Reprod Nutr Dev (1990) 30, 653-662

Original article

A quantitative histological study of adrenal development during late gestation and the perinatal period in intact and hypophysectomized fetal sheep

A Nicolle, MJ Bosc

INRA, Centre de Tours, Station de Physiologie de la Reproduction, 37380 Monnaie, France

(Received 3 March 1990; accepted 14 August 1990)

Summary – Growth of the sheep adrenal was studied during late gestation and the perinatal period. Adrenals were recovered from 28 fetuses taken at D_{100} (D_0: day of mating), D_{120}, D_{132} and D_{144}, and in 4 newborn animals 3 days after birth (equivalent to D_{151}). Animals were of the Ile de France breed. Cortex (without capsule) and medulla volumes increased respectively by 7.2 and 2.4 between D_{100} and D_{151}. The 2 parts had the same volume between D_{100} and D_{120}; thereafter the cortex became predominant, representing 74% of the whole gland at D_{151}. Hypertrophy and hyperplasia were shown after D_{132} in the cortex (zona fasciculata); they were observed later after D_{144} in the medulla (central zone). Fifteen fetuses hypophysectomized at D_{100} or D_{120} were recovered at D_{120} or D_{144}. The lack of pituitary inhibited cortical growth (zona fasciculata) and suppressed hypertrophy and hyperplasia. The medulla continued to grow after hypophysectomy but to a lesser extent than in controls.

sheep / adrenal / development / fetus / newborn / hypophysectomy

Résumé — Données quantitatives histologiques du développement des surrénales de mouton normal et hyphysectomisé, pendant la fin de la gestation et 3 jours après la mise bas. *La croissance de la surrénale de mouton de race Ile de France a été étudiée entre le 100e j de la gestation (J_{100}, J_0 étant le jour de la saillie), et le 3e jour après la naissance (équivalent à J_{151}). Les surrénales ont été récupérées chez 28 fœtus à J_{100}, J_{120}, J_{132} et J_{144} et chez 4 nouveau-nés à J_{151}. Le volume du cortex (capsule non comprise) et celui de la médulla sont respectivement multipliés par 7,2 et 2,4 entre J_{100} et J_{151}. Les 2 parties ont le même volume entre J_{100} et J_{120}, puis le cortex devient prédominant, il représente 74% de la glande totale à J_{151}. Hypertrophie et hyperplasie sont observées après J_{132} dans le cortex (zone fasciculée), après J_{144} dans la médulla (zone centrale). Quinze fœtus hypophysectomisés à J_{100} ou à J_{120} ont été récupérés à J_{120} ou à J_{144}. L'absence d'hypophyse inhibe l'augmentation du volume cortical et supprime l'hypertrophie et l'hyperplasie (zone fasciculée) constatées chez les témoins. La médulla continue à croître après hypophysectomie mais de façon moins importante que chez les témoins.*

mouton / surrénale / développement / fœtus / nouveau-né / hypophysectomie

INTRODUCTION

In domestic sheep, the exponential weight increase of the adrenals before birth (Comline and Silver, 1961; Liggins, 1969; Wrutniak *et al,* 1985) accounts for the dramatic changes of plasma corticosteroid levels observed at that time (Bassett and Thorburn, 1969; Nathanielsz *et al,* 1972; Magyar *et al,* 1981). This phase of adrenal growth mainly implies a thickness augmentation of the zona fasciculata of the cortex and changes in the morphology of the inner cortical cells (Boshier *et al,* 1980; Boshier and Holloway, 1989). Most of these changes can be induced by ACTH infusion, and inversely can be arrested by hypophysectomy (Liggins *et al,* 1973; Durand *et al,* 1980; Boshier *et al,* 1981; Robinson *et al,* 1983). During the same period, the medulla grows (Boshier *et al,* 1989; Coulter *et al,* 1989). Hypertrophy and hyperplasia have been observed in juxtacortical and central medullary cells (Boshier *et al,* 1989) and no change has been noted in the ratio of the areas of the adrenal occupied by adrenaline – and noradrenaline-containing cells (Coulter *et al,* 1989). Medullary growth does not depend on control by the fetal pituitary (Coulter *et al,* 1989). These estimations of adrenal growth during late gestation and the perinatal period have often been restricted to one region of the gland, and have been based upon measurements of mid-glandular sections sometimes with a rough estimation of the length of the gland. The aim of this study was to provide precise data on the growth of both regions and to observe the effects of hypophysectomy on their development. For this study, length, area, volumes of the cortex and medulla, number, size of cells and nucleocytoplasmic ratio were measured in adrenal glands taken at the end of gestation or just after birth.

