Rev. Chil. Anat.,
18(1):5-11, 2000.

GnRH NEURONES POPULATION IN THE DIENCEPHALON OF THE COYPU (*Myocastor coypus*)

POBLACIÓN DE NEURONAS GnRH EN EL DIENCÉFALO DEL COIPO (*Myocastor coypus*)

* Silva, L.
* Sánchez, H.
* Acosta, W.
** Portiansky, E.
* Zuccolilli, G.

SILVA, L.; SÁNCHEZ, H.; ACOSTA, W.; PORTIANSKY, E. & ZUCCOLILLI, G. GnRH neurones population in the diencephalon of the coypu (*Myocastor coypus*). ***Rev. Chil. Anat., 18(1)***:5-11, 2000.

SUMMARY: The goal of this study was to investigate the distribution and morphology of neurones that synthesise and store gonadotrophin releasing factor (GnRH) in the diencephalon of the coypu (*Myocastor coypus*), a South American rodent of the hystricomorpha suborder. For this purpose the encephalon of three mature male coypus were fixed by intra-arterial perfusion, using a paraformaldehyde and picric acid solution. The hypothalamic blocks were cut with freezing microtome to obtain 40µm thick coronal sections. The slides were exposed to an ultrasound antigen retrieval process in order to unmask hidden antigens. These sections were then processed using an immunohistochemical technique to show the GnRH neurones, using a monoclonal antibody (LRH 13). One group of serial sections was stained with cresyl violet (Nissl techniques) while the other sections were stained using Küver Barrera's method (luxol fast blue and cresyl violet), to show the nuclei and nervous tracts of the hypothalamus under microscopic observation. Morphometric and quantitative analysis of neuronal bodies were performed using an image analyser. GnRH immunoreactive neurones were bipolar and long. The total number of neuronal bodies for this species was estimated at 1072 ± 27. All the cells were located in the rostral hypothalamus, mainly in the preoptic area. A few neurones were also observed in the bed nucleus of the stria terminalis and hypothalamic preoptic medial nucleus. The immunoreactive fibres were observed in the external layer of the median eminence. According to the data obtained we inferred that the distribution of the GnRH neurones of the coypu mainly coincides with that of other rodents such as the rat.

KEY WORDS: 1. Coypu; 2. GnRH; 3. Hypothalamus; 4. Immunohistochemistry; 5. Image analysis.

INTRODUCTION

Gonadotrophin releasing hormone (GnRH), originally isolated from sheep and swine brains (FINK, 1988), is the neurotransmitter in charge of releasing the adenohypophysis follicle stimulating hormone (FSH) and the luteinizing hormone (LH), which govern reproductive functions of mammals (THIÉRY & MARTIN, 1991), through the activation of the hypothalamus-hypophysis-gonadal axis.

Several immunohistochemical analysis showed the population of GnRH neurones and have been used successfully in several species of mammals such as rats (KAWANO & DAIKOKU, 1981), rabbits (FOSTER & YOUNGLAI, 1991), goats (ZUCCOLILLI, G. *et al.*, 1994) sheep (LEHMAN *et al.*, 1986); (CALDANI *et al.*,1988), ferrets and monkeys (KING & ANTHONY, 1984; MARSHALL & GOLDSMITH, 1980).

* Institute of Anatomy.
** Institute of Pathology. School of Veterinary Sciences. National University of La Plata. Argentina.
This study was partially supported by a grant from the National Scientific Research Council (CONICET) and from the Secretariat for Science and Technology, National University of La Plata. ELP is a Research Carrier member of the CONICET.

The location of the GnRH neurones is similar in all the above-mentioned species, with the neuronal bodies distributed along the hypothalamus, from the septo-preoptic area to the mediobasal hypothalamus. However, a considerable variation exists among species regarding the proportion of neurones found in different hypothalamic areas (NOZAKI *et al.*,1988; ZUCCOLILLI *et al.*). In rats, all the immunoreactive GnRH neurones (GnRH-ir) were observed in the rostral hypothalamus, preoptic area (POA), medial hypothalamic preoptic nucleus (MPOA) and diagonal band of Broca (DBB) (JENNES & CONN, 1994). In ruminants, most of the GnRH-ir neurones were located in the same nuclei, but 6% of the immunoreactive cell bodies were specifically identified in the arcuate nucleus (ARC) of the mediobasal hypothalamus (ZUCCOLILLI *et al.*). In more phylogenetically evolved species such as ferrets, non-human primates and humans (KING & ANTHONY), the highest percentage of GnRH-ir neurones was found in the ARC.

