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Embryonic development of the bovine pineal gland (*Bos taurus*) during prenatal life (30 to 135 days of gestation)

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Summary. The ontogenesis of the pineal gland of 30 bovine embryos (*Bos taurus*) has been analysed from 30 until 135 days of gestation by means of optical microscopy and immunohistochemical techniques. For this study, the specimens were grouped into three stages in accordance with the most relevant histological characteristics: Stage 1 (30 to 64 days of prenatal development); Stage 2 (70 to 90 days) and Stage 3 (106 to 135 days). In the cow, it is from 30 days of gestation that the first glandular outline becomes differentiated from the diencephalic ependyma of the third ventricle. This differentiation includes the phenomena of proliferation and multiplication of the ependymal cells that form the epithelium of the pineal outline in development. At 82 days of intrauterine life, in the interior of the pineal parenchyma, we witnessed some incipient pseudoglandular structures that at 135 days were well differentiated. The pineal parenchyma displays a cytology made up of two cellular types of structurally distinct characteristics: pinealoblasts and interstitial cells. Both cellular types begin differentiation at 70 days of embryonic development, the pinealoblasts being greater in number than the interstitial cells. The glandular stroma is formed from the capsular, trabecular and the perivascular connective tissue, filling the interparenchymal space. A dense network of capillaries, which drive across the trabecular connective tissue towards the central glandular zone where their density increases and their calibre is reduced, complete the glandular structure. GFAP positive cells were observed in the embryonic pineal parenchyma in stage 3. At 135 days of gestation, NPY positive fibers entered the pineal gland through the pineal capsule occupying a perivascular localization. Morphological studies of this nature are vital for future use as parameters, indicative of the functional activity of the bovine pineal gland during embryonic development.

Key words: Ontogenesis, Pineal gland, Embryos, Bovine, Development

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Introduction

The pineal gland, which in mammals develops from the diencephalic ependyma and during embryonic development is located between the anterior and the posterior commissure (Redondo et al., 1996; Regodón et al., 1998), has been extensively studied with a variety of biochemical, physiological and pharmacological techniques. One of the least examined aspects of the pineal gland is its embryonic development. In past decades, only two papers have been published in relation to embryonic development of the bovine pineal gland (Brack, 1962; Anderson, 1965). Brack (1962) analyses, from a morphogenic as well as histological point of view, the evolution of the bovine pineal gland throughout its development. It is of note that this development begins in embryos of 4 cm long as a simple evagination of the diencephalic roof; an evagination that will later be surrounded by ependymal cells. He concludes by showing that the final size of the pineal gland reaches approximately 90 cm. Anderson (1965) points out the characteristic layout of the pineal cells in the shape of rosettes around a small central lumen. This arrangement mostly appears in the anterior, mid and posterior regions of the gland.

A detailed study of bovine pineal embryonic development may uncover basic features that are important for a better understanding of adult pineal morphology. In the present study we describe the structural development of the bovine pineal gland during prenatal life (30 days to 135 days of gestation).

Materials and methods

Animals

Thirty clinically healthy bovine embryos (*Bos taurus*) at different stages of development were used for this study. The embryos were obtained from a slaughterhouse in Olivenza (Badajoz, Spain), 5 min after the animals were killed. The animals were killed in the morning from June to November. The specimens were put inte three stages, each containing ten embryos, defined in terms of the most relevant histological

features: Stage 1 (1.7 to 7.8 cm, 30 to 64 days of prenatal development), Stage 2 (10.5 to 16 cm, 70 to 94 days of prenatal development) and Stage 3 (20 to 30 cm, 106 to 135 days of prenatal development). Once separated from maternal linking, embryos were euthanized by umbilical vein administration of 1 g Pentothal (thiopental sodium, Abbott, Madrid, Spain) in a 10% aqueous solution. Size-age estimates were performed according to the methodology proposed by Evans and Sack (1973).

Fixation of tissues

The heads of the specimens of ages between 30 and 54 days of gestation were processed whole, being set aside for the obtention of complete series of longitudinal cuts; parallel to the mid-cephalon plane. On the rest of the specimens of Stage 1 together with those of Stages 2 and 3, we performed direct dissections, by means of a skull autopsy. The skull and the bovine pineal gland with the adjacent epithalamic regions were immediately fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH= 7.4) at 4°C for about 48-72 hours. The skull was opened by means of an oscillating surgical saw and the brain was carefully removed.

Light microscopy

The bovine pineal gland and adjacent epithalamic regions were post-fixed in the same fixative for about 48 hours, and processed by the paraffin-embedding method. Sections 4 µm thick were cut sagitally and stained with hematoxylin and eosin (HE) and Gomori's method for reticulum fibres (RG). Finally, for detection of glial-type cells, staining with phosphotungstic acid hematoxylin (PTAH) was carried out.

Immunohistochemistry

ExtrAvidin Peroxidase Staining (EAS) was performed on deparaffinized pineal sections to detecte glial cell markers (glial fibrillary acidic protein -GFAP-) and markers of peptidergic innervation (neuropeptide Y -NPY-). First, tissue was deparaffinized, hydrated and treated sequentially with 0.5% hydrogen peroxide for 20 min in order to block endogenous peroxidase activity. Sections were blocked by incubation in diluted (1:50) normal swine serum (Dako, Madrid, Spain) for 15 min to reduce background. Samples were incubated with the following dilution in PBS of primary antisera: 1:500 rabbit anti-bovine GFAP (Dako, Madrid, Spain) and 1:500 rabbit anti-NPY (Sigma/Aldrich Química, Madrid, Spain) for 4 hours at 20°C. After the incubation, the sections were washed for 3x10 min in PBS. Biotinylated swine anti-rabbit IgG (1:200 dilution) (Dako, Madrid, Spain) was then added to the sections for 30 min. Sections were finally incubated with diluted (1:50) ExtrAvidin-Horseradish Peroxidase (Sigma/Aldrich Quimica, Madrid, Spain) for 1 hr. After diamino-benzidine reaction, nuclear counterstaining with Mayer hematoxylin was applied. Finally the sections were mounted with Entellan.

The specificity of the staining reaction was determined in control experiments. These comprised, substitution of the primary antibody by PBS or normal rabbit serum 1:100; or omission of both primary and secondary antibodies; and prior absorption of the primary antibody (overnight preincubation of the primary antisera with the respective peptide 50-100 µM). Next, the antibody/peptide mixture was applied to sections in the identical manner and concentration of the primary antibody.