MATERIALS AND METHODS

The animals were drawn from single or twin litters of Ile de France ewes (length of gestation, mean ± sd, 148 ± 2 d). Twenty-eight control fetuses were obtained at 4 stages of gestation by caesarian section or after slaughter of the ewes. Seven fetuses were taken at D_{100} (99-100-101), (D_0 being the day of mating or artificial insemination), 6 at D_{120} (119-120), 8 at D_{132} (130-132) and 7 at D_{144} (140-142-144). Four lambs were also obtained the third day after birth, expressed as D_{151} for convenience. Hypophysectomy was performed (Bosc *et al,* 1974) on 10 fetuses at D_{100} which were recovered at D_{120} (4) or at D_{144} (6) and on 5 fetuses at D_{120} recovered at D_{144}. Absence of pituitary was confirmed at autopsy and by the lack of prolactin in the plasma (Kann, 1971). Following dissection, adrenals were weighed, fixed in acetic Bouin Hollande solution and embedded in paraffin. They were cut perpendicularly to the longitudinal axis in serial sections (5 or 10 μm) and then stained by trichrome (Gabe, 1968; Nicolle and Bosc, 1989).

The total volume (without the capsule) of the gland *(Vt)* and volumes of the medulla *(Vm)* and cortex (*Vc* = *Vt-Vm*) were estimated, as previously described (Nicolle and Bosc, 1989), from elementary volumes delimited between 2 sections. Each elementary volume was calculated according to the formula: $V(mm^3) = 1/3\ L(S + S' + \sqrt{SS'})$, where S and S' (mm^2) were the respective areas of the 2 sections delimiting it, and L (mm) was the length between them. The areas were measured with a planimeter (Leitz microscope coupled with an integration table). The areas occupied by the central vein and the main blood vessels were included. Volumes were also estimated with the mean and the mid-glandular areas of the gland. The mean area represented the average of areas used for calculations of elementary volumes; the mid-glandular area represented a surface area taken from the central section measured in each gland. The lengths of the whole gland and medulla were estimated from the respective numbers of sections multiplied by the section thickness (10 μm).

The minimum number of elementary volumes necessary to obtain the 3 volumes *(Vt, Vm, Vc)* with precision was established on 3 pairs of adrenals taken at D_{120} (1 control and 1 hypophysectomized) and at D_{144} (1 control). One section out of 30 was sufficient to limit the variation of

the volumes to less than 5%, as in pigs (Nicolle and Bosc, 1989).

Data were used without taking into account shrinkage due to fixation. Shrinkage averaged 0.42 (± 0.07) when it was estimated by the total volume *(Vt)* divided by the weight; these 2 parameters were highly correlated ($r = 0.96$).

The number of cells per mm^3, the area of their nuclei, the nucleocytoplasmic ratio *(NCR)*, and the quantity of connective tissue and vessels were measured on sections cut at 5 μm within each third of the gland, in the zona fasciculata and in the central zone of the medulla. The total cell number was calculated according to Solari (1977). The nuclear areas were measured on 50 nuclei in cortex and medulla of 6 adrenals by stage. The nuclear and cellular volumes were calculated as previously described (Nicolle and Bosc, 1989).

The correlations and regression line equations were calculated after Pearson (Vessereau, 1960). One-way analysis of variance was applied to most parameters to compare normal and hypophysectomized fetuses of the same age and to compare different ages in the same type of animal. A two-way analysis of variance was used for nuclear areas according to age and to individual (Vessereau, 1960). All calculations were made without consideration of the sex of the animals ($P > 0.05$) or whether the left or right adrenal was used ($r = 0.94$ for the weight).

RESULTS

Control animals

Adrenal weight and total volume increased exponentially during the periods studied (table I). Adrenal weight increased significantly between the different ages ($P < 0.01$), except between D_{120} and D_{132} ($P > 0.05$). The increase in cortical volume was significant between the different ages ($P < 0.01$). For the medulla, the increase of the volume was significant between D_{100}-D_{120} and D_{132}-D_{144} ($P < 0.01$). Between D_{100} and D_{151} (3 d after birth), the total, cortical and medullary volumes were in-

Table I. Sheep adrenal of control and hypophysectomized fetuses during late gestation and the perinatal period: weight, volumes and number of cells in the cortex and medulla.

