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### Impact of thyroid status on the response to ram effect in ewes

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## ■ Impact of thyroid status on the response to ram effect in ewes.

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## ■ Συμβολή του θυρεοειδούς στην απάντηση στην "επίδραση του αρσενικού" στις προβατίνες.

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**ABSTRACT.** The role of thyroid hormones on the transition to anestrus has been well established in ewes, but their possible involvement in the induction of reproductive activity remains obscure. The aim of the present study was to investigate the role of thyroid hormones in the induction of the first ovulation in relation to "ram effect" in ewes. Forty-four ewes of the Greek Zackel mountain type breed were used. The ewes were housed separately from the rams until day 0 (entrance of rams). Blood samples were collected weekly for 4 weeks until 11 weeks after the ram entrance for the determination of thyroxin and progesterone levels. Thirty-five out of 44 ewes (group A) had a silent heat, while the rest 9 ewes (group B) exhibited a delayed estrus. Thyroxin levels rose immediately after ram entrance in animals of group A, but in group B the increase in thyroid hormone levels was evident only after the first week. The difference in  $T_4$  levels between week 0 and 1 for ewes of group A was significantly higher than that observed for animals of group B ( $p < 0.05$ ). The pattern of progesterone release was similar between the two groups, but a two week delay in the increase of progesterone concentration was observed in animals of group B compared to group A. It is concluded that thyroid hormones may act at the beginning of the breeding season to cause a modification in gonadotrophin secretion leading to early ovulation.

**Key words:** thyroxin, ewes, ram effect

**ΠΕΡΙΛΗΨΗ.** Ο θυρεοειδής αδένας είναι αποδεδειγμένα απαραίτητος για την έναρξη της άνοιξης περιόδου, ενώ δεν έχει αποδειχθεί ακόμη η σημασία του στην έναρξη της οιστρικής περιόδου στο πρόβατο. Σκοπός της παρούσας μελέτης ήταν η διερεύνηση των θυρεοειδικών ορμονών, καθώς και της προγεστερόνης σε σχέση με την έναρξη της ωθητικής δραστηριότητας μετά από «επίδραση του αρσενικού». Χρησιμοποιήθηκαν 44 προβατίνες Βλάχικης φυλής, οι οποίες ελέγχονταν καθημερινά για εμφάνιση οίστρου καθ' όλη τη διάρκεια του πειραματισμού. Δείγματα αίματος συλλέγονταν εβδομαδιαία για 4 εβδομάδες πριν

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έως 11 εβδομάδες μετά την είσοδο των κριών, οπότε όλα τα ζώα παρουσίασαν οίστρο. Στα δείγματα αυτά προσδιορίστηκαν τα επίπεδα της θυροξίνης και της προγεστερόνης με ραδιοανοσολογική μέθοδο. Οι 35 από τις 44 προβατίνες αντέδρασαν στην είσοδο των κριών με σιωπηλό οίστρο και χαρακτηρίστηκαν ως ομάδα Α, ενώ οι υπόλοιπες 9 που δεν αντέδρασαν και εμφάνισαν καθυστερημένα οίστρο αποτέλεσαν την ομάδα Β. Η συγκέντρωση της θυροξίνης παρουσίασε άμεση άνοδο (1η εβδομάδα) και διατηρήθηκε αυξημένη για 4 εβδομάδες στην ομάδα Α, που αντέδρασε στην είσοδο των κριών με σιωπηλό οίστρο. Στα ζώα της ομάδας Β η συγκέντρωση της θυροξίνης αυξήθηκε μετά την πρώτη εβδομάδα (από την είσοδο του κριού) και διατηρήθηκε αυξημένη για τις δύο επόμενες εβδομάδες. Η διαφορά στη συγκέντρωση της  $T_4$  μεταξύ της εβδομάδας 0 και 1 για τα ζώα της ομάδας Α ήταν στατιστικώς σημαντική ( $p < 0.05$ ), ενώ δεν παρατηρήθηκε διαφορά μεταξύ αυτών των τιμών για τα ζώα της ομάδας Β. Το πρότυπο της μεταβολής της συγκέντρωσης της προγεστερόνης ήταν παρόμοιο και στις δύο ομάδες με τη διαφορά ότι στα ζώα της ομάδας Β παρουσιάστηκε καθυστέρηση δύο εβδομάδων στην αύξηση των επιπέδων της. Διαφορά παρουσιάστηκε και στις γόνιμες ορχείες, οι οποίες για τις προβατίνες της ομάδας Α έγιναν στις  $4.6 \pm 0.3$  εβδομάδες ( $X \pm SEM$ ), ενώ σε αυτές της ομάδας Β στις  $8.4 \pm 0.8$  εβδομάδες, μετά την είσοδο του κριού. Συμπερασματικά, φαίνεται ότι η θυροξίνη κατά την «επίδραση του κριού» επηρεάζει την έκκριση του GnRH αυξάνοντας την ευαισθησία των υποθαλαμικών νευρώνων στη δράση των οιστρογόνων και επομένως στην πρόκληση της ωοθυλακιορρηξίας.

