قسم : الفسيولوجيا .
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# تأثير هرمون التيروكسين على الدورة الخلويه المنويه في الأرانب

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جرعت ثلاث مجموعات من الأرانب البلدى (كل مجموعة مكونة من ١٣، ١١، ١٠ أرنب على الترتيب) بثلاث جرعات من هرمون التيروكسين (٥ر٦ ، ١٣ ، ٥ر١٩ مجم/كجم من وزن الجسم) عن طريق الفم على الترتيب . ذبحت الأرانب بعد ١٢ يوم من بداية التجريع. وصبغت شرائح من أنسجة الخصيه بصبغة باص وقد حسب كل من نسبة خلايا سارتولي للدورة الخلوية المنوية وعدد خلايا ليدج وقطر الأنابيب المنوية.

وثبت احصائيا أن الجرعه الأولى غير مؤثرة . بينما الجرعة الثانية والثالثة اثرت على خلايا سارتولي . وانقسام وتميز خلايا الاسبر ماتوجونيا والاسبرما توسيت والاسبرماتيد وزيادة فسيولوجية في عدد خلايا ليدج .

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# *EFFECT OF THYROXINE ON NORMAL SPERMATOGENIC CELL CYCLE IN RABBIT*

*(With 5 Tables)*

*By*
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## *SUMMARY*

Three doses of thyroxine 6.5, 13, 19.5 mg/Kg body weight were given orally to three groups of Baladi rabbits, each group was 13, 11, 10 rabbits respectively. Five rabbits were used as control. The animals were slaughtered after 12 days. Testicular tissue sections were stained by P.A.S. The Sertoli cell ratio of the spermatogenic cell cycle, the number of leydig cells and the diameter of semineferous tubules were calculated. The first dose was statistically non significant. The seconed and third doses proportionally stimulated Sertoli cell, the division and differentiation of spermatogonia, spermatocyte and spermatid. Physiological hyperplasia of Leydig cell was prominant.

## *INTRODUCTION*

The relation between thyroid hormone and testicular function was based on clinical data and attempts to improve male fertility with thyroid hormone.

The data was still conflecting. Mild dose of thyroxine was harmful to sperm (FARRIS and COLTON, 1958). Small doses of thyroxine improved subfertile rabbit (MAQSOOD, 1951). Still the exact site or action of thyroid harmone on the testis is unknown.

The aim of this work was to study the effect of thyroid therapy on the different stages of spermatogenic cell cycle and Leydig cell in order to clarify the nature and site of thyroxine effect on testicular structure.

## *MATERIALS and METHOD*

Thirty nine adult Baladi male rabbits of about 11/2 : 2 Kg body weight were classified into four groups.

**Group 1** : included five rabbits kept as control.
**Group 2** : included 13 rabbits, which were given 6.5 mg/Kg body weight thyroid hormone.
**Group 3** : included 11 rabbits, which were given 13.0 mg/Kg body weight thyroid hormone.
**Group 4** : included 10 rabbits, which were given 19 5 mg/Kg body weight thyroid hormone.

Thyroxine was given orally in water daily for 12 days. At the end of the experiment the animals of all groups were slaughtered.

Testicular samples from both testicles were fixed in Bouns, embeded in paraffin. Serial sections were staind by P.A.S. stain.

Differential count for all cells occuping whole cross section of ten rounded semineferous tubules representing different stages of the cycle, and their Sertoli cell ratio were calculted according to SWIESTRA and FOOTE (1963).

The diameter of 10 cross - section of semineferous tubules were measured for each case. Also number of Leydig cells in 10 intertubular clusters were calculated for each animal.

The difference between groups were analysed statistically according to SNEDECOR (1964).

## *RESULTS*

**First dose :** There was significant increase in Sertoli cell number and spermatid type c. The Sertoli cell ratio was increased for spermatogonia, but it was decreased for total spermatocytes and spermatids.

The number of Leydig cells and diameter of semineferous tubules increased but statistically proved to be non significant (Tables 1 & 3).

**Second dose :** There was significant increase in Sertoli cell number, total spermatogonia and spermatids.

The increased total number of spermatogonia was due to significant increase in the types of spermatogonia. Spermatocyte cell number non significantly increased. The diplotene form only siginificantly increased. For spermatids, total number and type C were significantly increased.