Results

Group 1 (30 to 64 days of embryonic development)

The pineal outline appears at around 30 days of gestation (1.7 cm crown to rump, C-R). This first outline appears as a small dorsal evagination located in the midline of the diencephalic roof, between the habenular (anterior) and posterior commissures (Fig. 1). The apical part of the pineal epithelium appears delimited by the lumen of the third ventricle and the clear outline of the pineal recess. Large venous sinuses are situated on the base of this epithelium. In these early embryonic phases, the parenchyma of the pineal outline appears, formed by a recess of wide lumen – called the pineal recess, which extensively connects to the third ventricle (Figs. 1, 2). In the epithelium pineal recess abundant forms of cellular mitoses can be seen. The pineal epithelium shows no detectable morphologic differences with the adjacent neuroepithelium. The pineal epithelium is pseudostratified, highly vascular in its base from where the capillaries penetrate towards the interior. The epithelial cells displayed a perpendicular layout, with an elongated and highly euchromatic nucleus (Fig. 2).

At 40 days of embryonic development (4 cm C-R) the pineal recess appears covered by a pseudostratified epithelium of ependymal cells, bizonally structured in a central zone with high nuclear density (nuclei superimposed at various levels) and a clear predominance of elongated forms, and another apical zone free of nuclei. Near the recess lumen there are numerous mitotic forms (Fig. 3). In the base of the epithelium, we can see an epithelial proliferation, morphologically round and composed of a high cellular density with round nuclei. This cellular build up juts out towards the venous sinuses (Fig. 3).

As development progresses, 5.5 cm C-R, from the thickening of the embryonic neuroepithelium, which represents the first glandular outline (Fig. 4) (at around 52 days of gestation), pineal evagination appears more perpendicularly developed, displaying an extensive lumen connection with the third ventricle in the pineal recess. The pineal epithelium appears compressed, displaying high cellular density and filled with nuclear elements in division, with low cytoplasm (Fig. 4). The

basal edge of this epithelium is clearly delimited by the walls of the venous sinuses. However, the epithelium of both commissures is seen as being highly developed in comparison to that of the pineal recess.

At 7.8 cm C-R (64 days of gestation), the pineal recess, close to its definitive form, appears more developed with an extensive lumen in connection with that of the third ventricle. The pineal epithelium, at this age, is transformed into a markedly stratified epithelium with a large quantity of cellular elements (Fig. 5). The glandular outline appears surrounded by a lax connective tissue, with numerous blood vessels in its cavity. In the epithelium, we observed two cellular types: one made up of cells with elongated nuclei and another with rounded nuclei. In both, there is an abundance of mitotic phenomena. The apical edge of this epithelium is shown to be well delimited, clear and with almost no cellular elements (Figs. 5, 6).

Group 2 (70 to 94 days of embryonic development)

At 70 days of gestation (10.5 cm C-R) we observed for the first time how the glandular outline got progressively closer to its definitive morphological form, showing a rounded compact form penetrating towards the cerebral-cerebellar area. The progressive growth of the pineal outline is accompanied by a progressive reduction of the lumen of the recess. The habenular commissure appears well developed and close to a choroid plexus. The posterior commissure is shown as being well differentiated and in direct contact with the pineal gland. The choroid plexus are shown perfectly developed in the cerebral-cerebellar area. The third ventricle is displayed at this age as defined, maintaining its connection to the pineal recess (Figs. 7, 11).

In the pineal parenchyma (Fig. 8), we saw a net difference between the epithelium that covers the pineal recess and the interior of the gland. This epithelium appears well developed with an abundance of cells of elongated nuclei and a well-defined apical edge of the epithelium. The internal zone of the pineal parenchyma is the dominant one in terms of size and extension.

The gland remains surrounded on all surfaces by a cover (Figs. 8, 9), formed by a lax connective tissue. Within it, we found a high quantity of vascular structures. Towards the interior of the pineal parenchyma, these capillaries radiate a number of extensions in order to irrigate the parenchyma, at the same time as they delimit pseudolobes where an abundance of mitotic phenomena was observed.

The glandular cytology (Fig. 10) displays clear cell differentiation. On one side, we can appreciate a cellular type whose characteristics correspond to morphologically rounded cells with abundant vacuolar cytoplasm, oval euchromatic nuclei and a well-differentiated nucleolus that we refer to as the pinealoblasts. These cells are distributed widely throughout the glandular parenchyma, without maintaining any kind of regularly defined position. Meanwhile on the other side we observed other cells, less numerous with low cytoplasm, with rounded and intensely stained nuclei that correspond to the future interstitial cells.

At 13 cm (82 days of gestation) we witnessed the glandular outline at its most highly developed, with a highly accentuated rounded form, clearly delimited by the posterior and habenular commissures (Fig. 11). The gland is surrounded on all surface areas by a now well-defined capsule. The habenular commissure is well developed and close to a choroid plexus, also well defined. The posterior commissure, however, appears less well developed as compare with the habenular

Fig. 1. Photomicrograph of a sagittal direction section of the embryo at 1.7 cm C-R, 30 days. Outline pineal (Op) between the habenular (HC) and posterior (PC) commissures. H-E. Bar: 55 µm.

Fig. 2. Photomicrograph of a sagittal direction section of the embryo at 1.7 cm C-R, 30 days. Substantial connection between the pineal recess (R) and the third ventricle (V). H-E. Bar: 30 µm.

Fig. 3. Photomicrograph of a sagittal direction section of the embryo at 4 cm C-R, 40 days. Pineal recess (R) covered by a pseudostratified epithelium of ependymal cells. H-E. Bar: 30 µm.

