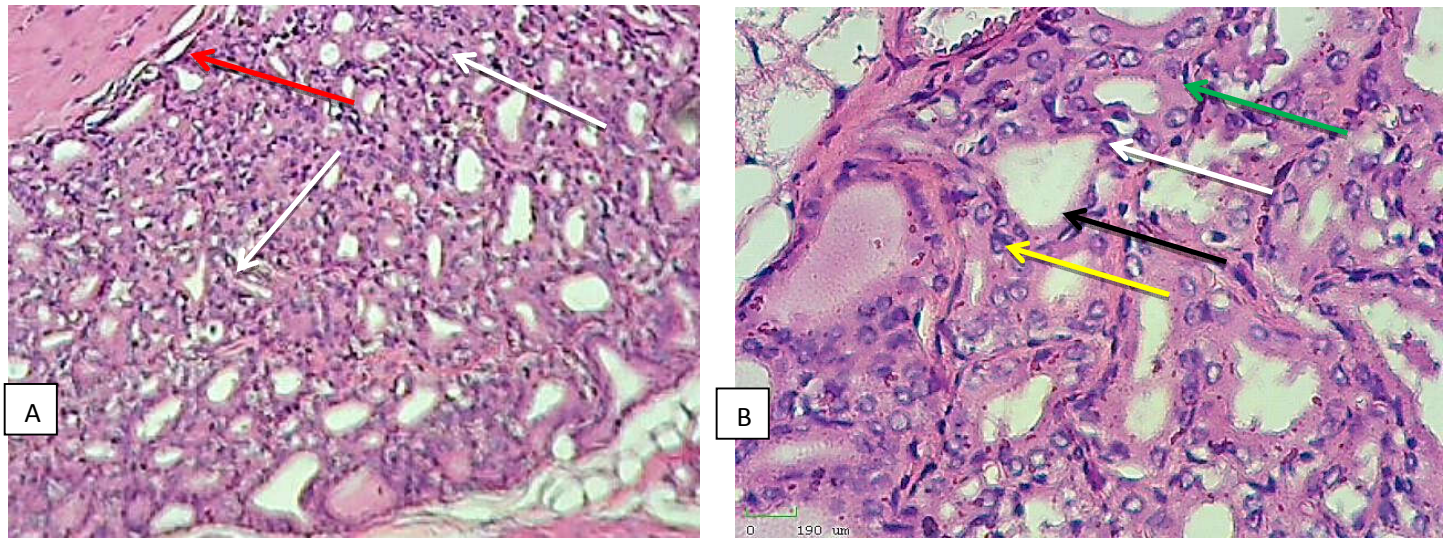

Fig.3: Thyroid gland of lactating female rats treated with Dex for 20 days, showing architectural changes, micro-thyroid folliclesfilled with small amount of colloid and lined by cuboidal cells(white arrow), some area of hyperplasia of follicular cells(red arrow), vacuolation of some thyroid follicles(green arrow), other distended thyroid follicles , filled with colloidand lined by low cuboidal or squamous thyrocytes(yellow arrow), (A&B). Stain (H&E) (A)100X , (B)400X

