Jurnal Kedokteran Hewan
P-ISSN : 1978-225X; E-ISSN : 2502-5600

March 2019, 13(1):19-23
DOI: https://doi.org/10.21157/j.ked.hewan.v13i1.12044

DEVELOPMENT OF THE CORTICAL AND MEDULLARY CELLS OF ADRENAL GLAND IN THE LONG-TAILED MACAQUE (*Macaca fascicularis*) DURING PRE- AND POSTNATAL PERIOD

Danang Dwi Cahyadi[1], Nurhidayat[1]*, Supratikno[1], Savitri Novelina[1], Chairun Nisa'[1], and Heru Setijanto[1]
[1]Department of Anatomy Physiology and Pharmacology, Faculty of Veterinary Medicine, IPB University (Bogor Agricultural University), Bogor, Indonesia
*Corresponding author: nhdayat@apps.ipb.ac.id

ABSTRACT

The study aimed to analyze the dynamics of the development of cortical and medullary cells of adrenal gland in the long-tailed macaque (*Macaca fascicularis*) during pre- and postnatal period. The samples of adrenal glands were taken from 5 fetuses aged 70, 85, 100, 120, and 150 days of gestation, a newborn aged 10, and an infant aged 105 days old. The samples of the adrenal glands were stained with hematoxylin-eosin and Masson trichrome (Goldner's modification). The results showed that the fetal adrenal cortex consisted of a definitive and fetal zones, but in the late gestation (fetus aged 150 days of gestation), the transitional zone appeared between both zones. At the postnatal period, the definitive zone changed to glomerulosa zone, while the outer cells of the transitional zone changed to fasciculata zone which radially arranged. The results indicated that the fetal zone was present until birth and it will disappear gradually after birth. The medullary cells were found at the fetal stages as an irregular structure of small islands of chromaffin cells in the fetal zone. This structure migrated slowly to the center of the adrenal glands. The characteristics of mature chromaffin cells were found in the adrenal glands at postnatal period. In conclusion, the cortex adrenal of the newborn of long-tailed macaque consisted of zona glomerulosa, zona fasciculata, zona transitional, and the remaining fetal zone that has dissapeared gradually after birth.

Key words: adrenal gland, cortical cells, development, medullary cells, monkey

ABSTRAK

Penelitian ini bertujuan mempelajari perkembangan sel-sel penyusun korteks dan medula kelenjar adrenal monyet ekor panjang (Macaca fascicularis) pada masa pra- dan pascalahir. Sampel kelenjar adrenal didapatkan dari 5 fetus berumur 70, 85, 100, 120, dan 150 hari kebuntingan dan seekor bayi monyet berumur 10 hari serta anak monyet umur 105 hari. Sampel kelenjar adrenal diwarnai dengan pewarna hematoksilin-eosin dan Masson trichrome (modifikasi Goldner). Hasil penelitian menunjukkan bahwa korteks adrenal fetus tersusun atas zona definitif dan zona fetus yang pada akhir masa kebuntingan (fetus umur 150 hari kebuntingan), zona transisional dapat diamati antara kedua zona tersebut. Pascalahir, zona definitif berkembang menjadi zona glomerulosa dan sel-sel bagian luar zona transisional berkembang menjadi zona fasikulata yang tersusun secara radial. Hal tersebut mengindikasikan bahwa zona fetus masih ditemukan pascalahir, kemudian menghilang secara perlahan setelah lahir. Sel-sel penyusun medula adrenal berupa struktur ireguler pulau-pulau sel chromaffin ditemukan di zona fetus pada tahap fetus. Struktur ini bermigrasi secara perlahan menuju bagian pusat kelenjar adrenal. Karakteristik sel-sel chromaffin dewasa ditemukan pada kelenjar adrenal pada masa pascalahir. Dapat disimpulkan bahwa adrenal kortek dari monyet ekor panjang yang baru lahir terdiri atas zona glomerulosa, zona fasciculata, zona transitional, dan zona fetus yang tersisa yang menghilang secara bertahap.

