Prenatal Development of the Pars Intermedia of the Pituitary Gland in the Water Buffalo (*Bubalus bubalis*).

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**Abstract**

This study was carried out on pituitary glands collected from buffalo embryos and fetuses with a crown vertebral rump length (CVRL) ranging from 1.5 to 95 cm. The specimens were immediately immersed in the fixatives at least 3 days and paraffin embedded for routine histological work. The primordia of the pars intermedia (PI) appeared at 1.5 cm CVRL as a group of undifferentiated mesenchymal cellular band. This band was completely enclosing the pars nervosa (PN) at 3.2 cm CVRL. It appeared thick ventrally around the bottom of the PN. It was separated from the pars distalis (PD) via the hypophyseal cleft and from the neural lobe via thick reticular connective tissue fibers. These cells were differentiated to chromophils and chromophobes at 24 cm CVRL. The chromophils were exclusively basophils. Melanotrophs (MSH-cells) began to appear as polyhedral cells; seen scattered among the follicular cells at 35 cm CVRL. Adrenocorticotrophs (ACTH-cells) first appeared at 75 cm CVRL. The numbers of MSH- and ACTH-cells increased toward the full term age. In conclusion, this numerical increase might prepare the foetal adrenals to face the stresses during and after parturition; and the foetal skin to protect itself (by melanin) from the solar insults.

**Key words**

Pituitary gland, Water buffalo, Pars intermedia, Prenatal development

**Introduction**

The pars intermedia (PI), as one of the three compartments of the pituitary gland (Dellmann, 1998; Dorton, 2000), exists during developmental stages in most vertebrates including the human fetus. In the adult human, the PI is no longer identifiable as a distinct layer (Dellmann, 1998). However, isolated groups of such cells can be found invading a short distance in the pars nervosa (PN). On the other hand, it is large especially in the camel and llama (Martin, 1976).
The PI lies adjacent to the neural lobe; from which it is incompletely separated by a thin layer of connective tissue (CT), while it is separated from the PD by the hypophyseal cleft (Dianne et al., 2006) or cavum which is usually filled with colloid material (Perry et al., 1981; Prasad and Singh, 1980). However, it was reported that the PD is being separated from the PI by a narrow band of CT, which is intermittently broken with vesicles of different sizes (Salama and Deeb, 1975). The PI contains three types of cells: basophils, chromophobes, and cells forming the colloid-filled cysts (Perry et al., 1981). These cysts are the remnants of Rathke’s pouch.

The development of PI was extensively studied in fishes (Amano et al., 2005; Einarsdóttir et al., 2006), amphibians (Heer et al., 2009; Kawamura and Kikuyama, 1998), reptiles (Batista et al., 2004), rodents (Boer et al., 1980; Dupouy and Dubois, 1975; Eurenius and Darskar, 1975; Negm, 1970; Wilson and Morgan 1980), rabbit (Chatterjee, 1976; dog (Sasaki and Nishioka 1998), sheep (Liggins, 1968; Silman et al., 1979) pig (Sasaki et al., 1992), camel (El-Ghribawy, 1990) and human (Dubois, 1973, Solov’ev et al., 2008).

Little information is available about the development of the PI in the water buffalo. Thus, in a continuing study of the ontogeny of the pituitary gland in the buffalo (Attia, 2008), the present study aimed to shed light on the prenatal development of the PI in this economically important species.

**Materials and methods**

The pituitary glands of 70 buffalo embryos and fetuses with their CVRL ranging from 1.5 to 95 cm were collected from many abattoirs in Cairo, kalyobia and Giza Governorates. The embryos and fetuses were freed from their embryonic membranes just after collection and carefully examined for exclusion of any abnormalities. While the embryos with CVRL ranged from 1 to 6 cm were taken as a whole to the fixatives, the heads from fetuses with CVRL ranged from 7 to 95 cm were cut and opened and the pituitary glands were carefully removed and immediately immersed in the fixatives. Different fixatives were used; Susa, Bouin’s and Neutral buffered formalin. The specimens were dehydrated, cleared, embedded in paraffin and sagittally sectioned. The specimens were stained with general, special and histochemical stains. These included Harris haematoxylin, Lead haematoxylin, Crossman’s tri-chrome stain, Gomori reticulin method, Periodic acid Schiff, Alcian blue, Alcian blue/ PAS, Acid fuchsin aniline blue, Bromin-Alcian blue-Orange G-Fuchsin (BR-AB-OFG) method and Peracetic acid Alcian blue PAS orange G method (PA-AB-PAS-OG). These methods were after Bancroft et al., (1994).

