Reprod. Nutr. Dev. (1989) 29, 237-245

Original article

Relationship between the thyroidal and gonadal axes during the estrous cycle of ewes of different breeds and ages

R. Peeters [1], N. Buys [1], I. Pauwels [1], E.R. Kühn [1], E. Decuypere [2], O. Siau [2] and J. Van Isterdael [2]

[1] *Laboratory of Comparative Endocrinology, Zoological Institute, Naamsestraat 61, B-3000 Leuven, K.U.L., and*
[2] *Department of Animal Husbandery, Kardinaal Mercierlaan 92, B-3030 Heverlee, K.U.L., Belgium*

(received 31 August 1988, accepted 15 February 1989)

Summary — In order to define the patterns of TSH, T_4, T_3, rT_3, GH and cortisol during the estrous cycle of sheep, pluriparous and primiparous ewes were synchronized with progestagen-impregnated pessaries (Veramix) at the start of the normal breeding season. After the pessaries were removed (day 0), daily blood sampling was carried out in cannulated ewes during the ovulatory cycle. Hormonal analyses of TSH, T_4, T_3, rT_3, GH, cortisol, LH and progesterone (P) were performed by RIA.

P and LH levels during the cycle were conform to the literature and were not different between the primiparous and pluriparous ewes of different breeds used in this study. Neither age nor breed influenced the hormone patterns. A significant negative correlation was found between TSH and P during the cycle, although the correlation between P and T_4 was not significant; during the estrous period, low P levels were paralleled by high T4 levels, whereas the reverse was observed during the luteal phase. Higher T_3 levels and T_3/T_4 ratios were observed during the luteal phase. No obvious pattern of rT_3 and cortisol during the cycle was found. The GH concentration increased during the 17 days of the cycle. A positive correlation with P was calculated.

During the estrous cycle obvious changes in thyroid hormones, GH and TSH occurred. However, this study shows no causal relationship between the thyroid and the gonadal axes.

ewe — estrus — progesterone — thyroxine — thyrotropin — breed — age

Résumé — Relation entre l'axe thyroïdien et l'axe gonadique pendant le cycle œstral de la brebis. *Dans cette étude, les modifications des hormones thyréotrope (TSH), thyroxine (T_4), triiodothyronine (T_3), reverse triiodothyronine (rT_3), hormone de croissance (GH) et cortisol ont été étudiées pendant le cycle œstral des brebis, ainsi que les corrélations entre les hormones thyroïdiennes et gonadiques.*

Après la synchronisation du cycle (éponges vaginales imprégnées d'un progestagène) et la cannulation de la veine jugulaire des brebis, une prise de sang est réalisée toutes les 2 h pendant les 3 premiers jours puis à 17 h jusqu'au 17e jour après le retrait des éponges vaginales. Les dosages des hormones TSH, T_4, T_3, rT_3, GH, cortisol, de l'hormone lutéinisante (LH) et de la progestérone (P) sont effectués par des procédés de RIA.

Les concentrations de P et LH ne sont pas différentes de celles données dans la littérature. Une corrélation négative et significative est observée entre TSH et P. Pendant la période d'œstrus, les valeurs basses de P correspondent aux valeurs hautes de T_4. Pendant la phase lutéale, une image inverse est trouvée entre ces deux hormones. Les concentrations de T_3 et le rapport T_3/T_4 sont élevés en début de phase lutéale. Aucun changement évident de concentration en rT_3 et cortisol n'est observé pendant le cycle œstrien. Les concentrations de GH augmentent pendant les 17 jours du cycle. Une corrélation positive entre GH et P est retrouvée.