**Λέξεις ευρετηρίασης:** θυροξίνη, προβατίνα, επίδραση κριού

## INTRODUCTION

The involvement of the thyroid in the regulation for seasonal reproduction has long been recognized. Thyroid hormones have crucial role in seasonal reproductive cyclicity in a number of species (Jallegeas and Assenmacher, 1979; Follet & Nochols, 1984; Shi and Barrel, 1992; O'Callaghan et al., 1993; Dahl et al., 1995; Wilson and Reinert, 1995). In ewes thyroid hormones are of physiological significance to generation of the seasonal reproductive cycle (Karch et al., 1995). Thyroidectomy was found to block the transition from the breeding season to anoestrus by preventing the seasonal increase in responsiveness to the negative feedback action of oestradiol on episodic GnRH secretion, thus interfering with a key neuroendocrine process necessary for anoestrus to develop (Webster et al., 1991a; Moenter et al., 1991a; Anderson et al., 2002). Furthermore, reduced thyroxin secretion, following treatment with methyl - thiouracil (Follett and Potts, 1990; Saleh et al., 1998) or propylthiouracil (Hernandez et al., 2003; Wells et al., 2003) has been shown to extend the breeding season, albeit marginally. Thyroxin exhibits a seasonal fluctuation, rising gradually from a nadir just prior to the onset of the breeding season to peak concentrations shortly before the transition to anestrus (Dahl et al., 1994).

Although the role of thyroid hormones on the transition to anestrus has been well established, their possible involvement in the induction of reproductive activity remains obscure. When intact, thyroidectomized (THx) and THx supplemented with thyroxin ( $T_4$ ) groups of animals were examined, a delay in the

onset of the breeding season was observed in animals of the THx group compared to other two groups. Although the difference did not reach significance, the great variability detected in these animals probably indicates that thyroid gland may also exert an effect on the onset of reproductive activity. These findings were observed when animals were driven by the endogenous rhythm that underlies the seasonal changes of reproductive activity (Webster et al., 1991b).

Data on rats support a role of thyroid hormones in ovulation, since it has been shown that prepubertal hypothyroidism inhibits the first ovulation in eCG-primed immature female rats and this blockage of ovulation is mainly mediated through the inhibition of preovulatory LH surge from the pituitary (Tamura et al., 1998).

The introduction of rams in anestrus ewes, previously completely isolated from the rams, brings about increases in tonic LH secretion, preovulatory LH and FSH surge and –in many instances- ovulation. The ewe response to the ram-effect is believed to be mediated by pheromones, which appear to reverse the photoperiod effects on the mode of LH secretion (Cushwa, et al., 1992; Martin and Scaramuzzi, 1983).

Therefore the aim of the present study was to investigate the role of thyroid hormones in the induction of the first ovulation in relation to "ram effect" in ewes.

## MATERIALS AND METHODS

### Animals and experimental design

Forty-four ewes of the Vlachiko breed (Greek Zackel mountain type) were used.



The experiment was carried out from March 18<sup>th</sup> to May 20<sup>th</sup> at the Institute of Animal Production in Giannitsa (40° 47'N 22° 24'E), North Greece. Animals were kept outdoors under the influence of natural changes in photoperiod and temperature, fed a balanced diet and allowed free access to water. All animals gave birth in December and were suckling until February. Ewes were kept separated from rams until April 15<sup>th</sup> (ram entrance, week 0). Following ram entrance ewes were checked for estrus, accompanied by matings, daily. The experiment lasted until all animals exhibited overt estrus (11 weeks after ram entrance). Fertile estrus was retrospectively confirmed by the lambing date.

Blood samples were collected weekly from jugular vein during the whole experimental period. Blood collection continued in the non-pregnant ewes until positive pregnancy determination by serum progesterone. Samples were stored until analyzed.

### Hormone determinations

Thyroxine levels were determined by radioimmunoassay, using a commercial RIA kit (Amerlex-M T<sub>4</sub>, of Amersham, UK). The sensitivity of the assay was 2.6 ng/ml. Inter- and intra-assay coefficients of variation were 2.6% and 3.6%, respectively.

