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VARIATIONS IN PITUITARY AND BLOOD LH DURING PUBERTY IN THE MALE LAMB. RELATION TO TIME OF BIRTH

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SUMMARY

Testicular weight, pituitary LH concentration, blood plasma LH were measured in *Ile-de-France* lambs born in the sexual season (September) or out of season (February) to see how these characters are modified at puberty.

During the period of low testicular growth, the impuberal period, there was a large increase in pituitary LH concentration and a significant linear increase in blood plasma LH ; the half-life of the latter is the same as in the adult. In the period of rapid testicular growth, the prepubertal and pubertal period, pituitary LH stabilised or decreased according to the season, and blood LH remained at a level lower than that of the peak reached at the end of the preceeding period. Thus spermatogenesis commenced after blood LH reached peak values.

In the lamb, a species in which spermatogenesis starts two to three months after birth (WATSON, SAPSFORD and MCCANCE, 1956 ; COUROT, 1962 ; SKINNER *et al.*, 1968) it has been demonstrated that the impuberal testis is dependent upon pituitary hormones and especially upon LH since hypophysectomy induces testicular weight regression while LH reverses the effects of this operation (COUROT, 1970). Thus, it is of interest to know the evolution of the pituitary content and that of the blood plasma levels of this hormone in the lamb during a period including the establishment of spermatogenesis. Such results are reported in the present paper. As the sheep has a well defined sexual season, the work dealt with lambs born either at the end of February or September.

MATERIAL AND METHODS

A. — *Animals*

Ile-de-France lambs were raised indoors with their mother under natural daylight duration and progressively weaned at hundred days of age. There were two groups, those born in September, those born in February.

B. — *Sampling*

— *Pituitary glands* were collected at autopsy in the morning from lambs selected according to their body weight as representative of the mean value of the flock. Animals were slaughtered at the ages shown in figure 2. The glands were immediately dissected, weighed and placed in cold grade A acetone (— 15°C). After dehydratation the anterior lobes were dissected free and pooled according to age of animals and season (10 to 20 glands per group), powdered and stored at — 15°C until assay.

— *Testes* were weighed at autopsy.

— *Blood samples* were collected once a week from birth onwards in two groups of lambs, 10 born at the end of February and 9 born at the end of September. 5 ml of blood were collected into heparinised syringes by acute jugular venepuncture at 9 a.m. ; blood plasma was immediately separated by centrifugation at 2 500 g at 4°C and stored in the frozen state until assay.

C. — *Assay*

The concentration of LH in the anterior pituitary was measured by the OAAD test as modified by PELLETIER (1963). The sensitivity has been established as 0.2 µg LH/mg. Results are given in µg NIH-LH-S 11 (0.81 NIH-LH-S 1) per mg of anterior pituitary preparation for each group of lambs.

The concentration of blood LH was determined on 3 × 50 µl of plasma by RIA using the double antibody technique (PELLETIER *et al.*, 1968). The sensitivity has been established as 0.5 ng LH/ml. Results are given in ng CNRS-LH-M3 (1.8 NIH-LH-S1) per ml of blood plasma. The mean value of the LH concentration in the blood plasma ($m \pm s_e$) was calculated for each week of age in both groups of lambs ; correlation and regression between these parameters were established.

D. — *Half-life of exogenous LH*

This was calculated in three 50 day old lambs after an acute injection into the jugular vein of purified ovine 131 I-labelled LH (1.5 µg CNRS-LH-M3, 15 µCi/animal). Blood samples were taken from the contralateral jugular vein every 5 minutes during the first 30 minutes following injection, and then every 15 minutes during the next 2.30 hours. The radioactivity of the blood plasma, and that of the immunoprecipitable fraction of the latter by anti-ovine LH antibody in excess was measured. Half-life was estimated in the period of rapid decrease of radioactivity as the time taken for the latter to decrease by half.