Simultaneously, morphological studies carried out in different species have attempted to find neuronal subtypes that could be associated to different functions. Even though the GnRH neurones have been classified as bipolar and multipolar (ZUCCOLILLI *et al.*), it was impossible to establish the subtype hypothesis associated to a specific function (DEES *et al.*, 1981).

There are numerous studies about GnRH neurones in various species of rodents, however, hystricomorpha rodents bear special interest because the anatomical features of this suborder are similar to those of the oldest extinguished rodents (PATTERSON & PASCUAL,1972); (HERSHKOVITZ, 1969). Some authors consider this group as the origin of all rodents (NOVASEK, 1992). Thus, present study's goal was to establish the location and distribution of the GnRH structures in a South American hystricomorpha (*Myocastor coypus*) and to quantify and characterise the neuronal subtypes observed using image analysis.

MATERIAL AND METHOD

Animals: The brains of three mature coypu males (6.200g average body weight) obtained from an established colony at the Institute of Anatomy, were used. The animals were anaesthetised with ketamine hydrochlorate (Ketalar®) and chlorpromacine maleate (Acedan®) by intramuscular administration followed by a perfusion with buffered saline and bleeding to white. The animals were then fixed by perfusion of paraformaldehyde-picric acid solution (ZAMBONI & MARTINO, 1967). The encephalon was removed, measured and postfixed in the same solution. The block containing the diencephalon and mesencephalon was cut in 40 µm coronal slices, using a freezing microtome.

Inmunohistochemistry: Antigen retrieval process based on ultrasound (PORTIANSKY & GIMENO, 1996) was performed in order to counteract the time-dependent cross-linking effects formaldehyde has on proteins (LEONG & GILHAM, 1989). Briefly, section slides were dipped into a glass dish containing citrate buffer. The tip of an ultrasound disrupter (Branson Ultrasonics model 250, USA) was immersed in the solution. The blocks were exposed to 40 W potency during 40 seconds and then transferred to buffered saline. Serial sections were processed using the monoclonal antibody LRH 13 (PARK & WAKABAYASHI, 1986). A commercial Kit (Venctastain ABC Kit, Vector Laboratories Inc., USA) was used as a detection system. The horseradish peroxidase activity was demonstrated using diaminobezidine, as a chromogen. The sections were finally countercoloured with toluidine blue, dehydrated and mounted for observation under an optic microscope. Another series of sections were stained either with cresyl-violet or with Küver Barrera method to show the nuclei and nervous tracts.

Image processing and analysis: The images of the different portions of the midbrain were captured from a microscope (Olympus BX50) with a microscopic magnification of 200x, through an attached analogical RGB videocamera (Sony DXC-151A CCD). The images were then digitised with a frame grabber (Flashpoint 128, Integral Technologies Inc) connected to the computer (PC Pentium II, 64Mb RAM, software: ImagePro Plus - Media Cybernetics, Silver Spring, MA, USA), with a pixel depth of 24 bits, RGB and TIFF format. To separate the positively stained neurones from other objects, colour segmentation based on the optic density of the object was performed. The selected object was then characterised by means of the quantification of values such as major and minor axis, aspect and roundness, all measured in micrometers. The immunoreactive area was expressed in square micrometers. Table I describes the morphometric values evaluated.

The total number of cellular bodies was estimated using the following equation (ZUCCOLILLI *et al.*):

$$N = \frac{d}{n.s} \sum_{i=1}^{n} x$$

N = total estimated number of cellular GnRH bodies;
d = distance of the area (mm) in the rostro-caudal axis;
n = number of slices per area;
s = thickness of the section (40 µm);
x = number of cells counted per slice

Statistical analysis: Student t test was used to compare the estimated number of cellular GnRH bodies in each area or hypothalamic nucleus. The student t test for two samples with different variances was performed to determine the significance of the area, aspect and roundness of the evaluated neurones.