Progesterone plasma concentrations were measured by a coated-tube RIA kit (Coat-A-Count, DPC, USA). The sensitivity of the method was 0.02 ng/ml and the inter- and intra-assay coefficients of variation were 4.0% and 5.7%, respectively.

Progesterone values greater than 1ng/ml were considered as indicative of corpus luteum function, while after mating, progesterone concentrations greater than 1 ng/ml were followed by at least four more consecutive high values as indicative of pregnancy.

All data are presented as least square means  $\pm$  SEM and were analysed using the SAS Statistical Programme (SAS 2005). Data for hormone levels were analysed using a General Linear Model (GLM) for repeated measures. All other data were analysed using chi-square test. Statistical significance was set at  $P < 0.05$ .

## RESULTS

Thirty-five out of 44 animals responded to ram effect by exhibiting silent heat, as was indicated by the first progesterone increase, different from the undetectable levels, which occurred at a mean time of

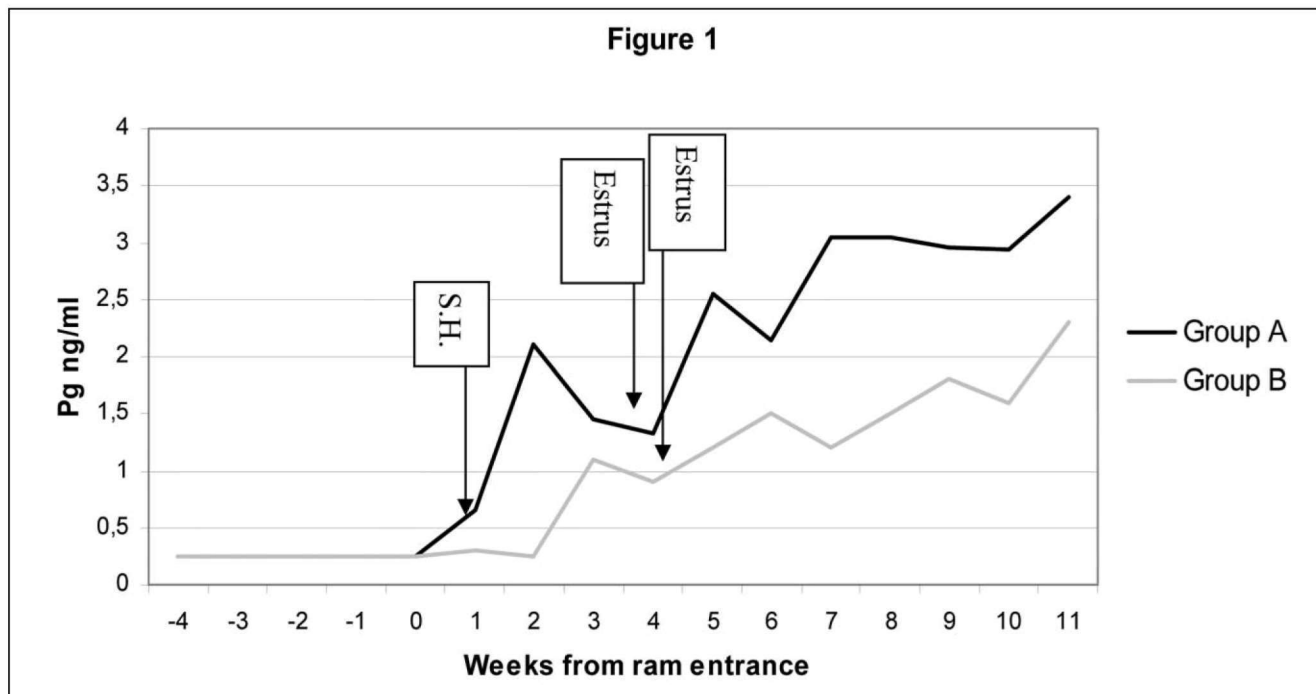
$0.4 \pm 0.1$  weeks following ram entrance. These animals were classified as group A. For the remaining nine animals this increase occurred at a mean time of  $3.9 \pm 0.6$  weeks and were classified as group B. This difference was found to be significant ( $p < 0.001$ ). Ewes of group A exhibited overt estrus at  $3.5 \pm 0.1$  weeks (mean  $\pm$  SEM). The number of animals that conceived was 33/35 and fertile estrus took place at  $4.7 \pm 0.8$  weeks following ram entrance, as was estimated by both weekly progesterone levels ( $\geq 1.5$ ng/ml) and lambing data. In group B overt estrus was detected at  $4.6 \pm 0.3$  weeks and fertile estrus at  $8.4 \pm 0.8$  weeks after ram entrance. The number of animals that conceived was found to be 7/9. Significant differences were detected for the time of estrus occurrence ( $p < 0.05$ ) and for the time of fertile estrus ( $p < 0.001$ ) between the two groups of ewes.