The adrenocorticotrophs (ACTH-cells) and melanotrophs (MSH-cells) were counted in 5 different tissue sections for each age (35-95 cm CVRL) group. For each section, cells were counted in 10 fields (x10) and the mean value for each section was calculated. The
numbers (means ± SD)\(^1\) of cells were then calculated for each age group (table 1). Photomicrographs of the PI were captured and processed by an image processing program.

### Results

The primordia of the PI appeared as early as at 1.5 cm CVRL as an evagination from the stomatodoeum (Rathk’s pouch) represented by group of undifferentiated mesenchymal cells (Fig 1a). A pronounced cellular proliferation was more evident in the posterior wall of Rathk’s pouch than in its other regions. The cells increased in size and the PI appeared as a thin band surrounding the PN (Fig 1b). The blood vessels and the fine collagen fibres began to appear at the margin between PN and PI and separate the PI from the architecture of the PN (Fig 1b). These cells were characterized by deeply stained cytoplasm and large central nuclei. Some cells appeared with irregular outline. The nuclei were spherical with peripheral condensed chromatin granules and a prominent nucleolus. Other cells contained more nucleoli at 4.5 cm CVRL. These cells were located underneath the epithelium of the hypophyseal cleft. Mitotic figures were prominent at this stage (Fig 1c). The cells lining the hypophyseal cleft start to differentiate to columnar epithelium (Fig 1d).

At 14-18 cm CVRL, the undifferentiated cells of the PI increased in size and number.

These cells were arranged in the form of short cords or single cells with granular acidophilic cytoplasm (Fig 1e). The staining intensity of these cells was less than the neighbouring cells in PN.

At 24 cm CVRL, two types of cells were observed in the PI, chromophils and chromophobes. Chromophils were only basophilic with oval to ovoid centrally located nuclei. These cells were arranged perpen-dicular to the cleft in the form of light and dark islands of cells (Fig 1f).

At 30-50 cm CVRL, The cells of the PI were arranged in the form of irregular anastomosing cords or follicles surrounded by CT. Polyhedral cells with oval or spherical, centrally located nuclei and slightly basophilic cytoplasm were seen among the follicular cells represent-ing the melanotrophs (Fig 2a). Chromophobes were spherical or oval cells scattered along with the basophilic cells in the follicles with unstained cytoplasm and vesicular eccentric nuclei (Fig 2b).

At 60-80 cm CVRL, the melanotrophs increased in number and size and appeared in the form of collected groups or follicles (Fig 2c). The follicles separated from each others by CT septa that seem to divide the PI into incomplete compartments (Fig 2d, 3c). Tall columnar cells with oval nuclei and granular cytoplasm were appeared at 75 cm CVRL that represent the ACTH

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1 Microsoft Excel 2003 program
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numbers of adrenocorticotrophs &
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stomatodoeum in the form of undiffere-
cm CVRL as evagination from the
The primordia of the PI  appeared at 1.5
to the PN and stained dark blue or
fibers separated the PN from the PI (Fig
2f, 3c).
At 90-95 cm CVRL, the PI appeared as
thin band of tissue completely
surrounding the PN. It appeared thick at
the parts that surround the bottom of
the PN. Blood capillaries were rarely
distributed among the follicles while it
appeared numerous at the junction
between the PN and PI (Fig 2f). The
cells of the PI appeared oval, columnar
or polygonal in shape with large
centrally located nuclei and prominent
one or two nucleoli (Fig 3b). Melanotrophs appeared as large
polyhedral cells that were distributed
among the follicles (Fig 3b). The cells of
the PI showed variable PAS reactivity
(Fig 3d). The ACTH and MSH cells
represented the bulk cells of the PI
while the chromophobes represent-ted
the less frequent cell type. The
numbers of adrenocorticotrophs &
melanotrophs increased toward the full
term age (table 1). A structureless homogenous mass (colloid) was
located in the center of the follicles
especially in full term fetuses (Fig 3e ,
3f).