Kata kunci: adrenal, sel korteks, perkembangan, sel medula, monyet

INTRODUCTION

Adrenal glands in mammals are composed of cortex derived from lateral mesoderm layer and medulla derived from the differentiation of neural crest cells (Turner, 1976; Lohr *et al.*, 2006). The development of primate adrenal glands starts by the formation of fetal adrenal cortex, consisting of definitive zone and fetal zone, which then differentiate to form adult adrenal cortex (Coulter, 2004). The structure of primate adrenal cortex is similar to that of other mammals, composed of zona glomerulosa, zona intermedia, zona fasciculata, and zona reticularis.

Adrenal cortex plays important roles in the body physiology in terms of the mechanism of hormon action, affected by pituitary gland which secretes adrenocorticotropic hormone (ACTH). This hormone plays a role in stimulating the development of cortical cells and inhibiting the maturation of fetal zone to adult conformation (Friedrich, 1996). Hypophysectomy in *Macaca mulatta* (*M. mulatta*) was reported to cause cessation in the development of adrenal cortex (McNulty *et al.*, 1981), which is fatal to the body (Lin and Achermann, 2004). In prenatal period, fetal adrenal cortex plays a role in cortisol synthesis (fetal cortisol), related to the maturation of fetal organ system. In addition, cortisol also functions as regulator at birth (Messiano and Jaffe, 1997). Fetal adrenal gland cortex secretes steroid hormones which have crucial roles in the regulation of intrauterine homeostasis and maturation of fetal organ system for the postnatal life (Dumitrescu *et al.*, 2007; Ishimoto and Jaffe, 2011).

The adrenal medulla as a separate structure cannot be found in primate fetal adrenal gland during most of gestation period. Nevertheless, during the gestation period, structures of small islands of chromaffin cells could be found, scattered in the cortex (Messiano and Jaffe, 1997). The cells of adrenal medulla, chromaffin and ganglion cells, develop from sympathoadrenal cells originating from neural crests (Kmieć and Kaczmarczyk, 2004; Huber, 2006).

Information about the development of adrenal gland is important as a basis for scientific research in health and biomedicine. Many biomedical studies are known to use nonhuman primates as animal model. Nonhuman primates have similar characteristics to human in terms of genes, anatomy, physiology, behavior, and susceptibility to various infectious and degenerative diseases (Iskandriati and Pamungkas, 2008). *Macaca fascicularis* (*M. fascicularis*) from the family Cercopithecidae is one of the nonhuman primates that are commonly used as animal model in biomedical research (Fortman *et al*., 2002).

Several studies have analyzed the postnatal development of the adrenal glands in marmoset monkey (Pryce *et al.*, 2002) and rats (Lohr *et al.*, 2006), as well as the pre- and postnatal period in *M. mulatta* fetus and infants (McNulty *et al.*, 1981). However, data on the development of the adrenal glands in *M. fascicularis* are very limited, thus studies on the pre- and postnatal development of the adrenal glands in *M. fascicularis* are still needed. This study aimed to analyze the dynamics of the development of cortical and medullary cells of adrenal gland in the long-tailed macaque during the pre- and postnatal period.

MATERIALS DAN METHODS

Sample of Adrenal Glands

This study used the adrenal glands of *M. fascicularis* fetuses aged 70 days (F70), 85 days (F85), 100 days (F100), 120 days (F120), and 150 days (F150) of gestation, and a newborn aged 10 days (A10) and an infant aged 105 days (A105). The samples were obtained from the Primate Research Center, Bogor Agricultural University (PSSP IPB). The procedure used to obtain the *M. fascicularis* fetuses, a newborn, and an infant referred to Pangestiningsih *et al.* (2009). The procedures for sample collection were carried out under the permission by the Animal Care and Use Committee of Primate Research Center in Bogor Agricultural University (No. 02-0030IR).

Histological Staining Procedure

The adrenal glands were placed in tissue cassette and dehydrated by sequentially immersing in graded alcohols solutions at 70%, 80%, 90%, and 95%. The glands were immersed in each alcohol for 6 hours at a room temperature followed by three immersions in absolute alcohols for 1 hour at a room temperature. The following process was clearing using xylene for 30 minutes three times. After clearing, the infiltration of paraffin was done in three repetitions, 30 minutes for each repetition, at 60° C. Further, the adrenal tissues were placed embedded in parafin.