Animals Type	Age (d)	*Adrenal weight* (mg)	*Cortical volume* (mm^3)	*Medulla volume* (mm^3)	*Cell number x 103/mm^3* Cortex	Medulla
Control	100	(14) 82 ± 13	15 ± 3 (12)	16 ± 4	1188 ± 135 (12)	884 ± 53
Control	120	(12) 125 ± 15	24 ± 4 (10)	26 ± 3	1081 ± 99 (9)	797 ± 40
Hx*		(7) 106 ± 11	22 ± 4 (6)	18 ± 5	1150 ± 187 (5)	787 ± 220
Control	132	(14) 141 ± 27	31 ± 5 (12)	27 ± 8	967 ± 103 (12)	785 ± 80
Control		(12) 250 ± 31	71 ± 8 (12)	42 ± 5	749 ± 116 (10)	681 ± 102
Hx*	144	(12) 124 ± 25	21 ± 3 (12)	32 ± 11	1075 ± 121 (12)	803 ± 86
HX**		(10) 142 ± 17	27 ± 2 (10)	35 ± 6	1202 ± 119 (10)	723 ± 101
Control	151	(8) 305 ± 25	107 ± 14 (7)	38 ± 7	740 ± 101 (6)	788 ± 55

D_0 = day of mating; D_{151} = equivalent to 3 days after birth. Hx : hypophysectominzed at D_{100}* and D_{120}**. Values are mean ± *sd* (number).

creased respectively by 4.7, 7.2 and 2.4 (table I). The adrenal growth was therefore mainly due to the cortex. Two periods of growth were shown by the ratios *Vc/Vt* and *Vm/Vt* (fig 1). The first occurred between D_{100} and D_{120}, when cortex and medulla increased at the same rate, and represented respectively 48 and 52% of the total volume. During the second, the cortex grew more rapidly than the medulla and its relative volume reached 63 and 74% respectively at D_{144} and D_{151}.

The mean areas of medulla and of the whole gland increased respectively by 1.3 and 1.5 between D_{100}-D_{132}, by 1.2 and 2.2 between D_{132}-D_{151}. The length of medulla represented 88% (*sd* = 6%) of the length of the whole gland. These lengths increased at about the same rate and increased by 1.6 from D_{100} to D_{151}. The ratio $L/(\sqrt{S/\pi})$, (equivalent to the ratio of length per thickness), fluctuated around a mean value of 6.4 for the whole gland; it was highest at D_{132} (7.1) and lowest at D_{120} (6.1) and D_{151} (5.9), ($P > 0.05$). The same ratio increased from 7.4 at D_{100} to 8.6 at D_{151} for

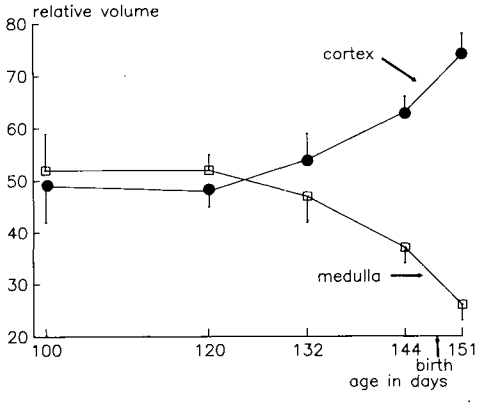

Fig 1. Relative volume of the cortex (*Vc/Vt* : ●), and medulla (*Vm/Vt* : ❑), according to the gestational age in control adrenal sheep. Vc, Vm and Vt are respectively the volumes of the cortex, medulla and whole gland. D_0 = day of mating; D_{151} is equivalent to 3 d after birth (m ± sd).

the medulla, particularly between D_{120} and D_{132}.

At the 5 ages studied, the mean areas of the whole gland and medulla were less than their respective cross-sectional areas measured in the middle of the gland (mid-section area). Consequently, the volumes calculated with the mean areas, were less than volumes calculated with mid-glandular areas ($P < 0.05$ at D_{151} and $P < 0.01$ at the other ages). The volumes resulting from the sum of elementary volumes (*Vc* and *Vt*) were generally highly correlated with the mean or mid-section areas or with volumes calculated with these respective areas. The lowest correlation was observed between *Vm* and its mid-section area (table II).

