J Endocrinol Reprod 16 (2012) 2 : 65-70 ***Original Article***

Pituitary colloid in Indian mouse-tailed bat, *Rhinopoma hardwickei hardwickei* (Gray) in relation to reproductive cycle

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Summary

Follicular colloids were examined in pituitary gland of Indian bat, *Rhinopoma hardwickei hardwickei* during annual reproductive cycle. Colloids were distributed throughout the pars distalis, pars intermedia and pars tuberalis. The colloid droplets were round or oval in shape. In some colloids outer border was irregular. These droplets were of various sizes and many of them were vacuolated. Pituitary colloids were observed during the different stages of the reproductive cycle. No change in number, size and shape was observed during anestrus, oestrus, and early pregnancy. At mid- and late pregnancy the number and size of colloid droplets increased. The increasing trend in number and size of colloid droplets was continued during early lactation also. At late lactation, colloid masses showed decrease in number and size. More colloid masses were observed in male pituitary than those observed in the female pituitary.

Key words **:** Bat, pituitary colloid, reproductive cycle.

Introduction

Extracellular accumulations of colloid have often been described as normal components of the vertebrate pituitary gland. They have been reported within the pars distalis, pars intermedia and pars tuberalis (Benjamin, 1981; Kameda, 1990). Numerous reports made by different authors suggest changes in colloid accumulations following experimental treatments such as gonadectomy and adrenalectomy (Vila-Porcile, 1972), thyroid inhibition (Dingemans and Feltkamp, 1972) and melatonin administration (Mohamed, et al., 2000). In the pars distalis of bats, the follicular cells have the ability to form colloid-containing follicles (Nunez and Gershon, 1982; Anthony and Gustafson, 1984; Singh and Krishna, 1993). The colloid-containing follicles of bats show seasonal changes (Richardson, 1980; Badwaik, 1988; Nunez and Gershon, 1982; Anthony and Gustafson, 1984; Singh and Krishna, 1993) and the colloid content increases gradually from birth through sexual maturity (Anthony and Gustafson, 1984). However, the precise proximate stimuli promoting colloid formation remain unclear. Nevertheless, since colloid accumulations exhibit considerable variability with endocrine condition, it has been suggested that these elements may be related to the secretory activity of adenohypophysis (Nunez and Gershon, 1982). This is a great deal of controversy about origin, formation, composition or function of pituitary colloid. The present work was undertaken to study distribution, histochemical characteristics and seasonal variations of pituitary colloid of *Rhinopoma hardwickei hardwickei*.

Materials and Methods

R. hardwickei hardwickei (Gray) is one of the largest Indian microchiropteran bats, a seasonally monoestrous species, and has a strictly defined annual reproductive cycle, breeding only once in a year (summer-breeding bat). For the present study 36 specimens were collected from Sikandara, Agra Fort and Fatehpur Sikri, about 40 km from Agra (UP, India) for one year representing different phases of the reproductive cycle. They are colonial in habit. The colonies range from about hundred to thousand animals. They were caught with the help of butterfly net. The animals were brought alive to the laboratory with minimum stress and maintained under constant supply of food and glucose water. The animals were sacrificed by cervical dislocation and the brain along with the pituitary was immediately dissected out and fixed for 24 hrs in various fixatives such as formal sublimate, neutral formalin, Rossman's, Bouin's and Carnoy's. After fixation pituitaries were washed in running water for 24 hrs, dehydrated in different grades of ethanol, cleared in xylene and embedded in paraffin wax. Sections were cut at 3-5 μm thickness, some horizontal and some sagittal, to ascertain the distribution of different cell types in the gland. A battery of histochemical techniques was employed to identify the different cell types and find the characteristics of the pituitary colloids (Pearse, 1968).

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Results

During all the reproductive stages of the males and females, extracellular colloids were observed throughout the pars distalis. The colloid masses were also frequently observed in the pars intermedia and the pars tuberalis. These were not observed in pars nervosa. The pituitary of the non-porous females contained notably less colloid masses than those of porous females. The colloid content increased progressively from birth through sexual maturity.

