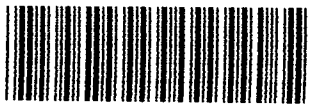
REPRODUCTION IN THE SPOTTED HYAENA CROCUTA CROCUTA (ERXLEBEN).

BY

MALAN LINDEQUE

Submitted in partial fulfilment of the
requirements for the degree of

M.Sc.(Zoology)



Faculty of Science
University of Pretoria
Pretoria

JULY 1981

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M. LINDEQUE

Supervisor : Professor J.D. Skinner
Mammal Research Institute
Department of Zoology
University of Pretoria
Pretoria

ABSTRACT

The reproductive biology of spotted hyaenas was studied from a sample of 39 females and 36 males collected in game reserves in southern Africa. A method for age estimation, based on the relative attrition of PM_2 was developed. Female spotted hyaenas attain sexual maturity at three years of age, are polyoestrus with recurring

oestrous cycles throughout the lactation period. Females were still reproducing when 16 years old. Breeding was not restricted to a particular season, as births occurred throughout the year and sexually active males were present in all four seasons.

Androgens were produced in significant amounts by the ovary and adrenal, with the ovary capable of a greater secretory response during acute stimulation, than the testis. The foetal ovary was the site of androgen production in the female foetus with the resulting masculinization of the female external genitalia. Reproductive status of female hyaenas could be predicted from the plasma levels of the sex steroids.

SAMEVATTING

'n Monster van 39 wyfies en 36 mannetjies verkry vanuit wildtuine in Suidelike-Afrika, is gebruik in 'n studie op die voortplantingsbiologie van gevlekte hiënas. 'n Metode vir ouderdomskatting is ontwikkel op grond van die mate van slytasie van PM_2. Wyfie-hiënas word geslagsryp op drie jaar, en is poli-estries met opeenvolgende oestrus-siklusse tydens die duur van die laktasieperiode. Wyfies kon steeds teel op 'n ouderdom van minstens 16 jaar. Vrugbare mannetjies en geboortes het regdeur die jaar voorgekom, en teling was nie beperk tot 'n bepaalde seisoen nie.

Androgene hormone is in aansienlike hoeveelhede deur die ovarium en bynier geproduseer, en die sekretoriese kapasiteit tydens akute stimulering was groter in die ovarium as in die testis. Die eksterne genitalieë van wyfie-foetusse vermanlik as gevolg van androgene hormone geproduseer deur die foetale ovarium. Plasmakonsentrasies van geslagsteroïedes was 'n aanduiding van voortplantingstatus van volwasse wyfies.

ACKNOWLEDGEMENTS

I would like to express my gratitude towards my supervisor for his enthusiasm and guidance. His interest in the well-being of his students is acknowledged with particular gratitude.

The Board of Trustees, National Parks Board of South Africa, and the Game and Fisheries Preservation Board of Natal kindly cooperated in this project. I would especially like to thank Prof. R. P. Millar, Department of Chemical Pathology, Medical School, University of Cape Town, for his advice and interest in my work, and for the use of radioimmunoassay facilities in his Department as well as the Gynaecology and Endocrine Services Laboratory, Depart= ment of Obstetrics and Gynaecology, University of Cape Town. A special word of thanks to the personnel of these institutions for their hospitality and assistance.

The use of facilities in the following institutions is gratefully acknowledged : Department of Radiology, Veterinary Faculty, Department of Geography, and the X-ray Diffraction Unit, Department of Geology, University of Pretoria. Pharma= ceuticals were donated by Ciba-Geigy Ltd (SA), Prof. R.P. Millar and Dr. N. Fairall. The advice and assistance of the following persons is appreciated : Drs. V. de Vos, R. Benges, G.L. Smuts, H. Ebedes, Messrs. I.J. Whyte, T. Yssel, A. Schreiber, A. Whateley, I. Espé, D.A. Els, T.J. Robinson, C. Tobler, R.J. van Aarde, R. Carr, M.G.L. Mills, and all my friends in the Department of Zoology, University of Pretoria. Mrs. L. Grobler prepared some of the figures, and Mrs. H. Botha typed the manuscript with great care.

During the course of this study I was the recipient of a CSIR postgraduate bursary and the Charles Astley Maberley Memorial Scholarship of the Transvaal Branch of the Wildlife Society of Southern Africa, and I am grateful to these bodies for their support.

CONTENTS

Page number

PREFACE xviii

CHAPTER 1 AGE DETERMINATION 1

INTRODUCTION 1
MATERIALS AND METHODS 2
RESULTS 4

Size frequency analysis 5
Assigning chronological ages to wear classes. 8
Comparisons with the age class schedule of Kruuk (1972) 10
Skull growth characteristics 11
Body morphometrics 12

DISCUSSION 14

CHAPTER 2 FEMALE REPRODUCTIVE BIOLOGY 17

INTRODUCTION 17
MATERIALS AND METHODS 19
RESULTS 22

Ovarian morphology 22
Follicular atresia 22
Formation and regression of corpora lutea of the oestrous cycle or pregnancy 23
Parameters of growth and regression of the corpus luteum 25
Changes in the corpus luteum during pregnancy and lactation 28
Occurrence of corpora lutea and follicles ... 32
Alternate ovulation 39
Sexual maturity 41
Pregnancy 42
Lactation 44
Reproductive senility 45
Seasonality 46

DISCUSSION 49

CHAPTER 3 MALE REPRODUCTIVE BIOLOGY 57

INTRODUCTION 57

MATERIALS AND METHODS 58

RESULTS 59

Testicular morphology 59

Age-related fertility 66

Seasonality 66

DISCUSSION 68

CHAPTER 4 PRODUCTION AND SECRETION OF SEX HORMONES 71

INTRODUCTION 71

MATERIALS AND METHODS 73

Husbandry 73

Experimental procedures 74

Tissue cultures 77

Radioimmunoassay 78

Histochemistry 79

RESULTS 81

Gonadal stimulation 82

LH-RH administration 82

HCG administration 87

Adrenal stimulation 95

ACTH administration 95

The effects of dexamethazone and HCG on the peripheral plasma steroid concentrations in a single brown hyaena female 103

Summary of results 106

Short term incubations 111

Histochemistry 113

Plasma, gonadal and adrenal steroids in the foetus 113

DISCUSSION 118

CHAPTER 5 ENDOCRINE CORRELATES OF REPRODUCTION 130

INTRODUCTION 130

MATERIALS AND METHODS 131

RESULTS 132

Age-related changes in the steroid pool 134

Interspecific sex steroid relationships 137

Predicting reproductive status from circulatory levels of sex steroids 140

DISCUSSION 144

SUMMARY 149

OPSOMMING 152

REFERENCES 155

APPENDICES 171

LIST OF TABLES

		Page number
1.	Mean areas of occlusal surface (PM_2) frequency modes	7
2.	Closure rate of the pulp cavities of C^1_1 (minimum and maximum diameters (mm) of pulp cavities from radiogram)	8
3.	Assigning chronological ages to wear classes ...	10
4.	Von Bertalanffy equations for growth in age of the skulls of spotted hyaenas, with means and standard errors for measurements at each age ...	11
5.	Von Bertalanffy equations for growth in head-body length and body mass with age	15
6.	Age differences between consecutive generations of corpora lutea in pregnant and lactating spotted hyaenas	28
7.	Mean numbers (± SE) of corpora lutea and generations of corpora lutea in females with corpora lutea	33
8.	Mean numbers (± SE) of corpora lutea in the first, second, third and fourth most recent generations of corpora lutea	35
9.	Mean numbers (± SE) of follicles and generations of follicles per female in each reproductive category	37
10.	Mean number (± SE) of cystic follicles and luteinized atretic follicles per female in each reproductive category	38
11.	Mean number (± SE) of corpora lutea in left and right ovaries	39
12.	Frequency percentage of occurrence of 39 female hyaenas in each reproductive category per age class	41

13. Quarterly distribution (%) of theoretical parturitions and conceptions from (A) present sample (n=14), and (B) composite sample (n=35). 47

14. Testicular composition analysed following Chalkley (1943), for each age class 64

15. Analysis of seasonal testicular composition of mature spotted hyaenas 68

16. Central nervous system depressants used in this study for immobilization and general anaesthesia 73

17. Stimulants and suppressants used in experimental procedures 75

18. Reagents used in tissue cultures 78

19. Intra- and interassay variation (%) 79

20. Responses to luteinizing hormone-releasing hormone (LH-RH) administration 86

21. Responses in male spotted hyaenas to injections of human chorionic gonadotrophin (HCG) 89

22. Responses in female spotted hyaenas to injections of human chorionic gonadotrophin (HCG) 93

23. Responses in male spotted hyaenas to injections of adrenocorticotrophic hormone (ACTH) 97

24. Responses in female spotted hyaenas to injections of adrenocorticotrophic hormone (ACTH) 101

25. Responses in an adult brown hyaena (Hyaena brunnea) female to injections of dexamethazone and human chorionic gonadotrophin (HCG) 105

26. A comparison between the ratio androstenedione (nmol/ℓ) : testosterone (nmol/ℓ), in the peripheral circulation of male and female spotted hyaenas before and after treatment with LH-RH, HCG and ACTH (juveniles excluded) . 107

27. A comparison between the maximum response in the peripheral plasma concentrations of testosterone (T) and Δ^4 androstenedione (A) in male and female spotted hyaenas (juve= niles excluded) following treatment with LH-RH, HCG and ACTH 108

28. A comparison between the mean percentage in= crease in the peripheral plasma concentrations of testosterone (T) and Δ^4 androstenedione (A) in male and female spotted hyaenas (juveniles excluded) following treatment with LH-RH, HCG and ACTH 109

29. Trial incubation experiment with rat testicular tissue 112

30. Short term incubations of testicular tissue 112

31. Short term incubations of the ovarian cortex ... 114

32. Short term incubations of the adrenal cortex ... 114

33. The presence of enzyme systems involved in steroid conversion 115

34. Gonadal and adrenal concentrations of testos= terone (T) and Δ^4 androstenedione (A) in the spotted hyaena foetus 116

35. Plasma concentrations of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2), cortisol (C) and luteinizing hormone (LH) in spotted hyaena foetuses 117

36. Ranges of testosterone, Δ^4 androstenedione, progesterone, oestrone, oestradiol-17β, cortisol and luteinizing hormone (LH) in the peripheral circulation of spotted hyaenas 132

37. Mean (± SE) peripheral circulatory concentrations of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestrone (E_1), and oestradiol-17β (E_2) of male and non-pregnant female hyaenas per age class 135

38. Mean (± SE) peripheral circulatory concentrations of testosterone (T), Δ^4 androstenedione (A), oestrone (E_1), oestradiol-17β (E_2) and progesterone (P) in male and non-pregnant female spotted hyaenas 136

39. The ratios testosterone (T) : Δ^4 androstenedione (A), and oestrone (E_1) : oestradiol-17β (E_2) in male and non-pregnant female spotted hyaenas per age class 138

40. The ratios testosterone (T) : Δ^4 androstenedione (A), and oestrone (E_1) : oestradiol-17β (E_2) in male and non-pregnant female spotted hyaenas 139

41. A comparison between the peripheral plasma concentration of testosterone (T), Δ^4 androstenedione (A), oestrone (E_1), oestradiol-17β (E_2) and progesterone (P) of nulliparous and parous female spotted hyaenas 140

42. A comparison between the peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), oestrone (E_1), oestradiol-17β (E_2) and progesterone (P) of parous non-pregnant and parous pregnant spotted hyaenas 141

43. A comparison between the peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), oestrone (E_1), oestradiol-17β (E_2) and progesterone (P) of parous lactating and parous anoestrous spotted hyaenas 142

44. A comparison between the peripheral plasma concentrations of testosterone (T), Δ^4 andro= stenedione (A), oestrone (E_1), oestradiol-17β (E_2) and progesterone (P) of nulliparous A and nulliparous B female spotted hyaenas 143

45. A comparison between the peripheral plasma concentrations of testosterone (T), Δ^4 andro= stenedione (A), oestrone (E_1), oestradiol-17β (E_2) and progesterone (P) of lactating spotted hyaenas with (D) or without (A) developing follicles 143

46. The ratio testosterone (T) : Δ^4 androstenedione (A), and oestrone (E_1) : oestradiol-17β (E_2), in each reproductive category of female, as well as in male spotted hyaenas 144

LIST OF FIGURES

		Page number
1.	Cumulative percentage frequency of the area of the occlusal surface of PM_2, in 50 mm^2 classes	5
2.	Inferred age-classes, separated by the area of the occlusal surface of PM_2 into discrete distributions	6
3.	Progress of fusion of four crainal sutures (curves fitted by eye)	9
4.	Von Bertalanffy curves for growth in skull measurements (mm) with age	12
5.	Von Bertalanffy curves for growth in head-body length (HB) and body mass (BM) with age	14
6.	A stylized sketch through an optical micrometer, of a corpus luteum (400x) illustrating the tran= sect method of measuring luteal cell diameter. Only shaded cells were measured, according to the technique described	21
7.	A plot of fibrocyte density against the age of the corpus luteum, assigned through extrapolation of the relationship expressed by the points not included by A-B	27
8.	Histological changes in the corpus luteum of pregnancy (curves fitted by eye)	29
9.	Changes in luteal cell density during pregnancy and the first half of lactation	30
10.	Changes in luteal cell diameter during pregnancy and the first half of lactation	30
11.	Changes in the density of vacuolated luteal cells during pregnancy and the first half of lactation.	31
12.	Changes in the volume of the corpus luteum during pregnancy and the first half of lactation	31
13.	The frequency distribution of the number of corpora lutea per ovary (n=38)	32

14. The frequency distribution of the number of corpora lutea per female 32

15. The relationship between the number of corpora lutea and the number of generations of corpora lutea with age, in parous pregnant females 34

16. The relationship between the number of corpora lutea and the number of generations of corpora lutea with age in parous lactating hyaenas with developing follicles (D) 34

17. The relationship between the number of corpora lutea and the number of generations of corpora lutea with age in parous lactating hyaenas with atretic follicles only (A) 35

18. The mean number (± SE) of generations of follicles per female in each age class 36

19. The frequency distribution of the number of corpora lutea per ovary 40

20. The frequency distribution of the number of generations of follicles per ovary 40

21. The percentage of females in each age class that were breeding (pregnant or lactating) 42

22. The percentage of females in each age class that were pregnant 43

23. The effects of progressive gestation and age on body mass 43

24. The percentage of females in each age class that were lactating 45

25. Seasonal distribution of females in each reproductive category 47

26. Per cent distribution of theoretical parturitions and conceptions per annum 48

27. Per cent distribution of parturitions and conceptions per annum found by combining theoretical dates with published data by Fairall (1968) 49

28. The relationship between combined testicular mass and the average diameter of the seminife= rous tubules 60

29. The relationship between seminiferous tubule diameter and combined testicular mass cor= rected for body mass 61

30. The relationship between combined testicular mass and body mass 61

31. The relationship between combined testicular mass and head-body length 62

32. The relationship between mean seminiferous tubule diameter and body mass 62

33. The relationship between mean seminiferous tubule diameter and head-body length 63

34. The relationship between thickness of the seminiferous epithelium and age 63

35. The relationship between Leydig cell density (A : extra-tubular areas only, B : total area of the testis) with age. Regression C in= dicates the percentage composition of the testis as extratubular areas, with age. (A : y = 7,62 x -0,97; r = -0,96; B : y = 2,80 x -0,37; r = -0,87; C : y = 43,9 x -5,17; r = -0,97) 65

36. Age-related changes in combined testicular mass, mean seminiferous tubule diameter, combined epididymal mass and sperm content per pair of epididymides in male spotted hyaenas 67

37. Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4 androstenedione (A) after LH-RH administration in a 51 kg male spotted hyaena (age class II) 83

38. Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4 androstenedione (A) after LH-RH administration in a 57 kg male spotted hyaena (age class II) 84

39. Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4 androstenedione (A) after LH-RH administration in a 44 kg female spotted hyaena (age class VII) 84

40. Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4 androstenedione (A) after LH-RH administration in a 56 kg female spotted hyaena (age class VII) 85

41. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and luteinizing hormone (LH) after HCG administration in a 41 kg male spotted hyaena (age class II) 88

42. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after HCG administration in a 76 kg male spotted hyaena (age class IV) 88

43. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after HCG administration in a 68 kg female spotted hyaena (age class VI) 90

44. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after HCG administration in a 79 kg pregnant female spotted hyaena (age class V) 91

45. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestradiol-17β (E_2) and cortisol (C) after HCG administration in a 75 kg pregnant spotted hyaena (age class V) 92

46. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after ACTH administration in a 51 kg male spotted hyaena (age class II) 95

47. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after ACTH administration in a 17 kg male spotted hyaena (age class I) 96

48. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after ACTH administration in a 43 kg male spotted hyaena (age class II) 96

49. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after ACTH administration in a 18 kg female spotted hyaena (age class I) 99

50. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after ACTH administration in a 31 kg female spotted hyaena (age class I) 99

51. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A) and cortisol (C) after ACTH administration in a 56 kg female spotted hyaena (age class II) 100

52. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after dexamethazone administration in a 67 kg female spotted hyaena (age class V) 103

53. Peripheral plasma concentrations of testosterone (T), Δ^4 androstenedione (A), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after dexamethazone and HCG administration in a 37 kg female brown hyaena 104

54. A schematic representation of the LH-RH-mediated LH-induced (a), HCG-induced (b) and ACTH-induced (c) gonadal and adrenal secretion of testosterone (T), Δ^4 androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) during acute stimulation in male and female spotted hyaenas 110

55. The relationship between peripheral plasma concentration of progesterone (P) and days pregnant in spotted hyaenas 133

56. A flow diagram for the preliminary determination of reproductive status of female spotted hyaenas . 145

PREFACE

Spotted hyaenas have survived many onslaughts in Africa, being the object of many inspired myths and seen as pests rather than a species worth conserving. Despite ruthless attempts to exterminate hyaenas in many parts of Africa, they are still the most common large carnivore on this continent. Since the pioneering work of Kruuk in East Africa, the spotted hyaena has been recognized as the very efficient and very adaptable carnivore that it is. Hyaenas are no more just mysterious hermaphrodites or unsavoury robbers of corpses, nor the cowardly killers of the sick and disabled, but have emerged as efficient hunters of African game, a vital link in the recirculation of biotic elements from producer back to substrate, and an indispensable warden of field sanitation. Above all, hyaenas are extremely well adapted to their environment, a true product of African severity and abundance alike.