Sertoli cell ratio for total spermatogonia was increased specially type B spermatogonia. For spermatocytes, it was decreased specially the ratio of early stages (Zygotene and Pachytene), but it was increased in advanced stages (diplotene). Sertoli cell ratio for total spermatids was increased specially for type C and D spermatids.

Leydig cells significantly increased. The diameter of semineferous tubules was increased but non significantly (Tables 1, 2 & 4).

**Third dose :** The number of Sertoli cells significantly increased. Also the spermatogonia particulary its B type. The total number of spermatocytes non significantly decreased. Its pachytene form was significantly decreased and diplotene increased. Total number of spermatids significantly incresed specially type D spermatid.

Sertoli cell ratio of total spermatogonia was incresed. Sertoli cell ratio of type A decreased and that of B type increased. Sertoli cell ratio of total spermatocyte decreased mostly in zygotene and pachytene while that of Diplotene was increased. Sertoli cell ratio of spermatids was inreased specially type C.

The number of Leydig cells significantly increased. Also the diameter of semineferous tubules was significantly increased (Tables 1, 2 & 5).

## *DISCUSSION*

The presented data had proved that the dossage in hormonal therapy is influential. Thyroid hormone has an augmental effect on the interstitial cells and the spermatogenic cell cycle. 6.5 mg/Kg body weight was statistically non effective. The standered stimulating effect was recorded within the seconed dose 13.0 mg/Kg body weight and proportionally increased within the third dose 19.5 mg/Kg body weight.

The stimulating effect of the thyroid involved Sertoli cell, spermatogonia, spermatocytes and spermatid. The effect was highly prominant on the interstitial Leydig cells.

Sertoli cell number was increased proportionally from the seconed to the third dose. The spermatogonial division was augmented as Sertoli cell ratio increased proportionaly.

Although the Sertoli cell ratio of the spermatocyte decreased proportionaly to the seconed and third dose. This decrease was explained on the basis of rapid differentiation to spermatid rather than suppressive effect on spermatocytes.

This fact is judged by the increased Sertoli number of total spermatid which result from the spermatocyte pool. The spermatid differentiation was also increased.

The Leydig cell responded by hyperplasia to thyroxine injection. 13 mg/Kg thyroxine caused double increase and 19.5 mg/Kg caused double and half increase in Leydig cells as compare with normal. Semilar results were demonestrated by EL-SHERRY, EL-NAGGAR and NASSAR, 1980, that thyroxine injection in summer stress caused Leydig cell hyperplasia.

The interstitial cells secreate testicular and circulating androgens (RONALD, SWERDLOFF and DAVID, 1981). Testicular androgens are essential for normal spermatogenesis (GERE and RICHARD, 1981).

Improvement in Sertoli cell ratio of spermatogenic cell cycle and its differentiation under thyroxine stimulation, indirectly proved a good level of testicular androgens secreted by the hyperplastic Leydig cells. In addition, the increased diameter of semineferous tubules was another testicular index for improved testicular function as whole under thyroid injection.

The mode and site of action of thyroxine on testis is a matter of controversy in literature. The following proposals are included. Thyroxine may affect gonadal function due to its action on metabolic processes in all body tissues (BARKER and SCHWARTZ, 1953). Levels of circulating thyroid hormones may affect secretion rates of the gonadotrophic hormones which control testicular function (CHU, 1944). Thyroxine may have modulating effects on the sensitivity of testis to gonadotrophic hormones (MEITES and CHANDRASHAKER, 1949) or it had direct effects on testes (HARA, 1963). In addition, thyroxine may affect responses to androgens (TONNOSN, GOMES and VAN DEMARK, 1970).

Our data visualized much more the role of thyroid therapy on interstitial cells and their androgens.