The colloids were typically located at the centre of acini or follicles formed by secretory cells and so they were mostly intrafollicular. Some colloid droplets were observed in interfollicular region. In most of the follicles, follicular cells were not in contact with the colloid droplets. The colloid accumulations were spherical, oval or sometimes irregular in shape. The colloids were of various sizes and many of them were vacuolated. No relationship was detected between the distribution of the colloid and any particular type of cells. In both the sexes, during all the reproductive phases, colloids stained with PAS, AB, AF, light green, aniline blue, acid Fuchsin and lead hematoxylin.

The colloids were strongly positive to PAS. In many colloid droplets, the inner central area was faintly stained and the outer ring was darkly stained. The colloids were stained dark pink in the combined histochemical procedure of LB/PAS/OG (Fig. 1) and blue in CL/OG/aniline blue/ acid alizarine blue technique (Fig. 2). In Crossmans technique, it was stained pink at the core and the periphery was stained blue or green (Fig. 3). In AF/LG/OG staining the core stained yellowish pink and the periphery stained purple (Fig. 4). In AB/PAS/OG technique, the central part of the colloid stained pink but the outer boundary stained in blue (Figs. 5, 6, 7, 8).

The colloid droplets were observed in the pituitary gland during all stages of the reproductive cycle of females. At anestrus and estrus, no change in number of colloid masses was observed. But at estrus, there was slight increase in size of colloid masses. At early pregnancy, the number of colloid droplets appeared near about the same as in the previous stages but there was slight increase in size. At mid- and late pregnancy the number and size of colloid droplets increased. It was observed that two colloid droplets of adjacent follicles are connected with each other to form large coupled irregular droplets. Many of the droplets became enlarged and took to irregular shape. The increasing trend in number and size of the colloid droplets continued during early lactation also. At late lactation, the colloid masses showed decrease in number and size.

In juvenile and immature males, the pituitary contained less colloid droplets than in adult males. In adult males, the colloid droplets were observed during inactive, active and regressed stages of the reproductive cycle. There was no change in number of colloid masses during the different stages of the reproductive cycle except in the regressed phase. During this phase there was a slight increase in number of colloid masses. There were variations in the size of colloid masses. The size of colloid masses increased during the active and the regressed stages. Some of the colloid masses were irregular.

Discussion

Pituitary colloids were observed in the pars distalis, pars intermedia and pars tuberalis of this species of bat during all stages of the reproductive cycle of both sexes. The presence of colloids in pars anterior was reported in *Myotis lucifugus lucifugus*, (Anthony and Gustafson, 1984), three species of Hipposiderid bats (Patil, 1974), *Megaderma lyra lyra* (Bhalchandra, 1979), *Cynopterus sphinx giganticus* and *Taphozous melanopogon* (Badwaik, 1988, 1991). Colloids were typically located at the centre of acini or follicles formed by secretory cells. Sometimes they are present in the interfollicular region. The colloid accumulations are mostly round or oval or sometimes irregular in shape. They are of various sizes and many of them are vauolated. The findings in *R. hardwickei hardwickei* are supported by other authors who studied bats (Anthony and Gustafson, 1984; Patil, 1974; Badwaik, 1991).

No relationship was detected between the distribution of colloid and any particular type of cells in pars distalis of this species of bat, similar to that reported in a Hipposiderid bat (Patil, 1974). In this species of bat colloids were stained with alcian blue, aldehyde Fuchsin light green, aniline blue, acid Fuchsin and lead hematoxyline and were distributed throughout the pars distalis. Similar reactivity of pituitary colloids to different stains was reported in other species of bats (Patil, 1974, Badwaik, 1991, Bhalchandra, 1979, Singh and Krishna, 1993).