Reproduction in spotted hyaenas is marked by many strange anomalies. Female hyaenas, having male-like external genitalia and enough of the male hormones to render them indistinguishable from males, produce only two well-developed cubs which are in the care of the mother for the first year of life at least. The nutrimental requirements of the litter throughout the first year, are met through lactation only. A further important role of the mother is protection of the cubs against predation and the cannibalistic tendencies of other hyaenas. Cub mortality is nevertheless extremely high, despite all maternal efforts to the contrary. After the loss of a litter, the female might conceive again and is thus not restricted by a particular season. If the litter survives to independence, a new litter will be born at most twice every three years. In conclusion, reproduction seems to proceed in a most conservative fashion in this species.

It is therefore remarkable that hyaenas have been able to recover so soon following drastic reductions in numbers through disease and hunting, as was experienced in the Kruger National Park during the 1950's. There is, however, some evidence that the natural increase in numbers through reproduction was substantially supplemented by hyaenas from surrounding areas immigrating into the then vacant areas in the KNP. This reservoir has subsequently been destroyed, as hyaenas are incompatible with human settlements and farming practices in the Lowveld region of southern Africa. It is doubtful whether immigration could still buffer hyaena populations in any southern African game reserve at present. The true extent of damage done to this buffer could be seen by the slow rate of recolonization experienced in areas sub= jected to recent population control measures in the KNP since 1976.

Once the reservoir population outside the KNP had been destroyed, population increase could only be achieved by way of reproduction, which is considered to be conservative if not perilously slow. The present study was therefore in part conducted to examine the effects of population reduction by artificial means, on reproduction. Exploited populations are known to respond by achieving a greater rate of reproduction, either through a lowering of the age of first reproduction, increased litter sizes or a decrease in the interval between births. Such compensatory mechanisms often result in a change in the basic population parameters, such as the age distribution, and could therefore have a profound effect on management policies.

It is doubtful whether large-scale control programmes would ever be reinstated in the KNP, and the large numbers of hyaenas culled in the past would probably never again be available for research. It is nevertheless advisable to monitor the rate of reproduction of all populations confined

to game reserves, especially when these have been exter= minated outside reserve boundaries, but this is not easily accomplished with a secretive nocturnal carnivore like the spotted hyaena. An alternative method was there= fore in demand, whereby adequate information on the repro= ductive status of individual hyaenas could be obtained, without the necessity of killing any. Recent progress in live-stock farming has shown that sex hormones can be used to distinguish between individuals in different reproductive categories. A similar approach for spotted hyaenas could perhaps be quite useful, especially since trapping techniques have been perfected.

Factors involved in secretion of the sex hormones were therefore studied, in view of the observed peculiar hormonal milieu of males and females. Once the origin of secretion could be established, peripheral levels of the sex steroids could in turn be related to the reproductive processes, enabling thus theoretically the monitoring of reproduction in the wild by predicting reproductive status of individuals, based on hormone levels in a single blood sample. The findings of the present study would hopefully be of some value in management considerations, even only in future when the need for population monitoring might be more pressing.

A limited number of hyaenas were available for this study, and not all age classes or reproductive categories were equally well represented as individuals were taken at random. The 75 individual hyaenas used in this study are nevertheless valuable, since it is unlikely that similar numbers will again be culled in the near future. The aims of the present study were therefore to re-examine some of the basic features of reproduction, with regard to any changes that might have been brought about by population control, and to solve the riddle of the 'androgenic' female spotted hyaena, with regard to the origin of secretion and the factors influencing secretion of the major sex steroids and the androgens in particular.

Trivial names used in text

Δ^4 Androstenedione (A)	: 4-Androstene-3,17-dione
Cholesterol	: 5-Cholesten-3β-ol
Cortisol (C)	: 11β,17,21-Trihydroxy-4 pregnene-3,20-dione
Dehydroepiandrosterone (DHEA)	: 3-Hydroxy-5α-androst-17-one
Dexamethazone	: 9α-fluoro-11β,17,21-trihydroxy-16α-methyl-$\Delta^{1,4}$-pregnadiene-3,20-dione
Metyrapone	: Su 4885 Metopirone
Oestradiol-17β (E_2)	: 1,3,5(10)-Estratriene-3,17β-diol
Oestrone (E_1)	: 3-Hydroxy-1,3,5(10)-estratrien-17-one
Progesterone (P)	: 4-Pregnene-3,20-dione
Stilboestrol	: Diethylstilbestrol
Testosterone (T)	: 17β-Hydroxy-4-androstene-3-one

CHAPTER 1

AGE DETERMINATION

INTRODUCTION

To provide an infallible ageing method applicable to spotted hyaenas, is beyond the scope of the project and material available. An attempt was made nevertheless, to estimate the ages of a select series of individuals for which body morphometrical information is available, complementary to information obtained from their skulls, such as the amount of tooth wear and progress of coalescence of the components of the cranium. This subsample was used to serve as standard representatives for a number of age classes, to facilitate age estimation of individuals that were released or when collection and measurements were incomplete.

Few ageing methods seem to be infallible and few results absolute, especially when the parameter measured is not solely dependent on age, but also on other aspects of the life history of that species (Dapson 1980). The only way to avoid predictions or interpolations based on mathematically weak assumptions is to subject results, obtained by a certain method, to thorough statistical analysis, especially when no known age material is available (Dapson 1980).

Ageing criteria for spotted hyaenas were first mentioned by Grimpe (1916), who described the gradual change from the completely black neonatal pelage to the spotted adult pelage, later substantiated and elaborated on by Deane (1962), Pournelle (1965), Golding (1969) and Kruuk (1972). Accurate estimation of ages of individuals older than approximately nine months could not be achieved by this method only (Kruuk 1972). Matthews (1939a) used relative tooth wear as an index of age, which was later developed to establish

age classes with chronological age limits by Kruuk (1972). He does not state, however, if chronological age per class was derived from known-age material or estimates.

No known age skulls are available in southern Africa, to my knowledge, and the chronological ages assigned by Kruuk (1972) to his age classes therefore had to be used as a guide. Annuli found in both canines and premolars are not *de facto* indicators of age in this species (N. Fairall, pers. comm.), and the traditional method of tooth attrition was therefore followed. Quantification of the amount of tooth attrition complemented by other age-related characters, resulted in a statistically sound method of ageing spotted hyaenas, the greatest advantage being that the age of live hyaenas could be estimated by relatively simple means.

MATERIALS AND METHODS

Thirty-five skulls collected from adult hyaenas (permanent dentition) for which body morphometrical data were available, were used in this analysis. The area of the occlusal surface of the mandibular PM_2, was measured using an electronic pla= nimeter (AAC-400, Hayashi Denkoh Co Ltd, Tokyo), after a 12 x magnification drawing on cardboard with the aid of a drawing tube fitted to a stereoscopic dissecting microscope.

The area (mm^2) of occlusal surface was taken as the mean of four consecutive readings (standard errors varied from 1-5 percent), corrected for magnification by dividing with the product of ocular, objective and drawing tube magnification factors. Mean values were grouped into 21 size frequency classes covering the range of observations. Multimodality in the size frequency distribution was analyzed according to the method of Harding (1949), as modified by Cassie (1954), following Caughley (1965).

Cumulative percentages of occlusal surface areas in the size frequency classes, were plotted on probability paper

(normal-probability scale) and points of inflections in the resulting curve represented boundaries between modes. Each mode was treated as a separate distribution to allow standard deviations to be calculated and means to be de= rived from the graph, as in Cassie (1954). Corrections for overlap between classes were not considered necessary in this case.

In an attempt to compare results from this analysis with that of Kruuk (1972), the same series of skulls were divided into the four age classes of Kruuk (1972), based on a visual estimate of the amount of wear in the third lower premolar. Occlusal surface areas of individuals belonging to each of these classes, were correlated with each age class, following Dapson (1980).

In addition, several other age-dependent characters were used to determine the validity and accuracy of the size fre= quency analysis. A series of ten cranial sutures was examined for coalescence. Sutures are defined as 'closed' when coalescence has progressed along the full length of the suture, although the outline may still be visible. Cranial dimensions, indicating both transversal and longi= tudinal growth, as defined by De Blase & Martin (1974), were measured with a vernier calliper or ruler.

The rate of closure of the canine pulp cavity was examined by X-ray. High definition amplification screen plates were used, with the anode to film distance of 95 cm, and an exposure of 1 second at 15 mA and 70 kV (Smuts, Anderson & Austin 1978). Canines of right maxillas and mandibles were extracted and mounted on clear perspex sheets (1,5 mm thick), with a pliable adhesive. Teeth were mounted with the plain of curvature parallel to the sheet, the lingual side facing the same direction. The diameter of the pulp cavity at the ventral limit of the enamel layer, and at its maximum width, were measured with a vernier calliper directly

on the X-ray negative. Von Bertalanffy growth equations (Sager 1980a,b), were derived for six skull growth para= meters, as well as head-body length and body mass using the relationship

$$x_t = X_\infty \ (1-e^{-k(t-t_o)})^3$$

where

x_t = measurement at time

X_∞ = asymptotic measurement

k = constant determining growth rate

t = age of individual

t_o = hypothetical age when measurement is zero.

Equations were solved according to the linear transformation method described by Hanks (1972).

Head-body length was measured as the distance over body curves, from the tip of the nose to the anterior end of the first caudal vertebrum. The mass of the stomach contents was subtracted from total body mass.

RESULTS

As soon as skulls dry out completely, teeth fall out easily, with the exception of the upper and lower premolars. These are conical in shape and consist of the principal cusp, the protoconid, lending the conical appearance to the teeth, flanked by the anterior and posterior accessory cusps (Hendley 1974), which only showed any sign of attrition when the principal cusp has been worn down to their level. These accessory cusps are more pronounced in PM_3, and are therefore worn earlier than in PM_2. The occlusal surface of the protoconid is oval in shape and easily measurable, because the surface in wear is consistent. When tooth

attrition is such that the occlusal surface begins to in= clude one or both of the accessory cusps, the shape changes drastically, and attrition is evident on various levels, thereby impeding measurement.

All measurements were made on the second lower premolar, because the worn surface remains more easily measurable with increasing wear, and because this tooth is the best developed of the bone-crushing premolars. Irregular abrasions, almost certainly due to earlier breakages, commonly occur in the series. It was also apparent that one tooth was almost always more worn than the contralateral partner, indicating perhaps a preferred side for chewing or crunching bones. Teeth on both sides of the jaw had to be measured, and the mean taken as an estimate of tooth attrition.

Size frequency analysis

Figure 1 represents an attempt to recognize multimodality in the distribution of areas of the occlusal surfaces of

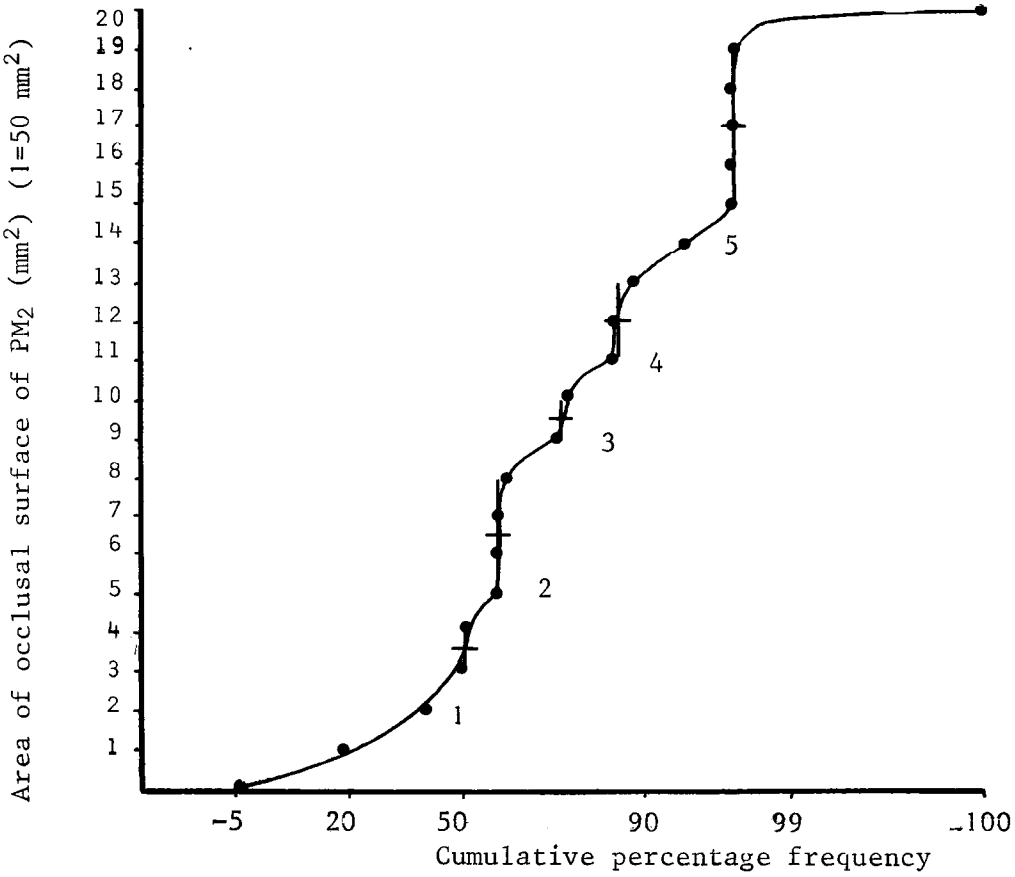


Fig. 1: Cumulative percentage frequency of the are of the occlusal surface of PM_2, in 50 mm^2 classes. Bars mark inflections, numbers in= dicate inferred modes.

PM_2, where cumulative percentages of frequency classes of identical interval, are plotted on a normal-probability scale. Inflections in the line of points serve to separate modes (Cassie 1954), which are developed into proper age classes, depending on the degree of overlap between classes. Overlap was considered to be negligible, according to examples given by Cassie (1954), and Caughley (1965). Dis= continuities are evident at values of 50%, 60%, 76%, 85,7% and 97,1%.

Each group of classes between inflections were treated as a separate distribution, according to Cassie (1954), which allows the mean of each distribution to be read off the graph as the surface area corresponding to the 50% cumulative frequency (Fig. 2). Standard deviations were calculated as

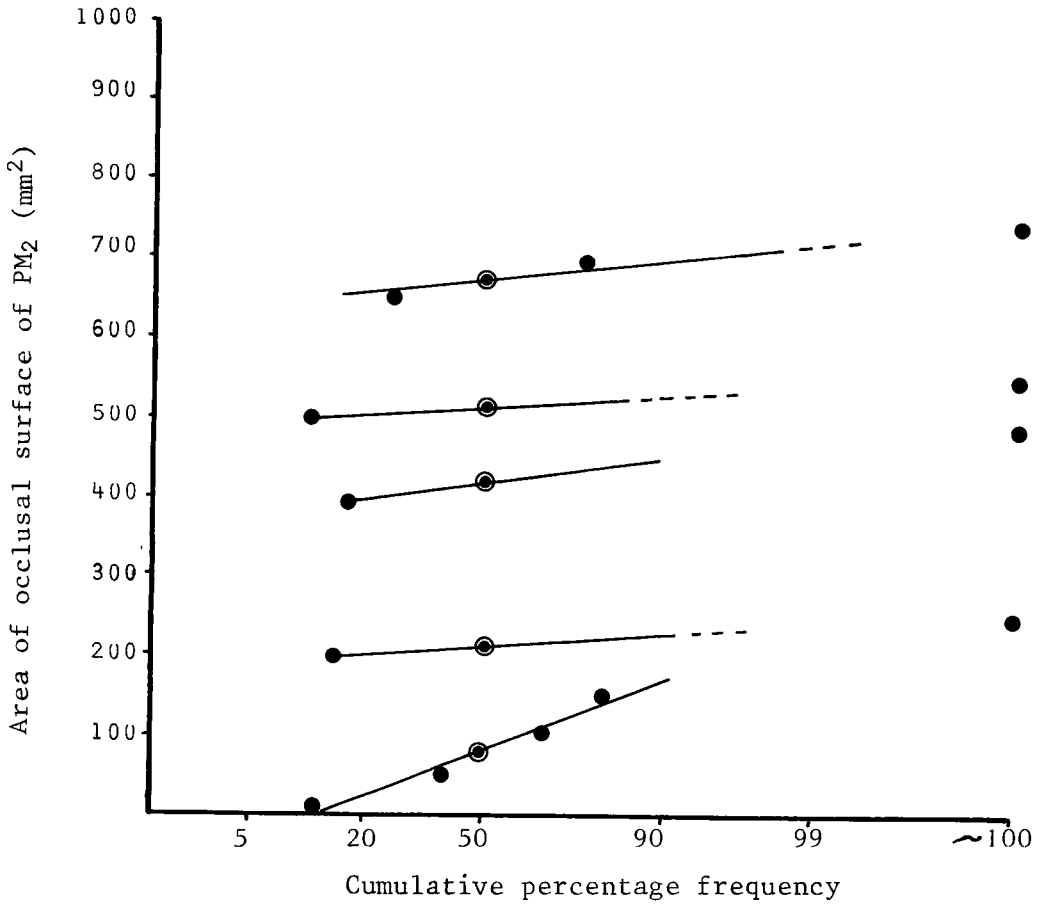


Fig. 2: Inferred age-classes, separated by the area of the occlusal surface of PM_2 into discrete distributions.

explained by Cassie (1954, 1962) and are presented in Table 1 together with the mean value of each class. Five frequency modes could be identified although a sixth, representing the oldest individual, could not be defined because of only a single representative. The first mode represents hyaenas with unworn teeth, but is not represent= ative of age class I, which is composed of individuals with deciduous premolars. Seven age classes could therefore be described.