Mean weekly concentration of thyroxine and progesterone levels for both groups are presented in figures 1 and 2, respectively.

During the time from week -4 until week 0 (ram entrance), thyroxine levels were nearly the same, fluctuating between  $90. \pm 3.5$  (week -4) to  $79.9 \pm 3$  (week 0) for group A. A significant increase in T<sub>4</sub> levels was detected between values of week 0 and those of weeks 1, 2 and 3 following ram entrance ( $98.5 \pm 3$ ,  $102.6 \pm 3$  and  $102.2 \pm 3$  ng/ml, respectively) ( $p < 0.05$ ). From week four onwards thyroxine concentrations fluctuated between  $60.4 \pm 3.7$  and  $86.5 \pm 3$  ng/ml.

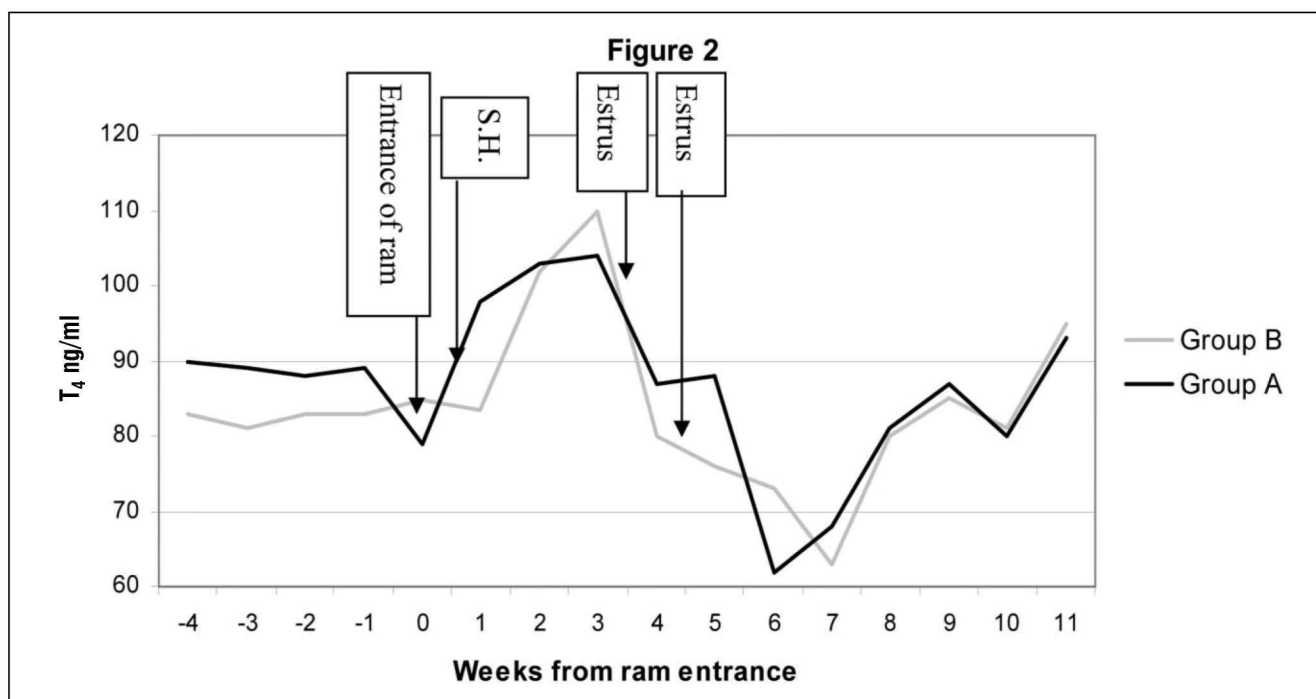
In group B, during the above period, T<sub>4</sub> concentrations were almost the same, fluctuating between  $84.8 \pm 3$  and  $83 \pm 3$  ng/ml ( $X \pm SEM$ ). In contrast to group A, the increase in thyroxine levels was evident only after the first week and significant differences were observed between values of week 1 and those of weeks 2 and 3 following "ram entrance". In animals of group A a significant difference for T<sub>4</sub> concentration was detected between week 0 and 1 ( $p < 0.05$ ), while in animals of group B no difference was observed. This finding indicates an advanced elevation in T<sub>4</sub> levels in ewes that have responded to ram effect. Thereafter (week 4 to 11) thyroxine levels in group B fluctuated from  $63.1 \pm 7.6$  to  $85.5 \pm 7.3$  ng/ml ( $X \pm SEM$ ) and were not different from those of group A.