Table I. Morphometric parameters used to characterize the immunoreactive neurons

Mayor axis	Reports the length of the main axis of the ellipse equivalent to the object (i.e., an ellipse with the same area, first and second degree moments)
Minor axis	Reports the length of the minor axis of the ellipse equivalent to the object (i.e., an ellipse with the same area, first and second degree moments)
Aspect	Reports the ratio between the major axis and the minor axis of the ellipse equivalent to the object (i.e., an ellipse with the same area, first and second-degree moments), as determined by Major Axis/Minor Axis.
Roundness	Reports the roundness of each object, as determined by the following formula: $(\text{perimeter}^2) / (4 * \pi * \text{area})$. Circular objects will have a roundness = 1; other shapes will have a roundness > 1.
Area	Reports the area of each object (minus any holes).

RESULTS

The use of a specific monoclonal antibody allowed visualisation of the GnRH neuronal bodies and fibres in all tested animals. The immunoreactive cell bodies were generally isolated; however, in highly concentrated areas they formed clusters. Only bipolar neurones were identified (Fig.1) because no multipolar neurones are present in this species. The immunoreactive neurones were located mainly in the preoptic area (POA), with few neuronal bodies scattered in the bed nucleus of the stria terminalis (BST), medial hypothalamic preoptic nucleus (MPOA), accumbens nucleus (ACB), diagonal band of Broca (DBB) and supraoptic nucleus (SON) (Fig.2 and Fig.3). A few immunoreactive neurones were also evident in the rostral hypothalamic area (RHA). However, no neuronal bodies were observed at the arcuate nucleus (ARC).

Fig. 1. Photomicrograph (200x) showing the preoptic area (POA) close to the fornix with several GnRH immunoreactive cell bodies and fibres (a). A bipolar neuron with its prolongations are seen in detail (b).

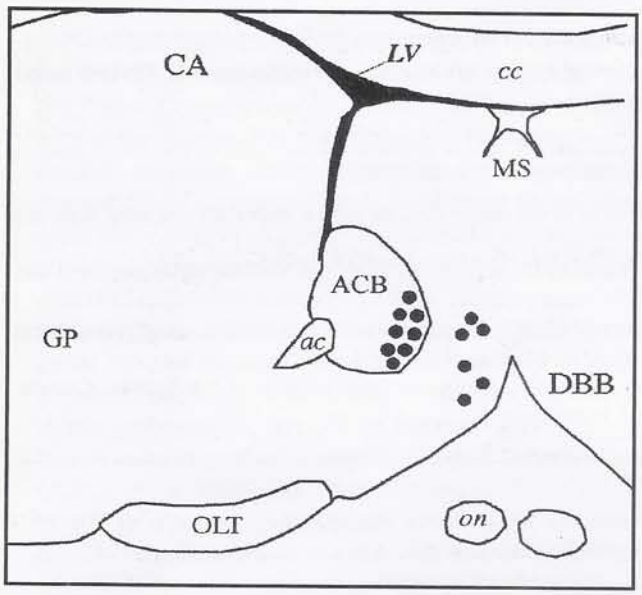

Fig. 2. Schematic distribution of immunoreactive GnRH neurones in a coronal section of the rostral hypothalamus, at the level of septo preoptic juntion. Points represent neuronal bodies. Abbreviations: Anterior commissure (*ac*); optical nerve (*on*); corpus callosum *(cc);* lateral ventricle *(LV)*; caudate nucleus (CA); medial septal nucleus (MS); accumbens nucleus(ACB); diagonal band of Broca (DBB); globus pallidus (GP); olfatory tubercle (OLT).

