REPRODUCTIVE BIOLOGY OF THE MALE AUSTRALIAN PARROT

Erica Lovas
School of Veterinary Science
The University of Queensland
St Lucia, Australia 4072.
and:
School of Animal Studies
The University of Queensland
Gatton, Australia 4343

Steve Johnston
School of Animal Studies
The University of Queensland
Gatton, Australia 4343

Lucio Filippich
School of Veterinary Science
The University of Queensland
St Lucia, Australia 4072.

INTRODUCTION

Parrots are a diverse family of birds comprising more than 350 species (Forshaw, 2006). Australia has been called *Terra Psittacorum,* 'land of the parrots' because it possesses approximately one-sixth of the world's species (Forshaw, 2002). Parrots are widely distributed throughout Australia occupying a diverse range of habitats from deserts to rainforests. Worldwide, avian species continue to come under threat by the actions of humans, with the principal threats being loss of habitat (e.g. food resources and availability of nesting hollows) and the illegal pet trade (Forshaw, 2002). Parrots are one of the most threatened groups of birds with approximately 13% of all species being classed as critically endangered or endangered and a further 89 species classed as vulnerable to near threatened (BirdLife International 2010). These conservation threats pose an uncertain future for the parrot taxon and highlight the need for further studies that allow assessment of reproductive status and the development of improved captive breeding programs.

While most Australian parrots are under some kind of conservation pressure there are other generalist species such as the galah, various corellas and the sulphur-crested cockatoo that have adapted well to the habitat changes associated with the use of agricultural development of the landscape for crops. These species can occur in large numbers and present as pests to farmers and threats to more endangered species by competing for resources. These species frequently raise 3 or more fledglings as an adaptation to cope with variable environments (Forshaw, 2002). Shooting, trapping or poisoning of parrots are typically promoted as mitigation measures despite there ineffectiveness in reducing actual crop damage (Forshaw, 2002).

Irrespective of dealing with endangered species or overabundant Australian parrot species there is a dearth of information on their reproductive anatomy, endocrinology and dynamics of sperm production; this is the case even in the most popular and domesticated species. A recent literature research revealed no descriptions of the gross reproductive anatomy of any Australian species, even in the overabundant species and only limited studies of spermatogenesis in parrot species worldwide,

yet such information is needed both for the conservation of parrots but also into the management of problematic species.

This study used the cockatiel (*Nymphicus hollandicus*) as a model to examine male parrot reproduction. The cockatiel is an endemic species to Australia and is the smallest member of the Cacatuidae family. The cockatiel is not only an exceptionally successful avicultural species but also an excellent model species given its availability, hardiness and willingness to breed in captivity (Yamamoto et al., 1989).

This project originally aimed to investigate semen collection and manipulation in the cockatiel. This was thought to be a relatively easy first up feat considering it had been done successfully in the much smaller budgerigar (Samour et al., 1988). However, despite persistent efforts and accounting for seasonal reproduction, semen could not be collected on a regular basis; leading to the question of how much is really known about general reproductive biology in male parrots and how different is it from a closely related species like the budgerigar? Therefore, based on this early set back the projects aims were refocused to explore more fundamental elements of cockatiel reproduction, including gross and micro anatomy of the male reproductive tract, spermiogenesis and testosterone secretion. Opportunistically, aspects of the comparative reproductive anatomy of the male galah (*Eolophus roseicapilla*), sulphur-crested cockatoo (*Cacatua galerita*), long-billed corella (*Cacatua tenuirostris*), pale-headed rosella (*Platycercus adscitus*) and rainbow lorikeet (*Trichoglossus haematodus*) were also investigated.

GROSS AND MICROSCOPIC ANATOMY

Not surprisingly, most studies on the reproductive anatomy of birds have focused on the economically important species for which reproductive anatomy has been studied in order to increase reproductive potential (Burke, 1993; Johnson, 2004); such species include the domestic fowl (*Gallus domesticus*), turkey (*Meleagris gallapavo*), duck (*Anas platyrhynchos*) and Japanese quail (*Coturnix coturnix japonica*). Unfortunately, much of the reproductive anatomical information collected from commercial species is not directly applicable to wild or endangered birds because production species have been genetically and environmentally manipulated to improve productivity (Johnson, 2004). For example, poultry can be made to reproduce throughout the whole year in a production system whereas most parrot species are highly seasonal breeders.