RESULTS

A. — *Testicular weight*

Testicular weights are given in text figure 1. Growth was low in both groups during the first 80-100 days. It then increased noticeably. Differences between groups of lambs appeared later ; the intensive growth of the testis occurred a little earlier in September than in February born animals. After 150 days of age, the

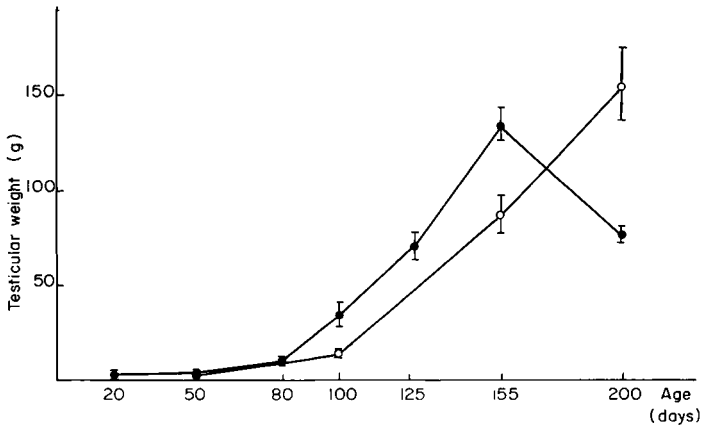

FIG. 1. — *Increase in testicular weight* ($m \pm s_e$) *in lambs born in September* (●———●) *or February* (○———○)

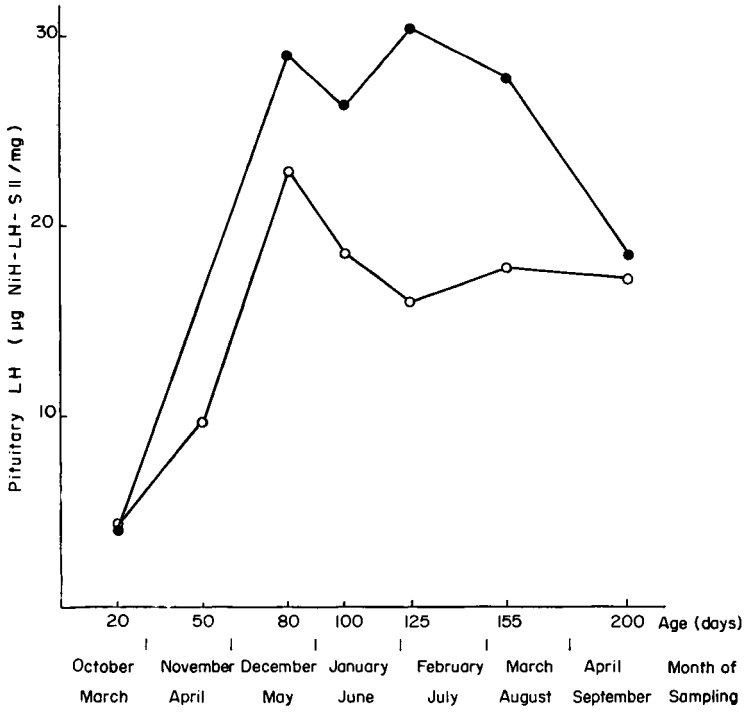

FIG. 2. — *Pituitary LH in lambs at different ages*
(●———● born in September; ○———○ born in February)

discrepancy in testicular weight in both groups was more evident. By 200 days, the September born lambs showed a statistically significant decrease in testicular weight while the gonads of lambs born in February continued to increase.

B. — *Pituitary LH content*

As shown in fig. 2, the LH concentration of the anterior pituitary tissue increased steeply, 6 to 7 times, from 20 to 80 days of age. After 80 days, the LH concentration in the pituitary was higher in the lambs born in September than in those born in February. It remained consistently higher than that of the 20-50 day old lambs. According to the weight increase of the pituitary from birth onwards (fig. 3), the total hypophyseal LH content varied more than the concentration (μg/mg) of this hormone in both groups of animals.

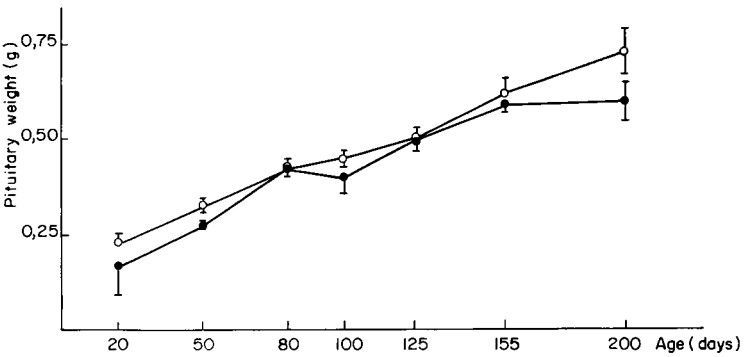

FIG. 3. — *Increase in pituitary weight* ($m \pm s_e$) *in lambs born in September* (●———●) *or February* (○———○)