Ainsi, une évolution opposée est constatée entre TSH, T_4 d'une part, et P d'autre part, pendant le cycle œstral de la brebis. Une relation de cause à effet entre l'axe thyroïdien et l'axe gonadique ne peut toutefois pas être déduite de cette étude.

brebis — œstrus — progestérone — thyroxine — thyrotrophine — race — âge

Introduction

Although thyroid hormone concentration in sheep have been extensively studied in relation to growth, temperature, metabolism and perinatal period, little information is available about the changes of these hormones during the estrous cycle of the ewe. Nevertheless, it is clearly demonstrated in different species that there is an interaction between the thyroidal and the gonadal hormones (Fisher and D'Angelo, 1971; Galton, 1971; Chen and Walfish, 1978; Sahwney *et al.*, 1978a,b). Taking into account the change in gonadal hormone concentrations during the estrous cycle of the ewe (Pant *et al.*, 1977) and the above-mentioned gonadal—thyroidal interaction, it was expected that the thyroid hormones would also vary during the cycle. Therefore, in the present study the patterns of circulating thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3) and reverse triiodothyronine (rT_3) were studied. Also growth hormone (GH) and cortisol patterns have been characterized during the 17 day estrus, since these hormones are known to interact with thyroid secretion or thyroidal peripheral metabolism in sheep (Wu *et al.*, 1978; Kühn *et al.*, 1986).

Breed and age are known to influence fertility (Lahlou-Kassi *et al.*, 1984) and ovulation rate (Hanrahan and Quirke, 1975; Driancourt *et al.*, 1985). In the following study, hormones of the thyroidal axis were assayed during the estrous cycle of ewes of different breeds and ages, together with GH and cortisol, and compared with luteinizing hormone (LH) and progesterone (P).

Materials and Methods

Animals

Twelve ewes obtained from the Zootechnical Center, K.U. Leuven, were divided into 3 experimental groups of 4 animals each, according to breed and age : pluriparous, 3 year old MS ewes and 1 year old primiparous MS ewes and primiparous F_2 ewes. These crossbreeds were selected from the following scheme :

Suffolk x Milksheep ram ↓ ewe — SM ram

Milkscheep x Suffolk ram ↓ ewe — MS ewe

SM ram ↓ MS ewe → F_2 ewe

(Van Isterdael *et al.*, 1987).

Experimental procedure

Estrus was synchronized at the start of the breeding season in all the ewes by progestagen pessaries (Veramix, Upjohn) inserted into the vagina of the ewes. The pessaries were withdrawn after 13 days (day 0) and the jugular vein was cannulated. Next day (day 1) and for 17 consecutive days, except on days 9 and 16, blood samples were taken at 5

p.m. In order to localize the preovulatory LH peak, 2 h sampling was also carried out during the first 3 days of the 17 day period.

On the basis of the analysis of LH and P, the different phases of the estrous cycle were determined : days 1—2 : estrus; days 3—14 : luteal phase; days 15—17 : follicular phase.

Hormone analyses

Blood was immediately centrifuged and plasma was frozen until analysis. The hormone concentrations were evaluated by radioimmunoassay (RIA) using commercial kits : T_4 (Abbott Diagnostics Division, Antwerp); T_3 (antisera from Mallinckrodt Diagnostica, Dietzenbach and tracer from Amersham); rT_3 (Mallinckrodt Diagnostica, Dietzenbach); and cortisol (Medica Diagnostics Inc, Cambridge). The GH levels were measured with a heterologous bovine RIA as described by Reynaert and Franchimont (1974). A good parallelism was obtained between the sheep plasma dilution curve and the bovine standard curve. P concentrations were evaluated by RIA following cyclohexane/ethylacetate extraction (v/v) and dextran-coated charcoal (DCC) separation of bound and unbound radioactivity. Liquid scintillation counting was done on the bound radioactivity which did not absorb to DCC (Verheyen *et al.*, 1987).

The immunoreagents of the oTSH and the oLH RIA were provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIADDK, USA). The purity of the antigens and the specificity of the antisera were guaranteed by the Institute.

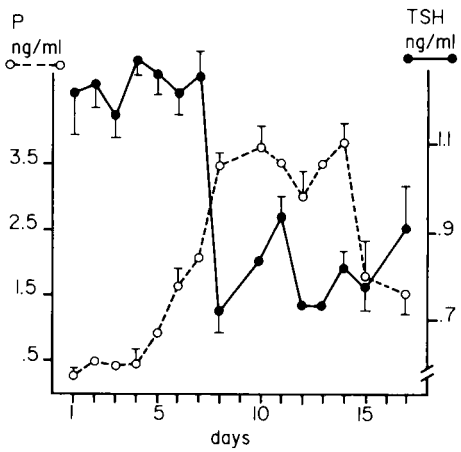

Fig. 1. Plasma concentration of P (---) and TSH (—), (mean ± SEM) during the estrous cycle of the ewe ($n = 12$).