To address the lack of information on the gross and micro anatomy of the male reproductive tract of Australian parrots, this study described gross male reproductive anatomy in the cockatiel, and to a lesser extent, the sulphur-crested cockatoo, galah and long-billed corella. The reproductive tracts of adult males were collected in aim of documenting the three main phases of their reproductive cycle (1) regeneration phase (2) acceleration phase and (3) culmination phase (Lofts and Murton, 1973). The coelomic cavity was opened and its visceral organs removed, allowing the reproductive tract to be visualised. To examine gross anatomy photographs were taken with the reproductive tract in situ to ascertain its relative position and the potential functionality of the ductus deferens and epididymis noted. Testicular size, colour, location and vascularisation were also noted. Testes were then removed as quickly as possible and measured for length and width to the nearest 0.1mm using vernier callipers. To examine micro anatomy the testis, adrenal gland, epididymal region and ductus deferens were rapidly excised and fixed in either Bouin's fluid for light microscopy or in 3% glutaraldehyde in 0.1M phosphate buffer for transmission electron microscopy.

Testis morphology varied with the reproductive cycle, particularly testis size. In the cockatiel testis

size varied from 3.4mm (length) x 2.8mm (width) during the regeneration phase to 10mm x 5.9mm at culmination. In the other parrot species examined increases in testicular size due to reproductive phase also occurred; 3.2mm x 2.1mm (regeneration) to 14mm x 6.1mm (acceleration) in the sulphur-crested cockatoo, 3.9mm x 1.2mm (regeneration) to 11.8 x 10mm (culmination) in the galah and 3.2mm x 1.1mm (regeneration) to 15.5 x 10mm (culmination) in the long-billed corella.

Cockatiel testes were white to yellowish in colour with a prominent vascular supply, especially at the height of the reproductive season (culmination period). The testes of the other species examined were pigmented. However, occasionally a white testis was observed in the long-billed corella. Pigmentation was most prominent in the sulphur-crested cockatoo and long-billed corella with the testes appearing black in colour. During the culmination period in the galah, the testes appeared grey in colour and the seminiferous tubules were visualised through the testis capsule.

The cockatiel testes were ellipsoidal in shape and in all cockatiels examined (n = 16) the testes were asymmetrical in size with the left testis larger (up to 5x) than the right irrespective of the breeding cycle. During the regeneration period in the other species examined the testes appeared 'bean-shaped' before taking on the characteristic ellipsoid shape encountered during the height of reproduction. In the majority of the other species examined the left testis was larger than the right, however, asymmetry was generally not as pronounced as in the cockatiel. Infrequently in the galah and long-beaked corella only the left testis was observed, with the right testis absent.

Initial studies on the microanatomy of the cockatiel reproductive tract have revealed that despite being essentially similar to that of many previously studied avian species some novel anatomy was discovered. For example, the ductus deferens was found to be ciliated and no seminal glomus was present in the cockatiel. It is clear that much is still unknown on the reproductive anatomy of birds.

SPERMIOGENESIS

Spermiogenesis can be defined as the series of sub-cellular events that leads to the formation of the spermatozoon from the haploid spermatid (Phillips, 1974). The process is often studied as a prerequisite for determining the stages of the cycle of the seminiferous epithelium (Jones and Lin, 1993). The morphological diversity of mature spermatozoa in parrots may also serve as a guide to understanding the phylogenetic and taxonomic relationships of this diverse group.

The current study examined spermiogenesis primarily in the cockatiel. Small blocks of testicular tissue were fixed in 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.0) and then post-fixed in 1% osmium tetroxide in the same buffer. After fixation, the specimens were dehydrated through a graded series of ethanol and embedded in Epon resin. Ultrathin sections were cut using an Ultracut ultramicrotome and sections were stained with uranyl acetate and lead citrate. All sections were examined and photographed using Jeol 1010 or 1011 transmission electron microscopes operating at 80kV.