C. — *LH in blood plasma*

LH was detectable soon after birth in the peripheral blood of the lamb (fig. 4). Thereafter, the mean level of LH increased linearly 3 to 4 times during the first two months after birth in both groups of lambs, independent of the season of birth, with a correlation coefficient of 0.92 and 0.95 between the weekly mean level of plasma LH and the age up to 63 and 70 days for lambs born in September and February respectively. At about 70-80 days of age, plasma LH tended to decrease, but the ratio of the mean LH at 125-165 days to the maximum LH at 63 or 70 days was higher in the February born lambs, 65 p. 100, than in those born in September, 33 p. 100.

D. — *Half-life of exogenous LH*

The mean halving time of radioactivity attributible to the immunoprecipitable LH was 24 mn. The same measurement done on the crude blood plasma gave a slightly higher value of 29 mn, the three lambs reacting in the same way.

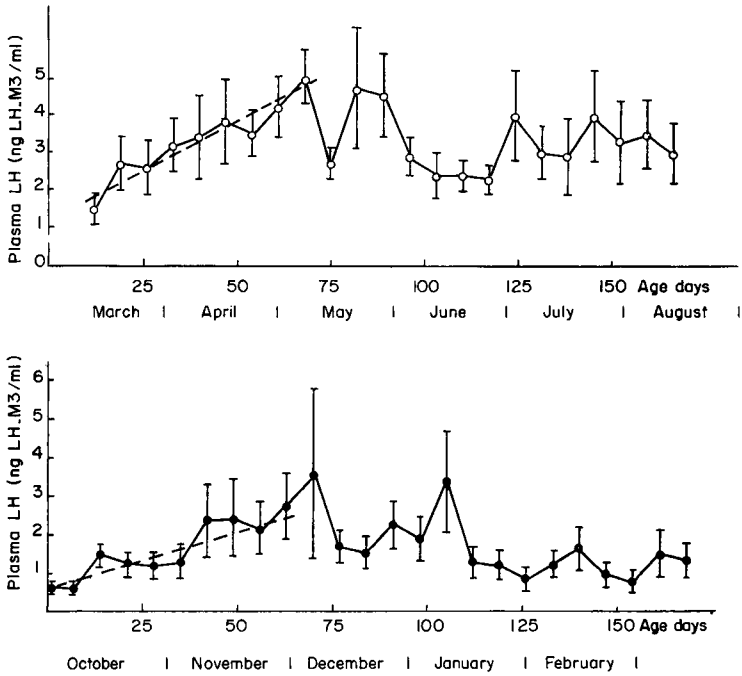

FIG. 4. — *Plasma LH concentration ($m \pm s_e$) in lambs at different ages*
Age and month of sampling are given on the abcissa.
Lambs born in September (●———●) or February (○———○)

DISCUSSION

The LH concentration in the lamb pituitary shows a large increase between birth and 80 days of age. This confirms the observations of SKINNER *et al.* (1968). This phenomenon is seasonally independent and the concentration at 80 days is significantly higher than that of the adult (PELLETIER, 1971). After 80 days, the pituitary content seemed to be related to the season of sampling, thus being secondarily dependent upon the birth season. The level of LH decreases rapidly in the February born lambs. For those born in Autumn, the decrease in LH commences at 155 days. Thus, the seasonal variation observed in the adult (PELLETIER and ORTAVANT, 1967) occurs only after the pituitary content of LH reached the peak value observed at 3 months.

Contrary to what has been described in the human (see review by FAIMAN and WINTER, 1974), in the chimpanzee (FAIMAN, REYES and WINTER, 1974) and in the bull (SCHAMS, BUTZ and KARG, 1972 ; GIMENEZ *et al.*, 1974), where blood LH remains at a constant and low level throughout the impuberal period, but in agreement with observations made in the guinea pig (DONOVAN, TER HAAR and PEDDIE, 1974), we found in the lamb that the blood LH increases quite regularly from birth onwards to the 9-10th week of age, where it reaches a maximum regardless of the season.

