

Sheep Fetal Thyroid Histological Development, with Adult Plasma T₄ and T₃ Hormones Concentrations

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Abstract: Thyroid hormones plasma concentrations values of 80 apparently healthy Iraqi sheep (40 ewes and 40 rams) were studied. The mean plasma T₄ and T₃ for ewes were 54.8±6.2 and 1.06±0.04 ng mL⁻¹, respectively. The mean T₄ and T₃ were decreases as the age of rams progress, while in late pregnant ewes the mean T₄ and T₃ were significantly p<0.01 more than that of lactating and old ewes. The mean thyroid hormones value of both lactating and old ewes were, also significantly (p<0.01) different. The fetal thyroid gland weight were increased from 18-972.6 mg as fetal CRL increased from 5-45 cm. Histologically thyroid follicles containing colloid were seen at 20 cm CRL and follicles increased in number, size and colloid content as fetal CRL increased toward birth. The effect of age and sex of sheep produce different degree of variation in the thyroid hormones concentrations. The high levels of T₄ and T₃ in late pregnancy is essential for fetal growth and development. Fetal thyroid is functioning during gestation.

Key words: Fetal, thyroid, histology, thyroxine, sheep, triiodothyronine

INTRODUCTION

The thyroid hormones plays an important role in regulating the process of growth, lactation, reproduction and the general health (Jainudeen *et al.*, 2000). Thyroid secretion rate of living intact animals has paved the way for study of normal variations of thyroid function in animals and correlation of observed variations with production performance (Coelho *et al.*, 2008). In adult sheep, Thomas and Nathanielsz (1983) reported that plasma T₄ and T₃ concentrations were 60 and 0.6 ng mL⁻¹, respectively. While Gluszk *et al.* (1989), found that the plasma T₄ and T₃ were increased from 36.6 and 0.32 ng mL⁻¹ by 7 weeks to 59.2 and 0.64 ng mL⁻¹ late in gestation, respectively. After parturition the values were 14.2 and 0.26 ng mL⁻¹ for T₄ and T₃ hormones. Thyroid hormones have been demonstrated for fetal development and adaptation to extra-uterine life and fetal plasma T₄ concentrations are greater than those of the mother during the last third of gestation (Thomas and Nathanielsz, 1983). Fetal sheep thyroid incorporates iodide and synthesizes thyroxine a bout 65 days after conception (Mellor *et al.*, 1976).

No in formations are available regarding the development of fetal thyroid gland and serum thyroid hormones value in the Iraqi sheep. It is seemed of interest to determine histological development and growth of fetal thyroid gland throughout the greater part of the intrauterine life.

MATERIALS AND METHODS

Blood samples were collected from 80 adult local sheep (40 rams and 40 ewes). Blood samples were centrifuged in tubes contain EDTA (Ethylenediamine Tetra Actic acid). The plasma was a liquated and stored at -20°C, until assay for total Thyroxine (T₄) and Triiodothyronine (T₃) were measuring by highly a specific Enzyme Immuno Assay (EIA) kits purchased from BioMeriux, Marcy-I Etoile, France). The intra-and inter-assay coefficient of variation were 3.8 and 7.0% for T₄ and 4.1 and 7.4% for T₃, respectively. No cross reaction with reverse Triiodothyronine (rT₃) could be observed.

A hundred and twenty four uterus of local ewes contained single fetus in various stages of gestation were collected from the abattoir. Fetuses were weight after separation from the placenta. Fetal Crown Rump Length (CRL) was measured following the technique of Lynyset (1968). Fetal thyroid glands were dissected free of connective tissues and their weights were taken on sensitive electric balance (PR 5003 comparator Mettler, Toledo) then fixed in 10% nutral buffered formalin. Cross section (5 nm thickness) and stained with hematoxylin and eosin. The study of thyroid glands were examined by light microscope.

Results were presented as Mean±SE data were analysis by statistical SAS analysis system. The level of significant was applied on (p<0.01).

RESULTS

Table 1 and 2 showed plasma levels of T_4 and T_3 hormones in male and female Awassi sheep. The average of T_4 and T_3 for growing males (Table 1) were 66.3 and 1.4 ng mL^{-1} , respectively. These levels of T_4 and T_3 reduced with age progress. The old mature rams showed $p < 0.01$ lower levels in both hormones compared with the younger rams at differed ages. In females, T_4 and T_3 levels were increased with pregnancy progress, then declined after birth (Table 2).

Table 3 represented, the mean body weight of the fetuses at different CRL. There was an increases in the body weight from 5-20 cm CRL, while there was a steadily increased from 25 cm CRL toward term. As well as, there was an increase in absolute fetal thyroid weight starting from 15 cm CRL of intrauterine development toward birth. Anatomically fetal thyroid gland has a compact thin structure and dark rose color at early stages.