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**Table (1)**

Average mean number for cells, their Sertoli ratio and Diameter of semineferous tubules for control rabbits, and doses (6.5, 13 and 19.5 mg thyroxin/kg body weight) treated rabbits

| Treatment | Sertoli | Spermatogonia | | Total Spermatogonia | Spermatocytes | | | | Total Spermatocytes | Spermatids | | | | Total Spermatids | Leydig cell | Diameter of semomefrpis tuules in U |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type A | Type B | | Zygotene | Pachytene | Diplotene | 2ry spermatocyte | | A | B | C | D | | | |
| Control | 3.6 ±0.1 | 15.9 ±2.2 | 1.9 ±0.8 | 17.8 ±2.5 | 26.4 ±6.5 | 29.5 ±2.8 | 3.5 ±0.9 | - | 59.5 ±9.2 | 67.5 ±4.2 | 4.2 ±2.8 | 2.2 ±0.8 | 6.2 ±2.9 | 89.1 ±4.6 | 17.4 ±2.3 | 202.6 ±6.1 |
| | S.R. | 4.4 | 0.5 | 4.9 | 7.3 | 8.2 | 0.9 | - | 16.5 | 21.3 | 1.2 | 0.6 | 1.7 | 24.8 | - | - |
| 1st dose 6.5 | 4.1* ±0.1 | 20.9 ±1.6 | 3.9 ±0.7 | 24.8 ±1.9 | 25.9 ±1.9 | 23.1 ±2.1 | 5.3 ±1.1 | - | 52.6 ±3.4 | 79.8 ±7.5 | 3.9 ±2.5 | 7.1* ±2.1 | 7.3 ±1.7 | 98.2 ±9.9 | 21.9 ±1.8 | 207.7 ±5.8 |
| M mg/kg | S.R | 5.1 | 0.9 | 6.1 | 6.3 | 5.8 | 1.5 | - | 12.8 | 19.5 | 0.9 | 1.7 | 1.8 | 23.9 | - | - |
| 2nd dose 13 | 4.6** ±0.2 | 21.4* ±0.9 | 6.5* ±1.2 | 27.9** ±1.7 | 23.4 ±2.1 | 31.5 ±3.4 | 10.1* ±1.3 | - | 61.8 ±3.1 | 105.6 ±7.1 | - | 8.9* ±3.2 | 111.1 ±1.8 | 126.1** ±10.6 | 36.2** ±2.7 | 221.6 ±6.1 |
| M mg/kg | S.R. | 4.7 | 1.4 | 6.1 | 5.1 | 6.8 | 2.2 | - | 13.4 | 22.9 | - | 2.7 | 2.4 | 27.4 | - | - |
| 3rd dose 19.5 | 4.9** ±0.09 | 19.0 ±1.2 | 8.5** ±0.9 | 27.5** ±1.6 | 23.6 ±2.7 | 28.8 ±1.9 | 7.7** ±1.3 | - | 52.1 ±3.6 | 129.0 ±11.4 | - | - | 29.4** ±4.7 | 161.5** 15.7 | 42.9** 3.9 | 230.8 * 7.9 |
| M mg/kg | S.R | 3.9 | 1.7 | 5.6 | 4.8 | 4.2 | 1.6 | - | 10.6 | 26.5 | - | - | 6 | 32.9 | - | - |

* : (P/_ 0.05) ** : (P/_ 0.01) S.R. : Sertoli ratio.

TABLE (2): Average number of cells, their Sertoli ratio and diameter of seminferous tubules in control normal rabbits.

| Case Number | Sertoli cell | Spermatogonia | | Total Sperma-togonia | Spermatocytes | | | 2ry sperma-tocyte | Total sperma-cytes | Spermatids | | | | Total sper-matids | Leydig cell | Diameter of semi nefrous tubules in μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type A | Type B | | Zygo-tene | Pachy-tene | Diplo-tene | | | A | B | C | D | | | |
| Mean | 3.6 | 15.9 | 1.9 | 17.8 | 26.4 | 29.5 | 3.5 | - | 59.5 | 67.5 | 4.2 | 2.2 | 6.2 | 89.1 | 17.4 | 202.6 |
| S.E. | ±0.1 | ±2.2 | ±0.8 | ±2.5 | ±6.5 | ±2.8 | ±0.9 | - | ±9.2 | ±4.2 | ±2.8 | ±0.8 | ±2.9 | ±4.6 | ±2.3 | ±6.1 |
| Sertoli-ratio | - | 4.4 | 0.5 | 4.9 | 7.3 | 8.2 | 0.9 | - | 16.5 | 21.3 | 1.2 | 0.6 | 1.7 | 24.8 | - | - |

* : S.E. : Stander error.

