

STUDY ON THE THYROID FUNCTION OF THOROUGHBRED
FEMALES IN VARYING STAGES OF PREGNANCY,
USING "IN VITRO" TESTS $^{125}\text{I-T}_3$ AND $^{125}\text{I-T}_4$.

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DE MARTIN, B. W. — *Study on the thyroid function of thoroughbred females in varying stages of pregnancy, using "in vitro" tests $^{125}\text{I-T}_3$ and $^{125}\text{I-T}_4$.* Rev. Fac. Med. vet. Zootec. Univ. S. Paulo, 12:121-6, 1975.

SUMMARY: *The author has studied sera of 26 thoroughbred female equines, aged between five through 12 years, collected in five different pregnancy stages.*

We have performed five bleedings: the first from the group between 49 and 90 days pregnancy; the second between 103 and 167; the third between 158 and 222; the fourth between 216 and 280; and the last between 271 and 335 days of pregnancy. As average results of T_3 and T_4 for ^{125}I -lyothyronine retention in resin and of thyroxin per 100 ml of serum, we found 44.22% and 2.13 mcg in the first collection; 44.02% and 2.21 mcg in the second; 46.67% and 2.60 mcg in the third; 43.51% and 2.56 mcg in the fourth; and, finally, 41.14% and 1.94 mcg in the fifth collection.

A sixth group was formed, composed of females from the first group that were between 49 and 55 days pregnant, for which we registered values 46.27% for T_3 Test and 0.93 mcg of T_4 Test.

We have also performed the variance analysis of the results obtained in tests T_3 and T_4 in the six groups, the first with females between 49 and 55 days of pregnancy and the others, each represented by six animals chosen by allotment, with 62 through 90, 138 through 167, 193 through 222, 251 through 280, and 306 through 335 days of pregnancy.

Analysing the results of T_3 Test we have found statistical significance for the animal group and verified, by Tukey's test that only one animal was responsible for the significance. The data referring to the variance analysis applied to the results obtained with the help of T_4 Test showed statistical significance for the varying periods; and Tukey's test revealed that such fact occurred because the first group differed from the others.

UNITERMS: *Thoroughbred horse*; Thyroid function*; Test "in vitro"*.

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INTRODUCTION

Once studied the possibilities of T_3 and T_4 Tests for the evaluation of the thyroid functional conditions of thoroughbred equines^{6,7,9,13,5}, we tried to observe the existence of variation in the value obtained from these Tests in sera of females of these animals in different stages of pregnancy.

LITERATURE

T_3 and T_4 Tests are used as proof to evaluate the functional thyroid conditions of human beings^{14,15,16,12}. Former "in vitro" tests^{10,11,12,19,18,17} that served as basis for these tests showed that there was a difference in values obtained from pregnant women^{10,11,12}. T_3 Test used in sera of normal women, pregnant women, and patients who had aborted, showed that around the tenth week of pregnancy the values increased and remained stable to the end of the pregnancy, going back to normal from three to twelve weeks after delivery, depending on the nursing time²⁰.

The study made with PBI in pregnant female equines showed that the values suffered no alteration⁸. This fact was also observed when the authors compared the results of T_3 and T_4 Tests from Dutch pregnant cows with the group of non impregnated ones.

It should be emphasized that the values of T_3 and T_4 Tests were not statistically significant when confronting the influence of sex and age of thoroughbred equines^{9,4,5,13}.

MATERIAL AND METHODS

We have analysed sera obtained from 26 pregnant female equines, housed in the Haras Jahu, in the municipality of São Roque, State of São Paulo, aged between five to 12 years.

Before feeding or any other activity, an average of 30 ml of blood was collected

from the jugular vein. After clot retraction centrifugation was done and then the serum was placed in test tubes and kept in freezer at -10°C .

To measure the activity of the ^{125}I bound to the thyroid hormones, the author has used the gamma spectrometric system, model 8725, from the Nuclear Chicago coupled to a well detector with a two inches NaI (Tl) crystal, with an inner well diameter of 16 mm, gauged for the ^{125}I power, with a 15 kev window.

Method to determine the percentage of the ^{125}I -lyothyronine retention in resin. (Test $^{125}\text{I-T}_3$ or T_3 Test), modified.*

We have used SCHOLER's¹⁷ method (1962) with some modifications and, in this work, we have altered the serum amount of 0.05, 0.1 and 0.2 ml directly deposited on the resin column after addition of the ^{125}I -lyothyronine, in order to measure the places not bound to the tri-iodothyronine.

*Method to determine the total thyroxin (Test $^{125}\text{I-T}_4$ or T_4 Test)***

For this we have employed the method suggested by MURPHY and PATTEE¹⁴ (1964).

We have established the averages and standard deviations, as well as Pearson's variability coefficients of the values included in the tables showing the results.

Later on we have equalled the values by allotment and, before treatment, these were converted to angles (angle = arc. sen. $\sqrt{\text{percentage}}$).

In order to verify the statistical significance to a 95% confidence coefficient, we have used the variance and regression analysis.