The cell number per mm^3 did not vary in the cortex between D_{100}-D_{120} and D_{120}-D_{132}. It decreased significantly between D_{132}-D_{144} ($P < 0.01$) and remained at the same level between D_{144}-D_{151}. In the medulla, this cell number decreased significantly between D_{100}-D_{120} ($P < 0.01$) and D_{132}-D_{144} ($P < 0.05$), and increased between D_{144}-D_{151} ($P < 0.05$) (table I).

The total number of cells increased between each stage in the cortex and medulla. It increased respectively by 1.7 and 1.6 between D_{100}-D_{132}, and by 2.7 and 1.4 between D_{132}-D_{151} (fig 2). In the zona fasciculata, the daily rate of mitoses estimated from the total number of cells (fig 2) averaged 0.075 between D_{100}-D_{120} and 0.095 between D_{120}-D_{132}, and reached 0.150 between D_{132} and D_{144} and 0.217 thereafter. In the central zone of the medulla, the daily rate of mitoses averaged 0.071 between D_{100}-D_{120}, 0.103 between D_{132}-D_{144}, and 0.163 between D_{144}-D_{151}.

The cortical nucleocytoplasmic ratio (NCR) was higher at earlier ages of gestation than later ($P < 0.05$ or 0.01 according to the age). It fluctuated around an ave-

Table II. Correlation coefficients *(r)* and parameters of the regression line equations *(Y = aX + b)*, where *Y* is a volume of sheep adrenal estimated from a sum of elementary volumes and *X* is a mean or mid-section area, or volumes calculated from these respective areas and lengths.

Y	*X*	*a*	*b*	*r*
Total volume *(Vt)*	Mean area = A_1	12.74	−17.26	0.97
	Mid-section area = A_2	9.73	−21.92	0.93
	Volume = A_1 x *lt*	0.95	0.57	0.99
	Volume = A_2 x *lt*	0.77	−1.10	0.99
Medulla volume *(Vm)*	Mean area = A_3	11.00	−7.49	0.89
	Mid-section area = A_4	6.48	−0.71	0.76
	Volume = A_3 x *lm*	1.03	0.21	0.99
	Volume = A_4 x *lm*	0.75	0.36	0.97

lt: total length; lm: medulla length. Calculations were made on control fetuses, taken at D_{100}, D_{120}, D_{132}, D_{144} and D_{151}.

rage of 0.28 in the medulla ($P > 0.05$). At no age, these was a significant difference between the 2 regions of the gland (table III). In the cortex and medulla, the cells filled respectively an average of 89 and 78% of the volume. The connective tisue and blood and/or lymphatic vessels represented respectively 1 and 10% in the cortex, 6 and 16% in the medulla whatever the age. The connective tissue was always more abundant in the medulla than in the cortex ($P < 0.05$ at D_{100} and D_{144}; $P < 0.01$ at D_{120} and D_{132}).

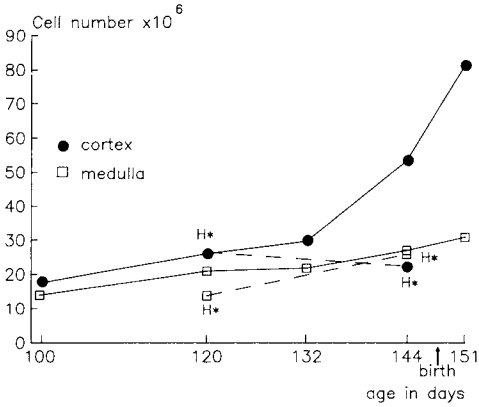

Fig 2. Total cell number estimations according to the gestational age in cortex (●) and medulla (□) of sheep adrenal in control (———) and hypophysectomized (– – –) animals. D_0 = Day of mating; D_{151} is equivalent to 3 d after birth. Hypophysectomy was performed at D_{100}.