TABLE (3): Average number of cells, their Sertoli ratio ad diameter of semineferous tubues in rabbits treated with 6.5 Ugm thyroxine/Kg body weight for 12 days.

| Case Number | Sertoli cell | Spermatogonia | | Total Sperma-togonia | Spermatocytes | | | 2ry sperma-tocyte | Total sperma-cytes | Spermatids | | | | Total sper-matids | Leydig cell | Diameter of semi nefrous tubules in μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type A | Type B | | Zygo-tene | Pachy-tene | Diplo-tene | | | A | B | C | D | | | |
| Mean | 4.1* | 20.9 | 3.9 | 24.8 | 25.9 | 23.1 | 5.3 | - | 52.6 | 79.8 | 3.9 | 7.1* | 7.3 | 98.2 | 21.9 | 207.7 |
| S.E. | ± 0.1 | ±1.6 | ±0.7 | ±1.9 | ±1.9 | ±2.1 | ±1.1 | - | ±3.4 | ±7.5 | ±2.5 | ±2.1 | ±1.7 | ±9.9 | ±1.8 | ±5.8 |
| Sertoli-ratio | | 5.1 | 0.9 | 6.05 | 6.3 | 5.8 | 1.5 | - | 12.8 | 19.5 | 0.9 | 1.7 | 1.8 | 23.9 | | |

* : (P / 0.5).

S.E. : Stamder errpr

TABLE (4): Average number of cells, their Sertoli ratio and diameter of semineferous tuules in rabbits treated wth 13.0 Ugm thyroine /Kg body weight for 12 days.

| Case Number | Sertoli cell | Spermatogonia | | Total Sperma-togonia | Spermatocytes | | | 2ry sperma-tocyte | Total sperma-cytes | Spermatids | | | | Total sper-matids | Leydig cell | Diameter of semi nefrous tubules in μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type A | Type B | | Zygo-tene | Pachy-tene | Diplo-tene | | | A | B | C | D | | | |
| Mean | 4.6 | 21.4 | 6.5 | 27.9 | 23.4 | 31.5 | 10.1 | - | 61.8 | 105.6 | - | 8.9 | 11.1 | 126.1 | 36.2 | 221.6 |
| S.E. | ±0.2 | ±0.9 | ±1.2 | ±1.7 | ±2.1 | ±3.4 | ±1.3 | - | ±3.1 | ±7.1 | - | ±3.2 | ±1.8 | ±10.6 | ±2.7 | ±6.1 |
| Sertoli-ratio | - | 4.7 | 1.4 | 6.1 | 5.1 | 6.8 | 2.2 | - | 13.4 | 22.9 | - | 2.7 | 2.4 | 27.4 | - | - |

*: ( P/ 0.05 ).
**: ( P / 0.1 ).
S.E. : Stander error.

TABLE (5): Average number of cells, their Sertoli ratio and diameter of semineferous tuules in rabbits treated with 19.5 Ugm thyroxine /Kg body weight for 12 days.

| Case Number | Sertoli cell | Spermatogonia | | Total Sperma-togonia | Spermatocytes | | | 2ry sperma-tocyte | Total sperma-cytes | Spermatids | | | | Total sper-matids | Leydig cell | Diameter of semi nefrous tubules in μ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Type A | Type B | | Zygo-tene | Pachy-tene | Diplo-tene | | | A | B | C | D | | | |
| Mean | 4.9** | 19 | 8.5** | 27.5 | 23.6 | 20.8* | 7.7* | - | 52.1 | 129.9 | - | - | 29.4** | 161.5** | 42.9** | 230.8 |
| S.E. | ±0.09 | 1.2 | ±0.9 | 1.6 | ±2.7 | ±1.9 | ±1.3 | - | ±3.6 | ±11.4 | - | - | ±4.7 | ±15.7 | ±3.9 | ±7.9 |
| Sertoli-ratio | - | 3.9 | 1.7 | 5.6 | 4.8 | 4.2 | 1.6 | - | 10.6 | 26.5 | - | - | 6 | 32.9 | | |

*: ( P/ 0.05)  **: ( P/ 0.01 )  D.E.: Stander error