However, histological sections showed small follicles with colloid which surrounded by cuboidal epithelial cells at 20 cm CRL (75 days old) (Fig. 1a). Then, the gland has progressive follicular development both in size and

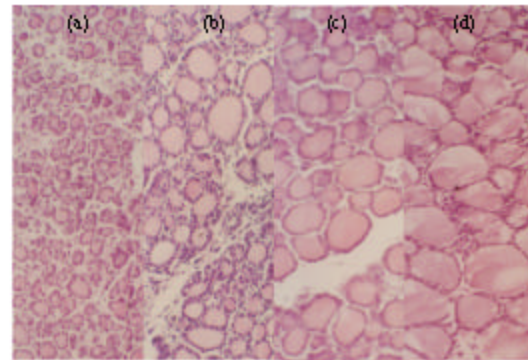


Fig. 1: Histological sections development of fetal thyroid gland at different CRL (H and E. $\times 200$). a): At 20 cm CRL (75 days old) sections shows the presence of small follicles some with colloid, surrounded by cuboidal epithelial cells. b): At 30 cm CRL (100 days old). Follicles are filled with homogenous colloid and surrounded by columnar epithelial cells. c): At 45 cm CRL (125 days old). Active thyroid gland contain small and large follicles filled with colloid. d): Near term fetus thyroid gland has larger follicles filled with colloid and lined by columnar epithelial cells

Table 1: The plasma concentration of T_4 and T_3 hormones in Rams Awassi Sheep (Mean \pm SE)

| Age of ram | No. of animals | Plasma level (ng mL^{-1}) | |
|----------------|----------------|--------------------------------------|-----------------|
| | | T_4 | T_3 |
| 3-6 months | 10 | 86.6 \pm 4.2A | 1.7 \pm 0.3A |
| 9-12 months | 10 | 65.3 \pm 3.4A | 1.5 \pm 0.3A |
| 2-3 years | 10 | 47.6 \pm 2.9B | 1.0 \pm 0.3B |
| All young rams | 30 | 66.3 \pm 5.5 | 1.4 \pm 0.4 |
| Old adult rams | 10 | 34.8 \pm 2.6C | 0.97 \pm 0.6C |
| All rams | 40 | 57.08 \pm 3.3 | 1.23 \pm 0.4 |

Table 2: The level of plasma T_4 and T_3 in Awassi ewes (Mean \pm SE)

| Ewes conditions | No. of ewes | Plasma level (ng mL^{-1}) | |
|-----------------|-------------|--------------------------------------|------------------|
| | | T_4 | T_3 |
| Early pregnant | 10 | 54.8 \pm 1.6AC | 0.9 \pm 0.1C |
| Late pregnant | 10 | 69.8 \pm 3.0A | 2.4 \pm 0.6A |
| Lactating | 10 | 60.6 \pm 2.7B | 1.3 \pm 0.2B |
| Ewes | 30 | 61.96 \pm 3.4 | 1.56 \pm 0.74 |
| Old ewes | 10 | 33.8 \pm 2.4D | 0.63 \pm 0.16D |
| All ewes | 40 | 54.8 \pm 6.2 | 1.06 \pm 0.04 |

Table 3: Relations between fetal CRL cm, fetal weight (gm) and thyroid weight (mg) in sheep (Mean \pm SD)

| No. of samples | CRL (cm) | Age (days) | Fetal weight | Thyroid weight | Thyroid body weight (%) |
|----------------|----------|------------|---------------------|------------------|-------------------------|
| 10 | 1-5 | 45 | 3.42 | - | - |
| 15 | 5-10 | 55 | 29.12 \pm 10.96 | 18 \pm 0.5 | 0.062 |
| 20 | 10-15 | 65 | 91.13 \pm 33.78 | 59.1 \pm 0.3 | 0.064 |
| 18 | 15-20 | 75 | 219.82 \pm 51.08 | 129.3 \pm 0.4 | 0.058 |
| 12 | 20-25 | 85 | 436.81 \pm 50.38 | 240.5 \pm 3.6 | 0.054 |
| 15 | 25-30 | 95 | 904.66 \pm 150.98 | 476 \pm 10.1 | 0.053 |
| 15 | 31-34 | 105 | 1130.80 \pm 97.45 | 470.7 \pm 3.01 | 0.042 |
| 13 | 35-40 | 105-115 | 1940.60 \pm 44.72 | 640.9 \pm 7.05 | 0.033 |
| 6 | 41-45 | 115-125 | 2490.30 \pm 241.0 | 972.6 \pm 3.07 | 0.039 |

number, at 30 cm CRL (Fig. 1b). The glands section showed resorption vacuoles in the colloid and follicles lined by columnar epithelium cells. The follicles were larger at 45 cm CRL (125 days old) and filled with homogenous colloid, surrounded by columnar epithelium cells (Fig. 1c). Near term (Fig. 1d), the gland had large follicles with an area of papillary infolding surrounded by columnar epithelial cells. Maternal gland had red congested thyroid gland during last third of pregnancy.

