AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE OVARY OF THE IMMATURE OSTRICH (*STRUTHIO CAMELUS*)

By

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A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in the Department of Anatomy and Physiology in the Faculty of Veterinary Science, University of Pretoria

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Table of contents

List of figures

List of tables

SUMMARY

AN IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE OVARY OF THE IMMATURE OSTRICH (*STRUTHIO CAMELUS*)

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The aim of this study was to investigate the components of the ovary in the sexually immature ostrich by using immunohistochemistry, light microscopy and electron microscopy. The light and electron microscopic studies carried out, revealed that the oocyte in the sexually immature ostrich is surrounded by seven layers which included the *zona radiata, lamina perivitellina, stratum granulosum, basal lamina*, thecal layers (theca interna and theca externa), connective tissue layer and superficial epithelium (see details in Chapter Two and Three). Several morphological and immunohistochemical changes occurred as the follicles developed and regressed, suggesting that ovarian follicles in the sexually immature ostrich undergo a cycle of growth and degeneration as reported in other avian species.

In the present study, thecal gland cells in the ovary of the sexually immature ostrich were common. In addition, interstitial gland cells were a notable feature in atretic follicles as described in the ovary of the crow, common myna and dove (Guraya and Chalana, 1976). Further investigations on the interstitial gland cells will provide an insight into the process of steroidogenesis in the sexually immature ostrich.

As discussed in Chapter five, various cells in the ovary showed immunoreactivity to oestrogen, progesterone and androgen receptors. These observations indicated that the ovarian tissue in the sexually immature ostrich is a potential target for gonadal hormones. Thus, it can be assumed that steroid hormones regulate ovarian functions in the ostrich.

The use of immunohistochemical procedures proved to be an excellent method to investigate the distribution of nerves in the ovary. The results of this study have shown that the ovary in the sexually immature ostrich is well-innervated. However, further studies are required to differentiate between cholinergic and adrenergic nerve fibres.

Declaration

I hereby declare that the work presented here is my original work. To the best of my knowledge, this work has never been published or submitted for a degree in this University. The University of Pretoria reserves the right of permission for duplication of the whole thesis or in part thereof.

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W.H. Kimaro

November, 2005.

Foreword

The main reason for conducting the present study was the lack of information on the morphology of the ovary in the sexually immature ostrich. A total of 26 sexually immature female ostriches aged between 12 and 14 months were used in the present study. Ovarian tissue samples were collected during the active reproductive phase (August to February), the regressive reproductive phase (March to early May) and the inactive reproductive phase (Late May to July). Tissue samples were processed routinely for light and electron microscopic studies. Immunohistochemistry was performed on either frozen or paraffin-embedded sections.

The objectives of Chapter Two and Chapter Three were to investigate the histological and ultrastructural organization of the ovary in the sexually immature ostrich. At the light microscope level, healthy and atretic primordial, previtellogenic and vitellogenic follicles were observed. The healthy follicles were composed of an oocyte surrounded by a granulosa cell layer and a thecal layer.

At the electron microscope level, granulosa cells in healthy follicles displayed apical cytoplasmic processes. Attached to the cytoplasmic processes were transosomes. A basal lamina separated the granulosa cell layer from the underlying thecal layer. The basal lamina closest to the granulosa cell layer was more electron dense than that adjacent to the thecal layer. The thecal layer contained undifferentiated (type I) and differentiated (type II) thecal gland cells, as well as vacuolated thecal cells. The type I thecal gland cells

contained a large oval or elongated nucleus, which exhibited clumps of heterochromatin. The nuclei of type II thecal gland cells were round to oblong in shape with a prominent nucleolus.

Non-bursting atresia was observed in all follicular sizes. The granulosa cells of atretic primordial and previtellogenic follicles contained numerous lipid droplets and electron dense bodies. Very few transosomes were observed in atretic follicles. Two forms of atresia were observed in vitellogenic follicles. Type I atresia resulted in the infiltration of the entire follicle by hyalinized connective tissue. In type II atresia, granulosa and theca interna cells differentiated into interstitial gland cells. These results indicate that the structural organization of the ovary in the sexually immature ostrich is similar to that reported in other avian species. In addition, it is apparent that ovarian follicles in the sexually immature ostrich undergo a cycle of growth and degeneration.

The objective of Chapter Four was to study the distribution of the intermediate filaments, desmin, vimentin and smooth muscle actin, in the ovary of the sexually immature ostrich. Positive immunostaining for desmin was observed in the granulosa cells of healthy primordial and previtellogenic follicles. Vimentin immunoreactivity was demonstrated in the granulosa cells of all follicles except the vitellogenic atretic follicles. Fibroblasts in healthy and atretic (type I) follicles exhibited strong immunostaining for smooth muscle actin. The results of this chapter suggested that the distribution of intermediate filaments changes during follicular development and atresia.

The objective of Chapter Five was to determine the distribution of steroid hormone receptors in the ovary of the sexually immature ostrich. Strong immunostaining for the oestrogen receptor, progesterone receptor and androgen receptor was observed in the nuclei of the germinal epithelium. Granulosa cells were immunopositive for the progesterone and androgen receptors, but not for the oestrogen receptor. However, positive immunoreactivity for the oestrogen receptor was exhibited in thecal gland cells. The distribution of steroid hormone receptors in the present study appears to be similar to that described in the domestic fowl.

The objective of Chapter Six was to describe the intrinsic innervation of the ovary using antibodies against neurofilament protein, protein gene product 9.5, and neuron specific enolase. Strong immunostaining for neurofilament protein, protein gene product 9.5 and neuron specific enolase was observed in nerve bundles, which coursed through the ovarian stalk and extended into the medulla and cortex. Neuron specific enolase immunoreactive nerve cell bodies were observed in the ovarian stalk and medulla. In addition, thecal and interstitial gland cells demonstrated neuron specific enolase immunostaining. Based on the results of this immunohistochemical study, it would appear that the distribution of immunoreactive nerve fibres in the ovary of the sexually immature ostrich resembles that of the domestic fowl.