The sections (10 µm) of the gland were then mounted on an aminopropyltriethoxysilane (APES)-coated glass slides. The slides were incubated at 37° C in a horizontal position for one night, deparaffinized by three washes in xylene, rehydrated in graded alcohols. The next process was staining with standard hematoxylin-eosin (HE) and Masson trichrome (Goldner's modification). After staining process, slides were washing-rinsing, dehydration, clearing, and mounting.

RESULTS AND DISCUSSION

Development of Adrenocortical Cells

The adrenal glands size of *M. fascicularis* in this study increased starting from F70, F85, F100, F120, and F150, then decreased at A10 (Figure 1). The decrease in size occurred because the cells in fetal zone experienced slow regression. In addition, the decrease in size of the adrenal glands is considered to be related to the decrease in weight of those glands, as reported in the adrenal glands of *M. mulatta* which grow rapidly at the second half of gestation period, but then decrease in weight by around one third at the first two week postnatal (McNulty *et al.*, 1981). During the development from the pre- to postnatal period, fetal adrenal glands experience morphological and functional changes into adult adrenal glands (Ishimoto and Jaffe, 2011).

The adrenal glands structure of F70, F85, F100, and F120 consisted of two different zones, namely definitive zone and fetal zone, similar to those in primates including human (McNulty *et al.*, 1981; Messiano and Jaffe, 1997; Coulter, 2004). The definitive zone in the adrenal glands of *M. fascicularis* is located in the superficial layer, right in the profundal of fibrous capsule. The structure of definitive zone is composed of small and dense layers of basophilic cells, while the fetal zone is composed of acidophilic cuboid or polygonal cells. The definitive zone of the glands in F85, F100, and F120 underwent a change-the cells grew in size. Some characteristics of the development of primate fetal adrenal cortex are rapid cell growth, high steroidogenic activity, and unique morphological development (Messiano and Jaffe, 1997).

The proliferation of the definitive zone cells of the glands of F85, F100, and F120 were already observable, while the cell differentiation was not observed. The proliferation of the fetal zone cells occurred more rapidly, making this zone have larger area compared to the definitive zone, around 80-90% from the cortical thickness (Messiano and Jaffe, 1997). This is similar to the condition observed in *M. mulatta*, which at the age of 145 days of the gestation period, the fetal zone has a thicker area along with an increased of the adrenal glands weight (McNulty *et al*., 1981).

The development that occurred in the gland of F150 can be observed due to the presence of transitional zone layer (dense band) located between the definitive zone and the fetal zone, similar to a condition in *M. Mulatta* (McNulty *et al.*, 1981). These transitional zone cells play a role in the secretion of cortisol nearing the end of gestation (Kempná and Flück, 2007). The proliferation of adrenal cortex cells decreases, followed by a differentiation of fetal adrenal cortex cells, causing an increase in steroid production (Dumitrescu *et al*., 2007).

The differentiation of the definitive zone cells was observed in the gland of A10, so that zona glomerulosa and zona fasciculata were identifiable (Figure 2). In humans, the definitive and transitional zones were completely differentiated at 3 years old (Kempná and