Then it decreases and stabilises at a relatively constant level between 125 and 200 days, being regulated by other factors, possibly seasonal ones, as shown by the ratio of the mean LH at 125-165 days to the maximum LH at 63 or 70 days. The initial increase in the blood plasma LH after birth has already been observed in the *Prealpes* and *Romanov* × *Prealpes* lambs (THIMONIER, PELLETIER and LAND, 1972) and in *Merino* × *Corriedale* lambs, but for a shorter period (LEE *et al.*, 1974) ; however, it was not noticed in preliminary results on *Ile-de-France* lambs born in Autumn where plasma LH had been found relatively constant (COUROT, de REVIERS and PELLETIER, 1972). On the other hand, the frequency of blood sampling — once a week — in the present work did not allow the study of the daily or hourly variations in blood LH as recently demonstrated in the lamb by FOSTER (1974). Such variations would probably explain the magnitude of the standard error and an exceptionnally elevated value (23 ng/ml) encountered in one sample of a 70 days old lamb. One may also point out that the mean levels noticed in the lamb are higher than those normally observed in the adult, whereas the half-life of the hormone, estimated by the disappearance from the blood of labelled LH, is very close in the lamb (see the present results), and in the adult (GESCHWIND and DEWEY, 1968 ; de KRETSER, ATKINS and PAULSEN, 1973). Thus it appears that during the first two to three months after birth there is a simultaneous increase of LH in the pituitary and blood plasma. This is indicative of a high synthetic and release activity by the pituitary gland.

At the same time, testicular growth is very low (COUROT, 1962), and it accelerates only after the pituitary concentration in LH reached its maximum and the blood plasma level has already decreased. The testicular development would thus appear to result from a previous hormonal stimulation. The more precocious testicular growth in Autumn born animals (fig. 1) could be explained by a larger hormonal impulse in these lambs born in the sexual season where photoperiodism influences the neuroendocrine system more favorably (PELLETIER, 1971) than it does in lambs born in February. This is quite clear with the pituitary content in LH (fig. 2) even if it has not been demonstrated in the plasma. Technical difficulties may have contributed to this ; because of the large number of blood samples it had not been possible to carry out all the assays simultaneously. The seasonally modulated hormonal impulse would also explain the difference in testicular weight at 200 days of age : this is in the sexual season (September) for the February born animals and in the non sexual season (April) for those born in September. This is supported by the pituitary LH for the latter (fig. 2) and the higher relative blood plasma LH for the former (LH at 150 days related to the peak at 70 days).

Testosterone has been assayed in the blood plasma samples of the September born lambs. It increased linearly during the first three months of life and is highly correlated with blood LH when the latter also increases linearly ($r = + 0.85$, COTTA *et al.*, 1974). Similar studies reported by SANFORD, PALMER and HOWLAND (1974) dealt only with measurements beginning at an older age. On the other hand, hemicastration provided experimental evidence for a negative feed-back in the young lamb (CRIM and GESCHWIND, 1972 ; FOSTER, COOK and NALBANDOV, 1972) and in the calf (ODELL and KIDDY, 1969). However, it has been shown that LH release in response to intravenous LRH injection was of the immature type before 60 days of age and changed to a mature type after 80-100 days of age (GALLOWAY and PELLE-

TIER, 1974). The present results with the decrease in plasma LH in the third month of life, could be related to the maturation of the feed-back mechanism in the lamb which changes to the adult type and becomes sensitive to modulation by photoperiodism.

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RÉSUMÉ

ÉVOLUTION DE LA LH HYPOPHYSAIRE ET SANGUINE PENDANT LA PUBERTÉ CHEZ L'AGNEAU MÂLE. RELATION AVEC LE MOMENT DE LA NAISSANCE

La concentration hypophysaire et la teneur du plasma sanguin périphérique en LH ont été mesurées chez des agneaux *Ile-de-France* nés pendant la saison sexuelle (septembre) ou en contre-saison (février) pour voir si des modifications particulières intervenaient au moment de la puberté. Les résultats ont été reliés à ceux du développement testiculaire.

Au cours de la période de croissance testiculaire lente, période impubère vraie, il y a une forte augmentation de la charge hypophysaire en LH (6 à 7 fois), indépendante de la saison, et une augmentation linéaire significative de la teneur en LH du plasma sanguin ; la demi-vie de LH plasmatique est la même que celle observée chez l'adulte. Dans la période de croissance testiculaire rapide, périodes prépubère et pubère, la concentration hypophysaire en LH est, selon la saison, stabilisée ou même en diminution. LH plasmatique, après le maximum observé à la fin de la période précédente, diminue et fluctue autour d'une valeur moyenne comparable à celle observée chez l'adulte. Ainsi, la spermatogenèse commence après que LH plasmatique ait atteint un maximum.

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