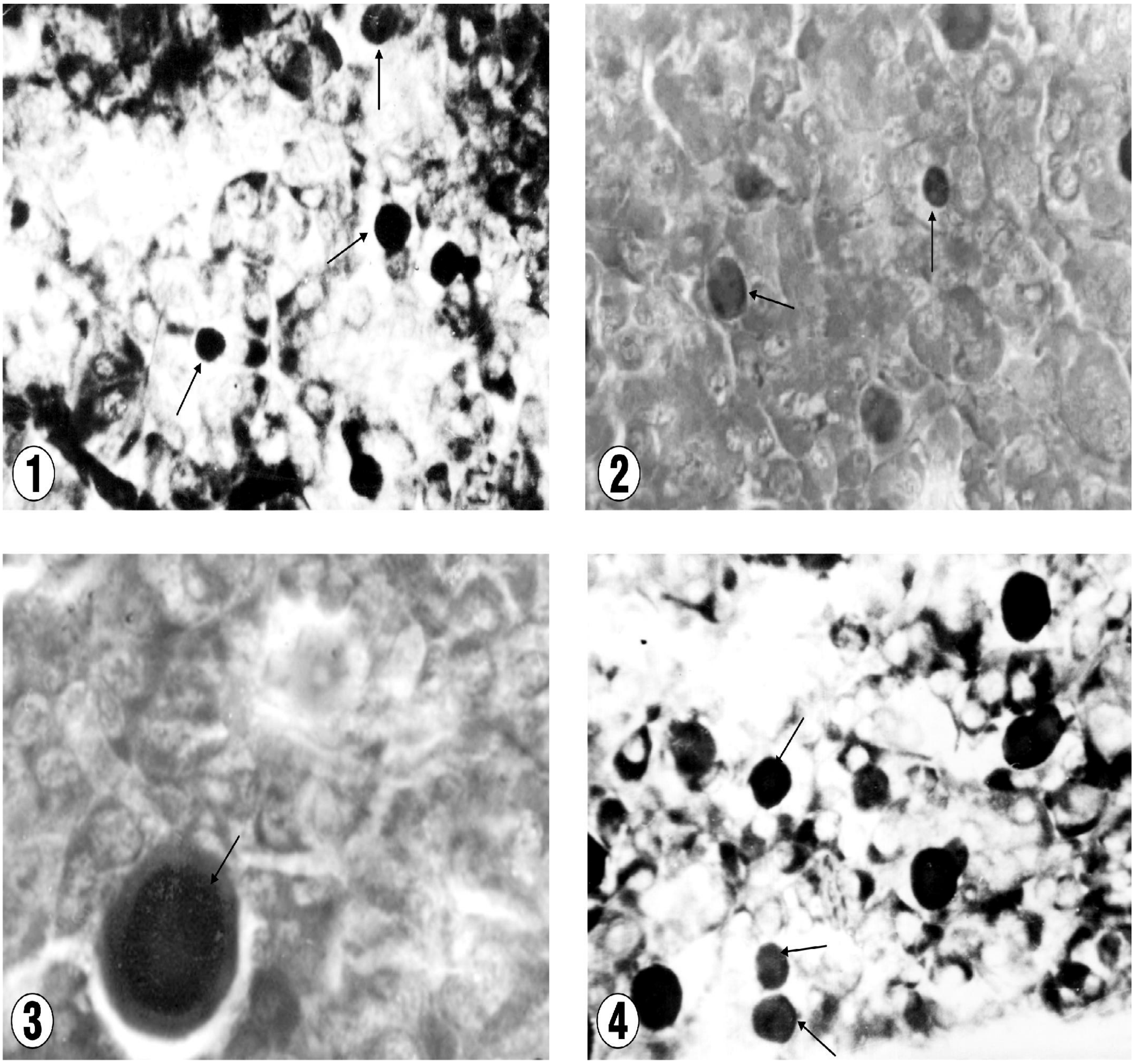
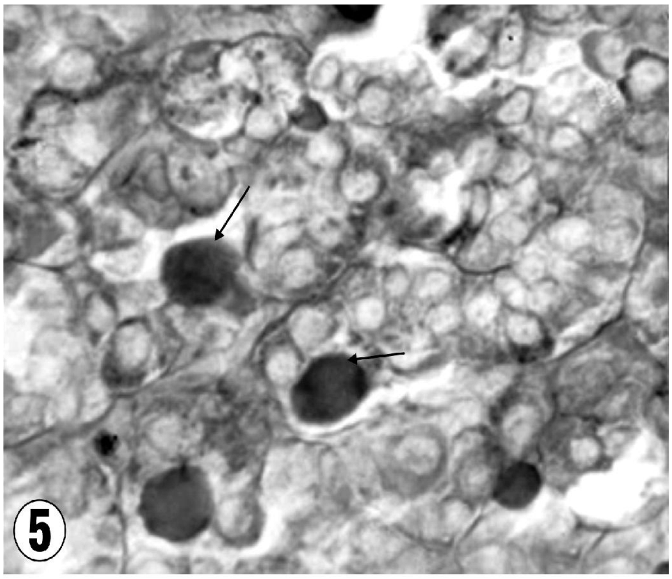