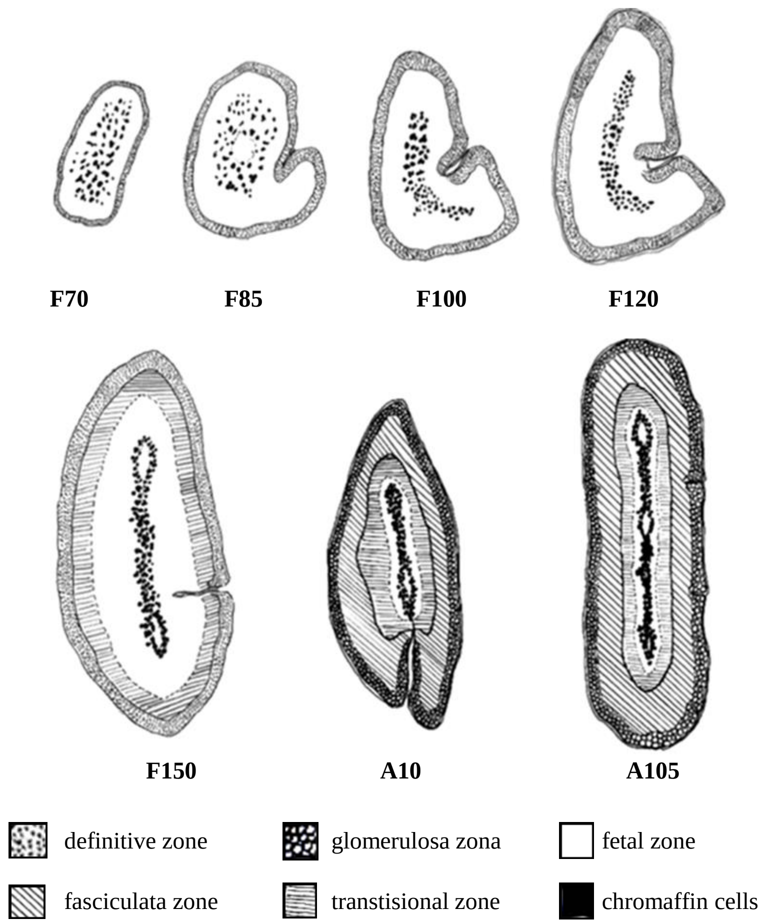

Figure 1. Schematic illustration of the adrenal gland development in the long-tailed macaque

Flück, 2007). The cells in the definitive zone of the fetal adrenal cortex at the end of gestation have similar functions to those in the zona glomerulosa of the adult adrenal cortex that playing a role in the secretion of enzyme for aldosterone (Messiano and Jaffe, 1997). Based on this function, the cells in the definitive zone are supposed to be origin of the development of zona glomerulosa cells. On the other hand, the cells in the transitional zone in the fetal adrenal cortex have the same function as those in the zona fasciculata in the adult adrenal cortex, for cortisol secretion (Sirianni *et al.*, 2005). In addition, the cells in the zona fasciculata have lots of fat vacuoles, so it is believed that the cells in the zona fasciculata develop from the acidophilic cells in the transitional zone (Messiano and Jaffe, 1997). The cells in the transitional zone have the same characteristics as those in the zona fasciculata of adult adrenal cortex (Scheys *et al.*, 2011).

The structure of the glands in A105 already developed like the adult adrenal glands. The adrenal cortex in A105 consisted of glomerulosa zone, fasciculata zone, transitional zone, and fetal zone (Figure 2). Morphologically, the zones in the adrenal cortex showed differences in their structures and cellular compositions. The homeostasis in the adrenal cortex and the cellular regeneration process are influenced by steroidogenic cell migration from the superficial layer to the more profundal layer, accompanied by the conversion of the cells in zona glomerulosa into the cells in zona fasciculata (Pignatti *et al.*, 2017).

Zona reticularis cells were not found in the A105 adrenal glands as in *M. mulatta* aged 180 days old (McNulty *et al.*, 1981). The zona reticularis cells in humans start to develop at 4 years old and differentiate perfectly at 15 years old (Kempná and Flück, 2007). Zona reticularis cells are believed to develop from the transitional zone (McNulty *et al.*, 1981). Functional differentiation and adrenocortical zonation are regulated by hormones which will then be involved in steroidogenesis, as a growth factor as well as transcription and cell cycle regulator (Kempná and Flück, 2007). The development of the transitional zone in baboon fetal adrenal cortex at the end of gestation period is affected by the secretion of ACTH, while the development and maturation of the definitive zone is not affected by ACTH (Leavitt *et al.*, 1999).