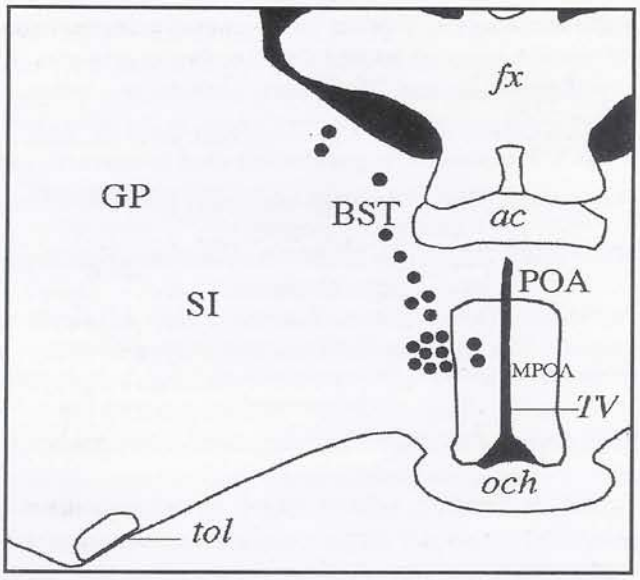

Fig. 3. Schematic distribution of immunoreactive GnRH neurones in a coronal section of the hypothalamus, at the level of optical chiasm. Points represent neuronal bodies. Abbreviations: Anterior commissure (*ac*); fornix (*fx*); optical chiasm (*och*); lateral olfactory tract (*tol*); third ventricle (*TV);* globus pallidus (GP); sustantia innominata (SI); hypothalamic preoptic area (POA); medial hypothalamic preoptic nucleus (MPOA); bed nucleus of the stria terminalis (BST);

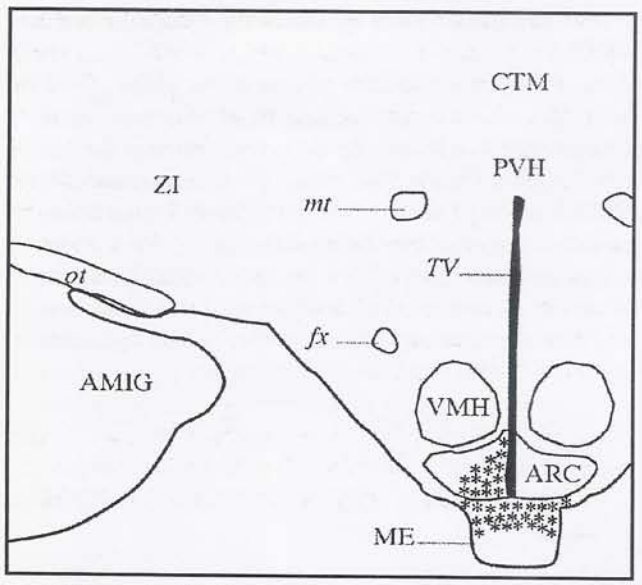

Fig. 4. Schematic distribution of immunoreactive GnRH fibres in a coronal section of the hypothalamus, at the level of median eminence. Asterisks represent the immunoreactive GnRH fibres. Abbreviations: median eminence (ME); arcuate nucleus (ARC); ventromedial hypothalamic nucleus (VMH); paraventricular hypothalamic nucleus (PVH); centromedial thalamic nucleus (CTM); amygdala (AMIG); third ventrículo (TV); zona incerta (ZI); mamillothalamic tract (*mt*); fornix (*fx)*; optic tract (*ot).*

The total estimated number of GnRH neurones was 1072 (Table II). As can be seen in Table III, no significant differences ($p>0.05$) were observed between the neuronal body area, roundness and aspect from POA (including MPOA) and the other examined areas (ACB, BST and DBB). The immunoreactive GnRH fibres were observed with their characteristic varicosis reflecting towards the median eminence (EM), forming a sheaf of amyelinic fibres next to the sagital plane and occasionally, in contact with the periventricular area of the third ventricle (TV). Most of the immunoreactive fibres had their terminal ends in the external layer of the EM where the highest concentration of immunoreactive material was visualised, surrounding the primary capillaries of the portal vessels (Fig.4).

DISCUSSION

The monoclonal antibody used has shown to be very effective in the immunostaining of GnRH neuronal bodies and fibres in this species. An antigenic homology was observed between the coypu's variety of GnRH and that of other species of mammals studied (NOZAKI *et. al.*). Considering the

Table II. Number of GnRH neurones distributed in the hypothalamic nuclei of the coypu.

Nuclei	Section 1*	Section 2*	Section 3*	Section 4*	Section 5*	Total Count	Total Estimate	%
ACB, BST y DBB	49 ± 11	19 ± 6	-	-	-	68 ± 14	504 ± 21	47
POA y MPOA	-	-	48 ± 9	20 ± 5	7 ± 2	75 ± 19	568 ± 27	53

* Mean number of neuronal bodies counted in each hypothalamic section (40μm) ± standard deviation

Table III. Morphometric parameters obtained from GnRH neurones from the hypothalamic nuclei of the coypu.

