

SOME FACTORS AFFECTING BLOOD PLASMA LEVELS OF THYROID HORMONES IN OSSIMI AND RAHMANI EWES DURING PREGNANCY

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Abstract:

The study was carried out using 42 ewes (18 Ossimi and 24 Rahmani) aged 1.5 to 3.5 years old. Blood samples were taken on 10, 20, 30, 50, 100, 120, 140 and 145 days of pregnancy period. The study aimed to determine plasma triiodothyronine (T3) and thyroxin (T4) during pregnancy period. The effect of breed, days of estimation within breed, parity number of ewes, sex and type of feti and regression on age and body weight of ewes were also studied. It was found that Rahmani ewes had higher plasma T3 (69.07ng/dl) and T4 (5.58 mg/dl) than Ossimi ones (66.37 ng/dl and 5.40 mg/dl, respectively). Days of pregnancy significantly affected plasma T3 and T4 level in both Ossimi and Rahmani ewes. Plasma T3 and T4 decreased with increasing ewes parity. The highest levels of T3 (94.42 ng/dl) and T4 (5.6 mg/dl) were found in ewes having 1st and 3rd parity, respectively. However, the lowest Levels of both two hormones (58.32 ng / dl and 5.43 mg/dl, respectively) were found in ewes having 4th parity. The highest level of plasma T3 level (73.50 ng/dl) was found in ewes having twins (male and female) feti, while in those having female and male, it was 66.50 and 63.15 ng/dl, respectively. On the other hand, ewes having male feti had the highest level of plasma T4 (5.84mg/dl) compared with female and twins 5.50 and 5.12 mg/dl, respectively. Ewes having single had higher values of T3 (69.39ng/dl) compared with twins (66.05 ng/dl). On the contrary, T4 concentration was significantly ($P < 0.001$) higher (5.88 mg/dl) in ewes having twins compared with 5.10 mg/dl in ewes having single feti. The linear and quadratic of coefficients regression of T3 and T4 hormones on age of ewes and also on weight of ewe were statistically highly significant ($P < 0.001$).

Introduction:

Thyroid hormones are known to play an important role in the adjustment of animals to environmental changes. Triiodothyronine (T3) is concerned, more, with thermogenesis while thyroxin (T4) is concerned with general metabolic activity (Anderson and Harnes, 1975 and Nathanielsz, 1975). Anderson et al., (1972) suggested that the thyroxin secretion rate of large animals may be related to body weight, so, the heavier animals need thyroxin secretion rate per body weight. In this respect, Mixner et al., (1966) reported that 1% increase in body weight resulted in 0.64 % increase in thyroid secretion rate. Also, Kudari (1992) indicated that, the linear and quadratic regression of both thyroid hormones (T3 and T4) on weight of ewe during pregnancy are statistically significant ($P < 0.05$ and $P < 0.01$, respectively). This result may emphasize the importance of ewe's bodyweight

for increasing the metabolic processes during pregnancy period. Salem et al., (1986) stated that the increased activity of the thyroid gland of ewe during follicular phase as well as late pregnancy (90-150 days) is probably due to the increased level of estrogen compared to the other phases of reproduction (Nada, 2004). Kudari (1992) found a significant ($P < 0.001$) variation in plasma T3 and T4 in Ossimi and crosses (Rahmani X Ossimi) ewes due to the pregnancy intervals. Nathanielsz et al., (1973 a and b) and Kudari (1992) reported that, plasma thyroxin levels varied significantly between 2.3 and 4.1 mg/100 ml during the period between 103 days of gestation and the day of birth, while the T3 concentration continued to rise during the period in which thyroxin concentration fall. Kudari (1992) with Ossimi and crossbred ewes showed significant ($P < 0.001$) effect on plasma T3 level due to the sex of embryo, and due to the interaction between periods of pregnancy and sex of embryo. This result indicates the change in thyroid activity according to the physiological status of the ewe. A linear and quadratic regression of both thyroid hormones (T3 and T4) on weight of ewes during pregnancy period are significant ($P < 0.005$ and $P < 0.01$), respectively (Kudari 1992). Increasing 1% in body weight results in 0.64 % increase in thyroid secretion rate (Mixner et al, 1966). In this respect, the thyroxin secretion rate of large animal maybe related to body weight, so, the heavier animals need more thyroxin secretion rate per body weight (Anderson et al. 1972). In addition, Ibrahim et al; (1993) showed that, level of thyroxin (T4) in blood plasma of Ossimi and crossbred lambs increased with advancing age (Mixner et al., 1966 and Kahl and Bitman, 1983).