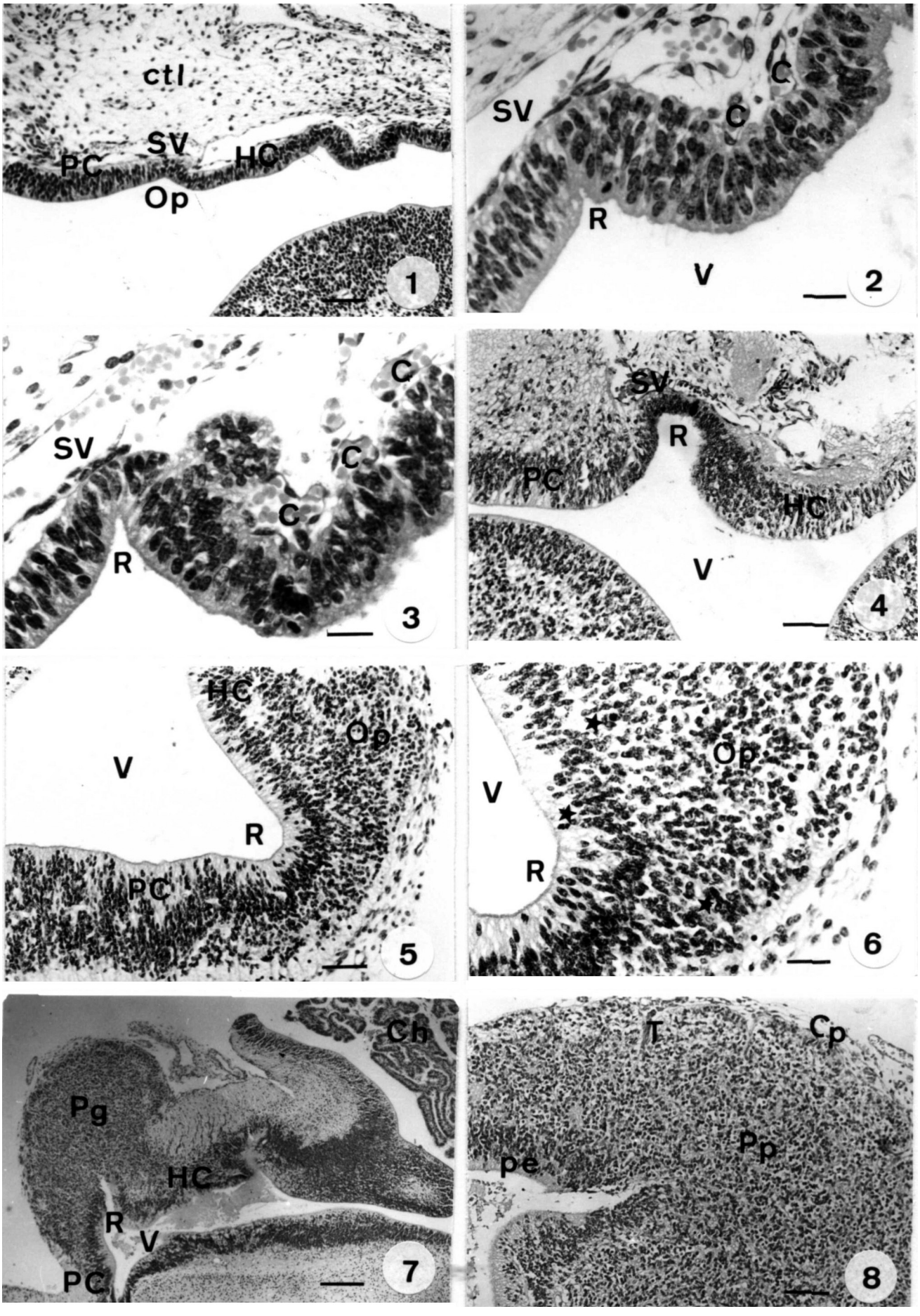
Fig. 4. Photomicrograph of a sagittal direction section of the embryo at 5.5 cm C-R, 52 days. High cellular density in the embryonic neuroepithelium. H-E. Bar: 5 5 µm.

Fig. 5. Photomicrograph of a sagittal direction section of the embryo at 7.8 cm C-R, 64 days. Stratified epithelium with high quantity of cellular elements. H-E. Bar: 55 µm.

Fig. 6. Photomicrograph of a sagittal direction section of the embryo at 7.8 cm C-R, 64 days. Glandular outline with high cellular proliferation. Presence of numerous mitotic phenomena (*). H-E. Bar: 45 µm.

Fig. 7. Photomicrograph of a sagittal direction section of the embryo at 10.5 cm C-R, 70 days. Round and compact morphology of the pineal gland (Pg). H-E. Bar: 134 µm.