The nuclear area (table III) varied only with age ($P < 0.01$) in spite of individual variations ($P > 0.05$). In the cortex, it did not change between D_{100} and D_{132} but increased significantly at D_{144} ($P < 0.01$) and after at D_{151} ($P < 0.01$). In the medulla, this area was higher at D_{151} than at all other stages ($P < 0.01$ or 0.05). When cortex and medulla were compared, this parameter differed at D_{144} and at D_{151} ($P < 0.01$). The mean volume of cells, estimated from the nuclear volume and the corresponding NCR (table III), varied as the nuclear area in each region of the gland. It increased by 63.6% in the cortex and by 14% in the medulla from D_{100} to D_{151}. The difference between the 2 regions of the gland was significant at D_{144} and D_{151} ($P < 0.05$).

Table III. Sheep adrenal of control and hypophysectomized fetuses during late gestation and the perinatal period: nuclear area, nucleocytoplasmic ratio, mean volume of cells in the cortex and medulla.

Animals		*Nuclear area (μm^2)*		*Nucleocytoplasmic ratio*		*Mean volume of cells (μm^3)*	
Type	*Age (d)*	*Cortex*	*Medulla*	*Cortex*	*Medulla*	*Cortex*	*Medulla*
Control	100	29.1 ± 1.0	29.0 ± 2.2	0.28 ± 0.03	0.27 ± 0.02	434 ± 68	448 ± 64
Control	120	27.9 ± 2.3	28.8 ± 1.9	0.29 ± 0.07	0.28 ± 0.04	401 ± 122	420 ± 39
Hx*(1)		24.5 ± 1.7	28.0 ± 0.9	0.33 ± 0.01	0.24 ± 0.05	280 ± 32	497 ± 134
Control	132	29.7 ± 1.4	28.4 ± 1.9	0.28 ± 0.02	0.26 ± 0.05	437 ± 37	454 ± 112
Control		33.7 ± 2.2	28.2 ± 1.0	0.26 ± 0.03	0.28 ± 0.06	574 ± 94	427 ± 101
Hx*	144	24.1 ± 2.1	29.5 ± 2.7	0.26 ± 0.05	0.22 ± 0.03	345 ± 65	558 ± 132
Hx**		26.0 ± 1.1	28.8 ± 2.7	0.28 ± 0.04	0.23 ± 0.04	366 ± 41	534 ± 169
Control	151	38.1 ± 1.0	32.4 ± 2.2	0.25 ± 0.04	0.29 ± 0.06	710 ± 72	510 ± 139

D_0 = day of mating; d151 = equivalent to 3 days after birth. Hx = hypophysectomized at D_{100}* and D_{120}**. Values are mean ± sd. The nuclear areas were the mean of 300 items of data (6 adrenals, except (1) = 5 adrenals). The nucleocytoplasmic ratios were the mean of 90 data (6 adrenals, except (1) = 5 adrenals). The mean cellular volume was the ratio of the mean nuclear volume to the nucleocytoplasmic ratio. The nuclei were considered as spherical and their volume were calculated from the nuclear area.

Hypophysectomized fetuses (Hx)

Adrenal weight, *Vc* and *Vm* were always less in *Hx* than in control fetuses of the same age. At D_{144} this difference was more marked in fetuses *Hx* at D_{100} than in *Hx* at D_{120} ($P < 0.01$ or 0.05 according to the case, table I). After hypophysectomy at D_{100}, there were increases in adrenal weight ($P < 0.05$) and cortical volume ($P < 0.05$, comparisons between controls at D_{100} and *Hx* taken at D_{120} or at D_{144}). This was also observed after hypophysectomy at D_{120} (comparison between controls at D_{120} and *Hx* taken at D_{144}, $P > 0.05$). The reduction of cortical volume corresponded to a reduction of mean areas ($P < 0.01$) and length of the whole gland ($P < 0.01$). In contrast, after hypophysectomy at D_{100} or at D_{120}, the volume of the medulla showed a pronounced increase at D_{144} ($P < 0.01$), (table I). At this stage, the medulla was not as long in *Hx* as in controls ($P < 0.01$), but the mean area was not altered ($P > 0.05$).

The cell number per mm^3 (table I) did not vary in *Hx* fetuses of different gestational ages in the cortex as in the medulla ($P > 0.05$). It was higher in *Hx* than in controls of the same age in the cortex, particularly at D_{144} ($P < 0.01$). The total cell number (fig 2) in the cortex was significantly lower at D_{144} in fetuses *Hx* than in controls ($P < 0.01$), and this was more pronounced in fetuses *Hx* at D_{100} than *Hx* at D_{120}. At the same age, this total cell number was not significantly affected in the medulla ($P > 0.05$) after hypophysectomy (fig 2).