Materials and methods:

This study was carried out during the period from September 2004 to May 2005 at a private farm in El – Sharkyea Governorate, Egypt. Hormonal assays were carried out in the laboratory of animal physiology Unit, Applied Radiobiology Department, Nuclear Research Center, Atomic Energy Authority. A total number of 42 ewes (18 Ossimi and 24 Rahmani) aged 1.5 to 3.5 years with body weight ranging between 45 to 55 Kg were used in this study. The study aimed to determine hormonal plasma T3 and T4 during pregnancy period. The effect of breed days of estimation within breed, parity number of ewes, sex and type of embryo and regression on age and weight of ewes were also studied. All ewes used in this experiment were fed on clover hay, crop residues and available green fodder by grazing conditions during summer and autumn months. While during winter and spring months, ewes were allowed to graze on Egyptian clover (*Trifolium alexandrinum*). Ewes were supplemented with an additional amount of a concentrated mixture 0.5 to 1 Kg/head/day, for two weeks before mating and during the last four weeks of pregnancy.

Blood Sampling:

Blood samples of approximately 7ml were collected from jugular vein in sterile evacuated tubes containing 10mg EDTA (Ethylene ediaminetetr acitic acid, sodium salt) as anticoagulant materials. During pregnancy, the blood samples were taken from each ewe in both breed at 10, 20, 30, 50, 100, 120, 140, and 145 days. Blood samples were chilled on ice and spun in cooling centrifuge at 3000 rpm for 15 minutes and plasma was separated and stored at -20°C in a deep freezer till analysis.

DURING PREGNANCY.

Hormonal determination:

Radioimmunoassay techniques (RIA) were performed for determining plasma Triiodothyronine (T₃) and thyroxine (T₄) using ready antibody coated tubes Kits manufactured by immunotech, Beckman counter company, France.

Statistical analysis:

The data were analyzed using SAS program (SAS 1996). Duncan's test (1955) was applied among means to test the significance between them. The following model was used:

$$Y_{ijklm} = M + B_i + D_{ij} + P A_k + S_i + P E_m + b_1 L (X_{ijklm} - X_1) + b_1 Q (X_{ijklm} - X_1) + b_2 L (X_{ijklm} - X_2)^2 + b_2 Q (X_{ijklm} - X_2)^2 + e_{ijklm}$$

Where:

Y_{ijklm} = The observation of $ijklm^{th}$ age and body weight of ewes.

M = The overall mean

B_i = The fixed effect of the i^{th} breed

D_{ij} = Days of pregnancy within i^{th} breed.

PA_k = The fixed effect of the j^{th} parity of ewe.

S_i = The fixed effect of the k^{th} sex of embryo.

PE_m = The fixed effect of the l^{th} number of embryo

$b_1, 2L$ and $b_1, 2Q$ = Partial linear and quadratic regression coefficients of a trial on age ewe and body weight, respectively.

X_1 and X_2 = The arithmetic mean of age and body weight of ewe respectively.

E_{ijklm} = Random error particular to the $ijklm^{th}$ observation and assumed to be independently random distributed (O, σ^2_e).

Results and Discussions:**1- Effect of breed :**

It was found that Rahmani ewes had higher T₃ and T₄ means (69.07ng/dl) and 5.58 mg/dl than Ossimi ones (66.37 ng/dl and 5.14mg/dl), respectively (table 1). Analysis of variance did not show any significant variation in either plasma level of T₃ and T₄ due to the effect of breed (table 3). These results agree with the finding of Kudari (1992) who found no difference in plasma level of T₃ and T₄ during pregnancy due to the effect of breed. It could be concluded that secretion rate of T₃ and T₄ have been reported to be affected by the reproductive status of ewes (Mourad et al, 1981; Pichaicharnarong et al; 1982 and Kudari, 1992).

2- Effect of days of pregnancy :

Data listed in (table 2) shows that the mean of both plasma T₃ and T₄ for either Ossimi or Rahmani ewes differed in the period of pregnancy. plasma Triiodothyronine (T₃) levels decreased up to 50th day of the pregnancy period (