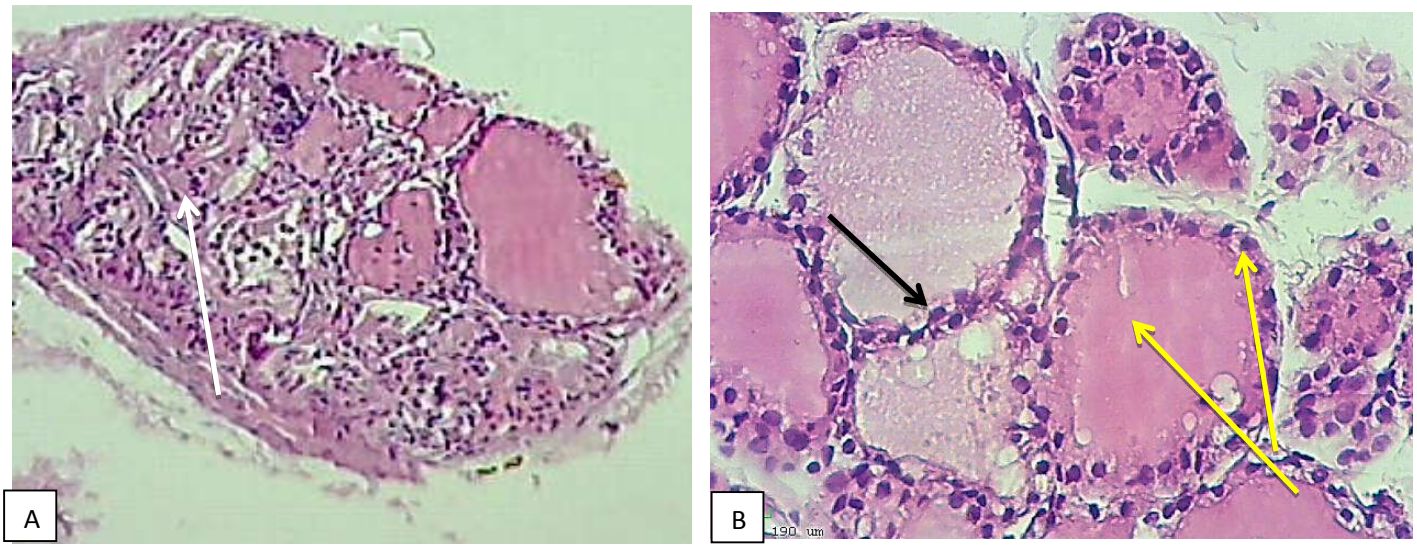

Fig.4: Thyroid gland of lactating female rats treated with Dexfor 30 days, showing , some thyroid follicles are distended, filled with colloidand lined by low cuboidal or appear squamous thyrocytes(yellow arrow) (black arrow), most other follicles lost its normal architecture (yellow arrow);Stain (H&E).(A) 100X, (B)400X

DISCUSSION

The results of the current study showed a significant decrease in serum TSH, T_4, and T_3 levels were recorded in female rats groups treated with Dex for 30, 20 and 30 and 10, 20 and 30 days during lactation respectively. These results are in agreement with (20) , who found that glucocorticoids causes reduction in serum Dex not suppressed TSH only but also reduced the TSH response to TRH treatment ,also long-term administration of Dex may be a cause of inhibition TSH level. A significant decrease in the levels of T4 and T3 in the current study was consistent with (21 ; 22) who indicated that the administration of Dex led to hypothyroidism associated with a decrease in the levels of T4 and T3 hormones compared with control group. Whereas, the same study recorded a significant increase in the level of TSH in Dex treated group, contrary to the results of the current study. The reduction of T3 and T4 Levels may be resulted from local effect of Dex on thyroid gland.

The results of the present study corroborated with the previous study which demonstrated that ewes treated with Dex resulted in reduction of plasma concentration of T_3 and T_4 and elevation of rT_3 without any change in tissue deiodinase activity (23). Numerous studies indicated

a significant decrease in the level of T_3 in animals subjected to nutritional stress (24). In the same way thermal stress lead to an elevation in GC levels and a reduction of both T3 and T4

Similarly, thermal stress causes an increase in GC levels and a decrease of both T3 and T4 (25; 26). The reduction in the level of biologically active thyroid hormones in the circulation of dams treated with Dex may be due to inhibition of hypothalamic- pituitary- thyroid axis activity.

In fact, in non-pregnant adult human, it was previously found that dexamethasone reduced the circulating concentrations of T 3 and T4 by reducing the pituitary sensitivity to thyroid releasing hormone and TSH secretions (27). The reduction in thyroid hormone levels in adult animals may be an acclimatization feature of endocrine system to stress stimulation leading to reduce the metabolic rate.

In men were injected intravenously with a single dose of betamethasone 8 mg causes a significant reduction of TSH level after 12 hours and a significant decrease in T3 after 16 to 40 hours after administration of betamethasone, while no significant change in the level of T_4 was observed. The decrease in the T_3 level may be attributed to the selective inhibition of glucocorticoids in the peripheral conversion of T_4 to T_3, the reduction in T_3 may be occurred due to the inhibition of TSH which induce the thyroidal secretion of T3 in preference to T_4 (28). The results of the current study are in agreement with those reported by(29) who reported that serum T_3 and T_4concentration decreased significantly after 3 injection in dog treated with multiple intramuscular injections of prednisone at dose of 2.2mg/kg bw every alternative day. The reduction in T4 and T3 levels in dogs received multiple injections of prednisone were resemble to reduction in thyroid hormones levels observed in persons and rats after GCs administration (29).

The thyroid function disorders in dogs treated with prednisolone is confirmed by histological changes of the gland, observed which include inactive areas characterize by distended thyroid follicles, with vacuolated thyrocytes (30). The prominent reduction in the number of lysosomes in thyroid gland of dogs treated with predinsone proposed to be causes reduced the colloidal

hydrolysis by lysosomal enzymes, which in turn lead sever decrement in thyroid hormones released into circulation (29; 30). Similar observation was reported by (31) who found that T3 and T4 levels decreased significantly after 12 days of treatment with multiple (i.m)injection of prednisone every alternative day accompanied with non-significant changes in TSH compared with control group in domestic rabbits.The difference in the results of TSH between the previous study and the current study which was observed only in the group that was treated with Dex for 30 days where no significant difference were observed in the 10 and 20 days of treatment. This indicates that the length of the treatment period influenced the level of the hormones, or the difference in the route of administration may be the cause of the differences in hormones level.