The nucleocytoplasmic ratio (table III) was significantly higher in the cortex at D_{120} than at D_{144} in fetuses *Hx* at D_{100} ($P < 0.01$), but did not change significantly in the medulla ($P > 0.05$). The comparison between the cortex and medulla showed

significant differences ($P < 0.01$ or 0.05 according to the case). In the cortex and medulla, the cells respectively filled an average of 93 and 83% of the volume. The connective tissue and blood and/or lymphatic vessels respectively represented 1 and 6% in the cortex, 4 and 7% in the medulla. The connective tissue was more abundant in the medulla than in the cortex ($P < 0.01$ at D_{120}, *Hx* at D_{100} and at D_{144} *Hx* at D_{120}; $P < 0.05$ at D_{144}, *Hx* at D_{100}).

The nuclear area was not different between control and *Hx* fetuses in the medulla ($P > 0.05$). The nuclear area was less in the cortex after hypophysectomy in fetuses of the same age ($P < 0.01$), whereas there was no significant difference at D_{144} between fetuses *Hx* at D_{100} or at D_{120}. The cell volume, smaller in the cortex in *Hx* than in controls ($P < 0.01$), decreased by 30% at D_{120} and by 40% at D_{144}. It fluctuated around an average of 530 μm^3 between D_{120}-D_{144} in the medulla (table III).

DISCUSSION

Between D_{100} and 3 d after birth, the cortex and medulla of fetal sheep adrenals grow continuously, but at different rates. From D_{100} to D_{120}, they have the same rate of growth and occupy about the same volume. After D_{120}, the cortex becomes increasingly more predominant and constitutes about 70% of the whole volume 3 d after birth. The development of both regions combines hypertrophy and hyperplasia which occur at different stages with respect to the region and account for the exponential weight increase during late gestation and the perinatal period. The destruction of the fetal pituitary suppresses hypertrophy and hyperplasia in the cortex but not in the medulla, even if it affects its increase of volume.

In the cortex of control fetuses, cell hypertrophy in the zona fasciculata begins after D_{132}, earlier than already described (Durand *et al,* 1978) as suggested by Boshier and Holloway (1989); it continues until D_{151}. It corresponds to a phase of protein synthesis (Durand, 1979), an increase in steroidogenic enzyme activities (Durand, 1987) and an increase in membrane receptors to ACTH (Durand, 1979). The explosive phase of hyperplasia between D_{132}-D_{151} corresponds to a phase of DNA replication observed in the whole gland (Durand *et al,* 1978). In the medulla, our results indicate that hypertrophy begins after D_{144} (table III). At that time it is associated with marked hyperplasia, but which is less important than in the cortex. Our results agree with recent observations on the central medullary cells which become predominant on the juxtacortical cells during late gestation and early postpartum (Boshier *et al,* 1989). It is likely that hypertrophy and hyperplasia concern cells producing noradrenaline and adrenaline since the ratio of areas occupied by each type of cells does not change significantly during the studied period (Coulter *et al,* 1989).

The ratio $L/(\sqrt{S/\pi})$ indicates that length and thickness do not vary in the same manner for the medulla and the whole gland. In the medulla, lengthening is faster than thickening during the period studied, particularly between D_{120} and D_{132}, the sole period during which this is observed in the whole gland. As the mean ratio $L/(\sqrt{S/\pi})$ varies with the stage of development, a sole area is not the most representative parameter of the volume of sheep adrenal during late gestation and the perinatal period.

In the cortex, the lack of development after hypophysectomy is particularly significant after D_{120}, agreeing with the trophic effects of ACTH (Durand *et al,* 1980) or related compounds (Durand, 1987). *In vivo,*

the perfusion of ACTH has been seen to increase the multiplication of inner cortical cells (Boshier *et al,* 1981; Robinson *et al,* 1983); it produces an increase in adrenal weight (Liggins, 1968; Cabalum *et al,* 1982; Lye *et al,* 1984) and provokes cellular hypertrophy (Durand *et al,* 1980; Robinson *et al,* 1983). Hypertrophy and hyperplasia coincide with times when ACTH secretion becomes pulsatile and thereafter increases substantially (Jack *et al,* 1975; Jones *et al,* 1977; Challis *et al,* 1977; Wintour *et al,* 1980; MacIsaac *et al,* 1985). It also coincides with the increase of ACTH-like activity seen in short-term cultured pituitary cells (Brieu and Durand, 1987). The low development which precedes the explosive phase of growth is also under pituitary control since hypophysectomy at D_{100} provokes hypotrophy at D_{120} as observed previously (Robinson *et al,* 1983), (table III).