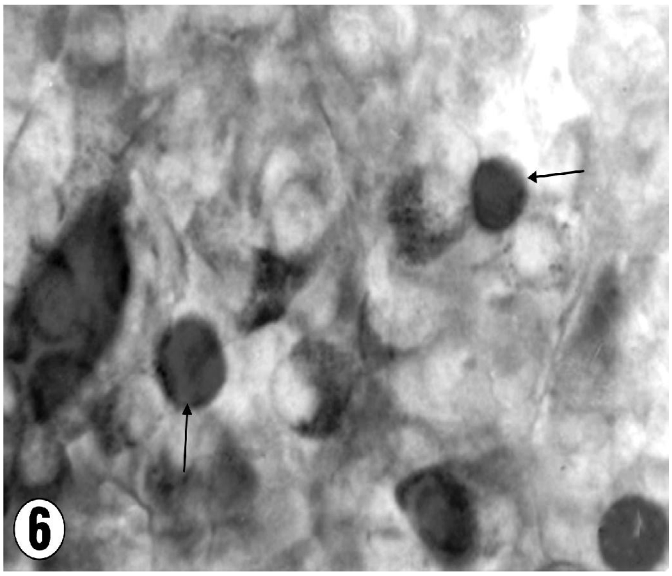
Fig.1. Section of pars distalis of female during mid-pregnancy showing extracellular colloid droplets (arrow). x450. LB/PAS/OG staining.

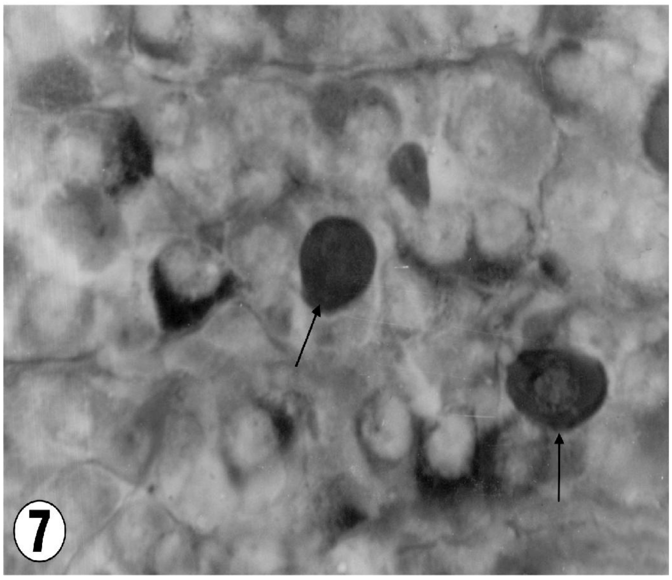
Fig.2. Section of pars distalis of female during late pregnancy showing large colloid droplets (arrow). x900. CL/OG/aniline blue/acid alizarine blue staining.

Fig.3. Section of pars distalis of female during mid-pregnancy. A large colloid droplet showing border stained with blue colour and centre with dark bluish orange colour is seen in the follicle (arrow). x675. Crossman's technique.

Fig.4. Section of pars distalis of male during the sexually quiescent period showing large number of colloid droplets (arrow). x640. AF/LG/OG staining.







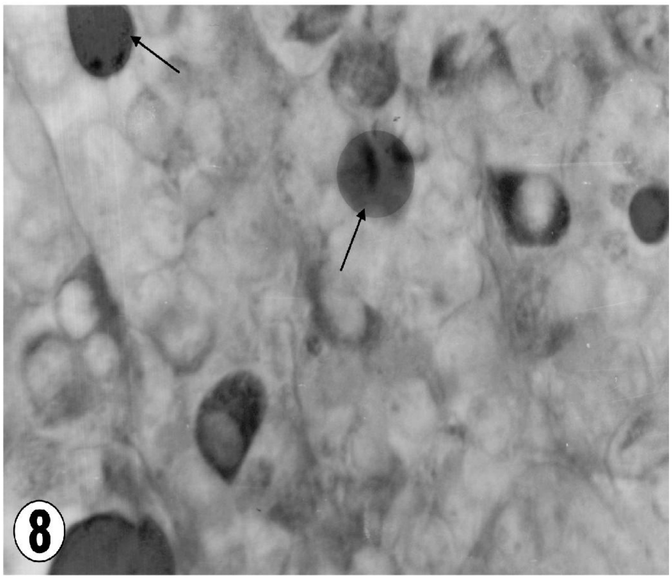


Fig.5. Section of the pars distalis of female during mid-pregnancy stained with AB/PAS/OG showing PAS positive colloid droplet (arrow). x675.