TABLE 1: MEAN AREAS OF OCCLUSAL SURFACE (PM_2) FREQUENCY MODES

Mode	Mean area of occlusal surface PM_2 (mm^2)	SD	SE	% of total (n = 35)	n Individuals per class
1	-	-	-	-	13
2	129,0	54,0	13,1	48,6	17
3	211,0	11,2	5,6	11,4	4
4	420,5	25,3	11,3	14,3	5
5	511,0	11,2	5,6	11,4	4
6	678,5	19,6	9,8	11,4	4
7	1 077,3	-	-	2,9	1

It is expected that if the above classification represents equal intervals in the life-span of the hyaena, that age-dependent characters such as the coalescence of cranial sutures and the closure of the pulp cavity (Table 2), will follow a normal distribution (Laws 1968). When such an age-dependent character is plotted against age, the distribution will follow a smooth ogive, its smoothness depending on sample size. If the ogive is irregular, then the age criteria used are not sufficiently accurate, or unequal intervals between classes are demonstrated.

TABLE 2: CLOSURE RATE OF THE PULP CAVITIES OF C_1^1 (MINIMUM AND MAXIMUM DIAMETERS (mm) OF PULP CAVITIES FROM RADIOGRAM)

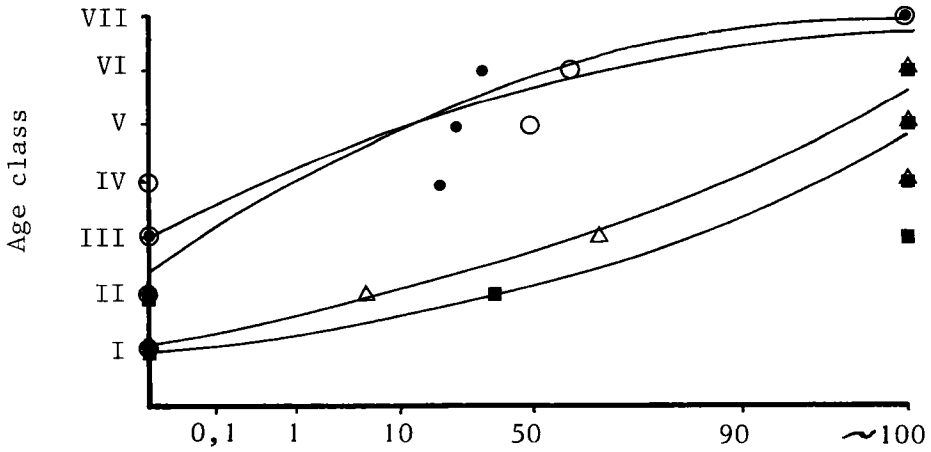
Age class	C^1		C^1	
	Min.	Max.	Min.	Max.
I	-*	-*	-*	-*
II	5,97 ± 0,68	8,78 ± 0,81	5,00 ± 0,53	8,03 ± 0,81
III	2,76 + 0,12	3,03 ± 0,11	2,25 ± 0,19	2,39 ± 0,17
IV	2,29 ± 0,35	2,66 ± 0,39	1,96 ± 0,13	2,38 ± 0,92
V	2,34 ± 0,11	2,63 ± 0,12	1,85 ± 0,19	2,08 ± 0,17
VI	2,20 ± 0,25	2,53 ± 0,34	1,89 ± 0,15	2,12 ± 0,26
VII	0,95	3,01	1,40	2,01

Such an analysis has been conducted, and the incidence of coalescence of four cranial sutures in each wear class, was plotted as percentage coalesced against age. None of these resulted in a smooth ogive, or a straight line when plotting incidence against age class on probability paper (Fig. 3). It can therefore be concluded that the number of years represented by each wear class, is not equal. A method had therefore to be developed to facilitate assignment of chronological ages to each wear class.

Assigning chronological ages to wear classes

The age class classification deducted from wear classes, as described in the previous section, resulted in the identification of seven classes, where class II corresponds to wear class II (1-3 years, Kruuk 1972) class III with class III (3-6 years), classes IV-VI with class IV (6-16 years) and class VII with class V (> 16 years) of Kruuk (1972).

A linear regression analysis of mean areas of occlusal surface against the minimum age of each age class, resulted in a



Percentage coalesced sutures.

- ● Baso-occipital-baso-sphenoidal suture
- ○ Naso-frontal suture
- △ Naso-premaxillary suture
- ■ Interfrontal suture

Fig. 3: Progress of fusion of four cranial sutures (curves fitted by eye).

coefficient of determination (r^2) of 1,00, indicating that all the variation in the minimum age per class, can be ascribed to changes in the area of the occlusal surface. Interpolation can therefore be validly used for projecting the chronological ages of each wear class. These are presented in Table 3. A similar analysis with maximum age as the dependent variable, also resulted in a coefficient of determination of 1,00, and chronological ages assigned to each wear class are also represented in Table 3.

Ages interpolated by means of these two ageing methods, differ, and it was decided to use the classification which would yield the highest coefficient of determination when the standard deviation in mean occlusal surface area is regressed against the number of years in each class. This coefficient has

been determined as 0,09 and 0,84 for the minimum and maximum limits per age class, as proposed by Kruuk (1972). The latter was therefore accepted and class IV therefore represents hyaenas 6-10 years old, class V 10-12 years, and class VI 12-16 years old (see Table 3).

TABLE 3: ASSIGNING CHRONOLOGICAL AGES TO WEAR CLASSES

n	Wear class	Age class (Kruuk 1972)	Interpolated class intervals (years) min.	max.	Mean area of occlusal surface of PM_2 (mm^2)
13	1	I (0-1)	0-1	0-1	-***
17	2	II (1-3)	1-3	1-3	129,0 ± 13,1
4	3	III (3-6)	3-6	3-6	211,0 ± 5,6
5	4	IV (6-16)	6-7*	6-10*	420,5 ± 11,3
4	5		7-10*	10-12*	511,0 ± 5,6
4	6		10-16*	12-16*	678,5 ± 9,8
1	7	V (> 16)	> 16	> 25**	1077,3

*Assigned according to a linear model (min.) $y = 0,02x - 0,65$ ($r^2 = 1,00$) and (max.) $y = 0,02x + 0,62$ ($r^2 = 1,00$)

**Maximum longevity (Flower 1931, Crandall 1964)

***Not erupted.

Comparisons with the age class schedule of Kruuk (1972)

In order to compare the new age class schedule with that of Kruuk (1972), occlusal surface areas of individuals assigned to his age classes, were regressed against each age class, as described by him. After different transformations, each followed by an analysis of residual variance, the best correllation coefficient (r = 0,996) was obtained by a weighted regression analysis (Steel & Torrie 1960).

That such a regression is of little predictive value, was shown by an analysis for homoscedasticity (equal array variances) (Dapson 1980), which was negative.

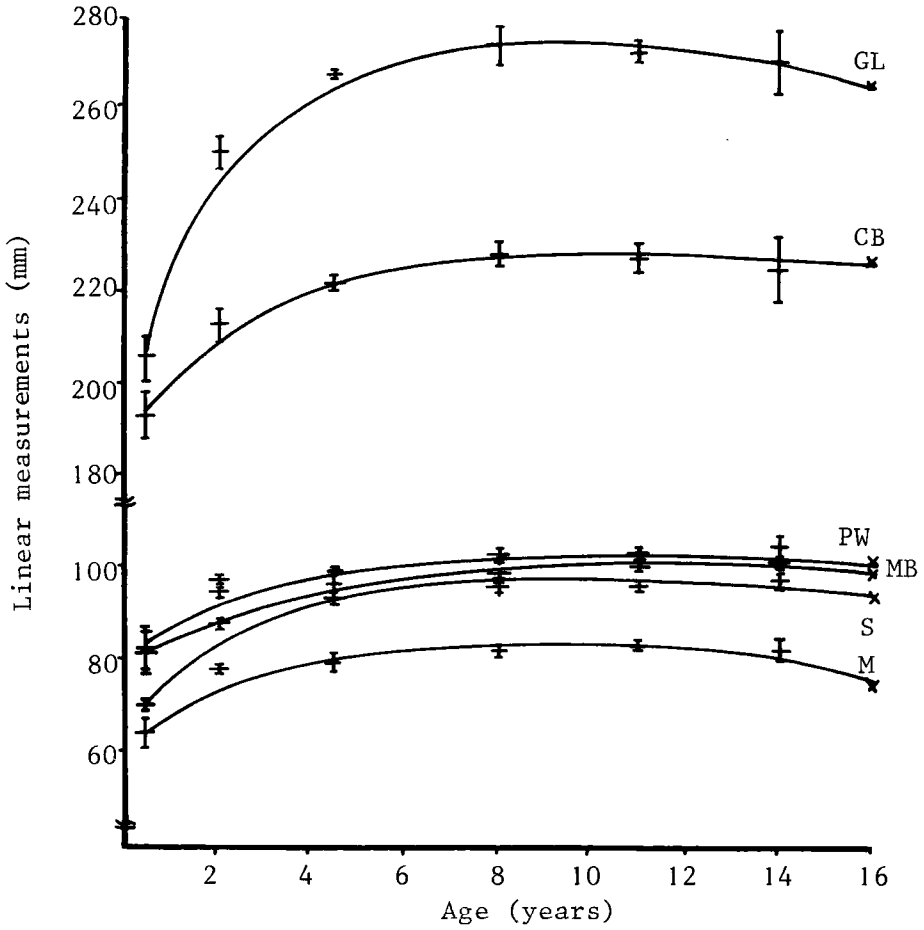
Skull growth characteristics

The present sample size did not allow separate equations to be calculated for males and females, but the mean values and standard errors of six linear measurements on the skulls of both sexes together, as well as the Von Bertalanffy growth equations, are presented in Table 4. Growth curves for these measurements are illustrated in Fig. 4.

TABLE 4: VON BERTALANFFY EQUATIONS FOR GROWTH WITH AGE OF THE SKULLS OF SPOTTED HYAENAS, WITH MEANS AND STANDARD ERRORS FOR MEASUREMENTS AT EACH AGE

Parameter (mm)	n	Von Bertalanffy equation
Greatest length	48	$L_t = 277,17\ (1-e^{-0,14(t+21,74)})^3$
Condylo-basal length	48	$L_t = 225,87\ (1-e^{-0,22(t+15,93)})^3$
Mastoid breadth	48	$L_t = 99,90\ (1-e^{-0,11(t+37,34)})^3$
Palatal width at PM^4	48	$L_t = 117,65\ (1-e^{-0,05(t+63,07)})^3$
Sagittal crest to condyle	48	$L_t = 97,34\ (1-e^{-0,14(t+23,6)})^3$
Maxillary tooth row	48	$L_t = 81,18\ (1-e^{-0,04(t-121,96)})^3$

Age (years)	Mean ± Standard Error (mm)					
	Greatest length	Condylo-basal	Mastoid breadth	Palatal width at PM^4	Saggittal crest to condyle	Maxillary tooth row
0-1	205,1 ± 5,4	193,7 ± 5,3	85,2 ± 2,6	81,8 ± 3,2	70,7 ± 1,1	65,0 ± 3,4
1-3	251,1 ± 3,0	214,7 ± 3,1	95,6 ± 0,9	98,1 ± 0,9	90,7 ± 1,2	80,4 ± 0,4
3-6	268,3 ± 1,0	222,5 ± 1,3	98,3 ± 0,7	96,9 ± 1,5	94,5 ± 0,9	80,2 ± 1,5
6-10	274,5 ± 4,2	228,8 ± 1,5	98,6 ± 0,9	102,9 ± 2,0	97,3 ± 1,8	82,6 ± 1,0
10-12	272,3 ± 1,0	227,0 ± 2,5	102,3 ± 2,8	102,7 ± 0,6	96,7 ± 0,6	84,0 ± 0,7
12-16	269,7 ± 7,0	224,7 ± 6,9	97,4 ± 2,1	99,1 ± 2,1	97,9 ± 3,5	83,0 ± 2,1
16+	265	227	100,7	102,3	94,1	75,2



GL : Greatest length
CB : Condylo-basal length
PW : Palatal width at PM^4
MB : Mastoid breadth
S : Sagittal crest to ventral edge of condyle
M : Length of maxillary tooth row

Fig. 4: Von Bertalanffy curves for growth in skull measurements (mm) with age.

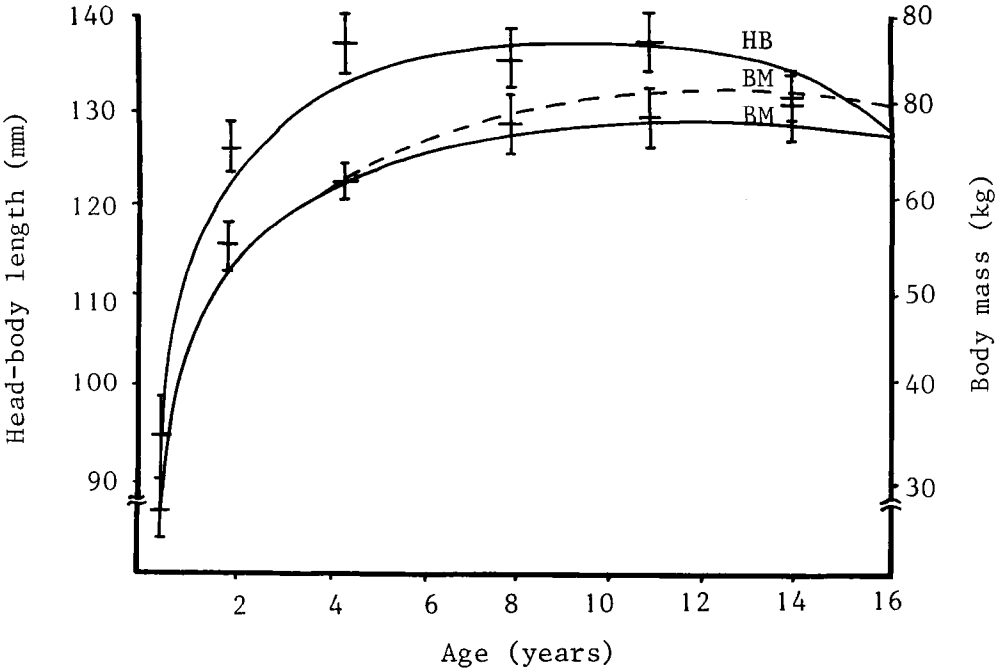
Body morphometrics

The means and standard errors for head-body lengths and body mass, as well as the respective Von Bertalanffy growth equations, are presented in Table 5. Growth curves for these measurements are illustrated in Fig. 5.

TABLE 5: VON BERTALANFFY EQUATIONS FOR GROWTH IN HEAD-BODY LENGTH AND BODY MASS WITH AGE

Parameter	n	Von Bertalanffy equation
Head-Body length (cm)	43	$L_t = 138,19\ (1-e^{-0,06(t+62,92)})^3$
Body mass (kg) (♂♂, ♀♀)	43	$W_t = 69,93\ (1-e^{0,18(t+10,34)})^3$
Body mass (kg) (♂♂, non-pregnant ♀♀)	38	$W_t = 67,92\ (1-e^{0,18(t+11,23)})^3$

Age (years)	Mean ± Standard Error		
	Head-body length (cm)	Body mass (kg) (oo, ♀♀)	Body mass (kg) (oo, non-pregnant ♀♀)
0-1	95 ± 4	27 ± 3	27 ± 3
1-3	126 ± 2	55 ± 2	55 ± 2
3-6	137 ± 3	61 ± 1	61 ± 1
6-10	135 ± 3	70 ± 3	68 ± 3
10-12	137 ± 3	73 ± 3	69 ± 3
12-16	130 ± 2	69 ± 1	68 ± 0
16+	128	66	66



.... pregnant females included

Fig. 5: Von Bertalanffy curves for growth in head-body length (HB) and body mass (BM) with age.

DISCUSSION

The use of a size frequency analysis, in this case of the area of the occlusal surface of PM_2, is particularly useful when known age material is not available (Caughley 1965), but is apparently dependent on the size of the sample to be analyzed. There is no doubt that the proposed age class intervals can be refined when more material becomes available.

More serious problems arise however, with the use of tooth attrition as an age-dependent variable. The rate of tooth attrition may vary in individuals and populations of different species (Spinage 1973) thereby leading to under- and over-estimations of age, or making comparisons between different populations unrealistic. Nevertheless, differences in diet are unlikely to affect attrition as much in carnivores as in herbivores.

Chronological ages assigned to individuals according to the described method, should not be regarded as absolute, because of possible interpopulation differences which would render the use of the chronological age limits of Kruuk (1972) for other populations, of little value. Neverthe= less, the diet of hyaenas in Kruuk's study was probably simi= lar to that of hyaenas in the Kruger National Park, at least as far as dietary influences on the rate of tooth attrition are concerned.

The measurement of the occlusal surface area although tedious, is nevertheless accurate and can be easily standardized. Teeth with fractured occlusal surfaces had to be excluded from the analysis, as wear in the ipsilateral tooth would have been accelerated and would therefore lead to an over= estimation of chronological age.