Statistical analysis

The least-squares means were used to analyze the differences in the hormone concentrations during the estrous cycle. Breed and age effects on the hormonal patterns during the 17 days were investigated using the general linear model for repeated measurements. Pearson correlation coefficients between the different hormone concentrations were also calculated.

Results

LH during the estrous cycle

In 11 ewes, the LH peak occurred during the first 2 days of the cycle. Peak levels of > 400 ng/ml were observed, while the basal LH concentrations fluctuated around 10—20 ng/ml.

P during the estrous cycle (Fig. 1)

The well-known pattern of P during the estrous cycle of the ewe was found. High (3—4 ng/ml) levels during the luteal phase were followed by low levels (*ca* 1.5 ng/ml) during the follicular phase (Fig. 2). Age and breed had no influence on mean P concentrations nor on the length of the P plateau (about 6 days).

TSH during the estrous cycle (Fig. 1)

The concentration of TSH remained significantly higher (1.0—1.3 ng/ml) during the first 7 days as compared with the following days of that cycle. The level decreased very sharply on day 8 to

0.6—0.8 ng/ml. The small increase from days 15 to 17 was not statistically significant. A negative correlation (r = –0.532, $P < 0.005$) was found between P and TSH. No influence of age or breed on the hormonal pattern of TSH was observed.

T_4 during the estrous cycle (Fig. 2a)

During estrus (days 1—2) low P levels were paralleled by high T_4 levels, whereas the reverse was observed during diestrus. The maximum values during estrus ranged from 76 to 110 ng/ml, whereas the lowest levels during the luteal phase fluctuated around 60 ng/ml. Age ($P < 0.02$) as well as breed ($P < 0.001$) influenced significantly the hormonal changes of T_4 during the 17 days.

T_3 during the estrous cycle (Fig. 2b)

All the breeds showed a similar pattern ($P < 0.001$) of T_3 variation during the cycle. The T_3 level began to increase on day 3 of the estrous cycle and reached higher values ($P < 0.05$) on day 5 (0.4—0.5 ng/ml). This T_3 increase was observed after the T_4 peak during estrus. After lower values from days 7 to 13, T_3 increased again on days 14 and 17 to 0.7 ng/ml ($P < 0.01$). Simultaneously augmented T_3/T_4 ratios were observed (Table I).

rT_3 during the estrous cycle (Fig. 2c)

During the second day of the estrous cycle, the rT_3 levels decreased markedly in the 3 groups. The following days of the cycle the concentrations decreased slowly (NS). The rT_3 levels on days 3 and 17 were not statistically different. Higher levels of rT_3 were found in the primiparous MS ewes throughout the estrous cycle compared with the other 2 groups ($P < 0.05$); however, the effect of breed on the hormonal pattern of rT_3 was not statistically significant.