Fig.6. Section of pars distalis of female during late pregnancy showing large and PAS positive colloid droplets (arrow). x675. AB/PAS/OG staining.

Fig.7. Section of pars distalis of male during the active breeding period. Colloid droplets are large, vacuolated and PAS-positive (arrow). x675. AB/PAS/OG staining.

Fig.8. Section of pars distalis of male during the preparatory period showing PAS-positive colloid droplets (arrow). x675. AB/PAS/OG staining.

Interesting feature about colloids in the *R. hardwickei hardwickei* is that in some colloid droplets the central core was stained pink and the outer boundary was stained blue in AB/PAS/OG, and the central portion of the colloid stained pink and the outer boundary was stained blue or green in Crossman's techniques. Probably, two cell types are releasing their contents or single cell type may be synthesizing two hormones simultaneously.

In the present study it was observed that the variations in the pituitary colloid content are closely related to age. The pituitary of the juvenile males and females showed significantly less colloid than those adult males and females. The non-porous females contained less colloid than the females. Similar age-related changes in pituitary colloid content have been reported in *M. lucifugus lucifugus* (Anthony and Gustafson, 1984). The findings in *R. hardwickei hardwickei* are supported by Anthony and Gustafson (1984) in *Myotis*.

The present study suggests that the amount of pituitary colloid changes in relation to annual reproductive cycle in this species of bat. The colloid content increased during hibernation in *M. lucifugus lucifugus*, (Anthony and Gustafson, 1984; Nunez and Gershon, 1982), winter dormancy in a Vespertilionoid bat (Singh and Krishna, 1993) and pregnancy in *Macrotus californicus* (Richardson, 1980), Hipposiderid bats (Patil, 1974), *T. melanopogon*, (Badwaik, 1988) and *C. sphinx giganteus* (Badwaik, 1991). On the other hand Cure et al., (1971) reported no change in pituitary colloid in pregnant hamster and cats. Anthony and Gustafson (1984), however, reported that the amount of colloid does not change in relation to annual reproductive cycle either in male or female. In *R. hardwickei hardwickei* the colloid content of pituitary increased during pregnancy and lactation and remained low during the sexually quiescent period. Pituitary colloid content of active male *Rhinopoma* was more than that of the inactive male. These results indicate that the effect of male or female reproductive activity on pituitary colloid may vary considerably between species.

There is great deal of controversy regarding the formation of colloid. Horrvath et al. (1974) postulated that colloids are derived from degenerating cells. On the other hand in a Vespertilionoid bat the cells surrounding the colloid lacked secretory granules but showed moderately developed Golgi apparatus and endoplasmic reticulum (Singh and Krishna, 1993). Recent studies have suggested that the colloid-containing follicles in the pars distalis may be a sign of storing unnecessary and waste product in the lumen (Kameda, 1991). The colloid may be the site of unused excess hormones. However, colloid content did not show immunoreactivity for the any of the anterior pituitary hormones recently investigated (Singh and Krishna, 1994). Thus, the material of the colloid may differ from the known active hormones.

Numerous investigators have attempted to clarify the functional significance of pituitary colloid by observing changes in colloid accumulation following experimental treatment such as gonadectomy (Vila-Porcile, 1972), adrenalectomy (Dingemans and Feltkamp, 1972), thyroid releasing hormone and L-dopa administration (Harisson et al., 1982 a, b) and melatonin administration (Mohamed et al., 2000). Primarily because almost any experimental manipulation seems to result in qualitative and/or quantitative changes in pituitary colloid, the precise proximate stimuli promoting colloid formation remain unclear. The colloid accumulations exhibit considerable variability with endocrine conditions. It has been suggested that these elements may in some way be intimately related to secretary activities of the adenohypophysis (Harrisson, et al., 1982 a; Nunez and Gershon, 1982). In the present study the colloid content of pituitary showed variation during the annual sex cycle of the bats. Therefore, it would be reasonable to suspect that changes in colloid content might accompany the extensive modifications in endocrine function that characterize the annual cycle of seasonal breeders (Mohamed et al., 2000).

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