from 73.99 and 82.45 ng/dl at the 10th day to 57.70 and 56.57 ng/dl at the 50th day in Ossimi and Rahmani ewes, respectively). Then it sharply increased reaching a level of 79.17 and 87.38 ng/dl at the 145th day of pregnancy period. Different trend was met in plasma thyroxin (T4) level. It decreased from 6.33 and 6.21 at the 10th day to 4.67 and 4.96 mg/dl at the 50th day, and it increased to 5.89 and 6.21 at the 140th days in Ossimi and Rahmani, respectively. Then plasma T4 level decreased to 5.95 and 5.08 mg/dl in the 145th day, respectively. An analysis of variance showed highly significant variation ($P < 0.001$ and $p < 0.05$) on T₃ and T₄, respectively due to the effect of days of pregnancy (table 3). These results may be also attributed to the different level of metabolic activity throughout the pregnancy period which depends on the different embryonic needs at different times of its embryonic development. The obtained results are in agreement with those found by Nathaniel's et al. (1973 a,b) who reported that, plasma thyroxin levels varied significantly between 2.3 and 4.1 mg/100 ml during the period between 103 days of gestation and the day of birth, while the T₃ concentration continued to rise during the period in which thyroxin concentration fall. In addition, the present findings partially agree, with those of Salem et al; (1986) who found that the secretory activity of the thyroid gland was higher during the last stage of pregnancy (90 to 150 days) compared to those of the early stage of pregnancy. In this respect, Kudari (1992) found a significant ($P < 0.001$) variation in plasma T₃ and T₄ in Ossimi and crosses (Rahmani X Ossimi) ewes due to the pregnancy intervals Plasma T₃ level which decreased up to the 90th day of the pregnancy period, then it sharply increased reaching a maximum level at the 120th day after which it decreased on the 150th day of the pregnancy period.

3- Effect of parity number of ewe:

Plasma T₃ level decreased significantly ($P < 0.001$) with increasing ewe's parity (table 1). T₃ level decreased from (91.42 ng/dl) in ewes having 1st parity to (58.32 ng/dl) in 4th parity. T₄ levels decreased not significantly by increasing ewes parity. The highest levels of T₄ was (5.61 mg/dl) in ewe's have 3rd parity. However, the lowest level (5.43 mg/dl) in ewes having 4th parity. Analysis of variance showed high significant effect of parities on plasma T₃ and insignificant on T₄ level (Table 3). The decrease in the level of plasma thyroid hormone with increasing the ewe's parity may lead to suggest that, this might be due to either decreasing level of thyroid activity as the ewe's grew older or to the increasing level of the metabolic processes to face the increasing demand for energy with age that accelerates the rate of thyroid hormones metabolism (breakdown) which results in decreasing their level in plasma. It was found by Kudari (1992) that ewe's breed showed it's significant ($P < 0.05$) effect on both the two thyroid hormones when interacted with ewes parity. Also he found that there were highly significant variation in both plasma T₃ ($P < 0.01$) and T₄ ($P < 0.01$) levels due to ewes' parity.

4- Effect of sex feti:

The highest level of plasma T3 level (73.50 ng/dl) was found in ewes having twins (male and female) feti, while those having Female and having male were found to be 66.50 and 63.15 ng/dl, respectively. While, the highest level of plasma T4 (5.84 mg/dl) was found in ewes having male feti, compared with 5.50 and 5.12 mg/dl in ewes having female and both male and female, respectively (table 1). Analysis of variance did not show a significant effect with sex of feti on T3, however it showed significant ($P < 0.001$) effect of sex on feti plasma T4 level only (table 3). It was found by Nathanielsz et al., (1973 a and b) that embryo starts to secrete its own thyroid hormones at about 50 days of its embryonic development period, and there is a high rate of fetal T4 secretion during the last trimester of gestation (Dussault et al., 1971). Other studies in Ossimi and crossbred ewes showed that the significant ($P < 0.001$) effect on plasma T3 level was due to the sex of embryo, and due to the interaction between periods of pregnancy and sex of embryo (Kudari, 1992).

5- Effect of type of feti :

Ewes having single feti had higher values of T3 level compared with twins (69.39 and 66.05 ng/dl, respectively). However, the T4 levels concentration was higher (5.88 mg/dl) in ewes having twins compared with ewes having single feti (5.10 mg/dl). Variation in plasma T3 did not show any significant effect while, variation in plasma T4 showed a highly significant ($P < 0.001$) due to the effect of type of feti (table 3). These results are quite scientifically logic since it was found that all metabolic processes are accelerated in case of twins. In addition, the amount of embryonic thyroid hormone, which may be transferred to the maternal circulation, may be involved in this aspect. Kudari (1992) found that ewes breed showed its significant ($P < 0.001$) effect on plasma T4 level when interacted with type of embryo. Also, he found a significant ($P < 0.001$) effect only for plasma T3 level due to the interactions between periods of pregnancy and type of embryo.