Development of Adrenal Medullary Cells

During fetal period, adrenal medulla has not yet formed in primate fetus, only chromaffin cells are found that forming a structure of islands of chromaffin cells and scatterred in the fetal zone (Messiano and Jaffe, 1997). The structure of islands of chromaffin cells in the glands in F70 was found in the fetal zone with randomly distributed. The chromaffin cells in

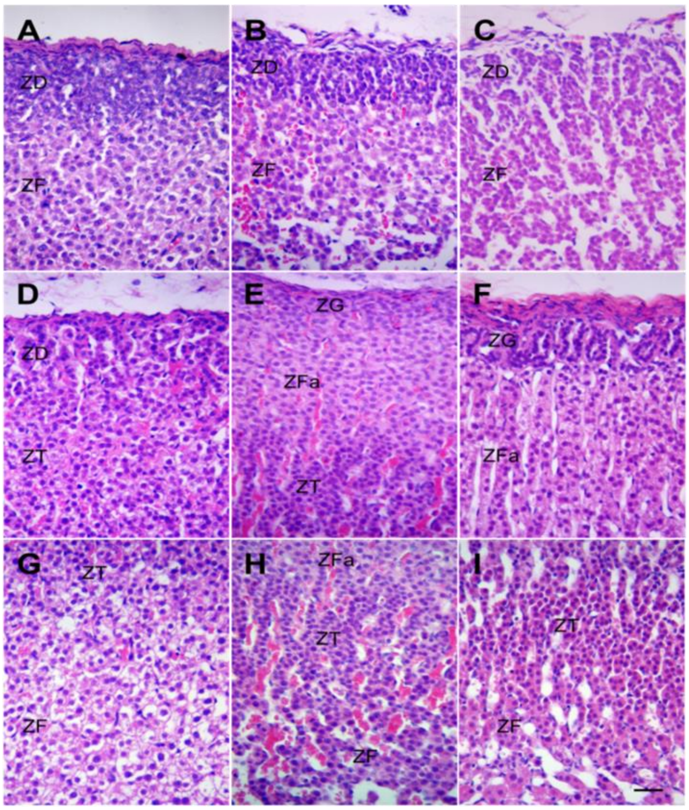

Figure 2. Representative micrographs of hematoxylin-eosin stained adrenal gland sections in A-D fetuses (F85, F100, F120, F150 respectively) and E-F infants (A10, A105) of long-tailed macaque, G-I profundal part of F150, A10, and A105 respectively. ZD= Definitive zone, ZF= Fetal zone, ZG= Glomerulosa zone, Zfa= Fasciculata zone, and ZT= Transitional zone. Scale bar= 30 µm

humans' fetal adrenal glands are found in the second trimester of gestation where those cells can identify due to the presence of catecholamines and neuropeptides (Zhou *et al.*, 2006).

An increased density of chromaffin cells was observed in the gland of F85, spread in the fetal zone to the center of the glands. The increased density of chromaffin cells occurred due to cell proliferation that took place at that age. According to Frödin and Gammeltoft (1994), the chromaffin cells proliferation in mammals occur during the prenatal and early postnatal period, however in adult adrenal glands, this proccess is inhibited by glucocorticoid compounds. The chromaffin cells in the gland of F85 were arranged circularly with large and bright cytoplasm after slides were stained with Masson trichrome staining. The cells in the fetal zone were found around the structure of the islands of the chromaffin cells.

The size of chromaffin cells increased in the gland of F100, the cytoplasm also grew larger, making the structure of the islands of the chromaffin cells denser. The constituent cells of the adrenal medulla in the gland of F120 started to gather and be concentrated in the center of the glands. At this age, the nuclei of the chromaffin cells were larger compared to those at younger age. This is believed to be due to an increased functional activity of the chromaffin cells. The structure of the islands of the chromaffin cells in the gland of F150 was concentrated around the vena centralis. In the postnatal adrenal glands of *M. fascicularis* (A10 and A105), structures similar to those in adult adrenal medulla were observable. The characteristics of the chromaffin cells in the postnatal adrenal glands in *M. fascicularis* changed, the nuclei grew larger yet the cytoplasm volume became smaller (Figure 3). The chromaffin cells formed the parenchyma of the adrenal medulla. In addition to chromaffin cells, ganglion cells were also found in the glands of A10 and A105. The ganglion cells have the granular cytoplasm as described in Kmieć and Kaczmarczyk (2004). In the first week postnatal, chromaffin cells in primate adrenal glands are concentrated around vena centralis, which then will form medulla. Chromaffin cell groups in primates are found in the center of the glands in the fourth week postnatal (Messiano and Jaffe, 1997), which will then form adrenal medulla after fetal zone regression (Lalli, 2010).