Development of other age-related characters examined, corres= ponded well with the age classes defined using tooth attrition. Unfortunately, only four out of a series of 10 sutures showed progressive coalescence over such periods that would allow sufficient discrimination between age classes. The same phenomenon was apparent in cranial measurements where asymptotes were attained at a young age. The small numbers of hyaenas representing each age class resulted in overlap= ping variances between classes which precluded absolute distinction between classes.

The rate of closure of the pulp cavity, although not suf= ficiently distinct to allow for discrimination between the older age classes, was nevertheless useful as a discriminant parameter for the younger classes. It was subsequently discovered that teeth need not be extracted, but could be X-rayed intact, with the same degree of definition as when removed (anode-film distance 85 cm, exposure 60 sec, 15 mA, 35 kV).

Although the other age-related characters examined, corres= ponded well with the ages derived as described, none could serve independently as an adequate ageing criterion due to insufficient discrimination of middle age and older in= dividuals. No obvious discrepancies between any other age related character and chronological ages derived from the amount of wear in PM_2 could be demonstrated, therefore suggesting an adequate representation of chronological age by the age classes developed as described.

The diagnostic value of the growth curves presented for both skull parameters and body measurements, is dubious. All these measurements are clearly curvolinear, and asymptotic values are attained at an early age. A possible exception is body mass which continues to increase with age, although field measurements on live hyaenas may be misleading because of the mass of the stomach contents, which may weigh a third as much as the body (Bearder 1977). The dip in the growth curves at the last age class can be ascribed to the single individual representing this class, which by chance proved to be a relatively small one. The length of the maxillary tooth row will also decrease in old age, when one or more tooth is lost, or when teeth are worn extensively. The use of relative growth for age estimation purposes is therefore limited, favouring the use of relative tooth attrition, as described, for age estimation.

Age estimation based on the relative degree of attrition of PM_2, facilitates the investigation of age-related phenomena in the reproductive biology of the spotted hyaena, such as age-related fertility and fecundity. Patterns of hormonal secretion are known to change with age, and such relationships could provide a new impetus towards the understanding of the functional role of the androgens in the establishment and maintenance of social relationships of the spotted hyaena.

CHAPTER 2

FEMALE REPRODUCTIVE BIOLOGY

INTRODUCTION

Information on reproduction in the female spotted hyaena resulted from observations on individuals breeding in captivity, as in Grimpe (1916), Schneider (1923,1926,1952), and Golding (1969) or from culled specimens as in Matthews (1939a) and Racey & Skinner (1979), or from behavioural anecdotes as in Deane (1962) and Kruuk (1972). Although most information was obtained through indirect methods, this does not imply that such information is necessarily inaccurate, but very often further qualification of previous observations is necessary.

Apart from the well-known masculine appearance of the external genitalia of the female, the most controversial aspect of reproduction is the length of the lactation period. The variability in the duration of lactation, generally accepted to last from 6 months to 18 months, is an indication of the variety of factors such as infant mortality and seasonal breeding, which can result in early or delayed termination of lactation. Lactating females are not necessarily anoestrous, as seen from the observations of Grimpe (1916) and Schneider (1926) of recurrent oestrus periods starting three weeks post partum. Whether such oestrus will lead to conception under normal circumstances is debatable, but females have been recorded as breeding again soon after a litter died or was removed. Any such overlap between successive reproductive attempts would be of considerable consequence in the productivity of the population.

Ovaries of spotted hyaenas frequently contained corpora lutea of different colours and sizes. A similar situation occurs

in the cervids, and it has been demonstrated that corpora lutea of different sizes or colours represent different ovulation events and therefore differ in age (Cheatum 1949). Corpora lutea in the spotted hyaena reputedly last longer than one reproductive cycle (Matthews 1939a) and if corpora lutea representing different cycles are present in the ovary, then the recent reproductive history of that particular female can be reconstructed, provided that structural differences between corpora lutea can be related to differences in age since formation.

Corpora lutea could not be classified by macroscopical appearance and the guidelines set by Matthews (1939a), Cheatum (1949), Golley (1957), Trauger & Haugen (1965), Kayanja (1969) and Hanks & Short (1972). Known histological parameters of growth and regression of the corpus luteum were measured to provide a more quantitative assessment of struc= tural differences, than just subjective assessments of colour and size. When more than one corpus luteum in the same hyaena exhibited the same values for the parameters that were measured, these were interpreted as being of the same age and degree of development since formation, and therefore consti= tute what is termed in this study, a generation of corpora lutea.

Corpora lutea in pregnant hyaenas were not homogenous with regard to structure, and all corpora lutea were therefore not of the current pregnancy. It was therefore assumed that distinct generations of corpora lutea represent con= secutive oestrous cycles. The aim of this part of the study was to investigate the phenomena associated with each phase of the reproductive cycle in the female hyaena, and especially to investigate the persistent nature of corpora lutea, as suggested by Matthews (1939a) with regard to the exceptionally long period of lactation in this species.

MATERIALS AND METHODS

Thirty nine females were available, the majority collected during this study. The annual predator control programme in the Central District of the Kruger National Park, con= ducted from 1974 until 1979, yielded approximately 330 hyaenas, of which only a small proportion was available. Nevertheless, the sample from the KNP was the main source of material, supplemented by smaller numbers from the Southern District of the KNP (8), Umfolozi Game Reserve, Natal (6), and Wankie National Park, Zimbabwe (2) collected specifically for the present study.

After the intact hyaena had been weighed and measured, the reproductive tract and adrenal glands were removed and stored in AFA, a mixture of ethanol (96%), formalin (40%), glacial acetic acid and distilled water, in the ratio of 3:1:1:5.

Ovaries and adrenals were post-fixed overnight in 70% ethanol, before being weighed on an electronic balance. Linear measurements were taken with a vernier calliper. Ovaries were sliced at approximately 2 mm intervals, and visible structures were identified and counted. Sections with particular structures suitably represented, were selected for detailed histological investigation.

After routine dehydration and paraffin embedding, tissue was sectioned at 5-8 µm and routinely stained with Delafield's/ Ehrlich's haematoxylin and alcoholic eosin, and following the staining methods of Mallory and Masson, in Lillie (1965).

Follicles were classified and counted according to criteria of development and atresia, following Matthews (1939a) and Weir & Rowlands (1977). In addition, several known age-related parameters for growth and regression of the corpus

luteum were defined for use in age estimation of corpora lutea of unknown age, as well as for discrimination between successive generations of corpora lutea. These were:

Volume: The two greatest diameters on any two perpendicular axes in a section of the corpus luteum were measured macroscopically with a vernier calliper. These values ($2r_1$ and $2r_2$) were used to estimate volume by means of Rashevsky's formula

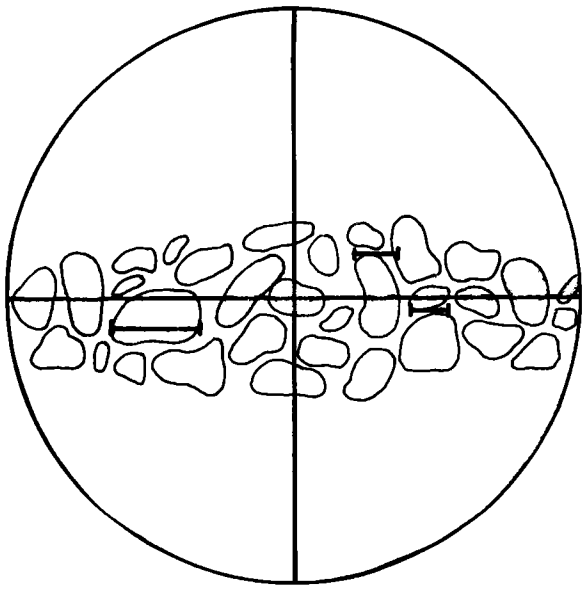
$$\frac{4}{3} \pi r_1 r_2 \left(\frac{r_1 + r_2}{2}\right)$$

Density of luteal cells: Measured on an ocular grid, as the number of vacuolated luteal cells/mm^2, under 100 x magnification. The mean from 25 fields, representing 1 mm^2 each, was taken.

Vacuolization: Measured as the number of vacuolated luteal cells/mm^2, under 400 x magnification. The mean from 25 fields was taken.

Luteal transect: The greatest diameter of luteal cells, regardless of orientation, that were bissected by the horizontal crossline of the ocular micrometer, were measured (Fig. 6). The micrometer was left stationary and only readings perpendicular to the horizontal crossline were taken. The mean diameter of 40 luteal cells thus randomly selected, was used.

Density of fibrocytic nuclei: measured as described for luteal cell density.



⟷ How measurements were made.

Fig. 6: A stylized sketch through an optical micrometer, of a corpus luteum (400x) illustrating the transect method of measuring luteal cell diameter. Only shaded cells were measured, according to the technique described.

Age of foetuses was estimated following Hugget & Widdas (1951), using a gestation period of 110 days (Schneider 1926) and foetal growth velocity 0,13 (Frazer & Hugget 1974). Theo= retical date of conception for each pregnancy, was calculated as the sampling date minus foetal age. Theoretical date of parturition was calculated as sampling date plus gestation period minus foetal age. Fairall (1968) summarized partu= rition times for different species in the KNP, including spotted hyaenas. In order to compare the present theoretical distribution of births of hyaenas with that given by Fairall (1968), a correction of one month was allowed representing the interval from birth after which cubs will accompany their mother, thereby becoming conspicuous (Van Lawick-Goodall &

Van Lawick 1970, Kruuk 1972). Although cubs are born in a highly precocial state of development (Grimpe 1916, Pournelle 1965), the first few weeks after birth are spent in underground shelters (Matthews 1939b, Kruuk 1972).

The present theoretical frequency distribution was tested for deviations from the corrected frequency distribution of Fairall (1968), but these were not significant ($\chi^2_{(11)} = 12,056$; $p < 0,01$). This justifies the lumping of the two distributions, which was done. Mean and median day of birth were calculated following Caughley (1971, 1977), the former using observed and theoretical dates to arrive at a mean day of birth. The second method utilizes the percentage of pregnant and lactating females throughout the year, to derive a median date of birth which is more meaningful as an indication of seasonality in breeding (Caughley 1977). In both cases, the mean or median day of conception could only be estimated by subtracting 110 days from the mean/median day of birth.

RESULTS

Ovarian morphology

For a detailed account of ovarian morphology vide Matthews (1939a) and only certain aspects relevant to the confirmation of reproductive status will be used to support conclusions about the reproductive pattern.

Follicular atresia

Atretic degeneration of follicles at all stages of maturation, was evident in all ovaries examined. Developing follicles are indeed the exception. As processing might result in the collapse or shrinkage of follicles, atresia could not be diagnozed macroscopically. Microscopical investigation

reveals that the oocyte of an atretic follicle has a cloudy appearance and shows the scattered remains of the fragmented nuclear material. The whole oocyte becomes more basophilic (eosinophilic) and stains much darker than a developing oocyte. Convolutions in the outer wall of the oocyte, a tightly enveloping zona pellucida, and a separation between the zona pellucida and surrounding cumulus oophorus, add further confirmation.

The appearance of the theca and granulosa layers is also diagnostic of atresia. Convolutions, separations and fractures with associated detachment from the adjoining layer, as well as mild hyalinization or transformation to fibrocytes in advanced cases, are indicative of atretic degeneration. In time the outline of atretic follicles becomes indistinct and eventually the only sign of an earlier follicle would be the shrunken, convoluted zona pellucida surrounded by cortical tissue consisting mainly of connective tissue.

Luteinization of atretic follicles was observed infrequently, but it would appear that elements of both the theca interna and zona granulosa, undergo transformation into luteal cells, although occasionally either layer was not luteinized at all. Luteinization and subsequent development of the luteal body may be confined to the original limits of the follicle, but one large corpus luteum, thought to have been of postovulatory origin, contained the remains of an oocyte in the centre of it. No evidence suggesting luteinization of developing follicles nearing ovulation, could be found.

Formation and regression of corpora lutea of the oestrous cycle or pregnancy

The youngest corpora lutea collected were from a female with two blastocysts approximately two weeks old. Bloody

ovulation scars on the ovarian surface were still evident but the corpus luteum, of the corpus haemorrhagicus type, had already attained a diameter of 2 cm, which was among the largest ever found. Two distinct types of cells are present in the corpus, which consists of loosely arranged clumps of granulosa lutein cells, intermingled with strands of a smaller, stronger eosinophylic cell type, presumably theca interna lutein cells.

The corpus luteum judged to be the second youngest collected, of unknown age, belonged to a parous lactating female. Consolidation of the clumps and strands of luteinized cells lends a solid texture to the corpus luteum, although the open, haemocoelic centre was still present. Numerous bloodvessels penetrating the whole of the corpus, were still present, and tiny strands of fibrocytes had appeared. No distinction was evident between the two formerly different types of cells. Connective tissue in the corpus luteum is suspected to arise in situ, as transformations of luteal elements to true fibrocytic tissue. Sequential elongation of the nuclei of former luteal cells to the final spindle-shape form of fibro= cytic nuclei, testifies to the origin of these cells. The sequence of development and regression from here onwards, seems to conform to the general mammalian pattern.

Following pregnancy, the bloodvessels once numerous in the core, gradually start moving towards the periphery. Fibro= cytes increase in number with age, until the corpus has changed to form a corpus albicans. A slight reduction in size during gestation could be seen, but otherwise one can not age a corpus luteum on the basis of size, or volume, alone. As in some species, the corpus luteum seems to attain its full size within a few days following ovulation. It is not known whether older corpora lutea of previous pregnancies become reactivated during later pregnancies, or merely retain the functional capability to produce progesta=

gens until the eventual disappearance from the ovary. Luteal cells of older corpora lutea from previous cycles did not differ structurally from the luteal cells of younger corpora lutea.

Corpora albicantia as such, are not common in the ovaries of spotted hyaenas. They are not pigmented, nor the only local disruptions in the cortex. Both the appearance and 'life-span' of corpora albicantia are believed to be extreme= ly variable. Sites of recent follicular activity resemble corpora albicantia and so further confound identification. It does however seem that corpora lutea need not eventually be transformed into proper corpora albicantia, but may get invaded by and intermingled with surrounding cortical tissue, while still retaining the glandular character of some luteal cells. The fate of luteal tissue therefore seems to be closely related to the formation of interstitial tissue in the ovarian cortex.

Parameters of growth and regression of the corpus luteum

A total of 90 corpora lutea were assessed as described. Known indices of growth, and therefore age, such as volume, cellular densities of both luteal cells and fibrocytes, and the size of luteal cells, were used to formulate an index of chronological age of individual corpora lutea since formation. This would enable the correct classification of corpora lutea of different ages in a female, into generations of corpora lutea where each generation is comprised of corpora lutea formed during the same oestrous cycle.

Ten corpora lutea of pregnancy from five pregnant females in different stages of gestation, were regarded as having been formed on day one of gestation, and were therefore of the same age as the foetuses from each pregnancy. Regressions of

different transformations of both dependent and independent variables were calculated for all five histological para= meters measured on the 10 corpora lutea of known ages. Each regression was followed by an analysis of residual variance and a test for homoscedasticity, as in Chapter 1. Only one regression was found where more than 80% of the variation in the dependent variable could be attributed to variation in the independent variable (age) (r^2 = 0,85; r = 0,92; y = 0,6570x + 0,0004; where y = density of fibrocytes and x = $\log_{10}$ age (in days)). Although not strictly homoscedastic, residual variances were always less than one unit of measurement (1 day), and were considered negligible. With the assumption of homoscedasticity, chronological ages were estimated through inter- and extra= polation for all other corpora lutea measured, based on the relative degree of transformation of luteal cells to fibro= cytes, measured as the density of the latter (Figure 7).

Age differences between consecutive generations of corpora lutea were considered to be representative of the interval between consecutive oestrous periods, and not between con= secutive pregnancies, because such age differences approximated observed oestrus intervals in spotted hyaenas more closely than observed intervals between consecutive pregnancies. This indicates that conception does not necessarily occur during each oestrous period, and that several "infertile" oestrous periods may occur during the interval between consecutive pregnancies. Age differences between consecutive generations of corpora lutea are presented in Table 6. It was apparent that the age differences between generations of corpora lutea in pregnant hyaenas were bimodal, namely one mode of approximately 100 days and another of approximately 20 days. The latter mode was also the only one found in parous non-pregnant females (Table 6).