After hypophysectomy, the medulla continues to grow but to a lesser extent than in controls (table I). This effect is due to a difference in length between *Hx* and controls but not to a change in the mean area, as already shown (Coulter *et al,* 1989). This effect of hypophysectomy on the medullary volume is probably an indirect consequence of the reduction in vascularization in both regions and the inhibition of cortical growth. The lack of pituitary does not change the ratio of the area occupied by cells containing adrenaline to the area occupied by cells with noradrenaline (Coulter *et al,* 1989). Our results show few effects of hypophysectomy on the measured cell parameters (tables I and II). Its effect on the juxtacortical and central medullary cells which have been described (Boshier *et al,* 1989) remains to be determined.

In conclusion, hypertrophy and hyperplasia are combined during late gestation in both cortex and medulla of sheep adrenal. They occur about 15 d before birth in the cortex and around the time of parturition in the medulla. Cortical development depends on fetal hypophyseal factors; it agrees with the roles attributed to ACTH or related molecules and with the corticotropin secretion described in other studies. Fetal hypophysectomy decreases the rate of medullary growth, probably as a consequence of the inhibition of cortical development.

ACKNOWLEDGMENTS

This work was supported by the Institut National de la Recherche Agronomique (France). We thank Dr MT Hochereau-de Reviers for her advice and helpful comments. We particularly thank Mrs Ducelliez for her technical assistance. We are indebted to our colleagues who put the planimetry equipment at our disposal. We also thank Mr Tanguy, who facilited computer use, and all the persons involved in animal care.

REFERENCES

Bassett JM, Thorburn GD (1969) Fœtal plasma corticosteroids and the initiation of parturition in sheep. *J Endocrinol* 44, 285-286

Bosc MJ, du Mesnil du Buisson F, Locatelli A (1974) Mise en évidence d'un contrôle fœtal de la parturition chez la truie. Interactions avec la fonction lutéale. *CR Acad Sci Paris Sér D* 278, 1507-1510

Boshier DP, Holloway H, Liggins GC (1980) Growth and cytodifferentiation of the fetal lamb adrenal cortex prior to parturition. *J Anat* 130, 97-111

Boshier DP, Holloway H, Liggins GC (1981) Effects of cortisol and ACTH on adrenocortical growth and cytodifferentiation in the hypophysectomized fetal sheep. *J Dev Physiol* 3, 355-373

Boshier DP, Holloway H (1989) Morphometric analyses of adrenal gland growth in fetal and neonatal sheep. I. The adrenal cortex. *J Anat* 167, 1-14

Boshier DP, Gavin CB, Holloway H (1989) Morphometric analyses of adrenal gland in fetal

and neonatal sheep. II. The adrenal medulla, with some observations on its ultrastructure. *J Anat* 167, 15-30

Brieu V, Durand P (1987) Changes in the ratio of bioactive to immunoreactive adrenocorticotropin-like activity released by pituitary cells from ovine fetuses and lambs. *Endocrinology* 120, 936-940

Cabalum TC, Oakes GK, Jansen CAM, Yu HK, Hammer T, Buster JE, Nathanielsz PW (1982) Induction of parturition in sheep by low dose intrafetal infusion of synthetic adrenocorticotrophin-(1-24) at 120-130 days gestation. *Endocrinology* 110, 1408-1415

Challis JRG, Jones CT, Robinson JS, Thorburn GD (1977) Development of fetal pituitary-adrenal function. *J Steroid Biochem* 8, 471-478

Comline RS, Silver M (1961) The release of adrenaline and noradrenaline from the adrenal glands of the fœtal sheep. *J Physiol* 156, 424-444

Coulter CL, Young IR, Browne CA, McMillen IC (1989) Effect of hypophysectomy on the growth and development of the sheep adrenal gland: a morphometric study. *Acta Endocrinol* 120, 301-307