Fig. 8. Photomicrograph of a sagittal direction section of the embryo at 10.5 cm C-R, 70 days. Pineal parenchyma (Pp), capsule (Cp), trabeculae (T) and pineal epithelium (pe). H-E. Bar: 55 µm.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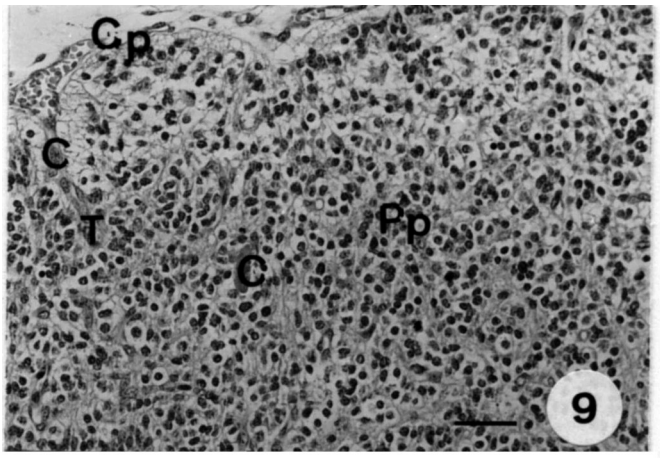
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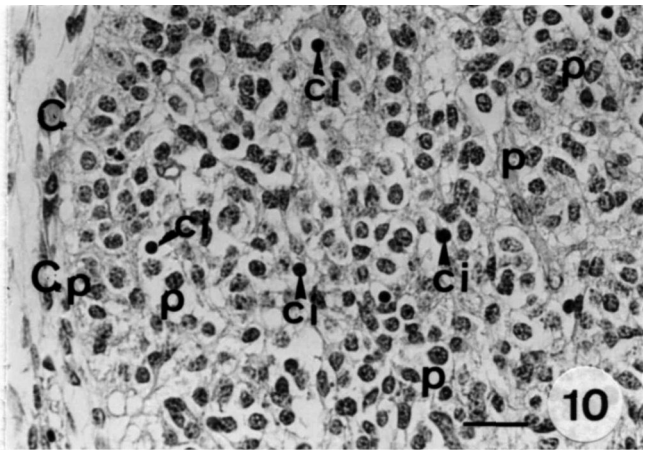
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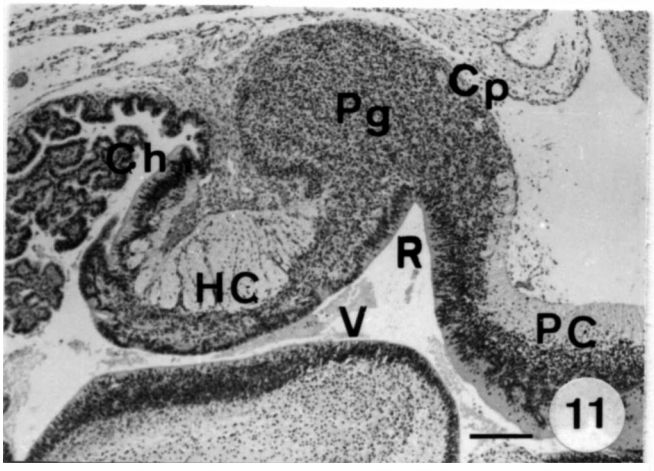
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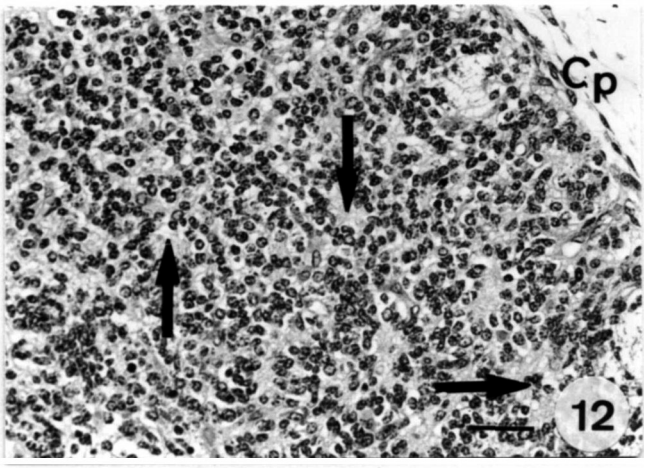
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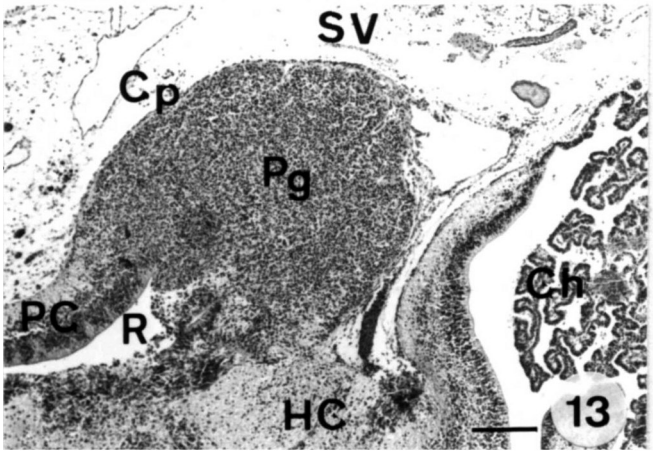
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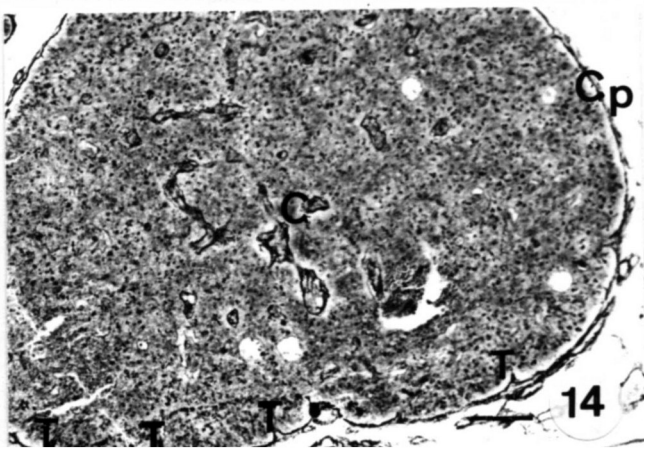
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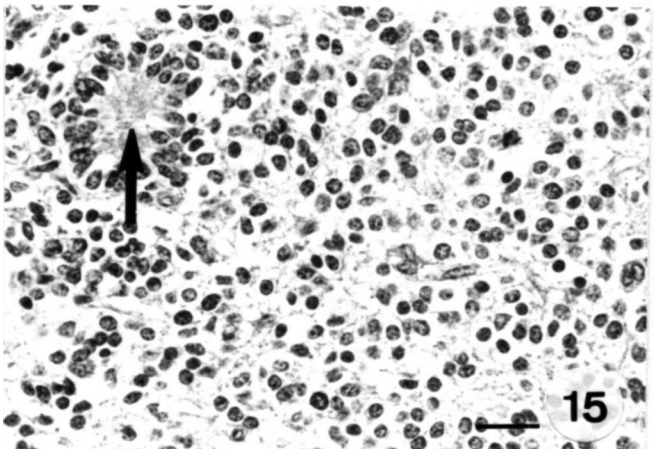
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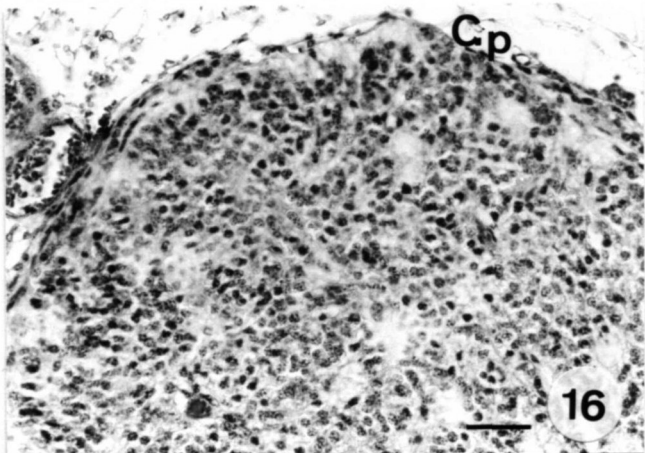
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Fig. 9. Photomicrograph of a sagittal direction section of the embryo at 10.5 cm C-R, 70 days. Pineal parenchyma (Pp), capsule (Cp), trabeculae (T) and capillaries (C). H-E. Bar: 45 µm.

Fig. 10. Photomicrograph of a sagittal direction section of the embryo at 10.5 cm C-R, 70 days. Pineal parenchyma where type cellular types can be observed: pinealoblasts (p) and interstitial cells (ci). H-E. Bar: 30 µm.

Fig. 11. Photomicrograph of a sagittal direction section of the embryo at 13 cm C-R, 82 days. Pineal gland (Pg), capsule of connective tissue (Cp), choroid plexus (Ch), third ventricle (V) and pineal recess (R). H-E. Bar: 134 µm.

Fig. 12. Photomicrograph of a sagittal direction section of the embryo at 13 cm C-R, 82 days. Pseudoglandular structures (arrows). H-E. Bar: 45 µm.

Fig. 13. Photomicrograph of a sagittal direction section of the embryo at 16 cm C-R, 92 days. Pineal gland (Pg), capsule of connective tissue (Cp), choroid plexus (Ch), venous sinus (SV) and pineal recess (R). H-E. Bar: 134 µm.