Tukey's test was made with the aim to verify differences between groups that presented significance through variance analysis.

* Trilute, Ames Company, Israel.

** Tetralute, Ames Company, Israel.

RESULTS

The values obtained in this work were registered in two tables.

In Table I we have examined the results obtained through T_3 and T_4 Tests in sera of females aged between six to seven years and that were between 49 and 55 days pregnant, that formed the first group. For the results of T_3 Test we have registered an average of 46.27% of ^{125}I -lyothyronine retention in resin and for T_4 Test 0.93 mcg of thyroxin per 100 ml of serum.

In Table II we have listed the results obtained through T_3 and T_4 Tests in sera of 26 females aged between five and twelve years. We have made five bleedings, the first from the group between 49 and 90 days of pregnancy, the second between 103 and 167, the third between 158 and 222, the fourth between 216 and 280 and the last one between 271 and 335 days of

pregnancy. As average results of T_3 and T_4 Tests for ^{125}I -lyothyronine retention in resin and of thyroxin per 100 ml of serum, we obtained 44.22% and 2.13 mcg in the first collection; 44.02% and 2.21 mcg in the second; 46.67% and 2.60 mcg in the third; 43.15% and 2.56 mcg in the fourth; and, finally, 41.14% and 1.94 mcg in the fifth collection.

We have performed the variance analysis of results obtained with T_3 and T_4 Tests in six groups, the first of which was formed by females between 49 and 55 days of pregnancy, shown in Table I, and the others, each represented by six animals chosen by allotment, with 62 to 90, 138 to 167, 193 to 222, 251 to 280 and 306 to 335 days of pregnancy, shown on Table II. The data referring to the T_3 Test results showed statistical significance for the animal group, having Tukey's test revealed that only one animal (nr. 11, of Table II) was responsible for the significance.

TABLE I - Averages, standard deviations and Pearson's variability coefficients of results obtained with the help of Tests T_3 and T_4 from sera of thoroughbred female equines in early pregnancy stage. Haras Jahu, São Roque. São Paulo, 1973.

ANIMAL		AGE (YEARS)	PREGNANCY (DAYS)	T_3 TEST (% DE RETENTION) SERUM 0,2 ml	T_4 TEST mcg $T_4/100$ ml
NO	NAME'S INITIAL				
1	P.	7	55	42,03	0,31
18	Q.	6	54	46,12	1,27
19	P.	7	53	47,07	1,07
21	O.	7	50	48,93	0,99
20	Q.	6	50	52,88	0,46
22	G.	6	49	40,62	1,53
\bar{x} (Mean)		6,5	51,81	46,27	0,93
\bar{s} (Standard deviation)		0,55	2,48	4,50	0,47
Pearson's variability coefficient		8,4	4,8	9,7	50,1

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The variance analysis applied to results obtained with the help of Test T_4 shows statistical significance for the several stages, Tukey's test revealing that this was due to the fact that the first group of animals was different from the others.

DISCUSSION

The study of the thyroid function of female equines, with the help of PBI, shows that there was no statistically significant difference among the several pregnancy periods, which was also observed in cows by means of "in vitro" T_3 and T_4 tests. However, for thoroughbred female equines, the T_4 values showed statistical significance when the group between 49 and 55

days of pregnancy was compared to those in more advanced stages. The confirmation of this results leads us to suggest the use of these tests as aids in the early diagnosis of pregnancy for this species.

Considering the pregnancy periods only, the T_3 values were not statistically significant. This fact does not coincide with the findings in the human species, where the Test T_3 values increase as of the tenth week of pregnancy.

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DE MARTIN, B. W. — *Estudo da função tiróidea em fêmeas de eqüinos Puro Sangue Inglês em diferentes tempos de gestação, mediante a utilização dos testes "in vitro" $^{125}\text{I-T}_3$ e $^{125}\text{I-T}_4$.* *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 12:121-6, 1975.

RESUMO: Foram estudados soros de 26 fêmeas de eqüinos Puro Sangue Inglês com idades entre 5 a 12 anos, colhidos em 5 diferentes períodos de gestação.

Realizamos 5 colheitas de sangue, a primeira com o lote entre 103 e 167, a terceira entre 158 e 222, a quarta entre 216 e 280 e a última entre 271 e 335 dias de gestação. Com resultados médios de T_3 e T_4 , para retenção de ^{125}I -liotironina em resina e de tiroxina por 100 ml de soro, obtivemos, na primeira colheita 44,22 e 2,13 mcg; na segunda, 44,02% e 2,21 mcg; na terceira, 46,67% e 2,60 mcg; na quarta, 43,51% e 2,56 mcg e, finalmente, na quinta, 41,14 e 1,94 mcg.

Constituí-se um 6.^o grupo, formado com éguas do 1.^o lote e que se encontravam entre 49 e 55 dias de gestação, para o qual registramos valores de 46,27% para o Teste T_3 e 0,93 mcg para o Teste T_4 .