Parameter	ACB	POA	Signification
Cellular area	180.47 ± 18.65	199.59 ± 21.34	N/S
Roundness	1.45 ± 0.57	1.45 ± 0.41	N/S
Major axis	21.37 ± 7.91	21.61 ± 8.28	N/S
Minor axis	9.31 ± 2.51	9.79 ± 2.89	N/S
Aspect	2.42 ± 1.00	2.31 ± 0 .99	N/S

Each number represents the mean parametric value ± standard deviation. N/S: non significant

last assumption, it could be assumed that all neurones evidenced in this study could correspond to those embryologically originated in the olfactory placoda and that they reach the rostral hypothalamus following a migratory route along the terminal nerve, as has been confirmed in previous studies for other mammals (ARAI *et. al.*, 1997; ZHENG *et. al.*, 1994).

The location of GnRH neurones in the coypu, largely coincides with the data reported in studies carried out on other rodents such as the rat (KAWANO & DAIKOKU), where most of these cells are located in the rostral hypothalamus. However, in the rat hypothalamus the highest concentration of GnRH neurones was described within the POA and MPOA. In the coypu the GnRH neurones were piled up within POA and ACB, showing few scattered neurones in BST, MPOA, DBB and SON. The immunoreactive neurones observed in the coypu showed a lengthened shape, were bipolar, and larger than those of the rat (KING & ANTHONY). On the other hand, there were no significant morphometric differences in the neuronal bodies of the POA with the neurones of other hypothalamic areas (BST, ACB, MPOA and SON), suggesting that would no functional or phylogenetic differences exist between the two groups of cells.

We have found differences between the estimated total number of neurones in the coypu (1072) and that reported for the rat (1300), mouse (750), hamster (800), papion (2400) (HOFFMAN *et. al.*, 1986), sheep and goat (2500) (ZUCCOLILLI *et. al.*). However, this study estimated the total number of immunoreactive neurones with a statistical analysis based on the amount of sectioned tissue as a base (ZUCCOLILLI *et. al.*), while other studies estimate the same figure based only on the quantity of processed sections (LEHMAN, *et al.*). The number of neurones for this species does not express a direct correlation with their encephalic size nor with their evolutionary level. Nevertheless, a numeric difference can be established between the data for the order rodentia and that obtained for ruminants and primates.

Primates (man and papion) (KING & ANTHONY) present a higher number of GnRH neurones in the mediobasal hypothalamus (ARC) while ruminants (sheep) (PENZIAS *et. al.*, 1995; LEHMAN *et al.* and CALDANI *et. al.*) and goats, (SILVA *et. al.*, 1996) only possess 6% of GnRH neuronal cells in this region. In contrast with these species, the coypu has demonstrated no GnRH immunoreactive neuronal bodies in this area. This finding allows us, not only to homologue the location of these neurones within the rodentia order, but also to infer that the coypu possesses a similar neural circuit to that of the rat for the government of sexual behaviour, where the pulsatil tonic liberation and release of GnRH in a preovulatory peak would be performed by neurones located in the rostral hypothalamus. On the other hand, the more common caudal location of this population in relation to phylogenetic evolution, suggests an anatomical modification of the rostral portion of the diencephalic floor, as can be observed in carnivores and primates in which a higher degree of visual system evolution is evident, mainly at the level of the optic chiasm and neighbouring regions.

Due to the fact that this study used only males, further studies in female individuals would confirm the homogeneity of the location and the morphometric values for the GnRH neurones.

ACKNOWLEDGMENTS

The authors wish to thank Silvina Viviana Macnie for her skilful technical assistance. This study was partially supported by a grant from the National Scientific Research Council (CONICET) and from the Secretariat for Science and Technology, National University of La Plata. ELP is a Research Carrier member of the CONICET.

SILVA, L.; SÁNCHEZ, H.; ACOSTA, W.; PORTIANSKY, E. & ZUCCOLILLI, G. GnRH neurones population in the diencephalon of the coypu (*Myocastor coypus*). ***Rev. Chil. Anat., 18(1)***:5-11, 2000.