Fig. 14. Photomicrograph of a sagittal direction section of the embryo at 16 cm C-R, 92 days. Intense reaction of reticular fibres of the capsule, trabeculae and vascular structures. RG. Bar. 55 µm.

Fig. 15. Photomicrograph of a sagittal direction section of the embryo at 16 cm C-R, 92 days. Pseudoglandular structures (arrow) of rounded morphology with central lumen. H-E. Bar: 45 µm.

Fig. 16. Photomicrograph of a sagittal direction section of the embryo at 16 cm C-R, 92 days. PTAH positive interstitial cells distributed around all the pineal parenchyma. PTAH. Bar: 55 µm.

commissure. In the glandular outline, we saw how the pineal recess penetrates towards the interior of the gland, covered by an epithelial layer similar to that described at 70 days of gestation.

In the interior of the pineal parenchyma, we continued to observe two cellular types, previously described, as well as some incipient pseudoglandular structures (Fig. 12). Moreover numerous mitotic forms were seen.

At 92 days of gestation (16 cm C-R), the glandular outline acquires its definitive morphological form: clearly rounded and compact in appearance. The increase in glandular size is due to the proliferation and multiplication of its cellular components. When we applied the argentic reaction (Gomori's methods -RG-), we saw all of the pineal outline delimited by a connective tissue of reticular fibres, with a strong argyrophil reaction that forms a delimiting capsule (Figs. 13, 14) in contact with large venous sinuses. From the periphery of the gland, coming from the capsule, some thin walls of connective tissue are emitted towards the interior of the pineal parenchyma. However, these connective septa did not display continuity along the glandular surface, but form pseudolobes (Fig. 14) that never achieve lobular integration in the pineal parenchyma. The intense argentine reaction is also seen in the reticular fibres that make up the vascular structures.

Fig. 17. Photomicrograph of a sagittal direction section of the embryo at 20 cm C-R, 106 days. Pseudolobular structures formed by walls of reticular fibres coming from the capsule. RG. Bar: 55 µm.

Fig. 18. Photomicrograph of a sagittal direction section of the embryo at 20 cm C-R, 106 days. Cellular groupings (arrows) formed by cells with oval and euchromatic nuclei. H-E. Bar: 30 µm.

Fig. 19. Photomicrograph of a sagittal direction section of the embryo at 25 cm C-R, 120 days. Cellular groupings (arrows). Pineal epithelium (pe). H-E. Bar: 55 µm.

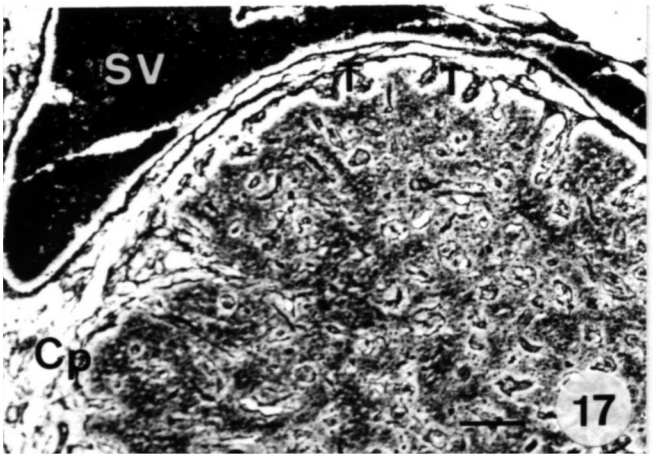
Fig. 20. Photomicrograph of a sagittal direction section of the embryo at 25 cm C-R, 120 days. Cellular groupings (arrows). Pinealoblasts (p) and interstitial cells (ci). H-E. Bar: 30 µm.

Fig. 21. Photomicrograph of a sagittal direction section of the embryo at 30 cm C-R, 135 days. Compact pineal gland. Pineal stalk (Ps). H-E. Bar: 134 µm.

Fig. 22. Photomicrograph of a sagittal direction section of the embryo at 30 cm C-R, 135 days. Pseudolobular configuration of the pineal parenchyma. H-E. Bar: 45 µm.

Fig. 23. Photomicrograph of a sagittal direction section of the embryo at 30 cm C-R, 135 days. Morphologically rounded (arrows)glandular structures. H-E. Bar: 45 µm.