Procedemos também a análise de variância dos resultados obtidos nos Testes T_3 e T_4 em 6 lotes, sendo o primeiro entre 49 e 85 dias de gestação e os demais, representados cada um por 6 animais escolhidos por sorteio, com 62 a 90, 138 a 167, 193 a 222, 251 a 280 e 306 a 335 dias de gestação.

Analisamos os dados referentes aos resultados do Teste T_3 e encontramos significância estatística para o grupo animal, tendo o Teste de Tukey esclarecido que apenas um animal foi o responsável pela significância. Os dados referentes à análise de variância aplicados aos resultados obtidos com o auxílio do Teste T_4 , mostraram significância estatística para os diversos períodos, revelando o Teste de Tukey que tal fato ocorreu por diferir o primeiro lote dos demais.

UNITERMOS: Eqüinos P.S.I.*; Função tiróidea*; Teste "in vitro*."

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BIBLIOGRAPHY

1. BAUER, R. et al. — The use of Sephadex G-25 in the estimation of total serum thyroxine. *Clin. Chem.*, 16:526, 1970.
2. BRAVERMAN, I. C. et al. — Evaluation of a simplified technique for the specific measurement of serum thyroxine concentration. *J. clin. Endocr.*, 32:497-502, 1971.
3. DEFINE, R. M. — Estudo da função tireóideia em fêmeas de bovinos da raça holandesa, através dos índices de triiodotironina e tiroxina obtidos com os testes $^{125}\text{I-T}_3$ e $^{125}\text{I-T}_4$. São Paulo, 1972. [Tese — Faculdade de Medicina Veterinária e Zootecnia da USP].
4. DE MARTIN, B. W. — Estudo da função tireóideia em eqüinos puro sangue inglês, através do $^{131}\text{I-T}$. B. I. *Rev. Fac. Med. vet. Zootec. Univ. S. Paulo*, 10: 35-44, 1973.
5. DE MARTIN, B. W. — Estudo da função tireóideia em eqüinos Puro Sangue Inglês, mediante a utilização dos testes "in vitro" $^{125}\text{I-T}_3$ e $^{125}\text{I-T}_4$. São Paulo, 1974. [Tese — Faculdade de Medicina Veterinária e Zootecnia da USP].
6. HIGHTOWER, D. & MILLER, L. F. — Thyroid function test in veterinary medicine. I. A review. *S. West Vet.*, 22:200-5, 1969.
7. HIGHTOWER, D. et al. — Thyroid function tests in veterinary medicine. *S. West Vet.*, 23:15-21, 1969.
8. IRVINE, C. H. G. — Thyroid function in the horse. In: *Ann. Conv. Amer. Assoc. Equine Practition*, 12th., Los Angeles, 1966. *Proceedings*, p. 197-206.
9. KALLFELZ, F. A. & LOWE, J. E. — Some normal values of thyroid function in horses. *J. Amer. vet. med. Ass.*, 156:1888-91, 1970.
10. MITCHELL, M. L. — Resin uptake of radiothyroxine in sera from non-pregnant women. *J. clin. Endocr.*, 18:1437-40, 1958.
11. MITCHELL, M. L. et al. — The in vitro resin sponge uptake of triiodothyronine- I^{131} from serum in thyroid disease and in pregnancy. *J. clin. Endocr.*, 20:1474-83, 1960.
12. MITCHELL, M. L. et al. — Resin uptake of radiothyroxine from serum in thyroid disease and in pregnancy. *J. clin. Endocr.*, 21:1448-54, 1961.
13. MOTLEY, J. S. — Use of radioactive triiodothyronine in the study of thyroid function in normal horses. *Vet. Med. Small Anim. Clin.*, 67:1225-8, 1972.
14. MURPHY, B. P. & PATTEE, C. J. — Determination of thyroxine utilizing the property of protein-binding. *J. clin. Endocr.*, 24:187-96, 1964.
15. MURPHY, B. P. & JACHAN, C. — The determination of thyroxine by competitive protein-binding analysis employing an anionexchange resin and radiothyroxine. *J. Lab. clin. Med.*, 66: 161-7, 1965.
16. MURPHY, B. P. et al. — Clinical evaluation of a new method for the determination of serum thyroxine. *J. clin. Endocr.*, 26:247-56, 1966.
17. SCHOLER, J. F. — A simple measure of thyro-binding by plasma: A test of thyroid function. *J. nucl. Med.*, 3:41-6, 1967.
18. STERLING, K. & TABACHNICK, M. — Resin uptake of I^{131} triiodothyronine as a test of thyroid function. *J. clin. Endocr.*, 21:456-64, 1961.
19. URELES, A. L. et al. — The erythrocyte uptake of I^{131} labeled triiodothyronine as a measure of thyroid function. *J. Lab. clin. Med.*, 54:178-85, 1959.
20. VISSCHER, R. D. — T_3 - I^{131} binding capacity of serum proteins. A test for evaluation of complications of pregnancy. *Amer. J. Obstet. Gynec.*, 86: 829-34, 1963.

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