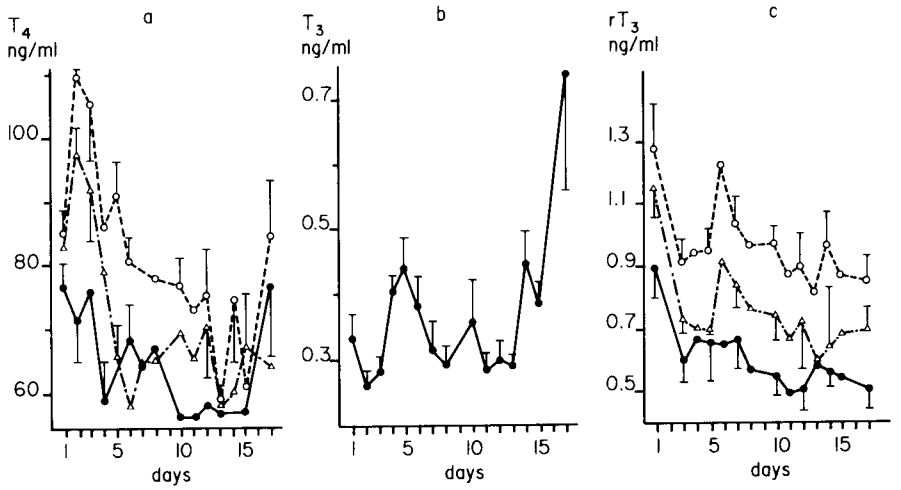

Fig. 2. Plasma concentrations of T_4, T_3 (overall mean) and rT_3 (mean ± SEM) during the estrous cycle of the ewe. 3 year old MS ewes (—), 1 year old MS ewes (- - -), 1 year old F_2 ewes (–•–).

Table I. The procentual T_3/T_4 ratio during the 17 days of the estrous cycle of the ewe.

Day	*1*	*2*	*3*	*4*	*6*	*7*	*8*	*10*	*11*	*12*	*13*	*14*	*15*	*17*
ratio	0.41	1.44	1.51	1.14	1.06	0.78	0.42	0.82	0.46	0.44	0.54	0.74	0.62	1.45
SEM	0.04	0.60	0.57	0.58	0.50	0.33	0.03	0.35	0.04	0.03	0.03	0.08	0.05	0.05

GH during the estrous cycle (Fig. 3b)

During the 17 days of the estrous cycle, GH decreased slowly. Significantly ($P < 0.001$) higher GH levels were observed on day 17 in comparison with day 1. The GH levels were influenced by ewe age : older ewes were characterized by significantly ($P < 0.001$) lower GH levels. Breed did not seem to have any influence on the GH changes.

Cortisol during the estrous cycle (Fig. 3a)

No relationship between the fluctuation of cortisol concentration and the estrous cycle was observed. The comparative study between the 2 breeds and ages did not result in significant differences.

Discussion

Although the basic endocrinology of the estrous cycle of the ewe is well known, to our knowledge no data are available concerning circulating concentrations of TSH, T_4, T_3, rT_3, GH and cortisol during the various phases of the cycle. Our results mainly indicate that an inverse relationship exists between P and TSH or T_4. They underline the importance of

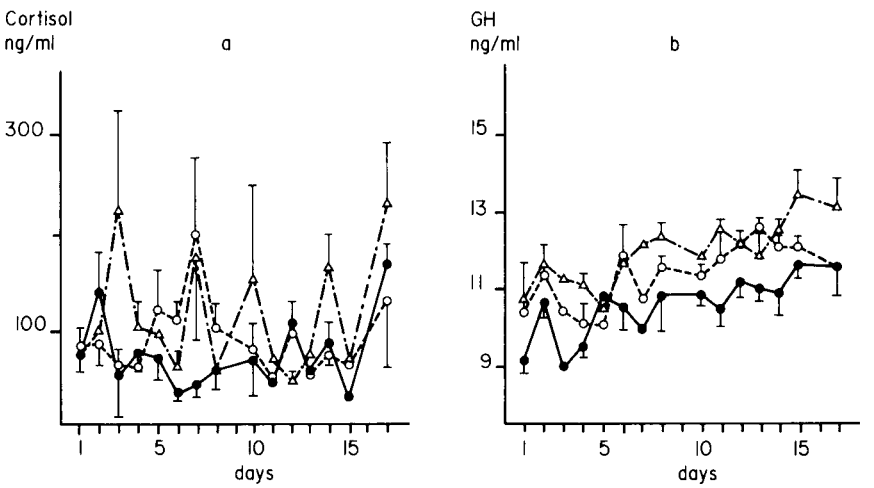

Fig. 3. Plasma concentration of GH and cortisol (mean ± SEM) during the estrous cycle of the ewe. 3 year old MS ewes (—), 1 year old MS ewes (- - -), 1 year old F_2 ewes (—•—).

considering the reproductive state of the animal during experimental investigation of the thyroid function.

Plasma concentrations of P and LH during the estrous cycle were conform to the literature (Hauger *et al.*, 1977).