Fig. 24. Photomicrograph of a sagittal direction section of the embryo at 30 cm C-R, 135 days. PTAH positive interstitial cells dispersed around all of the pineal parenchyma. PTAH. Bar: 55 µm.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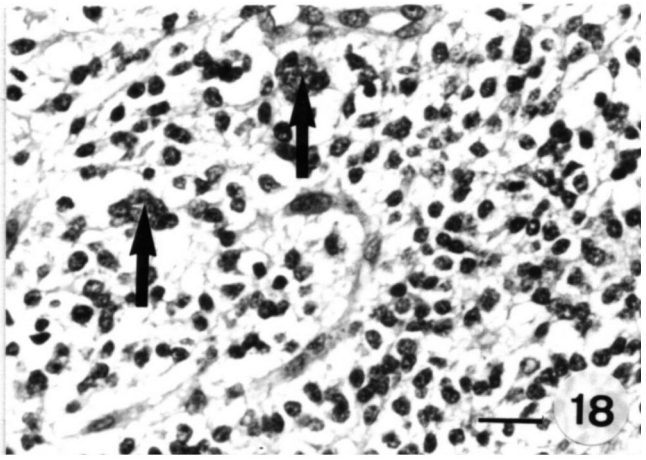
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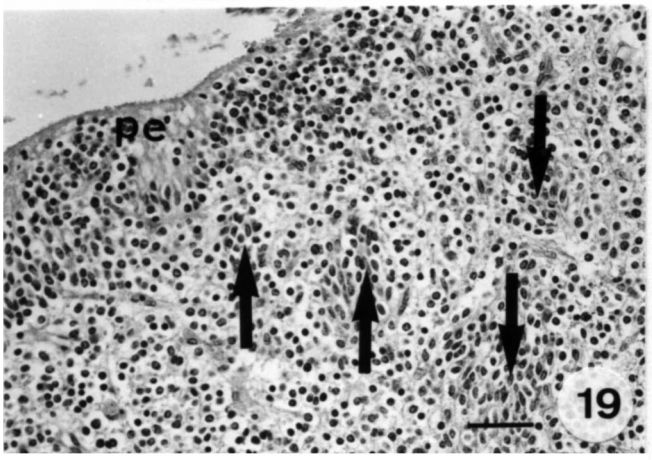
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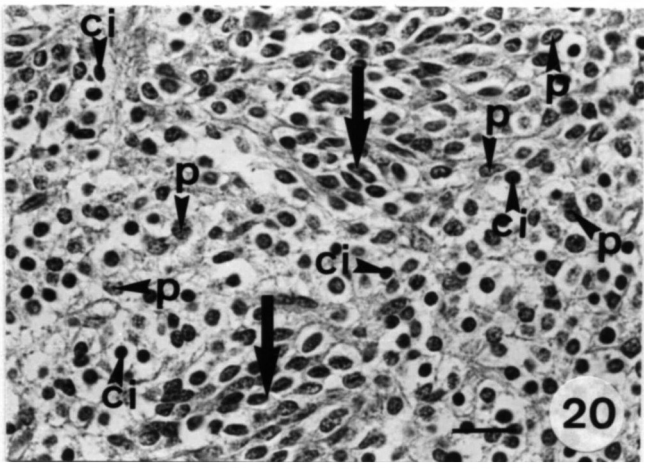
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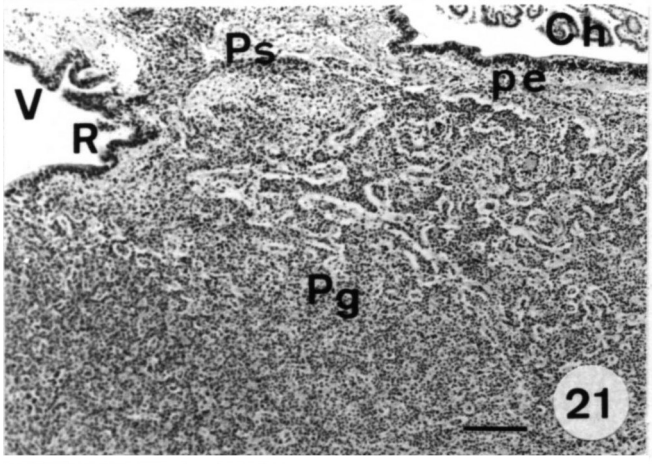
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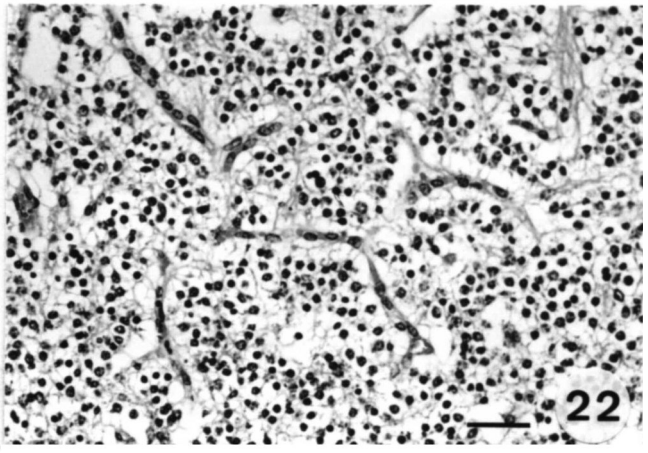
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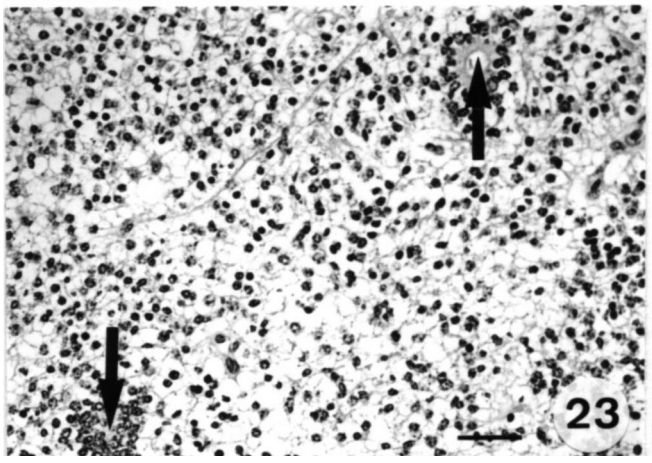
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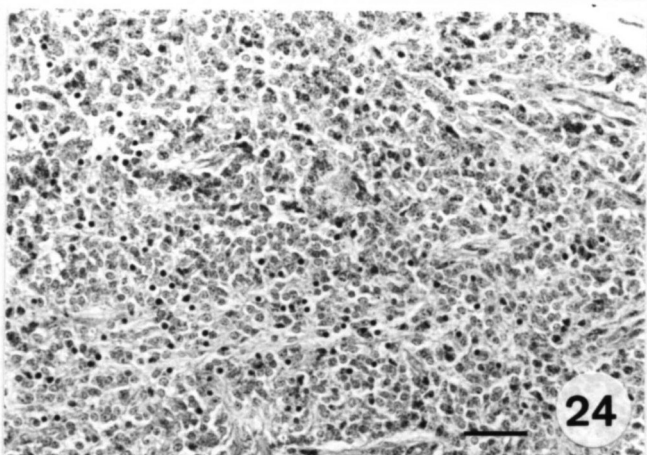
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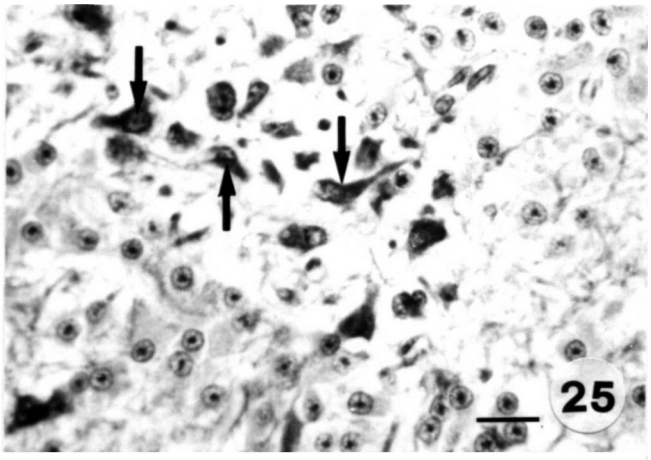
In the interior of the glandular parenchyma (Fig. 15) we saw how the pseudoglandular structures, displayed at 82 days of gestation, appear as a rounded structure with nuclei arranged in the periphery with a central lumen. The cellular groups were identical to those previously described. By means of the phosphotungstic acid-hematoxylin (PTAH) technique, we noted the existence of cells positive to this technique, which can be morphologically described as cells of low cytoplasm and highly stained nuclei, corresponding to the interstitial cells (Fig. 16).