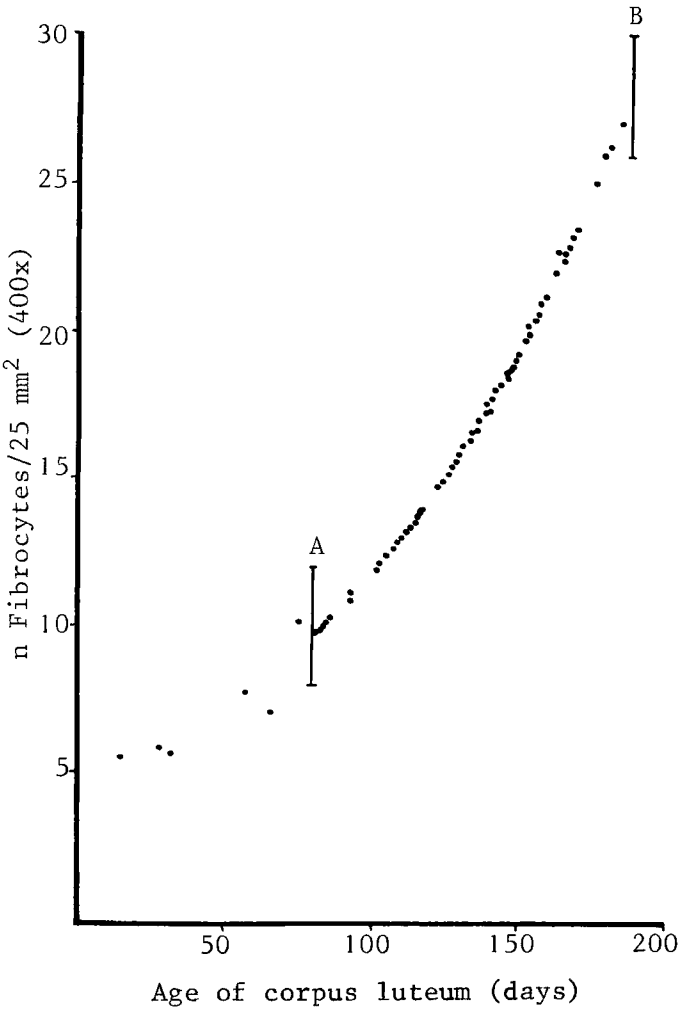


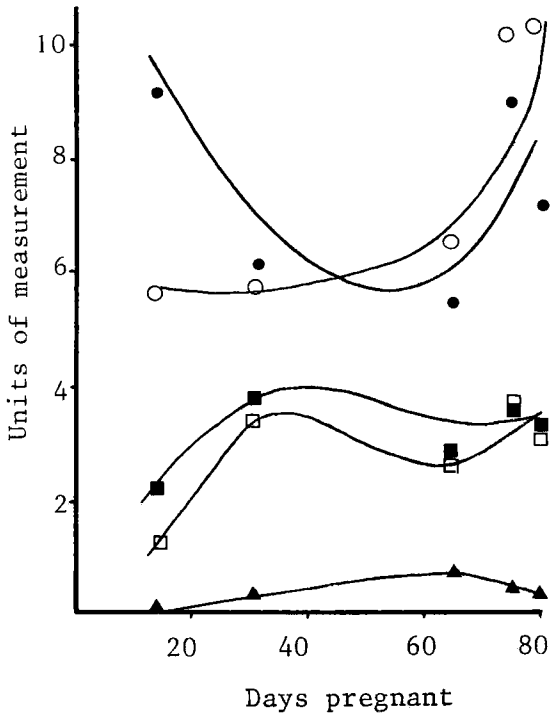
Fig. 7: A plot of fibrocyte density against the age of the corpus luteum, assigned through extrapolation of the relationship expressed by the points not included by A-B.

TABLE 6: AGE DIFFERENCES BETWEEN CONSECUTIVE GENERATIONS OF CORPORA LUTEA IN PREGNANT AND LACTATING SPOTTED HYAENAS

	n intervals between consec. gen. CL	$\bar{x}$ age difference between two consecutive generations of corpora lutea (days)
Pregnant ♀♀ (n=6)	10	55,3 ± 14,7
(n=2)	4	104,0 ± 16,7
(n=4)	6	22,7 ± 2,5
Parous lactating ♀♀ (with developing follicles) (n=4)	8	25,5 ± 1,6
Par. lact. ♀♀ (with atretic follicles) (n=7)	10	26,9 ± 3,2
All parous lactating ♀♀ (n=11)	18	26,3 ± 1,9

Changes in the corpus luteum during pregnancy and lactation

The present series lacks individuals in the last trimester of gestation, and all results therefore are only representative up to 80 days of pregnancy. Figure 8 illustrates changes in the five histological parameters during gestation, while Fig. 9-12 illustrate changes occurring from the time of formation, throughout pregnancy and the first half of lactation. It appears that the corpus luteum of pregnancy persists during the whole of gestation as well at least until the first half of lactation, without an obvious decline



- • Volume of corpus luteum ($mm^3 \times 10^{-2}$)
- ○ n Fibrocytes/25 mm^2 (400x)
- ■ $\bar{x}$ Luteal cell diameter ($\mu m \times 10^{-1}$)
- □ n Luteal cells/25 mm^2 (400x)
- ▲ n Vacuolated cells/25 mm^2 (400x)

Fig. 8: Histological changes in the corpus luteum of pregnancy (curves fitted by eye).

in volume, metabolic activity and luteal cell density. Perhaps the only significant feature of the ageing corpus luteum is that there is a gradual reduction in the size of luteal cells with advancing age, but this does not imply a total loss of secretionary functions.

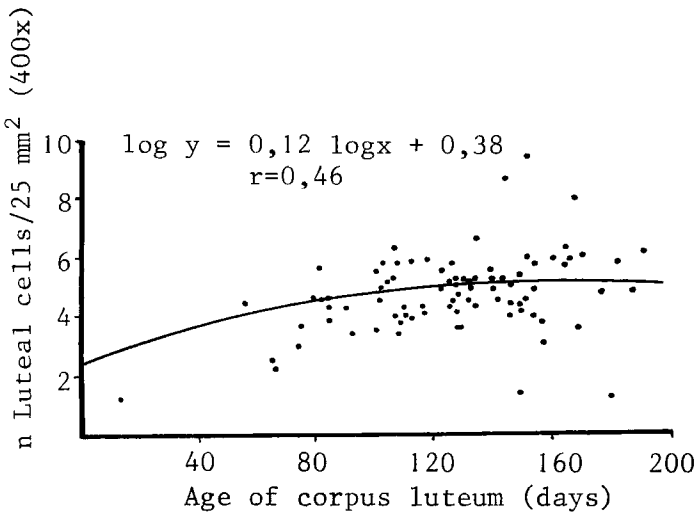


Fig. 9: Changes in luteal cell density during pregnancy and the first half of lactation.

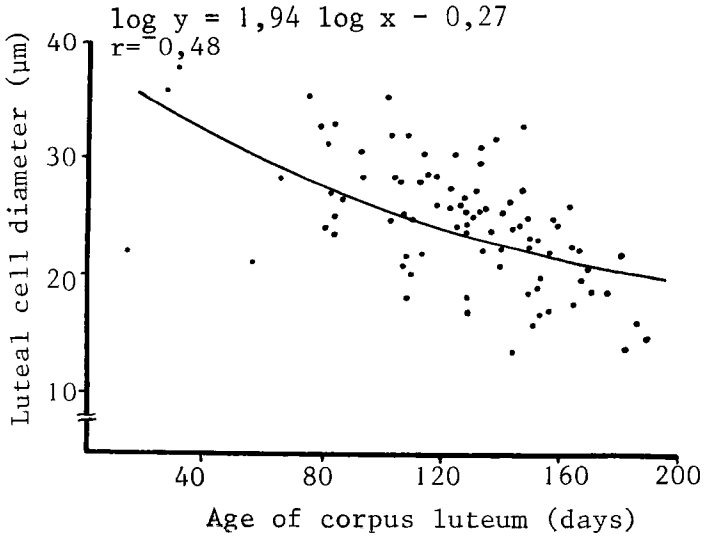


Fig. 10: Changes in luteal cell diameter during pregnancy and the first half of lactation.

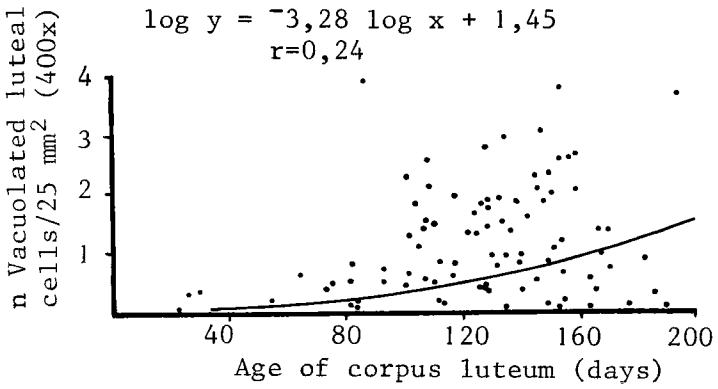


Fig. 11: Changes in the density of vacuolated luteal cells during pregnancy and the first half of lactation.

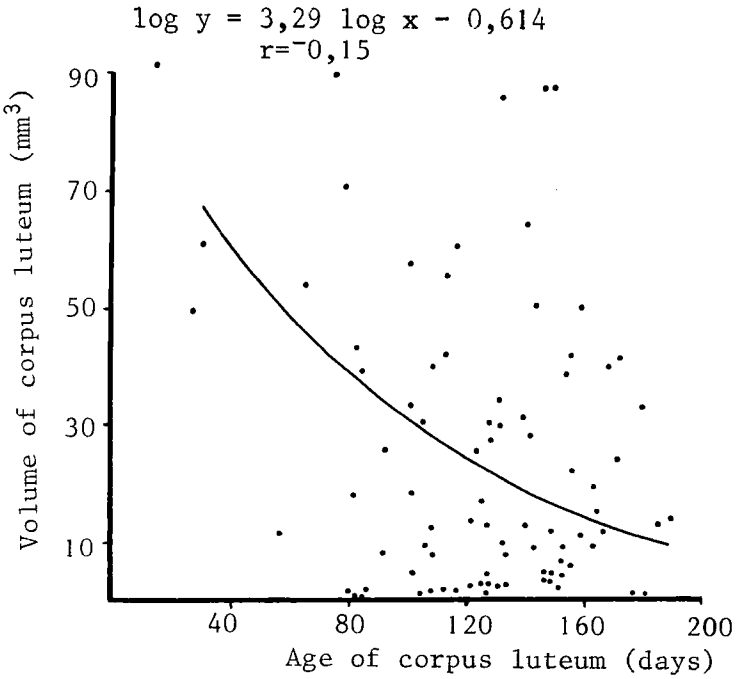


Fig. 12: Changes in the volume of the corpus luteum during pregnancy and the first half of lactation.

Occurrence of corpora lutea and follicles

Luteinization of atretic follicles occurred in only one nulliparous female. Otherwise, true corpora lutea were restricted to parous females, but not all parous females had corpora lutea. Corpora lutea were only found in pregnant and lactating females, and all such females had one or more corpora lutea in one or both ovaries. In the present series, a maximum of 14 corpora lutea per ovary, and 19 per individual, were found (18 corpora lutea and one corpus accessorius) (Fig. 13 and 14).

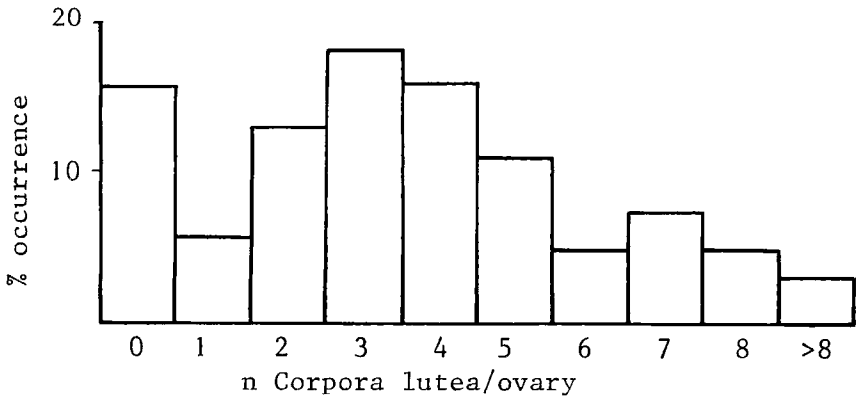


Fig. 13: The frequency distribution of the number of corpora lutea per ovary (n=38).

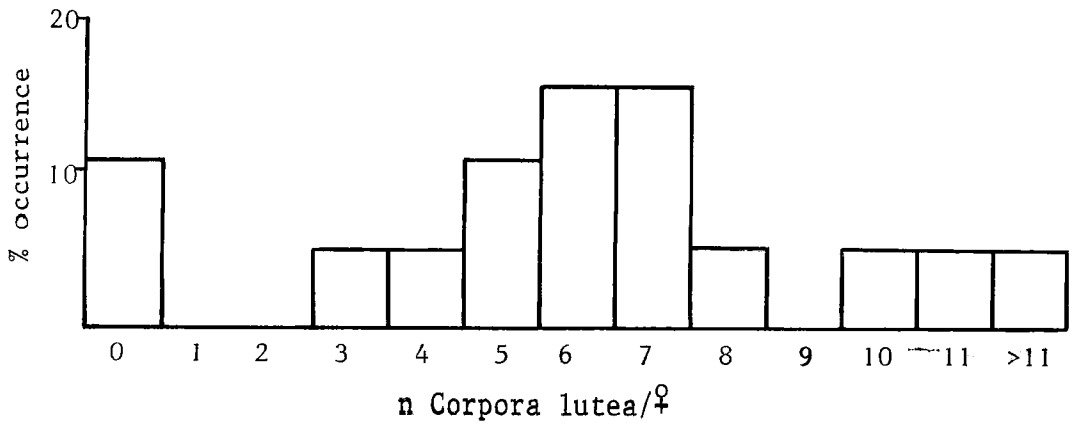


Fig. 14: The frequency distribution of the number of corpora lutea per female.

The mean number of generations for all females with corpora lutea was 2,5 ± 1,5, as in Table 7.

TABLE 7: MEAN NUMBERS (± SE) OF CORPORA LUTEA AND GENERATIONS OF CORPORA LUTEA IN FEMALES WITH CORPORA LUTEA

	$\bar{x}$ n. corpora lutea	$\bar{x}$ n. generations of corpora lutea	$\bar{x}$ n. corpora lutea per generation	n. accessory corpora lutea/♀
Parous pregnant	8,8 ± 3,2 (n=6)	2,7 ± 0,4 (n=16)	3,3 ± 0,6 (n=16)	0,7 ± 0,5 (n=6)
Parous lactating (D) Developing follicles	10,3 ± 3,2 (n=4)	2,8 ± 0,5 (n=11)	3,7 ± 0,5 (n=11)	0,5 ± 0,3 (n=4)
(A) Atretic follicles	6,6 ± 0,7 (n=7)	2,1 ± 0,3 (n=5)	3,1 ± 0,4 (n=15)	0,1 ± 0,1 (n=7)
All females with corpora lutea	8,2 ± 1,0 (n=17)	2,5 ± 0,1 (n=42)	3,3 ± 0,3 (n=42)	0,4 ± 0,2 (n=17)

In none of the three reproductive categories did the number of corpora lutea remain constant over age, as seen in Fig. 15-17, where the slopes of regressions calculated for the number of corpora lutea and the number of generations of corpora lutea are not identical. For the present sample, a slight reduction in the total number of corpora lutea produced per generation was evident in the older age classes. Differences in the number of corpora lutea in consecutive generations, described in Table 8, is thought to have been caused by the normal disappearance of older corpora lutea from the ovary, as well as the somewhat erratic development of accessory corpora lutea.

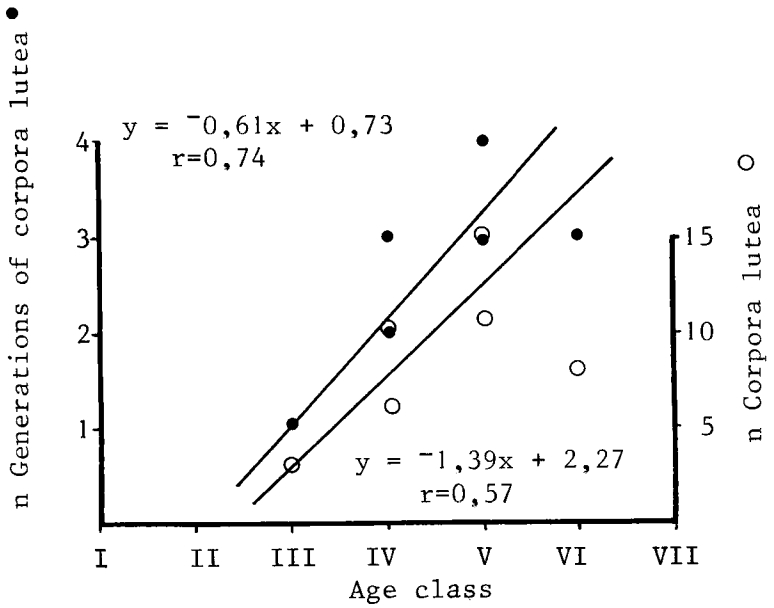


Fig. 15: The relationship between the number of corpora lutea and the number of generations of corpora lutea with age, in parous pregnant females.

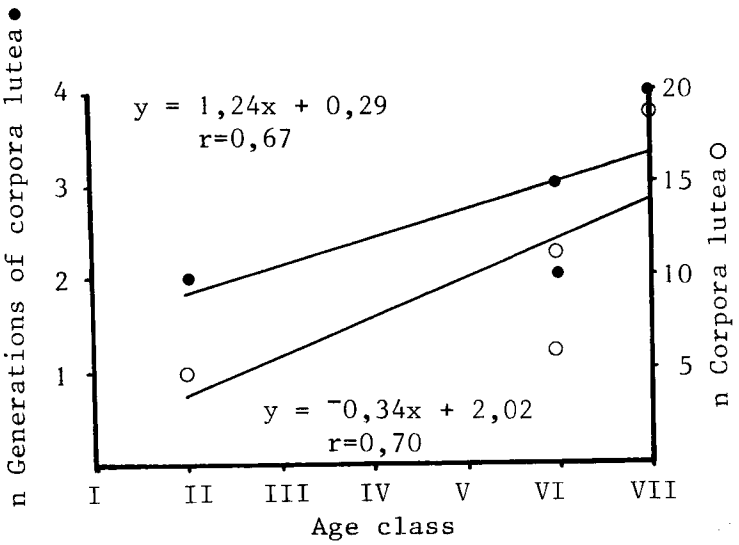


Fig. 16: The relationship between the number of corpora lutea and the number of generations of corpora lutea with age in parous lactating hyaenas with developing follicles (D).