Durand P (1979) ACTH receptor levels in lamb adrenals at late gestation and early neonatal stages. *Biol Reprod* 20, 837-845

Durand P (1987) Développement de l'axe hypophysosurrénalien au cours de la vie fœtale : modalités et régulations. *Ann Endocrinol* 48, 301-310

Durand P, Bosc MJ, Nicolle A (1978) Croissance des surrénales de fœtus ovin en fin de gestation : évolution de l'ADN et des protéines membranaires. *CR Acad Sci Paris Sér D* 287, 297-300

Durand P, Bosc MJ, Locatelli A (1980) Adrenal maturation of the sheep fetus during the late pregnancy. *Reprod Nutr Dev* 20, 339-347

Gabe M (1968) *Techniques Histologiques.* Masson, Paris

Jack PMB, Thomas AI, Nathanielsz PW (1975) Role of fœtal adrenocorticotropin during parturition in sheep. *Nature* 253, 274-275

Jones CT, Boddy K, Robinson JS (1977) Changes in the concentration of adrenocorticotropin and corticosteroid in the plasma of fœtal sheep in the latter half of pregnancy and during labour. *J Endocrinol* 72, 293-300

Kann G (1971) Dosage radioimmunologique de la prolactine plasmatique chez les ovins. *CR Acad Sci Paris Sér D* 272, 2934-2937

Liggins GC (1968) Premature parturition after infusion of corticotropin or cortisol into fœtal lambs. *J Endocrinol* 42, 323-329

Liggins GC (1969) The fœtal role in the initiation of parturition. *In: Fœtal Autonomy* (Wolstenholm GE, O'Connor M, eds) Churchill, London, 218-244

Liggins GC, Fairclough RJ, Grieves SA, Kendall JZ, Knox BS (1973) The mechanism of initiation of parturition in the ewe. *Recent Prog Horm Res* 29, 111-150

Lye SJ, Challis JRG (1984) *In vivo* adrenocorticotropin (1-24)-induced accumulation of cyclic adenosine monophosphate by ovine fetal adrenal cells is inhibited by concomitant infusion of metopirone. *Endocrinology* 115, 1584-1587

MacIsaac RJ, Bell RJ, McDougall JG, Tregear GW, Wang X, Wintour EM (1985) Development of the hypothalamic-pituitary-axis in the ovine fetus: ontogeny of action of ovine corticotropin-releasing factor. *J Dev Physiol* 7, 329-338

Magyar DM, Elsner CW, Fridshal D, Eliot J, Klein A, Glatz T, Lowe KC, Nathanielsz PW, Buster JE (1981) Time-trend analysis of plasma 11-desoxycorticosterone, corticosterone, cortisol and aldosterone in fetal and maternal sheep during the last 18 days of gestation. *J Steroid Biochem* 14, 1091-1099

Nathanielsz PW, Comline RS, Silver M, Paisey RB (1972) Cortisol metabolism in the fetal and neonatal sheep. *J Reprod Fertil* (suppl) 16, 39-59

Nicolle A, Bosc MJ (1989) A quantitative histological study of adrenal development during the perinatal period in intact and hypophysectomized pigs. *Reprod Nutr Dev* 29, 283-291

Robinson PM, Comline RS, Fowden AL, Silver M (1983) Adrenal cortex of fetal lamb: changes after hypophysectomy and effects of synacthen on cytoarchitecture and secretory activity. *Q J Exp Physiol* 69, 15-27

Solari A (1977) Etude quantitative d'organes ou de tissus. III. Méthodes d'estimation du

nombre de particules. *Ann Biol Anim Biochim Biophys* 17, 309-324

Vessereau A (1960) Méthodes statistiques en biologie et en agronomie. *In: Recherche et Expérimentation en Agriculture.* Baillères et Fils, Paris, vol 2, 539

Wintour EM, Coghlan JP, Hardy KH, Hennessy DP, Lingwood BE, Scoggins BA (1980) Adrenal corticosteroids and immunoreactive ACTH in chronically cannulated ovine fœtuses with bilateral adrenalectomy. *Acta Endocrinol* 95, 546-552

Wrutniak C, Cabello G, Bosc M (1985) Plasma free and total iodothyronine levels in hypophysectomized and intact lamb fœtuses during the last third of gestation. *Acta Endocrinol* 110, 388-394