Group 3 (104 to 135 days of embryonic development)

Macroscopically, the pineal gland of the fetuses that form this stage (20 to 30 cm C-R) displays an ovoid-rounded morphology. In general terms, the appearance of the epiphysis is that of a compact organ.



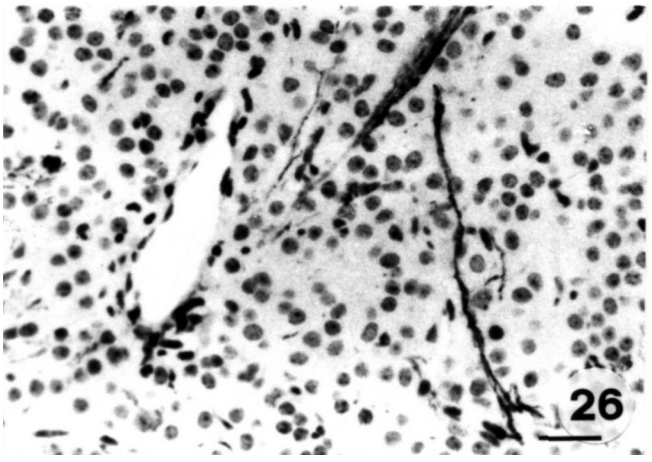


Fig. 25. Photomicrograph of a sagittal direction section of the embryo at 30 cm C-R, 135 days. GFAP positive cells (arrows). EAS. Bar: 20 µm.

Fig. 26. Photomicrograph of a sagittal direction section of the embryo at 30 cm C-R, 135 days. Perivascular disposition of NPY immunoreactive fibres. EAS. Bar: 45 µm.

At 20 cm (106 days of gestation) the pineal gland shows a similar morphology to that of Stage 2. The glandular surface appears surrounded by a well-developed capsule with a high quantity of highly argyrophil reticular fibres (Fig. 17). All of the capsular components appear delimited by an epithelium of flat cells that rest on top of a weak basal layer.

We also detected some connective septa (of a greater thickness that those observed in Stage 2) made up of reticular fibres of a high intensity argentic reaction (Fig. 17), which originate in the capsule and move towards the interior of the pineal parenchyma. However, these connective septa did not show any continuation across the whole glandular parenchyma, forming pseudolobular structures that never manage to completely wall this pineal organ.

The vascular structures (Fig. 17) are more differentiated than in stage 2. This greater vascular differentiation can be shown by the increased number of argyrophil reticular fibres and by the presence of more extensive and clearly defined lumina that continue in their journey towards the walls and septa that are projected from the capsule towards the interior of the parenchyma.

In the interior of the glandular parenchyma we saw some cellular groupings in the form of multinuclear cells made up of cells with oval and euchromatic nuclei (Fig. 18).

At 120 days of gestation (25 cm C-R) we witnessed, macroscopically, a considerable increase in the glandular size due to the substantial development of all its tissue structures. In the interior of the glandular parenchyma we saw more or less rounded cellular groupings, adjacent to the epithelium of the pineal recess and formed exclusively by long cells with long euchromatic nuclei (Figs. 19, 20). Nearby, we observed blood vessels throughout. The interstitial cells are also easily distinguished, of low cytoplasm with a rounded nucleus and highly basophilic, dispersed throughout the pineal parenchyma. If these cells in previous groups appeared in low numbers, at 120 days of gestation, they begin to become more abundant.

At 30 cm C-R (135 days of gestation) the gland continues its growth, the walls becoming more apparent and the blood vessels more developed, especially in the peripheral ventral zone towards the pineal stalk (Fig. 21). In the interior of the parenchyma, we saw some highly developed capillaries (Fig. 22) that even succeed in compartmentalising the parenchyma in pseudolobes. Similarly, we observed the presence of glandular structures (Fig. 23) formed by large cells of extensive cytoplasm with an elongated and euchromatic nucleus that with its apical zone delimit the glandular lumen. We also observed other compact structures.

In the parenchyma, we continued to observe the two types of previously described cells: pinealoblasts and interstitial cells, the latter, positive to PTAH (Fig. 24).

At 135 days of gestation we began to observe an incipient immune reaction to the glial fibrillary acidic

protein (GFAP) in the area around the habenular commissure. Immunopositive cells (whose appearance resembled that of the CNS astrocytes used as positive control) displayed small, dense and ovoid nuclei. The immunoreaction mainly affected the cytoplasm, a type of halo with no reaction, dividing the highly positive cytoplasmatic zone from the nuclear zone of negative reaction (Fig. 25).

Positive NPY nerve fibres begin to appear from 30 cm C-R. Generally, these fibres enter the pineal gland through the pineal capsule (Fig. 26), passing to the connective tissue of the glandular septae, occupying a perivascular localisation. Positive NPY fibres could also be seen dispersed among the pinealocytes.

Discussion

Determining the age of the specimens used in the experiment stands out as a significant factor to consider due to the importance that it has on the rest of the study. Of the chronological methods proposed by the various authors consulted (Postma, 1947; Kurnosov et al., 1960; Murillo Ferrol, 1972; Evans and Sack, 1973; Thomsen, 1975), we chose the method by Evans and Sack (1973) since they managed to combine and clarify all their findings, simplifying and presenting them with great precision and skill. Their growth curves and charts of external morphological characteristics are sufficiently precise to constitute a standard.

In our study, the corresponding age of the specimens that make up each age stage is fixed in specific days. The selection of the particular age stage (according to corporal length or C-R: crown to rump), was based always on the histological changes occurring throughout embryonic development. The number of specimens used (30 embryos in distinct stages of development – from 30 until 135 days of gestation) are sufficiently representative to carry out a chronological scheme of the most relevant histological aspects. It is worth pointing out that some authors subdivide the material by sex (Blin and Maurin, 1956; Boeckmann, 1980; Calvo et al., 1988) while others do not consider this factor (Brack, 1962; Anderson, 1965; Regodón et al., 1998; Calvo et al., 1990). We decided to not take sex into account given that we have not found differential results for either sex in relation to the ontogenesis of the pineal gland.

Using topographical criteria as a basis, we classified the bovine pineal gland (in these stages of development) such as type A in a similar way to the classification used for sheep by Vollrath (1979) and Regodón et al. (1998). This classification is based on the position, form, grouping of tissue and relations with nearby organs (the bovine pineal gland is situated during these first developmental phases in the proximity of the third ventricle).