The elevated levels of TSH observed during the beginning of the luteal phase in our sheep are to some extent comparable to the changes in TSH concentrations found during the cycle of the rat (Castro-Vazquez *et al.*, 1981; Miller *et al.*, 1977). Those authors explained the elevated TSH concentrations during proestrus and estrus in the rat by a long-lasting action of estradiol on the pituitary. The same mechanism may be involved in the elevated TSH levels found in ewes. In that case, however, we cannot explain the sharp drop of TSH on day 8.

On the other hand, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may be involved in the release of TSH and P. Indeed, $PGF_{2\alpha}$ is secreted with a complex series of peaks between days 13 and 17 of the estrous cycle (Nett *et al.*, 1976) and their frequency is increased as estrus approaches (the moment that TSH starts to augment). This increase of plasma $PGF_{2\alpha}$ is associated with a drop in P concentration. The results of Kasting and Martin (1984) show that administration of prostaglandin synthesis-inhibiting drugs decreases plasma levels and, hence, the secretion of GH and TSH. They suggest that prostaglandins play a role in the stimulation of TSH, probably by a postsynaptical interaction at the adrenergic synapse on TRH neurons.

The significant negative correlation between P and TSH may therefore be a secondary effect linked to the cyclic variation of both hormones without any direct causal relation, although that is not *a priori* totally excluded.

The T_4 decrease could be the result of ovulation itself or the formation of the corpus luteum. A first hypothesis has already been formulated in reptiles by Nilson (1982). In the adder, elevated thyroid gland activity concords with ovulation.

An increased turnover of T_4, resulting in the decrease of T_4 and the increase of T_3 levels and T_3/T_4 ratios, without altering the TSH concentrations, could, however, explain the observed hormonal changes. Indeed, the T_3 pattern during the estrous cycle shows an obvious increase of T_3 around day 4, clearly after the T_4 peak. Around this moment, elevated T_3/T_4 ratios are also observed, indicating that there could be a stimulation of T_4 T_3 conversion. This may also be the case during proestrus when elevated T_3/T_4 ratios were found. A stimulation of T_4 T_3 conversion, caused by the simultaneously augmented levels of estradiol during proestrus and metestrus of the estrous cycle might be responsible. An activation of the 5'-D after estradiol administration was reported in previous studies. Chen and Walfish (1978) demonstrated an increased T_4 T_3 conversion in ovariectomized rats treated with estradiol benzoate. Harris *et al.* (1979) obtained a similar result. Reimers *et al.* (1984), however, found elevated T_3 levels throughout diestrus. Monty *et al.* (1979) concluded that there are an increased thyroid gland synthetic and secretory activities during this reproductive state. On the other hand, Johnson (1986) found no fluctuations in T_4 and T_3 at any time of the reproductive cycle in the mare. These differences in results could be due to species differences.

The significantly elevated GH levels during proestrus could be related to the high estrogen secretion during that phase of the estrous cycle in ewes. Indeed, Davis and Borger (1974) demonstrated an enhancement of the GH secretory activity

after injection of estradiol benzoate in ovariectomized ewes. In support of this result, other authors observed increased plasma levels of GH after estradiol administration (Llyod *et al.*, 1971; Trenkle, 1970). The older ewes are characterized by significantly lower GH levels. A previous study by Frenkel *et al.* (1972) reported a similar result : a decrease in GH secretion concording with aging in humans. An endocrine failure with advancing age as a result of a reduction in hormone secretion or a reduction in the response of target cells to hormonal stimulation could be the cause. Morrison *et al.* (1981), however, found a completely inverse result. Aging was associated with an increase in baseline GH concentrations. The reason for the discrepancy could be related to species and/or sex differences.

The result of this study concerning cortisol concentrations during the cycle are confirmed by Reimers *et al.* (1984) in the dog. They found that the basal concentration of cortisol did not differ in the various reproductive states. In rats however, Buckingham *et al.* (1978) observed elevated levels of corticosterone and adrenocorticotrophin hormone (ACTH) during proestrus. This might be due to the stimulated hypophyseal synthesis of ACTH and the sensitization of the pituitary gland to the hypothalamic ACTH-releasing factor by increased estrogen levels (Naftolin *et al.*, 1972; Coyne and Kitay, 1969; Kitay, 1963).