The pattern of progesterone secretion was similar between the two groups, but a two week delay in the increase of progesterone concentration was observed in animals of group B compared to group A.



**Figure 1.** Weekly progesterone concentrations of groups A and B ewes.  
S.H. = Silent heats.

**Εικόνα 1.** Εβδομαδιαίες συγκεντρώσεις προγεστερόνης των προβατινών των ομάδων Α και Β.  
Weeks from ram entrance = εβδομάδες από την είσοδο των κριών.  
S.H. = σιωπηλοί οίστροι, Estrus = οίστρος



**Figure 2.** Weekly thyroxine concentrations of groups A and B ewes.  
S.H. = Silent heats.

**Εικόνα 2.** Εβδομαδιαίες συγκεντρώσεις θυροξίνης των προβατινών των ομάδων Α και Β.  
Weeks from ram entrance = εβδομάδες από την είσοδο των κριών.  
S.H. = σιωπηλοί οίστροι, Estrus = οίστρος, Entrance of rams = είσοδος των κριών



## DISCUSSION

The entrance of rams resulted in silent heats, as was indicated by a rise in progesterone levels in animals of group A. It is well established that a proportion of ewes exposed to "the ram effect" exhibit silent heat (Hawken et al., 2007; Ungerfeld et al., 2005; Ungerfeld et al., 2004b), accompanied by increased episodic LH pulses (Martin, 1984, Karsch et al., 1993; Ungerfeld et al., 2004a). Moreover, an elevated TSH concentration has also been detected during proestrus in hamsters (Howes et al, 1991). Peeters et al. (1989) found that plasma T<sub>4</sub> levels in ewes were higher during estrus and lower during the luteal phase. Also, in goats a rise in plasma T<sub>4</sub> was observed during induced or spontaneous estrus (Colavita and Malfatti, 1989). Taking together the above data support the hypothesis that an increase in LH may have enhanced TSH and thus T<sub>4</sub> release in ewes of group that responded earlier to ram effect by exhibiting silent heat. Data in humans and rats have reported that hCG increases TSH and consequently T<sub>4</sub> levels (Glinoe, et al., 1993; Glinoe, et al., 1990; Pekary, et al., 1983; Kimura et al., 1990; Grun. et al., 1997).

It has been shown that in ewes thyroidectomy blocks the transition from the breeding season to anestrus by preventing the seasonal increase in responsiveness to the negative feedback action of oestradiol on episodic GnRH secretion (Karsch et al., 1995a; Moenter et al., 1991). Furthermore, it has been postulated that in sheep the responsiveness of the reproductive neuroendocrine axis to thyroid hormones is season-dependent, hence, a strict time "window" plays a regulatory role on thyroid hormones in sheep reproduction (Thrun et al., 1997).

A number of neurotransmitters control the GnRH pulse generator and a number of in vivo and in vitro studies suggest that the thyroid hormones affect the

turnover, release and receptor binding affinity of these neurotransmitters. Therefore, changes in thyroid hormone concentration alter the control of GnRH secreting neurons (Harris et al, 1986), (Reymond et al., 1987, Velasquez et al., 1997).

Thyroid hormones have been shown to influence synaptic transition and are also involved in ovarian steroidogenesis and follicular maturation. In humans hypothyroidism causes reduced response of LH to GnRH, which increases after T<sub>4</sub> administration (Velazquez et al., 1997). Studies in vitro reported that thyroid hormones have a synergistic effect with FSH and lead to an increase 3-β hydroxysteroid dehydrogenase and aromatase activities (Maruo et al., 1987).

Data on rats have shown that after thyroidectomy a greater amount of estrogen is necessary to induce preovulatory LH surge than in intact animals (Freeman et al, 1976).

So, the results of the present study support a facilitatory action of thyroid hormones, which may be exerted at the level of hypothalamus by increasing the sensitivity of GnRH neurons to estrogen action. Thyroid hormones may act at the beginning of the breeding season to cause a modification in gonadotrophin secretion.

If this modified mode of secretion is the cause or the consequence of the cascade of physiologic events leading to resumption of breeding season, remains to be elucidated.

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