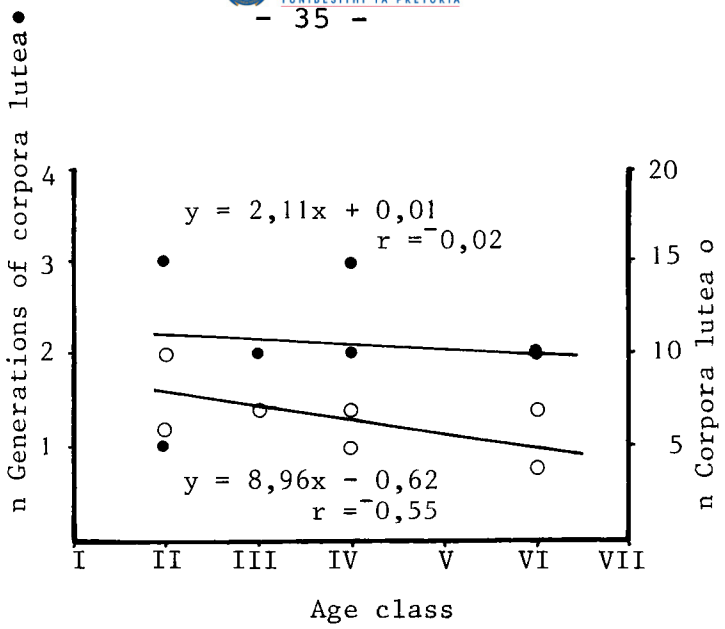


Fig. 17: The relationship between the number of corpora lutea and the number of generations of corpora lutea with age in parous lactating hyaenas with atretic follicles only (A).

TABLE 8: MEAN NUMBERS (± SE) OF CORPORA LUTEA IN THE FIRST, SECOND, THIRD AND FOURTH MOST RECENT GENERATIONS OF CORPORA LUTEA

	1st gen.	2nd gen.	3rd gen.	4th gen.
Pregnant ♀♀ (n=6)	[1] 2,3 ± 0,4	4,8 ± 1,5 *5,8 ± 1,4 (n=5)	1,2 ± 0,5 **2,3 ± 0,3 (n=3)	0,2 ± 0,2 1,0 (n=1)
Lactating ♀♀ D(n=4)	3,0 ± 0,8	4,5 ± 1,1 *4,5 ± 1,1 (n=4)	2,5 ± 1,5 **5,0 (n=2)	0,3 ± 0,3 ***1,0 (n=1)
Lactating ♀♀ A(n=7)	2,7 ± 0,6	2,7 ± 0,6 *3,2 ± 0,5 (n=6)	2,0 ± 1,2 **4,0 (n=2)	- -
All ♀♀ with corpora lutea (n=17)	2,7 ± 0,3	3,9 ± 0,6 *4,4 ± 0,6 (n=15)	1,6 ± 0,5 **3,4 ± 0,5 (n=8)	0,1 ± 0,1 ***1,0 (n=2)

* Only females with more than one generation of corpora lutea
** Only females with more than two generations of corpora lutea
*** Only females with more than three generations of corpora lutea

[1] Represents the mean number of corpora lutea of pregnancy.

Follicles in all stages of development were found in the ovaries of the youngest individual (less than a year old), and were still present in the ovaries of the oldest females (over 16 years of age). Few developing follicles were present in any one ovary, as most follicles undergo atretic degeneration. The remnants of destroyed follicles remain present in the cortex, in most cases only the zona pellucida of the non-ovulated follicle remaining.

Similar generations of follicular development were identified, the mean numbers of which are presented against reproductive category in Table 9, and age (Fig. 18). The occurrence of cystic follicles, luteinized follicles and developing follicles are described in Table 10.

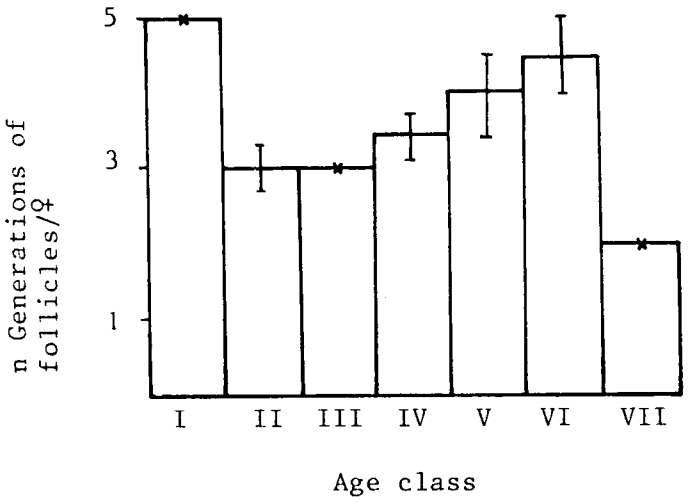


Fig. 18: The mean number (± SE) of generations of follicles per female in each age class.

TABLE 9: MEAN NUMBER (± SE) OF FOLLICLES AND GENERATIONS OF FOLLICLES PER FEMALE IN EACH REPRODUCTIVE CATEGORY

	$\bar{x}$ n. Atretic follicles			$\bar{x}$ n. generations of follicles	$\bar{x}$ n. Developing follicles		
	Prim.	Sec.	Tert.		Prim.	Sec.	Tert.
Nulliparous ♀♀ (n=4)	7,0 ± 1,8	7,8 ± 4,5	14,8 ± 4,6	3,8 ± 0,5	-	-	0,3 ± 0,3
Nulliparous ♀♀(A) (n=1)	12,0	20,0	25,0	5,0	-	-	-
Nulliparous ♀♀(B) (n=3)	5,3 ± 0,9	3,7 ± 2,7	11,3 ± 4,4	3,3 ± 0,3	-	-	0,3 ± 0,3
Parous pregnant ♀♀ (n=6)	8,5 ± 3,0	9,7 ± 1,6	18,5 ± 2,4	3,8 ± 0,4	-	-	-
Parous non-pregnant ♀♀ (n=13)	7,5 ± 2,5	8,1 ± 1,5	16,9 ± 2,7	3,5 ± 0,3	1,2 ± 1,2	0,1 ± 0,1	1,8 ± 1,0
Parous lactating ♀♀ (n=11)	7,9 ± 2,9	7,7 ± 1,7	17,6 ± 3,1	3,4 ± 0,3	1,4 ± 1,4	0,1 ± 0,1	1,8 ± 1,1
Parous lactating ♀♀ (A) (n=7)	3,9 ± 1,3	5,9 ± 1,8	17,0 ± 3,3	3,3 ± 0,2	-	-	-
Parous lactating ♀♀ (D) (n=4)	15,0 ± 6,7	11,0 ± 3,2	18,5 ± 6,9	3,5 ± 1,0	3,8 ± 3,8	0,3 ± 0,3	5,0 ± 2,5
Parous anoestrous ♀♀ (n=2)	5,5 (3,0-8,0)	10,0 (9,0-11,0)	13,5 (8,0-19,0)	4,0	-	-	1,5 (0,0-3,0)

TABLE 10: MEAN NUMBER (± SE) OF CYSTIC FOLLICLES AND LUTEINIZED ATRETIC FOLLICLES PER FEMALE IN EACH REPRODUCTIVE CATEGORY

	$\bar{x}$ n. cystic follicles	$\bar{x}$ n. luteinized atretic follicles
Nulliparous ♀♀ (n=4)	-	1,0 ± 1,0
Nulliparous ♀♀ (A) (n=1)	-	-
Nulliparous ♀♀ (B) (n=3)	-	1,3 ± 1,3
Parous pregnant ♀♀ (n=6)	-	0,8 ± 0,5
Parous non-pregnant ♀♀ (n=13)	-	0,7 ± 0,6
Parous lactating ♀♀ (n=11)	-	0,8 ± 0,7
Parous lactating ♀♀ (A) (n=7)	-	0,1 ± 0,1
Parous lactating ♀♀ (D) (n=4)	-	2,0 ± 2,0
Parous anoestrous ♀♀ (n=2)	1,0 (0,0-2,0)	-

Alternate ovulation

Synchronized development and maturation of follicles commonly occur in this species, yet not exclusively so. A comparison between the number of generations of corpora lutea and follicles in the left and right ovaries (Table 11, Fig. 19) showed that only 23,8% of corpora lutea originated as ovulations from alternate ovaries.

TABLE 11: MEAN NUMBER (+ SE) OF CORPORA LUTEA IN LEFT AND RIGHT OVARIES

	Parous pregnant (n=6)	Parous lactating D (n=4)	Parous lactating A (n=7)
Left Right	$\bar{x}$ = 5,2±1,0 $\bar{x}$ = 5,3±1,0*	$\bar{x}$ = 7,0±2,5 $\bar{x}$ = 3,3±0,9*	$\bar{x}$ = 3,9±0,8 $\bar{x}$ = 2,7±0,7*
$\bar{x}$ n. Corpora lutea per ovary	4,4 ± 0,7 (n=12)	5,1 ± 1,4 (n=8)	3,3 ± 0,5 (n=14)
$\bar{x}$ n. Corpora lutea per female	8,8 ± 1,7	10,3 ± 3,2	6,6 ± 0,5

*No significant difference between left and right ($p > 0,01$)

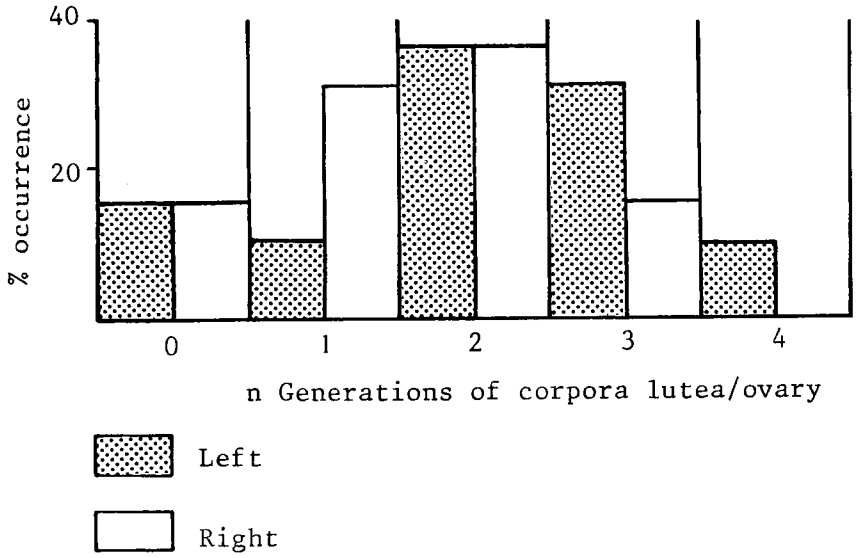


Fig. 19: The frequency distribution of the number of corpora lutea per ovary.

Of all consecutive generations of corpora lutea, only 4,8% occurred in one ovary only. The distribution of generations of follicles (Fig. 20) shows that only 20,2% of all ovulations are from alternate ovaries, while 9,6% of consecutive ovulations occurred in one ovary only.

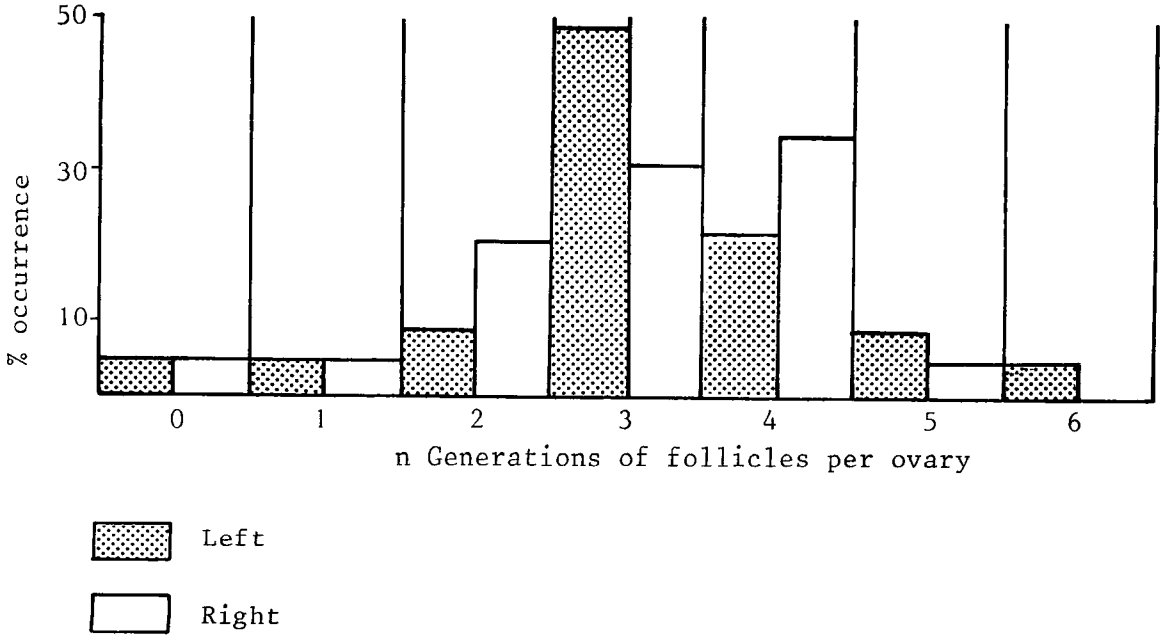


Fig. 20: The frequency distribution of the number of generations of follicles per ovary.

Sexual maturity

Development of follicles until the tertiary stage (antral follicles), already occurred in the youngest female examined, but the first indications of reproductive maturity or puberty were taken as the occurrence of luteinized follicles along with tertiary follicles. That all tertiary follicles event= ually become atretic at this stage, was evident from the absence of developing follicles in the four prepubertal females examined (one less than a year old, and three 1-3 years old). The distribution of reproductive categories with age is presented in Table 12, and Fig. 21 illustrates the progressive increase of the proportion of breeding females with age.

TABLE 12: FREQUENCY PERCENTAGE OF OCCURRENCE OF 39 FEMALE HYAENAS IN EACH REPRODUCTIVE CATE= GORY PER AGE CLASS

Age class	Nulli= parous	Anoes= trous	Preg= nant	Lacta= ting	Lacta= ting D	Lacta= ting A
I (n=4)	100,0	0,0	0,0	0,0	0,0	0,0
II (n=12)	58,3	0,0	0,0	41,7	33,3	25,0
III (n=4)	25,0	0,0	25,0	50,0	0,0	12,5
IV (n=6)	0,0	16,7	50,0	23,3	0,0	25,0
V (n=5)	0,0	20,0	60,0	20,0	16,7	0,0
VI (n=5)	0,0	0,0	0,0	100,0	33,3	25,0
VII (n=3)	0,0	0,0	33,3	66,7	16,7	12,5

Fig. 21: The percentage of females in each age class that were breeding (pregnant or lactating).

Of the pregnant females in the series, the youngest was 3-6 years old, but lactating females of 1-3 years old were also found (Table 12). That these young lactating females had actually been pregnant, were evident from the distended state of the uterus, typical of all parous females, and the presence of a generation of corpora lutea in their ovaries. A single generation of corpora lutea was found in 1-3 year old lactating females, indicating that this was their first reproductive attempt.

Pregnancy

Pregnant females were found in all of the last five age classes (III-VII), representing a period of > 13 years, which would be the maximum age difference between the youngest and oldest pregnant individual (Fig. 22). This implies that the reproductive lifespan of female spotted hyaenas, or the capability to conceive, would range over at least thirteen years. No definite relationship between body mass and

gestational age could be found, probably due to the added effect of age on body mass (Fig. 23). The youngest pregnant female was 3-6 years old and the oldest > 16 years.

Fig. 22: The percentage of females in each age class that were pregnant.

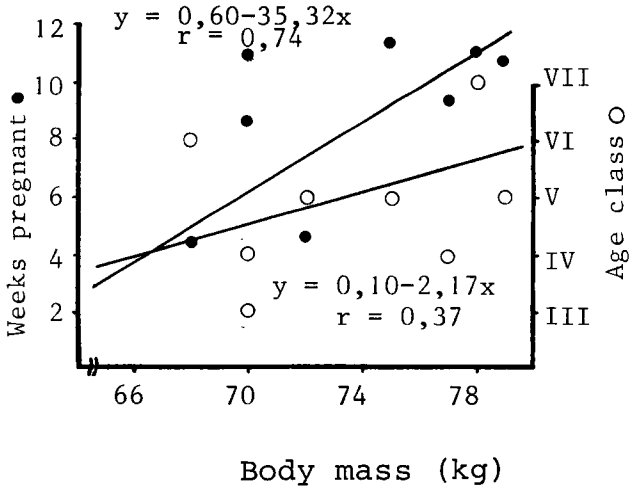


Fig. 23: The effects of progressive gestation and age on body mass.

With the exception of one female, all others (n=8) con=tained two foetuses. The singleton foetus may have occurred because the condition of the other uterine cornu resembled chronic endometritis, a condition commonly occurring in domes=tic mammals and man. For the present series (n=19) the foetal sex ratio (1:1,5; ♀♀ : ♂♂) seemingly biased towards males, does not differ significantly from equality ($\chi^2_{(1)} = 1,2$; $p > 0,01$).

Foetal wastage, in terms of the number of ova released com=pared to the number of conceptions, was calculated as 21,4%. In one case however, only one corpus luteum but two foetuses were found. This is possibly a case of twinning, identical in this case. The fact that both foetuses were males and that polyovular follicles were never observed to have developed further than the secondary stage, further substantiates a possible case of identical twinning. When this case was ignored, foetal wastage was calculated as 30,8%.

When two foetuses were present in an uterus, both uterine horns were occupied. Unfortunately, litter size never ex=ceeded two in the present series, so the distribution of larger litters as reported by Stevenson-Hamilton (1947), Golding (1969), Kruuk (1972) and Eloff (1975) remains unknown. The possible case of twinning mentioned above, also represents the only occurrence of transuterine migration of either blasto=cyst or unfertilized ovum. All other pregnancies were characterized by ovulation in both ovaries.