6- Regression on age and body weight of ewes:

Obtained data (table 3) indicates that, the linear and quadratic regression of both thyroid hormones (T3 and T4) on age of ewe and also on weight of ewe were statistically highly significant ($P < 0.001$). This result may emphasize the importance of ewe's body weight for increasing the metabolic processes during pregnancy period. A linear and quadratic regression of both thyroid hormones (T3 and T4) on weight of ewes during pregnancy period are significant ($P < 0.005$ and $P < 0.01$), respectively (Kudari 1992). Increase of 1% in body weight results in 0.64 % increase in thyroid secretion rate (Mixner et al, 1966). In this respect, the thyroxin secretion rate of large animal maybe related to body weight, so, the heavier animals need more thyroxin secretion rate per body weight (Anderson et al. 1972). In addition, Ibrahim et al; (1993) showed that, level of thyroxin (T4) in blood plasma of Ossimi and crossbred lambs increased with advancing age (Mixner et al., 1966 and Kahl and Bitman, 1983).

Table (1): Least square means $\pm$ Standard error of Factors affecting plasma Triiodothyronine and Thyroxine during pregnancy in Ossimi and Rahmani ewes.

Independent Variable	No. of Ewes	No. of Estimation	Triiodothyronine (T3) ng/dl	Thyroxine (T4) mg/dl
Breed:				
Ossimi	18	144	66.37 $\pm$ 2.53 ^a	5.14 $\pm$ 0.10 ^a
Rahmani	24	192	69.07 $\pm$ 1.86 ^a	5.58 $\pm$ 0.10 ^a
Parity No.:				
1 st parity	13	104	91.42 $\pm$ 4.68 ^a	5.46 $\pm$ 0.18 ^a
2 nd parity	12	96	63.90 $\pm$ 2.92 ^b	5.45 $\pm$ 0.11 ^a
3 rd parity	8	64	57.25 $\pm$ 3.53 ^{bc}	5.61 $\pm$ 0.14 ^a
4 th parity	9	72	58.32 $\pm$ 2.62 ^c	5.43 $\pm$ 0.21 ^b
Sex of Fetus:				
Male	20	160	63.15 $\pm$ 3.31 ^a	5.84 $\pm$ 0.13 ^a
Female	15	120	66.50 $\pm$ 2.82 ^a	5.50 $\pm$ 0.19 ^a
Male and Female	7	56	73.50 $\pm$ 4.10 ^a	5.12 $\pm$ 0.16 ^b
Type of fetus:				
Single	31	248	69.39 $\pm$ 2.50 ^a	5.10 $\pm$ 0.10 ^a
Twins	11	88	66.05 $\pm$ 3.52 ^a	5.88 $\pm$ 0.14 ^a
Overall mean	42	336	67.72$\pm$2.16	5.19$\pm$0.10

Means within any classification, followed by different letters are significantly different ($P < 0.05$) otherwise they are not.

Table (2): least squares means $\pm$ standard error of Triiodothyronine (T3) and Thyroxine (T4) during pregnancy in Ossimi and Rahmani ewes

Days of pregnancy	Triiodothyronine (T3) ng/dl		Thyroxine (T4) mg/dl	
	Ossimi	Rahmani	Ossimi	Rahmani
10 days	73.99 $\pm$ 5.74	82.45 $\pm$ 4.83	6.33 $\pm$ 0.22	6.21 $\pm$ 0.19
20 days	69.10 $\pm$ 5.74	74.10 $\pm$ 4.83	5.60 $\pm$ 0.22	5.32 $\pm$ 0.19
30 days	59.61 $\pm$ 5.74	63.61 $\pm$ 4.83	5.87 $\pm$ 0.22	6.12 $\pm$ 0.19
50 days	57.70 $\pm$ 5.74	56.57 $\pm$ 4.83	4.67 $\pm$ 0.22	4.96 $\pm$ 0.19
100 days	59.35 $\pm$ 5.74	56.74 $\pm$ 4.83	4.83 $\pm$ 0.22	5.07 $\pm$ 0.19
120 days	65.77 $\pm$ 5.74	69.12 $\pm$ 4.83	5.09 $\pm$ 0.22	5.24 $\pm$ 0.19
140 days	66.25 $\pm$ 5.74	62.61 $\pm$ 4.83	5.89 $\pm$ 0.22	6.21 $\pm$ 0.19
145 days	79.17 $\pm$ 5.74	87.38 $\pm$ 4.83	5.95 $\pm$ 0.22	5.08 $\pm$ 0.19

Table (3): F- ratio of analysis of variance for factors affecting Triiodothyronine and thyroxin during pregnancy in Ossimi and Rahmani ewes.