Spermiogenesis in the cockatiel was described based on the morphological development of the acrosome, nucleus and flagellum. Early acrosomal development in the cockatiel spermatid was comparable with the domestic chicken (Nagano 1962), the Japanese quail (Lin and Jones, 1993) and the turkey (Aire, 2003). However, after this phase, spermiogenesis in the cockatiel appeared to differ quite substantially from that of other avian species that have been previously described. In the cockatiel much of the early development of the perforatorium/acrosome occurred before the nucleus had condensed and/or elongated. Perforatorium development in the cockatiel is similar to that described for the budgerigar (Humphreys, 1975) in that a filamentous rod appears in the

subacrosomal invagination and continues to extend to the floor of the invagination. This differs from the observations made by Lin and Jones (1993) in the Japanese quail who show that the perforatorium begins as a dense droplet in the nucleoplasm. During spermiogenesis in the cockatiel, flagella where often seen coiling within the spermatid cell membrane rather than what is more typically described, extending into the seminiferous tubule. The presence of internal flagella was also observed in other parrot species examined in this study including the sulphur-crested cockatoo, long-billed corella and rainbow lorikeet. To our knowledge this is the first ultrastructural study of spermiogenesis in a parrot. This study closely parallels work conducted on acrosome differentiation in the budgerigar (Humphreys, 1975) and will form the template for further studies in the galah, sulphur-crested cockatoo, long-billed corella, pale-headed rosella and rainbow lorikeet

SECRETORY CAPACITY TEST

Fertility assessment in birds is often a complex issue incorporating both non-medical causes of infertility (e.g. immaturity, sexual inexperience, inappropriate housing, lack of stimulus, mate incompatibility) and medical causes (e.g. malnutrition, vision or foot problems, reproductive tract disorders or infections) (Speer, 1991). This study aimed to develop techniques that would allow the assessment of reproductive function in the male cockatiel by determining the maximum testosterone producing capacity of the testes using synthetic mammalian Gonadotropin-releasing hormone (GnRH) to ultimately cause a surge of testosterone secretion. Commonly known as a GnRH challenge it is possible to use synthetic GnRH to induce the release of luteinising hormone from the anterior pituitary, which will then subsequently result in the production of enzymes that convert cholesterol stored within the Leydig cell into testosterone. Confirmation of a significant increase in plasma testosterone following a GnRH injection is indicative of normal steroidogenesis. Testosterone is the primary sex steroid in the avian testis (Lake and Furr, 1971; Galli et al., 1973) and is one of the main steroid hormones that control development and functional activity of the accessory sexual organs and secondary sexual characteristics (Paster, 1991).

In the current study, a GnRH agonist (buserelin; 8.0 µg of peptide/kg bodyweight) was injected intramuscularly into 16 male cockatiels and 3 sulphur-crested cockatoos and serial blood samples collected at 0, 30, 60, 90 and 120 minutes after administration. Birds were anaesthetised for each venipuncture using 3% isoflurane in oxygen at a rate of 1.5 L/min for induction and 1.5% isoflurane for maintenance. Blood (0.5ml) was collected from the jugular vein using a 25-gauge needle attached to 1ml heparinised syringe. Blood samples were centrifuged at 6000 rpm for 10 minutes and the plasma stored at -20ºC until analysed using Spectria® testosterone radioimmunoassay kits (Orion Diagnostica, Finland). Once validated, the technique was subsequently used to examine seasonal changes (23 months) in the testosterone profile of a captive cockatiel population.

This study showed that a single injection of a synthetic mammalian GnRH (buserelin) provided a reliable index of testosterone secretion of the cockatiel testis, with maximum concentrations occurring at 60 to 90 minutes after injection. Despite no clear pattern of seasonal variation in testosterone secretion being detected in cockatiel plasma, blood samples taken 60 and 90 minutes after administration, showed a significant increase in all seasons. Injection of buserelin in the sulphur-crested cockatoo also resulted in an increase in testosterone secretion with maximal concentrations obtained after 90 minutes, further supporting the stimulation test as an investigative tool for exploring the reproductive status of a range of parrot species.