Lactation

Lactating females were found in age classes II to VII, repre=senting a period of > 16 years (Fig. 24). The youngest female found to be lactating was 1-3 years old, and the oldest > 16 years of age. All except two females were typically anoestrous, although 30,8% of lactating females (n=13) showed

signs of follicular development. The two exceptions were both pregnant females (one with blastocysts estimated to be a week old, the other at 75 days of gestation) which were also lactating, though in small quantity.

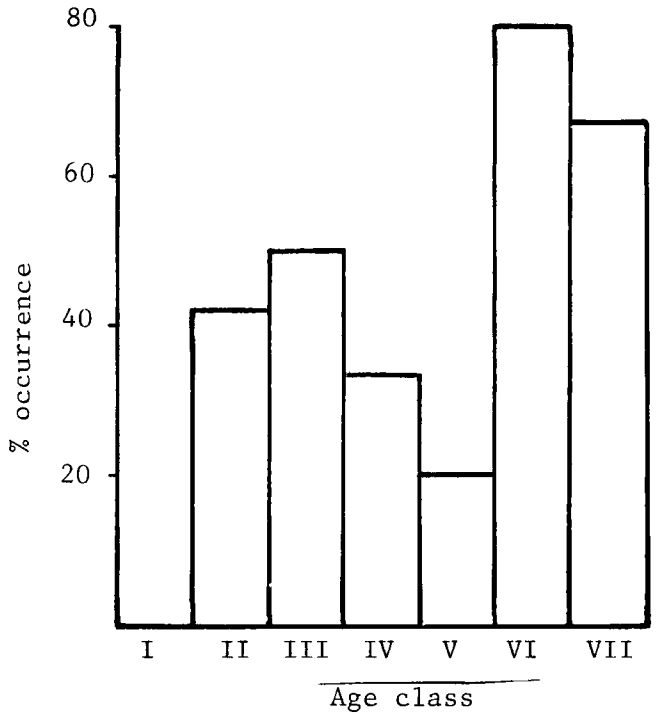


Fig. 24: The percentage of females in each age class that were lactating.

The reproductive histories of the lactating females were not identical, as seen from individual differences in numbers of recent generations of corpora lutea present. Neverthe= less, the number of corpora lutea in those generations judged to be the generations representing pregnancy, was of the same magnitude as for observed pregnancies (Table 8).

Reproductive senility

Of the three oldest females (> 16 years), two were lactating (one with atretic follicles, the other with developing

follicles), and one was pregnant. These females were in good health and condition, with perhaps the only sign of forthcoming senescence the relatively smaller numbers of follicles that reached the tertiary stage of development during the most recent episodes of follicular recruitment.

Two females however, 6-10 and 10-12 years old respectively, had never been pregnant, as indicated by the total absence of luteal tissue in the small recrudescent ovaries (pea-shaped). The 6-10 year old female had one comparatively large cystic follicle occupying most of each ovary.

Seasonality

Figure 25 presents the percentage frequency of occurrence of pregnant, lactating, pregnant and lactating, and non-pregnant, non-lactating (anoestrous) females. The occurrence of preg= nant females in the sample decreases through the year starting in February, while lactating females made up greater portions of the samples collected later in the year. A theoretical distribution of births and conceptions per annum, is given in Fig. 26. The highest frequencies of conceptions and parturitions were in December and March respectively. The median day of birth was calculated as 13 February (SE $\pm$ 54), and the median day of conception estimated as 25 October. In the lumped distribution (Fig. 27), the highest frequencies of conceptions and parturitions were in December and March respectively, the same as in Fig. 26. The mean day of birth was calculated as 17 January (SE $\pm$ 17), while the estimate for mean day of conception was 30 September.

From Table 13, it appears that most births occur in the first quarter of the year, and most conceptions in the last. If the three consecutive months with the highest frequencies are taken as "peaks", the period February-April accounts for 57,1% of the births in the theoretical distribution, and 38,8% in the composite distribution. Similarly the period

November-January accounts for 57,1% and 38,8% of the con= ceptions per annum. It seems that births and conceptions do occur at any time of the year, with slight peaks in late summer and early summer respectively.

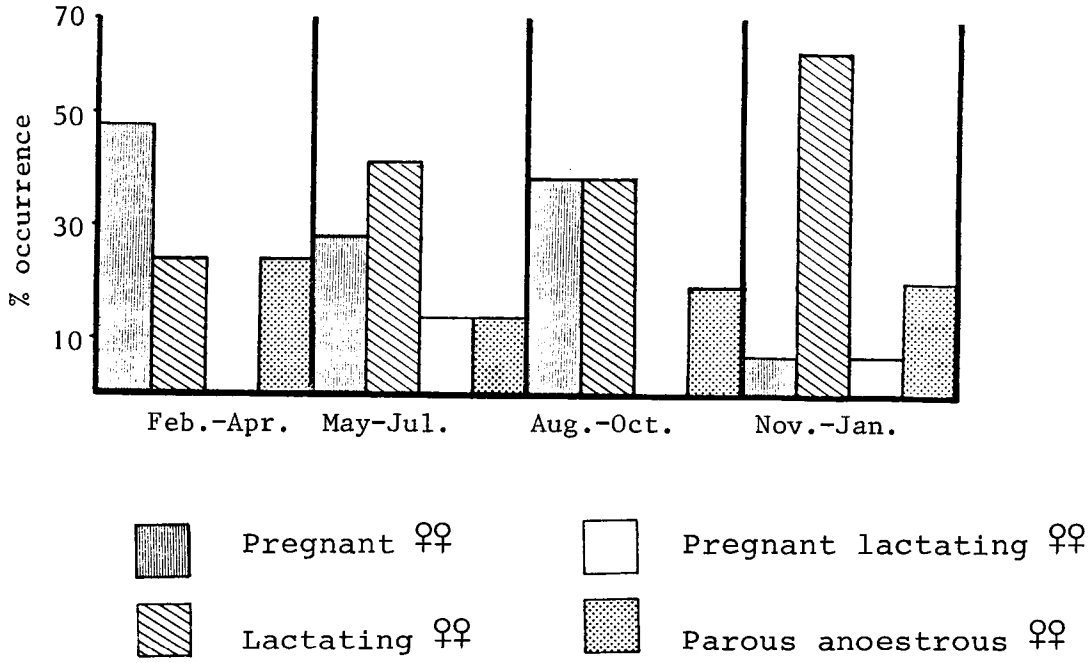
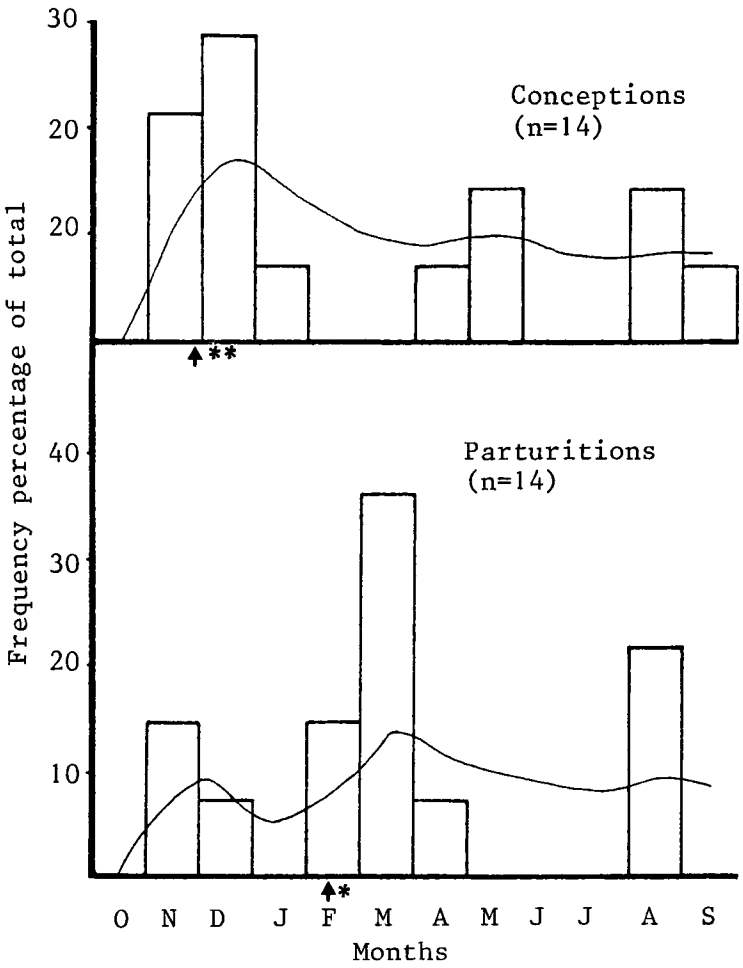


Fig. 25: Seasonal distribution of females in each reproduc= tive category.

TABLE 13: QUARTERLY DISTRIBUTION (%) OF THEORETICAL PARTURITIONS AND CONCEPTIONS FROM (A) PRESENT SAMPLE (n=14), and (B) COMPOSITE SAMPLE (n=35).

		Parturition	Conception
January-March	A	50,0	7,1
	B	32,3	31,4
April-June	A	7,1	21,4
	B	29,1	13,4
July-September	A	21,4	21,4
	B	16,1	28,6
October-December	A	21,4	50,0
	B	22,7	28,5

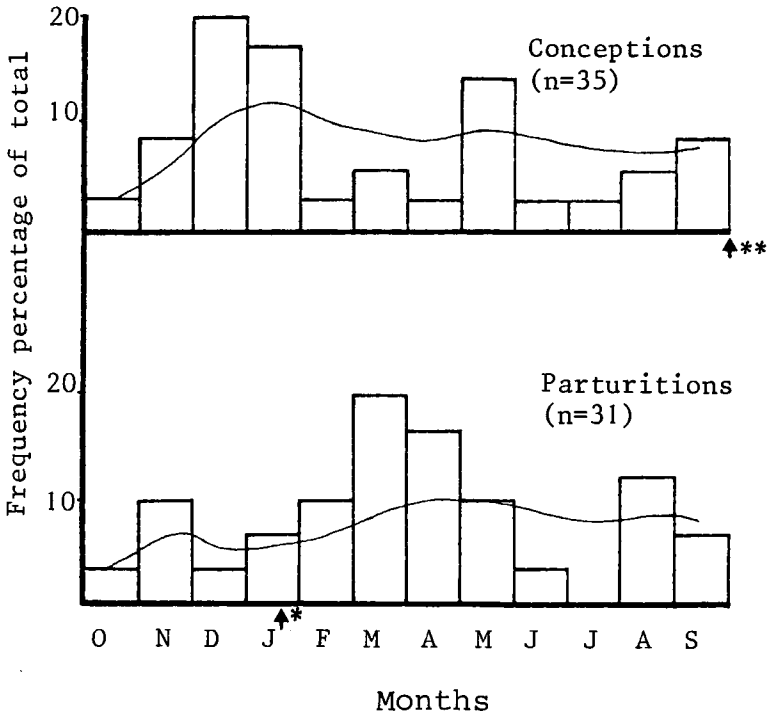


— Cumulative percentages

* Median day of birth 13 Feb. (SE ± 54)

** Estimated median day of conception 25 Oct.

Fig. 26: Per cent distribution of theoretical parturitions and conceptions per annum.



— Cumulative percentages

* Mean day of birth 17 Jan. (SE ± 17)

** Estimated mean day of conception 30 Sept.

Fig. 27: Per cent distribution of parturitions and conceptions per annum found by combining theoretical dates with published data by Fairall (1968).

DISCUSSION

Hitherto published information on the reproductive cycle of the spotted hyaena female, can be summarized as follows. Females become sexually mature at approximately three years of age, somewhat later than the male (Schneider 1926, Matthews 1939a, Kruuk 1972). The oestrous cycle is reported to be 14 days (Grimpe 1916, Schneider 1926), and is recurrent with intermittent anoestrous periods recorded as 14 days (Grimpe 1916), 21-60 days (Schneider 1926) and 45 days (Golding 1969). Females therefore have a polyoestrous cycle. The luteal phase lasts longer than half of the oestrous cycle as in most mammals, and corpora lutea are believed to be of the persis= tent type (Matthews 1939a).

Pregnancy lasts for approximately 110 days (99 days, Grimpe (1916); 109-111 days and 97-132 days, Schneider (1926)) and usually two young are born in an advanced state of develop= ment (Grimpe 1916, Schneider 1926, Deane 1962, Pournelle 1965, Golding 1969, Kruuk 1972). Litter size varies from one to four (Stevenson-Hamilton 1947, Golding 1969, Kruuk 1972, Eloff 1975), but the modal number appears to be two. Lactation lasts between six and 18 months (Matthews 1939a, Kruuk 1972), but the latter author believes that 12-14 months is the usual period. After the loss of a litter, through natural mortality or cub removal in captivity, females are capable of breeding again soon (21-35 days, Grimpe 1916, 45 days, Golding 1969), even while still lactating (Schneider 1926, Kruuk 1972). Following normal weaning, litter inter= vals of 9-26 months, on average 17,4 months, have been re= corded by Kruuk (1972).

In southern Africa, aseasonal breeding without any peaks, has been reported for Botswana (Smithers 1971), Kalahari Gemsbok Park (Eloff 1975), the Transvaal Lowveld (Pienaar 1963) and Zululand (Deane 1962). Reports of peaks in births are available for Malawi-Zimbabwe (April-July, Smithers 1966), Etosha, South West Africa (October-April, Van der Spuy in Deane 1962) and the Transvaal Lowveld (winter months Fairall 1968). Although conclusions must be qualified due to limited sample size, the one aspect on which there is agreement, is the occurrence of births throughout the year in the spotted hyaena, a point of contention being the occur= rence and validity of peaks at certain times of the year. The latter may be further confounded by reduced seasonal breeding of prey species the closer one comes to the equator. Thus Matthews (1939) and Kruuk (1972), demonstrated an absence of peaks in latter day Tanganyika and the present day Serengeti areas. Kruuk (1972) did, however, detect a slight peak in births during April in the Ngorogoro Crater in Tanzania.

It was seldom necessary to rely on the structure of the ovaries only, to determine the reproductive status of individuals examined in the field. Lactating females were identified as such when milk was extruded on pressure being applied to the mammae. The quantity was of no great significance, since greatly swollen udders seldom yielded much more than smaller ones. The effects of the immobilizing agent on milk let-down have not been studied, but are thought to be inhibitory. Pregnant females in the first trimester of pregnancy could only be diagnosed accordingly through dissection, which always provided confirmation when palpation of obviously extended abdomens indicated pregnancies.

The state of the external genitalia and mammae could not be regarded as conclusive evidence to distinguish between preg= nant, pregnant-lactating, or lactating females. Parous non-pregnant, non-lactating females could not be identified without dissection either, but pre-pubertal and nulliparous females all had clittori and urogenital orifices lacking signs of previous births. When dissection was resorted to, the uterus yielded little additional information on status in the parous non-pregnant category. No placental scars could be identified with certainty, and it appeared that the uterus quickly involutes, a fact borne out by Grimpe's (1916) observation of conception 35 days after parturition, followed by a normal pregnancy. Pre-pubertal females are easily identified by the thin, wiry appearance of the uterine horns, and the small mammae.

The life-span of the corpus luteum presents a problematic situation. If corpora lutea are persistent as Matthews (1939a) believes, and are present in the ovary for up to three years, one would expect far greater numbers of corpora lutea in the ovaries of animals old enough to have gone through several cycles, than was apparent from this study. In addition the remarkably small differences in appearance of corpora lutea

of different generations would imply the regular occurrence of infertile cycles, during which ovulation and corpus luteum formation take place, until the animal conceives. This suggestion, as well as the fact that more corpora lutea are formed out of atretic follicles, would not support Matthews' (1939a) conclusion that each distinct generation of corpora lutea will differ at least one year in age.

On the other hand, the fact that very few corpora albicantia were found by both Matthews and in the present study, would support the theory that corpora lutea are persistent. The fate of the corpus luteum as described, would however suggest that corpora albicantia are only rarely formed anyway, due to the fact that the corpus luteum eventually 'dissolves' into the surrounding cortex, and is not transformed in situ. An alternative explanation for the phenomenon of multiple generations of corpora lutea in some ovaries, would be the regular occurrence of infertile cycles as part of the poly= oestrous pattern in this species. These infertile cycles are not necessarily maladaptive, as Conaway (1971) maintains, for if a litter is lost, the female could conceive again with= in a relatively short period. It does appear that if the litter is successfully reared, lactation, or some factor related to lactation, causes the inhibition of conception at one or other point in that process. The female nevertheless continues to cycle.

It is also possible that interpopulation differences in breeding, such as the interval between litters, might explain the apparent contradiction between Matthews' (1939a) results and those of the present study. Perry (1953) suggested a method of estimating calving interval in ungulates, where the gestation period is divided by the ratio of pregnant females : all parous females. The gestation period is then added to this figure, to give an estimate of the calving interval. This method has subsequently been used by Buss &

Smith (1960), Laws & Parker (1968), Grimsdell (1973) and Hall-Martin & Skinner (1978). If this method is applied to Matthews' (1939) data, the litter interval of the 1939 population is 24,8 months, which is well within the range of observed litter intervals by Kruuk (1972) (9-26 months). The average litter interval in Ngorongoro in the late 1960's is quoted by Kruuk (1972) as 17,4 months (± 2,4), which according to him is a very conservative estimate. In captivity, Pournelle (1965) recorded an average litter interval of 11,6 ± 1,5 months, but did not state whether cubs were removed after birth or not.