Discussion

The primordia of the PI appeared at 1.5
cm CVRL as evagination from the
stomatodoeum in the form of undiffere-
natated mesenchymal cells. The current
study was in accord with previous
works recorded in buffalo (Wahba,
1985), in camel (El-Gharbawy, 1990)
and in rat (Negm, 1970). PI of water
buffalo enclosed the PN completely
from all sides. This observation goes in
line with that described by Salama and
Deeb (1975), Prasad and Singh (1980)
and Wahba, (1985) in the buffalo and
Paino et al., (1981) in cattle. Similar
findings were also described in other
mammals as in musk shrew (Naik and
Dominic, 1972). In the sheep the PI
appeared as a compact, incompletely
lobulated V-shaped region confined
between PD and PN, and is closely
associated with the PN (Perry et al.,

The PI was separated from the PD by a
prominent epithelium-lined, amorphous
material-containing hypophyseal cleft.
In areas where the cleft space was
absent, the junction between the PI and
PD was not clearly defined. Similar
observations were reported by Jubb
and McEntee (1955); Salama and Deeb
(1975) and Prasad and Singh (1980),
but it differed in other mammals as in
musk shrew, in which the hypophyseal
cleft separated the PI from the PN
anteriorly and ventrally (Naik and
Dominic, 1972).

Moriarty and Moriarty (1973) obser-ved
the ACTH- and MSH-cells in the rat
intermediate lobe by using the
peroxidase-antiperoxidase complex-
unlabeled antibody technique. MSH-
cells are most abundant in all domestic
mammalian species (Dellmann, 1998).
The PI produces MSH, which causes
the expansion of melanocytes in
amphibian and possibly stimulates the
melanocytes in mammals (Dellmann,
1998). In the present study, the melanotrophs firstly appeared at 35 cm CVRL while the adrenocorticotrophs appeared at a later stage (75 cm CVRL).

Although the region of the CT septum confined between the PI and the PN is richly vascularized, blood vessels were rarely observed in the PI. It is presumed that the PI is adequately nourished by diffusion from these nearby vessels (Howe and Maxwell, 1968). It is worth noted that the fenestrated blood vessels were not observed in the rabbit PI until five months after birth (Chatterjee, 1976).

The parenchyma of the PI was entirely devoid of acidophils. It consisted mainly of two types namely, basophils and chromophobes. The latter cells are small and have small mostly spherical nuclei (Salama and Deeb, 1975 and Wahba, 1985). On the other hand El-Gharbawy, (1990) reported acidophils located among the follicles near the hypophyseal cleft.

The basophilic cells were cuboidal or columnar cells arranged in groups with small patches of colloid present in the lumen. Their nuclei were oval to spherical and some of them have a very irregular outline. Similar findings were reported in ruminant (Perry et al., 1981; Wahba, 1985; Dellmann 1998).

The cells of the developing PI attained an alveolar appearance and were light stained. They conferred the PI an appearance of alternating dark and light areas. These results were supported by the findings of Wahba (1985) in the buffalo and El-Gharbawy (1990) in the camel.

In the rat PI, ACTH-cells and the α-MSH-cells first appeared on 18th gestational day while the β-MSH cells appeared on 17th gestational day (Dupouy and Dubois, 1975). Thus, it is noticeable that ACTH-cells appear in late pregnancy. Likewise, ACTH-cells were observed in the fetal porcine PD and PI at 40 days of gestation (Sasaki et al., 1992). In human, during the first half of pregnancy, some cells are easily recognizable owing to their cellular organelles. These cells may be responsible for the MSH secretion of human fetal anterior hypophysis (Dubois, 1973).

The cells of PI displayed a basophilic staining affinity. Similar findings were reported by Salama and Deeb (1975); Prasad and Singh (1980) and Wahba (1985). These cells have variable affinities for the PAS reaction. Therefore, whereas some cells took deeper stain the other some displayed a lighter staining with PAS/OG procedure. This was in agreement with the observation of Prasad and Singh (1980).