In summary, these results yield new data about the changes of hormones other than the reproductive hormones during the estrous cycle of the ewe. The circulating levels of TSH, T_4, T_3 and GH seem to vary according to the stage of the estrous cycle, while no obvious changes in rT_3 and cortisol were observed during that cycle. There is an inverse relationship between TSH, T_4 and P. However, further study is needed to clarify a causal relationship between the gonadal and thyroidal axes during the reproductive cycle.

Acknowledgments

The authors acknowledge the technical assistance of L. Noterdaeme, F. Voets, W. Van Ham and G. Nackaerts. R. Peeters was supported by I.W.O.N.L. (Instituut tot aanmoediging van het Wetenschappelijk Onderzoek van Nijverheid en Lanbouw). E. Decuypere was supported by the National Foundation for Scientific Research (N.F.W.O.). The antisera for P were kindly provided by Professor G. Verhoeven (Faculty of Medicine, K.U. Leuven).

References

Buckingham J.C., Dohler K.D. & Wilson C.A. (1978) Activity of the pituitary-adrenocortical system and thyroid gland during estrous cycle of the rat. *J. Endocrinol.* 78, 359-366

Castro-Vazquez A., Ojeda S.R., Krulich L. & McCann S.M. (1981) TSH release during the estrous cycle of the rat : variations in responsiveness to cervicovaginal stimulation. *Neuroendocrinology* 33, 193-198

Chen H.J. & Walfish P.G. (1978) Effects of estradiol benzoate on thyroid—pituitary function in female rats. *Endocrinology* 103, 1023-1030

Coyne M.D. & Kitay J.I. (1969) Effect of ovariectomy on pituitary secretion of ACTH. *Endocrinology* 85, 1097-1102

Davis S.L. & Borger M.L. (1974) Dynamic changes in plasma prolactin, luteinizing hormone and growth hormone in ovariectomized ewes. *J. Anim. Sci.* 38, 795-802

Driancourt M.A., Cahill L.P. & Bindon B.M. (1985) Ovarian follicular populations and preovulatory enlargement in Booroola and control Merino ewes. *J. Reprod. Fert.* 73, 93-107

Finkelstein J.W., Rofflang H.P., Bayar R.M., Kream J. & Hellman L. (1972) Age-related

change in twenty-four hour spontaneous secretion of growth hormone. *J. Clin. Endocrol. Metab.* 35, 665-671

Fisher J.S. & D'Angelo S.A. (1971) Stimulatory and inhibitory action of estradiol on TSH secretion. *Endocrinology* 88, 687-691

Galton V.A. (1971) Thyroxine metabolism and thyroid function in rats following administration of estrogen. *Endocrinology* 88, 976-982

Hanrahan J.P. & Quirke J.F. (1975) Repeatability of the duration of estrus and breed differences in the relationship between duration of estrus and ovulation rate of sheep. *J. Reprod. Fert.* 45, 29-36

Harris A.R.C., Vagenakis A.G. & Braverman L.E. (1979) Sex-related differences in outer ring monodeiodination of thyroxine and 3,3', 5'-triiodothyronine by rat liver homogenate. *Endocrinology* 104, 645-652

Hauger R.L., Karsch F.J. & Foster D.L. (1977) A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. *Endocrinology* 101, 807-817

Johnson A.L. (1986) Serum concentrations of prolactin, thyroxine and triiodothyronine relative to season and the estrous cycle in the mare. *J. Anim. Sci.* 62, 1012-1020

Kasting N.W. & Martin J.B. (1984) Endogenous prostaglandins affect growth hormone and thyrotropin release at a hypothalamic, not a pituitary level. *Neuroendocrinology* 39, 201-205

Kitay J.I. (1963) Pituitary—adrenal function in the rat after gonadectomy and gonadal hormone replacement. *Endocrinology* 73, 253-260