This study is important, as it provides a reliable technique for understanding male reproductive endocrinology of Australian Parrots; in particular, the GnRH challenge will allow us to elucidate

seasonal changes in testosterone secretion. The technique has the advantage of assessing testosterone secretion in single samples of small volume thereby removing the need for repeated venipuncture to account for the episodic nature of testosterone secretion. Establishment of a GnRH challenge also provides a means of assessing the efficacy of contraceptive chemicals that function by reducing testosterone.

CONCLUSION

Much remains to be discovered about the reproductive biology of Australian parrots so it is extremely important that fundamental studies of anatomy and endocrinology be encouraged in order to provide a baseline understanding from which more advanced breeding programs can be implemented. It is hoped that the research cockatiel model produced in this study will form a template on which future studies into assisted breeding techniques for endangered parrots can be developed.

REFERENCES

Aire TA. 2003. Ultrastructural study of spermiogenesis in the turkey, *Meleagris gallopavo*. *British Poultry Science.* 44: 674-682.

BirdLife International. 2010. *Search for species*, viewed 1st July 2010 http://www.birdlife.org/datazone/species/index.html

Burke WH. 1993. Avian Reproduction. *Dukes Physiology of Domestic Animals*. eds. Swenson MJ and Reece WO. Cornell University Press. USA. pp 728-750.

Forshaw JM. 2002. Australian Parrots. Alexander Editions. Australia.

Forshaw JM. 2006. Parrots of the World an Identification Guide. Princeton University Press. New Jersey.

Galli FE, Irusta O, Wassermann GF. 1973. Androgen production by testes of *Gallus domesticus* during postembryonic development. *General and Comparative Endocrinology*. 21: 262-266.

Humphreys PN. 1975. The differentiation of the acrosome in the spermatid of the budgerigar (*Melopsittacus undulates*). *Cell and Tissue Research*. 156: 411-416.

Johnson PA. 2004. Avian reproduction. *Duke's Physiology of Domestic Animals*, 12th edition. ed. Reece W-O. Cornell University Press. Ithaca. pp 742-753.

Jones RC, Lin M. 1993. Spermatogenesis in birds. *Oxford Reviews of Reproductive Biology*, 15: 233-264.

Lake PE, Furr BJA. 1971. The endocrine testis in reproduction. *Physiology and Biochemistry of the Domestic Fowl*. eds. Bell DJ and Freeman BM. Academic Press. London. pp 1469-1488.

Lin M, Jones RC. 1993. Spermiogenesis and spermiation in the Japanese quail (*Coturnix coturnix japonica*). *Journal of Anatomy*. 183: 525-535.

Lofts B, Murton RK. 1973. Reproduction in birds. *Avian Biology*. eds. Farner DS and King JR. Academic Press. New York. pp1-107.

Nagano T. 1962. Observations on the fine structure of the developing spermatid in the domestic chicken. *Journal of Cell Biology*. 14: 193-205.

Paster MB. 1991. Avian Reproductive Endocrinology. *Veterinary Clinics of North America: Small Animal Practice*. 21: 1343-1359.

Philipps DM. 1974. Spermiogenesis. Academic Press. New York.

Speer BL. 1991. A Clinical Approach to Psittacine Infertility. Proceedings Association of Avian Veterinarians, Chicago. pp 173-187.

Samour JH, Markham JA, Moore HDM, Watson PF. 1988. Semen cryopreservation and artificial insemination in budgerigars (*Melopsittacus undulates*). *Journal of Zoology*. 216: 169-176.

Yamamoto JT, Shields KM, Millam JR, Roudybush TE, Grau CR. 1989. Reproductive activity of force-paired cockatiels (*Nymphicus hollandicus*). *Auk*. 106: 86-93.