DISCUSSION

Thyroid hormones levels were decreased with animals age progress however, the growing males showed higher values of T_3 and T_4 compared with old males. Same trend were also recorded by Colavita *et al.* (2009). This result could be due to the process growth of animals, the growing animals showed high level of thyroid hormones compared with mature animals (Salah, 1996). In sheep, the hormones concentrations in plasma could falls as more hormones are required to regulate the metabolism process.

Plasma thyroid hormones concentration were increased with advance pregnancy and drops after birth, more decline in old ewes hormones were estimated (Table 2). This drop in thyroid hormones concentration were in agreement with the finding of Salah (1996) in goat, Saliman *et al.* (1999) in cows and Hashmar *et al.* (1984) in

she camel, this reduction could be due redistribution of the nutrients to the mammary gland in preparation for milk yield (Salah, 1996). However, Griffin *et al.* (1962) found high levels of thyroxin hormone in lactating period than in old ewes as it was found in this study.

Body weights of the fetuses at different ages were recorded as the same trend found by Evans and Sacks (1973), Robertson and Falconer (1980) using sheep fetuses and Kudu and Kaikini (1987) in goat. In this study, it was found a sharply increase in fetal thyroid weight and an increases in follicle size and colloid contents in fetuses at 20 cm CRL on ward. These observations on an increases in size and number of thyroid follicles with fetal development indicated that the sheep fetal thyroid gland is functioning during gestation, similar to those found in sheep by Thomas and Nathanielsz (1983) using pig by Fentener *et al.* (1983) and in monkey by Pickering (1968).

CONCLUSION

Thyroid hormones levels are affected by age of sheep and increasing during gestation and decreasing after birth. The thyroid hormones are important for fetal growth and development.

REFERENCES

- Coelho, L.A., A. Sasa, S.D. Bicudo and J.C.C. Balieiro, 2008. Plasma concentrations of testosterone, T₃ and T₄ in bucks submitted to heat stress.
- Colavita, G.P., A. Debenedetti, C. Ferri, B. Lisi and A. Lucaroni, 2009. Plasma concentrations of thyroid hormones in the domestic goat. Seasonal variation in Relation to age. *Boll. Soc. Italian. Sper. J.*, 59 (6): 779-785. PMID: 6349664.
- Evans, H.E. and W.O. Sack, 1973. Prenatal development of domestic and laboratory mammals. *Anatomia Histologia Embryologia*, 12: 11-45.
- Gluszek, A., T. Studzinski and A. Czarenecki, 1989. Changes in concentrations of T₃ and T₄ in the blood plasma during pregnancy. *Vet. Bull.*, 59 (15): 3071, 429.
- Griffin, S.A., H.A. Henneman and E.P. Reineke, 1962. The thyroid secretion rate of sheep as related to season, breed, sex and semen quality. *Am. J. Vet. Res.*, 23: 109-113.
- Hashmar, H.A., A. Taha, A.A. Ismail and M.B.A. Sami, 1984. Levels of thyroid hormones in the plasma of pregnant camels (*Camelus dromedaries*). *Indian J. Anim. Sci.*, 54: 663-665.
- Jainudeen, M.R., H. Wahid and E.S.E. Hafez, 2000. Sheep and Goats. 7th Edn. Reproduction in Farm Animals. In: Hafez E.S.E. and B. Hafaz (Eds.). Lippincott Williams and Wilkins, Philadelphia, USA, pp: 172-181.
- Kudu, M.S. and A.S. Kaikini, 1987. Functional activities of the ovaries and uterine horns of goats. *Indian Vet. J.*, 64: 945-949.
- Lynset, O., 1968. Studies on the reproduction in the goat. The normal genital organs of the non-pregnant goat. *Acta Vet. Scand.*, 12: 208-222.
- Mellor, D.J., I.C. Matheson, J. Small and H. Wright, 1976. Plasma T₄ concentrations in the ewes and their fetuses during the last 6 weeks of Pregnancy. *Res. Vet. Sci.*, 21: 102-103.
- Pickering, D.E., 1968. Thyroid physiology in the developing monkey fetus (*Macaca Mullatta*). *General and Comparative Endocrinol.*, 10: 182-190.
- Robertson, H.A. and I.R. Falconer, 1980. Reproduction and thyroid activity. *J. Endocrinol.*, pp: 133-142.
- Salah, M.S., 1996. Thyroid hormones during late pregnancy and earlylactation in the Aardi goats. *J. King Saudi Univ.*, 8 (1): 87-96.
- Saliman, F.A., H. Nasr and K. Zaki, 1999. Level of thyrotrophic hormone in the blood of Friesion cows at various reproductive stages. *J. Reprod. Fertility*, 6: 335-340.
- Thomas, A.L. and P.W. Nathanielsz, 1983. The fetal thyroid: Current topics in experimental. *Endocrinology*, 5: 97-116.