Vascularity in Adrenal Glands

Vascularization of the adrenal glands was already observable in the gland of F70 by the presence of sinusoid capillaries in between the rows of the fetal zone cells. Development of vascularization in primate adrenal glands is marked by the presence of sinusoids in the fetal zone in the ninth week of gestation (Messiano and Jaffe, 1997). A similar condition was found in the glands of F85, F100, F120, F150, A10, and A105, particularly near the center of the glands. Vascularization in fetal adrenal glands makes it as one of the hypervascular organs in primate fetus, which is dominant in the areas near the center of the glands (Messiano and Jaffe, 1997). The venous networks found in fetal adrenal cortex are also believed to play a

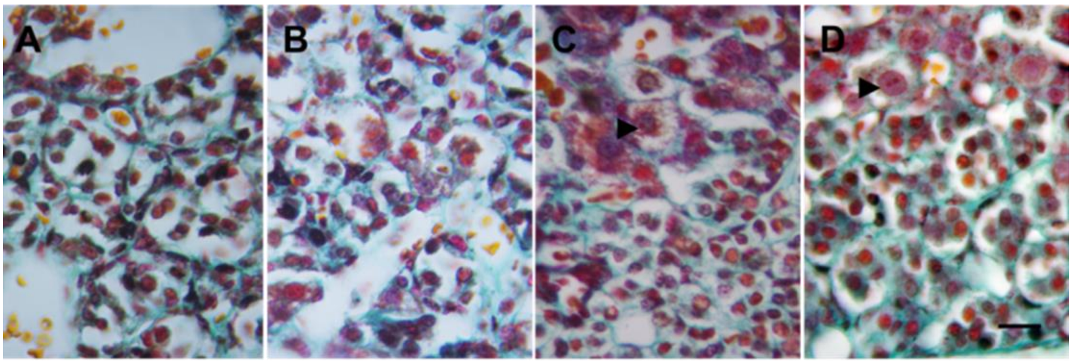

Figure 3. Histological characteristic of chromaffin cells of Masson-trichrome stained adrenal gland in long-tailed macaque. A= F120, B= F150, C= A10, and D= A105. At the postnatal period, the nucleus was larger while the cytoplasmic volume was smaller. Ganglion cells (►) were found in the medula adrenal at the postnatal periods. Scale bar: 20 μm

role in the migration of chromaffin cells (Lohr *et al.*, 2006). The vascular system of the fetal adrenal glands in *M. fascicularis* plays a role in the migration of sympathetic neuronal precursor cells. Artemin, a factor from blood vessels, is essential for the development of sympathetic neurons (Allmendinger *et al.,* 2003). Large venous structures in the center of the adrenal glands were observable in the glands of F150 and at later ages. Sinusoids among the radially arranged zona fasciculata cells were clearly observable in the glands in A105.

CONCLUSION

The cortex adrenal of the newborn of long-tailed macaque consisted of zona glomerulosa, zona fasciculata, zona transitional, and the remaining fetal zone that has dissapeared gradually after birth.

ACKNOWLEDGEMENT

We thank to Primate Research Center, Bogor Agricultural University (PSSP, LPPM IPB) for granting permits to use the animals from the research center, and for their valuable technical support on this research.

REFERENCES

Allmendinger, A., E. Stoeckel, M. Saarma, K. Unsicker, and K. Huber. 2003. Development of adrenal chromaffin cells is largely normal in mice lacking the receptor tyrosine kinase c-Ret. **Mech. Dev.** 120:299-304.

Coulter, C.L. 2004. Functional biology of the primate fetal adrenal gland: Advances in technology provide new insight. **Clin. Exp. Pharmacol. Physiol.** 31:475-484.

Dumitrescu, A., G.W. Aberdeen, G.J. Pepe, and E.D. Albrecht. 2007. Developmental expression of cell cycle regulators in the baboon fetal adrenal gland. **J. Endocrinol.** 192:237-247.

Fortman, J.D., A.H. Terry, and B. Taylor Bennet. 2002. **The Laboratory Nonhuman Primate**. CRC Press, Florida.