For the differentiation of the first glandular outline, we used topographical criteria as a basis, since histologically it is not possible to differentiate it from the adjacent neuroepithelium from which it comes, specifically from the diencephalic ependyma of the third ventricle (Calvo and Boya, 1981; Ueck, 1986; Regodón et al., 1998). For us, the chronological appearance of the first glandular outline (as a thickening of the mid diencephalic line, between the habenular and posterior commissures) is placed at 30 days of gestation. These observations do not coincide with those pointed out by Brack (1962) who describes the start of development of the pineal gland in bovine embryos at around 4 cm C-R (at approximately 40 days of prenatal development).

If we compare the timing of the appearance of the pineal outline in cow with other species of mammals, we can deduce that in the cow (Brack, 1962; Anderson, 1965), in the same way as in carnivores (Zach, 1960) and in sheep (Regodón et al., 1998), the pineal outline appears in the first stages of embryonic development. However, in species such as the hamster (Clabough, 1973); the rat (Clavo and Boya, 1981; Ueck, 1986) and the rabbit (Moller et al., 1975), this differentiation is much later, being placed at approximately the last days of prenatal development.

Quay (1974) describes two phases in the embryonic development of the ovine pineal gland: a morphogenic phase and another cellular proliferation phase. Both begin in the first few days of embryonic life and end at birth. Our results coincide with those proposed by Quay (1974) in relation to the growth of the bovine pineal gland from the pineal outline. This growth, as we have previously described, begins at 30 days of prenatal development and includes the phenomena of proliferation and multiplication of the ependymal cells in the outline of the pineal epithelium (Brack, 1962; Anderson, 1965; Redondo et al., 1996). At 64 days of gestation, we witnessed how a phase of cellular proliferation in the pineal outline in the cow was started. During this stage of development, mitoses, above all in the proximity of the lumen of the pineal recess, are common, a fact that has likewise been observed by Calvo and Boya (1978, 1981) and Regodón et al. (1998) in chicken, rat and sheep embryos respectively.

During prenatal development of the bovine pineal gland, in the interior of the pineal parenchyma, we witnessed some pseudoglandular structures at 82 days of gestation. These incipient pseudoglandular structures at 135 days of gestation appear well differentiated. Our results show that these structures are found formed by morphologically similar cells to those that form the epithelium of the pineal recess. Due to this, our findings do not coincide with the research of García-Mauriño and Boya (1992), who in the pineal gland of the rabbit point out that these structures (known as rosettes), are formed by Type II pinealocytes (interstitial cells). Nor do we coincide with Brack (1962) and Anderson (1965), who show that the rosettes of the pineal gland of sheep and cow are formed by ependymal cells coming from the third ventricle. Clabough (1973) and Calvo and Boya (1981) state that the rosettes that appear during the embryonic development of hamster and rat are formed by undifferentiated cells.

Jové et al. (1999) show that 85% of chicken embryos at 5 days old display small cellular rosettes, distinct in terms of morphology, size and number. The number of rosettes per square millimetre decreases between 10 to 15 days of incubation. This reduction in density could be due to an increase in size of the rosettes as well as an increase in the size of the glandular surface, without a change in the number of rosettes (Campbell and Gibson, 1970). The functional significance of these rosettes is unknown. However, they could represent different stages of pineal development, given that they are responsible for storing secretory products, produced by the pinealocytes (Greve et al., 1993; Nowak et al., 1997). The appearance of these rosettes could be indicative of the functional activity developed by the bovine pineal gland during embryonic stages.

In relation to glandular histogenesis, different terms have been used to define the cellular types present in the pineal gland of domestic mammals. Altar (1982) and Ueck (1986) in distinct species of mammals describe the pinealoblasts as cells that in their morphology and distribution by the pineal parenchyma follow a generic pattern, although with variations. Regodón et al. (1998) in ovine embryos in distinct phases of development show the presence of these cells at 15 cm C-R (69 days of gestation). They describe them as morphologically long cells with central, oval or rounded nuclei, with peripheral build-up of heterochromatin with a central nucleolus, widely distributed in all of the pineal parenchyma. Pevet (1977) proposes different names to define the pinealoblasts, such as: principal pinealocytes, clear pinealocytes, parenchymal cells or Type I pinealocytes.

Numerous terms have been used to designate the second type of pineal gland parenchyma: interstitial cells (Cozzi, 1986; Franco et al., 1997; Regodón et al., 1998); glial cells (Brack, 1962; Anderson, 1965; Calvo et al., 1988) and astrocytes (Boya and Calvo, 1993). In ovine embryos (Franco et al., 1997; Regodón et al., 1998) describe the presence of these cells at 98 days of gestation, showing a principally perivascular location. Brack (1962) marks its presence at around 27 weeks of gestation (189 days of prenatal development). There exists a proximity relation between these cells and the vascular structures (Calvo and Boya, 1983; Calvo et al., 1988; Boya and Calvo, 1993; Regodón et al., 1998). This would explain their possible functionality in the exchange of substances between the pineal parenchyma and the blood; it would also explain their support function (Brack, 1962; López-Muñoz et al., 1992; Franco et al., 1997).

During prenatal development of the ovine pineal gland, Franco et al. (1997) and Regodón et al. (1998) describe the existence, at 118 days of intrauterine life, of a third cellular grouping, which they call pigmented cells. They attribute a principal role in melanosynthesis to these cells during the stages of embryonic life in sheep, confirming that pointed out by Calvo et al. (1988, 1990) during postnatal development of dog.

Our results confirm the existence during bovine prenatal development of two cellular types: pinealoblasts and interstitial cells. Both cellular types appear in the pineal parenchyma from 70 days of gestation, the pinealoblasts being more numerous than the interstitial cells. As embryonic development advances both cell types increase progressively in number. This would explain the fact that the changes that occur in the pineal volume are in correlation with modifications in the number of pineal cells (Diehl et al., 1984; Matsushima et al., 1990; Jové et al., 1999).

The organisation of the glandular architecture appears made up of pseudolobes, delimited by walls that originate in the pineal capsule. Forming a part of these walls are blood vessels that penetrate it and that will later be responsible for irrigating the pineal parenchyma. These walls are formed by abundant reticulin fibres (Calvo and Boya, 1984; Calvo et al., 1990; Franco et al., 1997; Regodón et al., 1998) and a few collagen fibres (Franco et al., 1997; Regodón et al., 1998) that delimit the glandular tissue.

In the first days of embryonic development of the bovine pineal gland, we observed some poorly defined capillaries: from 106 days of gestation they were seen to be well developed. These capillaries have extensive lumina similar to those non-fenestrate capillaries described in carnivores (Calvo et al., 1990; Boya et al., 1995) and in ovine (Regodón et al., 1998).

We conclude by stating that morphological studies of this nature are vital so that they can be used in the future as parameters that will be indicative of the functional activity of the bovine pineal gland during embryonic development.

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