Source of variance	d-f	F – ratio	
		Triiodothyronine(T3) ng/dl	Thyroxin(T4) mg/dl
Breed:	1	0.20	0.26
Days of pregnancy / breed	14	3.83***	8.34***
Parity No.	3	11.30***	0.44
Sex feti	2	1.40	5.18***
Type of feti	1	0.42	15.08***
Regression on age of ewe:			
linear		9.02***	3.44
quadratic		10.13***	10.20***
	1		
	1		
Regression on body of ewe:			
linear	1	11.16***	15.53***
quadratic	1	10.72***	12.56***
Remainder : d.f	310		
Remainder : M.S.		545.79	0.82

***= P<0.001

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بعض العوامل المؤثرة في مستوى هرمونات الدرقية في بلازما دم نعاج الأوسمي والرحماني أثناء الحمل

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الملخص العربي:

أستخدمت في هذه الدراسة 42 نعجة (18 أوسمي ، 24 رحماني) تتراوح عمرها بين 1.5 - 3.5 سنة) وتم أخذ عنبات دم من هذه النعاج في فترة الحمل عند الأيام 10 ، 20 ، 30 ، 50 ، 100 ، 120 ، 140 ، 145) يوم حمل . وكان الهدف من هذه الدراسة هو تقدير هرمومات التراي أيودوثيرونين (T3) والثيروكسين (T4) ، وتمت دراسة تأثير سلالة النعاج ويوم القياس داخل النوع الواحد وعدد مرات الولادة وكذلك جنس وعدد الأجنة في البطن الواحدة ومعامل إختدار القياسات السابقة على عمر ووزن النعاج عند الحمل .

- وقد وجد إرتفاع مستوى كل من هرمون التراي أيودوثيرونين والثيروكسين في النعاج الرحماني (69.7 نانوجرام/ديسيلتر ، 5.58 ميكروجرام/ديسيلتر على الترتيب) مقارنة بالنعاج الأوسمي (66.37 نانوجرام/ديسيمتر ، 5.40 ميكروجرام/ديسيلتر على الترتيب) ولكن هذه الفروق لم تكن معنوية .

- وكان ليوم القياس خلال فترة الحمل داخل النوع (النعاج الأوسمي والرحماني) تأثيراً معنوياً (عند مستوى 0.1%) على تركيز هرمون التراي أيودوثيرونين والثيروكسين .

- إنخفض مستوى هرمون التراي أيودوثيرونين معنوياً (عند مستوى 0.1%) مع تتالي الإنجاب من 91.42 إلى 57.25 نانوجرام/ديسيلتر في النعاج ذات الإنجاب أو الحمل الأولى والرابع على الترتيب ، في حين كان إنخفاض مستوى هرمون الثيروكسين بدرجة غير معنوية بزيادة رقم البطن من 5.6 إلى 5.44 ميكروجرام/ديسيلتر عند رقم البطن الثالث والرابع على الترتيب .

- زاد مستوى هرمون التراي أيدوثيرونين إلى 73.50 نانوجرام/ديسيلتر في النعاج التي حملت توأم (ذكر + أنثى) بينما كان مستوى الهرمون 66.50 ، 63.15 نانوجرام / ديسيلتر في النعاج التي حملت إناث أو ذكور على الترتيب . بينما كان الوضع مختلفاً بالنسبة لهرمون الثيروكسين فقد أعطت النعاج التي حملت ذكور أعلى مستوى للثيروكسين (5.84 ميكروجرام/ديسيلتر) مقارنة بـ 5.50 ، 5.12 ميكروجرام/ديسيلتر في النعاج التي حملت إناث أو توأم (ذكر + أنثى) على الترتيب .

- النعاج التي حملت في فرادى كانت أعلى في مستوى هرمون التراي أيدوثيرونين (69.39 نانوجرام/ديسيلتر) مقارنة بـ 66.05 نانوجرام/ديسيلتر في النعاج التي حملت في قوائم على العكس من ذلك كان مستوى هرمون الثيروكسين أعلى (5.88 ميكروجرام/ديسيلتر) في النعاج التي حملت توأم مقارنة بـ 5.10 ميكروجرام/ديسيلتر في النعاج التي أعطت مفرد والفروق لم تكن معنوية .

- كان إنحدار كلاً من هرمون التراي أيدوثيرونين والثيروكسين على كلاً من العمر ووزن الجسم للنعاج كان عالي المعنوية عند مستوى 0.1% .

- وقد أعطي ذلك دلالة على أهمية وزن الجسم لزيادة العمليات التمثيلية أثناء الحمل وأكدت النتائج زيادة مستوى هرمون الثيروكسين في النعاج في الثلث الأخير من فترة الحمل (الفترة من 100 - 145 يوم من الحمل) وذلك لمجابهة إحتياجات الجنين للنمو المتزايد في هذه الفترة .