Friedrich, M. 1996. Influence of exogenous and endogenous ACTH on adrenal cortex activity in calves during the early postnatal period. **Br. Vet. J.** 152:315-320.

Frödin, M. and S. Gammeltoft. 1994. Insulin-like growth factors act synergistically with basic fibroblast growth factor and nerve growth factor to promote chromaffin cell proliferation. **Proc. Natl. Acad. Sci. USA.** 91(5):1771-177.

Huber, K. 2006. The sympathoadrenal cell lineage: specification, diversification, and new perspectives. **Dev. Biol.** 298:335-343.

Ishimoto, H. and R.B. Jaffe. 2011. Development and function of the human fetal adrenal cortex: A key component in the feto-placental unit. **Endocr. Rev.** 32:317-355.

Iskandriati, D. and J. Pamungkas. 2008. Skrining beberapa patogen penting untuk menghasilkan satwa primata sebagai hewan laboratorium. **Proceeding of KIVNAS 2008**. Bogor, Indonesia.

Kempná, P. and C.E. Flück. 2007. Adrenal gland development dan defects. **Best Pract. Res. Clin. Endocrinol. Metab.** 1(22):77-93.

Kmieć, B.L. and D. Kaczmarczyk. 2004. The cellular composition of the adrenal medulla of sexually mature guinea pigs. **Folia Morphol.** 63(1):75-78.

Lalli, E. 2010. Adrenal cortex ontogenesis. **Best Pract. Res. Clin. Endocrinol. Metab.** 24:853-864.

Leavitt, M.G., E.G. Albrecht, and G.J. Pepe. 1999. Development of the baboon fetal adrenal gland: Regulation of the ontogenesis of the definitive and transitional zones by adrenocorticotropin. **J. Clin. Endocrinol. Metab.** 10(28):3831-3835.

Lin, L. and J.C. Achermann. 2004. The adrenal. **Horm. Res. Paediatr**. 62(suppl.3):22-29.

Lohr, J., P. Gut, N. Karch, K. Unsicker, and K. Huber. 2006. Development of chromaffin cells in *Sf1* heterozygous mice. **Cell Tissue Res.** 325:437-444.

McNulty, W.P., M.J. Novy, and S.W. Walsh. 1981. Fetal and postnatal development of adrenal glands in *Macaca mulatta*. **Biol. Reprod.** 25:1079-1089.

Messiano, S. and R.B. Jaffe. 1997. Developmental and functional biology of the primate fetal adrenal cortex. **Endocrinol. Rev.** 18:378-403.

Pangestiningsih, T.W., A. Hendrickson, K. Sigit, D. Sajuthi, Nurhidayat, and D.M. Bowden. 2009. Development of the area postrema: an immunohistochemical study in the macaque. **Brain Res.** 1280:23-32.

Pignatti, E., S. Leng, D.L. Carlone, and D.T. Breault. 2017. Regulation of zonation and homeostasis in the adrenal cortex. **Mol. Cell. Endocrinol.** 441:146-155.

Pryce, C.R., R. Palme, and J. Feldon. 2002. Development of pituitary-adrenal endocrine function in the marmoset monkey: infant hypercortisolism is the norm. **J. Clin. Endocrinol. Metab.** 87(2):691-699.

Scheys, J.O., J.H. Heaton, and G.D. Hammer. 2011. Evidence of adrenal failure in aging Dax1-deficient mice. **Endocrinology.** 152(9):3430-3439.

Sirianni, R., K.S. Rehman, B.R. Carr, C.R. Parker, and W.E. Rainey. 2005. Corticotropin- releasing hormone directly stimulates cortisol and the cortisol biosynthetic pathway in human fetal adrenal cells. **J. Clin. Endocrinol. Metab.** 90(1):279-285.

Turner, C.D. and J.T. Bagnara. 1976. **General Endocrinology**. WB Saunders, Philadelphia.

Zhou, H., J. Aziza, J.C. Sol, M. Courtade-Saïdi, S. Chatelin, C. Evra, O. Parant, Y. Lazorthes, and S. Jozan. 2006. Cell therapy of pain: characterization of human fetal chromaffin cells at early adrenal medulla development. **Exp. Neurol.** 198:370-381.