Kühn E.R., Van Osselaer P., Siau O., Decuypere E. & Moreels A. (1986) Thyroid function in newborn lambs : influence of prolactin and growth hormone. *J. Endocrinol.* 109, 215-219

Lahlou-Kassi A., Schams D. & Glatzel P. (1984) Plasma gonadotrophin concentrations during the estrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. *J. Reprod. Fert.* 70, 165-173

Lloyd H.M., Meares J.D., Jacobi J. & Thomas F.J. (1971) Effects of stilbestrol on growth hormone secretion and pituitary cell proliferation in the male rat. *J. Endocrinol.* 51, 473-481

Miller W.L., Knight M.M. & Gorski J. (1977) Estrogen action *in vitro* : regulation of thyroid stimulating and other pituitary hormones in cell cultures. *Endocrinology* 101, 1455-1460

Monty D.E. Jr., Wilson O. & Stone J.M. (1979) Thyroid studies in pregnant and newborn beagles using ^{125}I. *Am. J. Vet. Res.* 40, 1249-1256

Morrisom M.W., Davis S.L. & Spicer L.J. (1981) Age-associated changes in secretory patterns of growth hormone, prolactin and thyrotropin and the hormonal responses to thyrotropin-releasing hormone in rams. *J. Anim. Sci.* 53, 160-170

Naftolin F., Brown-Grant K. & Corker C.S. (1972) Plasma and pituitary luteinizing hormone and peripheral plasma œstradiol concentrations in the normal estrus cycle of the rat and after experimental manipulation of the cycle. *J. Endocrinol.* 53, 17-30

Nett T.M., Staingmiller R.B., Akbar A.D., Diekman M.A., Ellingwood W.E. & Niswender G.D. (1976) Secretion of prostaglandin $F_{2\alpha}$ in cycling and pregnant ewes. *J. Anim. Sci.* 42, 876-880

Nilson G. (1982) Thyroid activity and experimental evidence for its role in reproduction in the adder *Vipera berus*. *Gen. Comp. Endocrinol.* 47, 148-158

Pant H.C., Hopkinson C.R.N. & Fitspatrick R.J. (1977) Concentration of œstradiol, progesterone, luteinizing hormone and follicle-stimulating hormone in the jugular venous plasma of ewes during the estrous cycle. *J. Endocrinol.* 73, 247-255

Reimers T.J., Mummery L.K., McCann J.P., Cowan R.G. & Concannon P.W. (1984) Effects of reproductive state on concentrations of thyroxine, 3,5,3'-triiodothyronine and cortisol in serum of dogs. *Biol. Reprod.* 31, 148-154

Reynaert R. & Franchimont P. (1974) Radio-immunoassay of bovine growth hormone. *Ann. Endocrinol.* 35, 139-148

Sawhney R.C., Rastogi I. & Rastogi G. (1978a) Effect of estrogens on thyroid function. I. Alternations in Rhesus plasma thyrotropin and its kinetics. *Endocrinology* 102, 1310-1316

Sawhney R.C., Rastogi I. & Rastogi G. (1978b) Effect of estrogens on thyroid function. II. Alternations in plasma thyroid hormone levels and their metabolism. *Metabolism* 27, 279-288

Trenkle A. (1970) Plasma levels of growth hormone, insulin and plasma protein-bound

iodine in finishing cattle. *J. Anim. Sci.* 31, 389-391

Van Isterdael J., Moreels A. & Michels H. (1987) 3. Schapenhouderij. 3.1. Voortplanting, groei en karkaskwaliteit, 3.2. Bijdrage van kruisingen tot de optimalisatie van de schapenhouderij. *Landbouwtijdschrift* 3, 569-614

Verheyen G., Decuypere E., Chiasson R.B., Vervloesem J., Kühn E.R. & Michels H. (1987) Effect of exogenous LH on plasma concentrations of progesterone and œstradiol in relation to the cessation of egg laying induced by different moulting methods. *J. Reprod. Fert.* 81, 13-21

Wu S., Klein A.H., Chopra I.J. & Fisher D.A. (1978) Alternations in tissue thyroxine-5'-monodeiodinating activity in perinatal period. *Endocrinology* 103, 235-239