SILVA, L.; SÁNCHEZ, H.; ACOSTA, W.; PORTIANSKY, E. & ZUCCOLILLI, G. Población de neuronas GnRH en el diencéfalo del coipo *(Myocastor coypus)*. ***Rev. Chil. Anat., 18(1)***:5-11, 2000.

RESUMEN: El objetivo del presente estudio fue investigar la distribución y morfología de las neuronas que sintetizan y almacenan el factor de liberación de gonadotrofinas (GnRH), en el diencéfalo del coipo (*Myocastor Coypus*), roedor sudamericano del Suborden histricomorpha. Para tales fines los encéfalos de tres coipos machos, adultos, fueron fijados por perfusión intra-arterial, utilizando una solución de paraformaldehído y ácido pícrico. Los bloques del hipotálamo fueron separados del resto del encéfalo y seccionados en micrótomo de congelación, obteniendo láminas coronales de 40µm de espesor. Las secciones fueron sometidas a un proceso de recuperación antigénica por ultrasonido, para desenmascarar los antígenos ocultos, luego las secciones fueron procesadas utilizando una técnica de inmunohistoquímica para evidenciar las neuronas GnRH, usando un anticuerpo monoclonal (LRH 13). Un grupo de secciones seriadas fue coloreada con violeta de cresilo (técnica de Nissl), mientras que, las otras secciones fueron coloreadas usando el método de Küver Barrera (azul luxol rápido y violeta de cresilo), para observar los núcleos y tractos nerviosos del hipotálamo, con el microscopio óptico. Los análisis morfométrico y cuantitativo de los cuerpos neuronales fue realizado usando un analizador de imágenes. Las neuronas GnRH inmunoreactivas observadas eran bipolares y alargadas. El número total de cuerpos neuronales para esta especie fue estimado en 1072 ± 27. Todas las células fueron localizadas en el hipotálamo rostral, principalmente en el área preóptica. Pocas neuronas fueron observadas en el núcleo de la estría terminal y en el núcleo medial preóptico hipotalámico. Las fibras inmunorreactivas fueron visibles en la capa externa de la eminencia media. De acuerdo a los datos obtenidos, se concluye que la distribución de las neuronas GnRH en el coipo coincide con la de otros roedores, tales como la rata.

PALABRAS CLAVE: 1. Coipo; 2. GnRH; 3. Hipotálamo; 4. Inmunohistoquímica; 5. Análisis de imágenes.

REFERENCE

ARAI, Y.; MURAKAMI, S.; SEKI, T; MIYAKAWA, T. & KAMIYA, M. Origin and migration of GnRH neurons in the chick embryo: an experimental study. In GnRH neurons: gene to behavior. ed. Tokyo. Brain Shuppan, 1997.

CALDANI, M.; BATAILLER, M.; THIERY, J.C. &. DUBOIS, M.P. LHRH immunoreactive structures in the sheep brain. *Histochemistry,* 89: 129-39, 1988.

DEES, W.L.; SORENSEN, A.M. Jr.; KEMP, W.M. & McARTHUR, N.H. Immunohistochemical localization of gonadotropin-releasing hormone (GnRH) in the brain and infundibulum of the sheep. *Cell. Tiss. Res.*, 215: 181-91, 1981.

FINK, G. Gonadotropin secretion and its control. The physiology of reproduction. ed. New York, Raven Press, 1988.

FOSTER, W.G. & YOUNGLAI, E.V. An immunohistochemical study of the GnRH neuron morphology and topography in the adult female rabbit hypothalamus. *Am. J. Anat.,* 191(3): 293-300, 1991.

HERSHKOVITZ, E. The resent mammals of the neotropical region: a zoogeographic and ecological review. *Quart Rev. Biol.;* 44: 1-70, 1969.

HOFFMAN, G.E.; PHELPS, C.J.; KHACHATURIAN, H. & SLADEK, J.R. Jr. Neuroendocrine projections to the median eminence. *Top. Neuroend.* 7: 161-87, 1986.

JENNES, L. & CONN, P.M. Gonadotropin-releasing hormone and its receptors in rat brain *Front. Neuroendocrinol.,* 15: 51-77, 1994.