Histopathological examination of thyroid glands in lactating female rats treated with Dex for different periods showed different structural changes in thyroid glands. The histopathological finding of the current study are in consistent with those of (29) who demonstrated that dogs treated with multiple injections of prednisone showed colloid and cytoplasmic droplets in the follicular thyrocytes. This result indicates that prednisone may interfere with the secretion of basal thyroid hormone by inhibiting lysosomal colloidal hydrolysis in the thyroid follicular cells. Thyroid follicles lumen was filled with colloidal material that has been vacuolated in some follicles at the periphery. In general, two types of follicles are found in the thyroid gland section, and the follicles in the periphery of the gland had larger diameter than those in the middle part. Furthermore, the peripheral follicles had small cuboidal epithelial cells compared to those in the central follicles. Similarly (32) showed that the histological changes of the thyroid gland of Female rabbit treated with Dex for 15 days include a decrease in the height of the follicular cells to become low cuboidal and even squamous, and the follicles distended with colloid accumulation. These changes include each of the peripheral and central thyroid follicles. In accordance with the present results it was found that male rats exposed to stress induced by exposure to constant light (24 h/day, light intensity of 600 lux) for 4 weeks and 3 months showed that exposure to light for long durations causes architecture alteration included hypertrophy with hyperplasia, and the thyroid follicles lined by multiple layers of thyrocytes or lined by vacuolated cells. Some thyroid follicles appeared cystic hyperplasia, congested blood capillaries were seen between the follicles (33). Similarly light microscopic findings thyroid

gland of rats exposed to stress induced by exposure to constant of light for one month revealed that the thyroid gland was stimulated, as evidenced by microfollicular thyroid structure and the thyroid follicles have little or no colloid. Similar results were recorded by (34). The vacuolation of the thyrocytes cytoplasm could be attributed to apoptosis. (35) Found that exposure to constant light increases the cellular oxidative stress, reduces the activity of antioxidants and increases apoptosis activity inside the glandular tissue.

CONCLUSIONS:

From the results of the current study we can deduce that dexamethasone treatment during lactation causes disturbances in thyroid hormoneslevel , accompanied by histological changes in thyroid gland, which gives a picture of hypothyroidism.

تأثيرالمعاملة بالديكساميثازون أثناء الرضاعة على وظيفة الغدة الدرقية في إناث الجرذان

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الخلاصة

تم تصميم هذه الدراسة لتقييم تأثيرالمعاملة بالديكساميثازون أثناء الرضاعة على وظيفة الغدةالدرقية في إناث الجرذان. تم تقسيم ستة وثلاثون من إناث الجرذان المرضعة تراوح وزن الجسم فيها (٢٠±٢٥٠ غم) عشوائيا إلى ست مجموعات متساوية (n=6). ثلاث مجموعات سيطرة (١-٣) حقنت تحت الجلد بمحلول الملح الفسيولوجي من اليوم(١-١٠،١-٢٠ و ١-٣٠) أثناء الرضاعة على التوالي. وثلاث مجموعات معاملة(T_1,T_2&T_3)حقنت تحت الجلد ١٥٠ ميكروغرام / كغم / يوم ديكساميثازون من اليوم(١-١٠ ، ١-٢٠ و ١-٣٠) أثناء الرضاعةعلى التوالي. في نهاية التجربة ،تم التضحية بالحيوانات وأُخذت عينات من الدم لتقييم مستوى هرمونات الغدة الدرقية وعينات من نسيج الغدة الدرقية حفظت في ١٠ ٪ من الفورمالين المحايد للفحص النسجي المرضي. أظهرت النتائج انخفاض معنوي ($P\leq 0,05$) في مستوى هرمون المحفز للدرقية (TSH)في المجموعة المعاملة بالدكيساميثازون (30-1)يوم وهرمون الدرقين(T_4)في المجموعتين (30-20,1-1) يوم وهرمون الثايرونين ثلاثي اليود(T_3) في جميع المجموعاتالمعاملة بـ Dexمقارنة معمجموعات السيطرة. بالإضافةإلى ذلك،أظهرت الدراسة النسجية المرضيه التغيرات الشكلية تضمنت تكوين جريبات دقيقة واخرى متسعة ممتلئة بالغروان ومبطنة قليلة الارتفاع او حرشفية مع تفجي الغروان وبعض الخلايا الدرقية مع مناطق منفرطة التنسج.

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