The present findings showed that the PI was well differentiated and exhibited relatively high ratios of adrenocorticotrophs in the last trimester of gestation. This supports the possibility that the fetal PI may play a role in the initiation of parturition (Liggins et al. 1967). Additionally, the parturition can be induced within a few days following
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synthetic corticotropin infusion into the intact (Liggins 1968) or hypophysectomized (Jones et al. 1978) fetus.

In conclusion, the numbers of the MSH- and ACTH-cells increased toward the full term age. These cells represented the majority of cells of the PI while the chromophobes represented the less frequent cell type. It is worth noting that the increase in melanotrophs’ numbers is simultaneous with the change in the skin and hair color in the full term calves through its effect on melanocytes, while the increased numbers of adrenocorticotrophs set up the adrenals of the newborn animals for facing the potential stresses during and after parturition.

References


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<p>| Table(1): Numerical values (means ± SD) of melanotrophs and adreno-corticotrophs. |</p>
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<td>29.6 ± 1.95</td>
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<td>85</td>
<td>41.8 ± 1.48</td>
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Fig (1)

(a) Pituitary gland, buffalo, H&E; 1.5 cm CVRL, showing the primordial of the pars nervosa (N) and pars intermedia (I) separated from the pars distalis (D) by Rathke’s pouch (R). X40

(b) Pituitary gland, buffalo, Crossmon’s trichrome; 3 cm CVRL. Pars nervosa (PN) and intermedia (PI) separated from pars distalis (PD) by Rathke’s pouch (R); developing blood vessels (arrowheads). X40

(c) Pituitary gland, buffalo, H&E; 4.5 cm CVRL; deeply stained cells with mitotic figures (arrowheads). X20

(d) Pituitary gland, buffalo, BR-AB-OFG; 6 cm CVRL, the columnar cells (arrows) of the hypophyseal cleft (HC), pars intermedia (PI), pars nervosa (PN). X20

(e) Pituitary gland, buffalo, OFG, 18 cm CVRL, cells arranged in short cords (arrowheads) in pars intermedia (PI), pars nervosa (PN), blood vessel (arrow). X10

(f) Pituitary gland, buffalo, H&E; 24 cm CVRL, the differentiated cells: chromophobes (c) and chromophils (s). X4
Fig (2)

(a) Pituitary gland, buffalo, H&E, 35 cm CVRL, the polyhedral melanotrophs (M) in pars intermedia (PI), pars nervosa (PN). X40

(b) Pituitary gland, buffalo, H&E, 50 cm CVRL, the different forms of the PI cells. Chromophobes (arrow), melanotrophs (arrowheads). X20

(c) Pars intermedia, buffalo, H&E, 62 cm CVRL, the increased size of melanotrophs (M). X40

(d) Pituitary gland, buffalo, Crossmon’s trichrome, 70 cm CVRL, the incomplete compartements (asterisks) of the PI, separated by thin CT septa (arrowheads). X40

(e) Pituitary gland, buffalo, Lead haematoxylin, 75 cm CVRL, pars nervosa (PN), the adrenocorticotrophs (arrows) of pars intermedia (PI). X40

(f) Pituitary gland, buffalo, Crossmon’s trichrome, 80 cm CVRL, blood capillaries (B) in the CT septum (arrows) separating pars intermedia (PI) from pars nervosa (PN). X100
Fig (3)

(a) Pituitary gland, buffalo, Lead haematoxylin, 91 cm CVRL, the adrenocorticotrophs (A). X100

(b) Pituitary gland, buffalo, PA-AB-PAS-OG, 90 cm CVRL, the arrangement of the adrenocorticotrophs (A), melanotrophs (M) and chromophobes (c). X40

(c) Pituitary gland, buffalo, Gomori reticulin, 93 cm CVRL, pars nervosa (PN); the stroma of the pars intermedia (PI), represented by thin reticular partitions (arrowheads). X40

(d) Pars intermedia, buffalo, PAS, 95 cm CVRL, variable PAS reactivity in the different PI cells: melanotrophs (arrowheads) and chromophobes (notched arrowheads). X40

(e) Pituitary gland, buffalo, BR-AB-OFG, 95 cm CVRL, the colloid materials (asterisk) in the follicle (F). X40

(f) Pars intermedia, buffalo, Toludine blue, 95 cm CVRL, the colloid materials (asterisk) in the follicle (F) and different cell types. X100