KAWANO, M. & DAIKOKU, S. Immunohistochemical demonstration of LHRH neurons and their pathways in the rat hypothalamus. *Neuroendocrinology,* 32: 179-86, 1981.

KING, J.C. & ANTHONY, E.L. LHRH Neurons and Their Projections in Humans and other Mammals: Species Comparisons. *Peptides,* 5(1): 195-207, 1984.

LEHMAN, M.N.; ROBINSON, J.E.; KARSCH, F.J. & SILVERMAN, A.J. Immunocytochemical localization of luteinizing hormone-releasing hormone (LHRH) patways in the sheep brain during anestrus and the mid-luteal phase of the estrous cycle. *J. Comp. Neurol.,* 244: 19-35, 1986.

LEONG, A.S.Y. & GILHAM, P.N. The effects of progressive formaldehyde fixation on the preservation of tissue antigens. Pathology, 21: 266-68, 1989.

MARSHALL, P.E., & GOLDSMITH, P.C. Neurosecretory and neuroendocrine GnRH pathways in the hypothalamus and forebrain of the baboon. *Brain Res.* 193: 353-72, 1980.

NOVASEK, M. J. Mammalian phylogeny: Shaking the tree. *Nature, 365*: 121-25, 1992.

NOZAKI, M.; TSUKAHARA, T. & KOBAYASHI, H. Neuronal system producing LHRH in vertebrates. In: Endocrine correlates of reproduction, K. Ochiai and al, ed. Japan Science Society Press Springer, Tokyo. p. 3-27. 1984.

PARK, M.K. & WAKABAYASHI, K. Preparation of a monoclonal antibody to common amino acid sequence of LHRH and its application. *Endo-crinology,* 33(2):257-72, 1986.

PATTERSON, B. & PASCUAL, R. The fossil mammals fauna of South America. In: Keast A., Erik F.C., Glass B., ed. Evolution, Mammals and Southern Continents. New York: The University of New York Press, Albany, p. 247- 309, 1972.

PENZIAS, A.S.; GOODMAN, S.R.; ROSSI, G. et al. Preliminary evidence that GnRH has the properties of a growth hormone-releasing factor in vivo in the rat. *J. Soc. Gynecol. Invest.; 2*:623-9, 1995.

PORTIANSKY, E.L. & GIMENO, E.J. A new epitope retrieval method for the detection of structural cytokeratins in the bovine prostatic tissue. *Appl. Inmunohistochem.,* 4:.208-14, 1996.

SILVA, L.; SANCHEZ, H. & ZUCCOLILLI, G. Estudio sobre la Morfología de las Neuronas GnRH identificadas por inmunohistoquímica en el hipotálamo de Cabras Domésticas. *Res. 1ras Jorn. Cient. Soc. Cien. Morf. La Plata.*, 47, 1996.

THIÉRY, J.C. &. MARTIN., G.B. Neurophysiological control of the secretion of gonadotropin-releasing hormone and luteinizing hormone in the sheep - A review. *Reprod. Fertil. Dev.,* 3:137-73, 1991.

ZAMBONI, L. & MARTINO, D. Buffered picric acid paraformaldehyde: a new rapid fixative for electron microscopy. *J. Cell Biol.,* 35:148, 1967.

ZHENG, L.M.; PFAFF, D.W. & SCHWANZEL-FUKUDA M. Electron microscopic identification of luteinizing hormone-releasing hormone-inmunoreactive neurons in the medial olfactory placode and basal forebrain of embrionic mice. *Neuroscience,* 46:407-18, 1991.

ZUCCOLILLI, G.; HAMADA, M. &. MORI, Y. Gonadotropin-releasing hormone (GnRH) neuron in the forebrain of male Shiba goat. *J. Reprod. Dev., 40*:19-25, 1994.

Dirección para correspondencia:
Prof. Dra. Liliana B. Silva
Instituto de Anatomía
Facultad de Ciencias Veterinarias
Universidad Nacional de La Plata
Calle 60 y 118
1900 La Plata - ARGENTINA

Teléfono: 54-221-4236664 ext 423
Fax: 42-221-4253276
Email:guoszucc@fcv.medvet.unlp.edu.ar

Recibido : 09-11-1999
Aceptado: 22-01-2000