The estimate for litter interval in the Central District of the KNP is 19,3 months, and 13,2 months in the Southern District, giving an average of 15,9 months for the KNP population. The KNP population is an exploited one, and has been so for more than 80 years (Pienaar 1963, 1969) and one would expect to find differences in fecundity between a natural and an exploited population (Laws 1973). The effects of the extensive reduction of the population in the Central District in 1974-1980 (365 hyaenas culled out of an estimated population of 1800 (Smuts 1976, 1978a,b)), have not been studied as yet, but the greater litter interval in the Central District as compared to the Southern District where the population was left untouched, indicates perhaps a local effect of a lower fecundity caused by population control measures in the Central District. The litter interval in the Central District was nevertheless much lower than in the 1939 Serengeti population.

In conclusion, it would seem that corpora lutea in the spotted hyaena are of the persistent type, but almost certainly are not visible after much longer than one birth interval. Multiple generations of corpora lutea, as was found in the present study, therefore represent recurring oestrous cycles (infertile), and only occasionally more than one breeding cycle or pregnancy. This can be seen as an adaptation to

the high infant mortality, as recorded by Kruuk (1972), that allows the female hyaena to conceive again rapidly after the loss of a litter.

In southern Africa, studies by Pienaar (1969), Bearder (1977), Smuts (1979), Tilson, von Blottnitz & Henschel (1980) and Skinner & Van Aarde (in prep.), have indicated that spotted hyaenas utilize a great variety of food items ranging from berries and quelea finches, to large ungulates. The mere variety in food items, suggests that spotted hyaenas are opportunistic feeders, easily switching from one food source to the next, presumably the more abundant. In the past, and still in some areas, fewer types of prey might have been utilized, if hyaenas had migrated with the dominant ungulate species. It seems that at present, spotted hyaenas are sedentary throughout most of their range in southern Africa, notably the wetter eastern half. Under these conditions, and certainly when small cubs are present, the local residents would have to make do with what is available in that area. When calves or lambs are plentiful, these make up most of their diets, otherwise even unlikely animals such as lions, African wild cats, eagles and rodents have to be utilized.

If there is agreement on the opportunism in the feeding strategy of the spotted hyaena, then it may well be reasoned that they might be able to exploit periods of abundance in a reproductive context, especially since they seem to be such conservative breeders. The mechanism by which a peak in births could result is suggested to be as follows. Periods of abundance and over-abundance of food might ease the energy burden on the lactating female, either through easier replenishment of energy lost in milk production, or faster weaning of the litter. The female could then theoretically embark on a next reproductive attempt. This would explain Kruuk's (1972) finding of a higher incidence of conceptions after a period

of food abundance. Since these conditions would only apply to females in the required phase of lactation, the resulting peak would only be slight, as observed.

Results from the present study support the generally accepted conclusion of all-year-round breeding. Fluctuations in fertility levels in the male population throughout the year could not account for deviations from a year-round pattern as in Matthews (1939a). The aseasonal occurrence of oestrus and the absence of any oestrus synchronization, only confirm all-year-round breeding. Since the work of Matthews (1939a), and Kruuk (1972), the importance of the lactation period in the ecology of this species has received much attention, and is suspected of being the limiting factor in female productivity. The peculiar strategy of sustaining cubs on milk alone (Kruuk 1972) limits the female to one reproductive attempt at any stage. The female seems to be incapable of breeding again until the previous litter is weaned, which may occur at 6-18 months (Van Lawick-Goodall and Van Lawick 1970, Kruuk 1972), or after the loss of a litter (Grimpe 1916, Schneider 1926). The extreme variation in the lactation period suggests that food availability might cause early, or delayed weaning, and therefore a delay in, or acceleration of, the onset of the next reproductive attempt.

Kruuk (1972), explains the occurrence and absence of a peak in births in two equi-latitudinal areas, the Ngorongoro Crater and Serengeti, in terms of food abundance. In Serengeti, the food supply remains constant throughout the year, while the Ngorongoro hyaenas experience a period of abundance during January-February, followed by a higher incidence in conceptions and a resulting peak in births in April although this peak is very slight.

In the Kruger National Park, and also in the Lowveld region of southern Africa in general, periods of abundance do occur.

All three major prey species, impala, wildebeest and zebra (Pienaar 1969, Bearder 1977, Mills 1978, Smuts 1979), lamb/calve at roughly the same time of year, with peaks in the November-December period (Fairall 1968, Smuts 1974). This period coincides exactly with the period showing the highest incidence in conceptions (Figs. 26 & 27). I suggest that slight peaks in births might occur resulting from a response in that sector of the population able to do so as a result of females nearing the end of lactation with litters nearing minimum weaning age and thus able to respond locally to periods of abundant food supply.

This will differ from region to region, depending on the local configuration of food supply, temporal versus continual, and the occurrence of peaks should be interpreted accordingly. Lastly, the problematic situation of adaptation to annual periods of abundance should cause little concern. This could never have developed in the spotted hyaena, since the minimum duration of the normal female cycle is slightly longer than one year in any case (Matthews 1939a), as well as the fact that infant mortality seems to be universally very high (Kruuk 1972), and females breed again soon after the loss of a litter. Every year a different group of females will be able to take advantage of any seasonal bonus such as the impala lamb crop.

CHAPTER 3

MALE REPRODUCTIVE BIOLOGY

INTRODUCTION

Earlier reports, as mentioned in the previous chapter, indicated seasonal breeding in certain regions in southern Africa. This could result from fluctuating fertility in male hyaenas. Matthews (1939a) did indeed find individual differences in the extent of sexual activity in males al= though births occur throughout the year in most regions, thereby indicating that there are always sexually active males present.

The role of the male component in the spotted hyaena popu= lation, regarding breeding strategy and social structure, remains somewhat enigmatic. Kruuk (1972) observed that although a number of males were attracted to an oestrous female, only one individual achieved all the matings, with little or no competition or aggression from the other attend= ant males. Furthermore, it appears that females exercise some degree of mate selection, although this is apparently not influenced by the age and social standing of individual males (Kruuk 1972). In one instance, an oestrous female did not allow a male from the same clan to mate with her, although the male was willing to copulate, as seen from its redirected attentions to a nearby cub, and repeated spontaneous ejaculations. The impression derived from such beha= vioural anecdotes is reminiscent of the situation occurring in the closest relative of the spotted hyaena, namely the brown hyaena (*Hyaena brunnea*), where again only one male will do all the matings. This individual always seems to be outside the social relations of the female involved, i.e. from another family (Mills, Gorman & Mills 1980, M.G.L. Mills, pers. comm.).

Male spotted hyaenas are known to be less attached to a particular clan, than females, and are prone to wander between neighbouring or even distant clans (Kruuk 1972, Whateley 1980). It is conceivable that these individual males might perform a function similar to the brown hyaena male, where only an outsider would mate, while the local or resident males are not preferred to some degree.

MATERIALS AND METHODS

Thirty-six males (29 adults) were available for this study. From October 1975, hyaenas were culled on six occasions in the Central District of the Kruger National Park (17 adults, 2 juveniles), the Southern District of the KNP (11 adults, 2 juveniles), Umfolozi Game Reserve, Natal (1 adult, 1 juvenile captured and released) and in Wankie National Park, Zimbabwe (2 adults). In addition, material collected from three adult males taken in 1971 in the KNP, was also avail= able.

After individuals were weighed and measured, reproductive tracts were removed. Epididymides, after being dissected free from the testis, were kept frozen. Testes were weighed on a triple-beam-balance, after which a small piece was stored in formalin-calcium solution, and the bulk in AFA. Post-fixation weights of fixed material were measured on an electronic balance, while linear measurements of the diameter and length of the testis, were taken with a vernier calliper.

Following routine dehydration and paraffin embedding tissue was sectioned at 5-8 µm and sections were routinely stained with Delafield's/Ehrlich's haematoxylin and as counterstain Chromotrope 2R, eosin or eosin-phloxine. Cryostat sections of 8 µm, sectioned at -30^{o} C, of tissue samples fixed in

formalin-calcium, were dessicated with silica gel at 0° C for at least 48 h before being used. Neutral lipids and triglycerides were demonstrated by treatment with Methyl Green, Oil Red O and Sudan Black, the latter according to a modification of Threadgold's (1957) method I.

Seminiferous tubule diameters were measured in cross section with a micrometer eyepiece calibrated with a micrometer slide, and the mean value for 25 tubules was used. The random observation technique of Chalkley (1943) for the determination of the cellular composition of the testis, was used, involving random sampling of elements appearing at the tips of four pointers set into the occular lens of a microscope, in a minimum of 50 randomly selected fields. Densities of interstitial cells were measured on an occular grid at 400 x magnifications. The mean value of 40 fields was used, after trials with greater sample sizes resulted in negligible variation. Interstitial cells of Leydig were identified by their large nuclei, vacuoles and high lipid content, as demonstrated with Sudan Black.

Epididymal contents, were recovered following Dott & Skinner (1967) for fresh-frozen material, and through homogenization of fixed epididymides and subsequent centrifugation and dilutions of suspensions. An attempt was made to obtain semen from immobilized animals through electrical stimulation. Electro-ejaculation was achieved with a rectal probe and variable output stimulator, but yields were low and consisted only of the secretions of the accessory glands of the re= productive tract. This technique was therefore discarded.

RESULTS

Testicular morphology

The histology of the testis and epididymis has been described in great detail by Matthews (1939a) whose findings and those

of the present study confirm that the testicular histology conforms to the general mammalian pattern.

Relationships between the diameter of the seminiferous tubules and combined testicular mass (Fig. 28), and the ratio of combined testicular mass (g) to body mass (kg) x 10^{-1} (Fig. 29), indicate that all the hyaenas were not equally sexually active, showing some individual variation, possibly due to season, age and size. Figures 30 and 31 illustrate the poor relationship between combined testicular mass and head-body length or body mass, and Figures 32 and 33 illustrate a similar poor relationship between the average diameter of seminiferous tubules and head-body length or body mass.

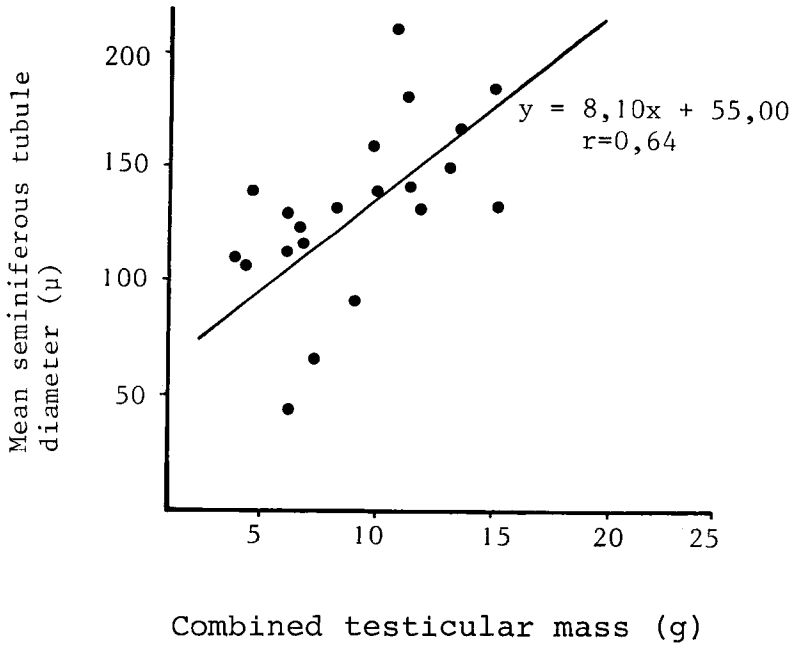


Fig. 28: The relationship between combined testicular mass and the average diameter of the seminiferous tubules.

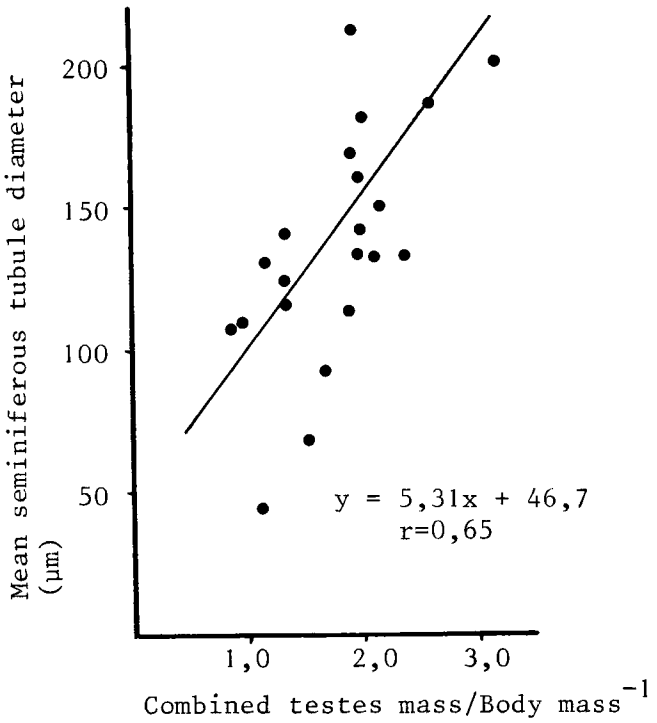


Fig. 29: The relationship between seminiferous tubule diameter and combined testicular mass corrected for body mass.

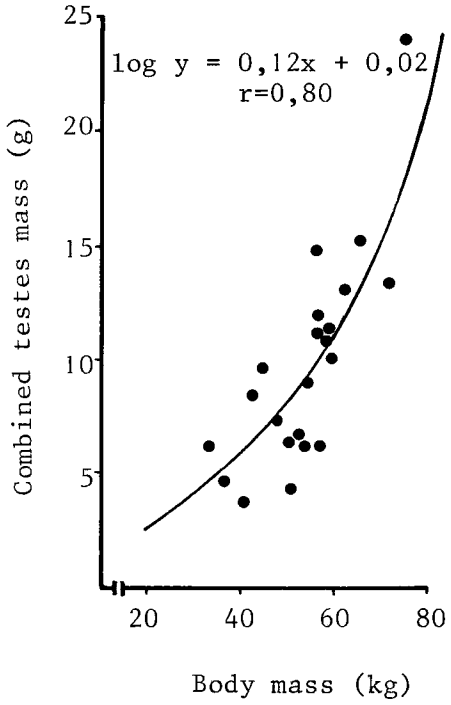


Fig. 30: The relationship between combined testicular mass and body mass.

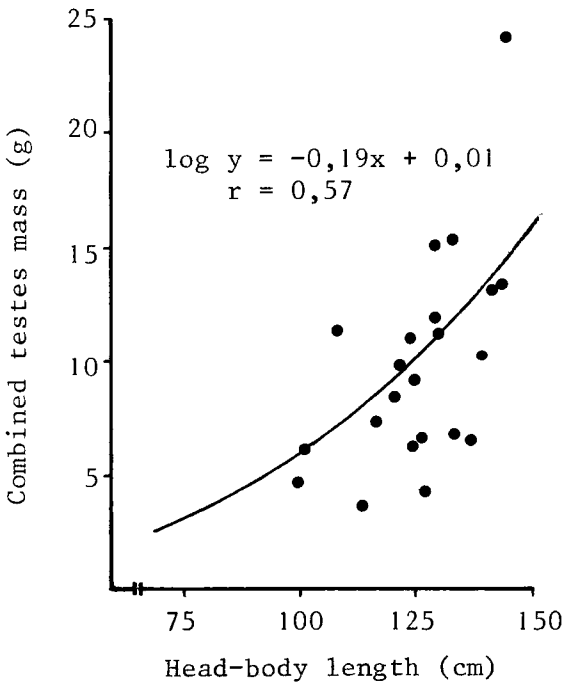


Fig. 31: The relationship between combined testicular mass and head-body length.

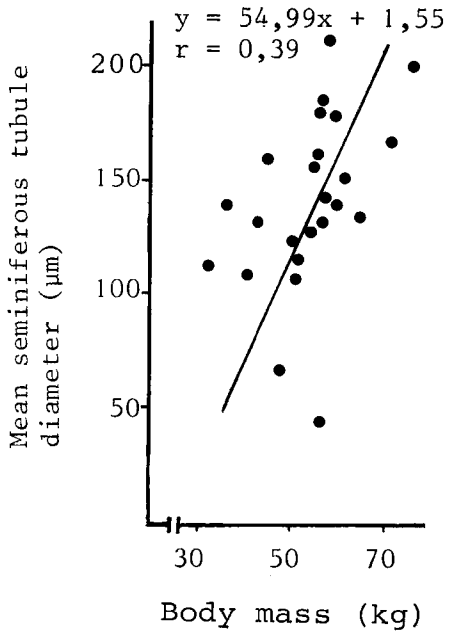


Fig. 32: The relationship between mean seminiferous tubule diameter and body mass.

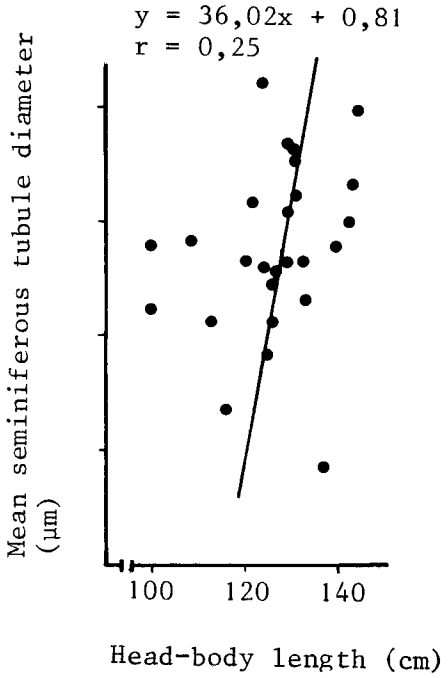


Fig. 33: The relationship between mean seminiferous tubule diameter and head-body length.

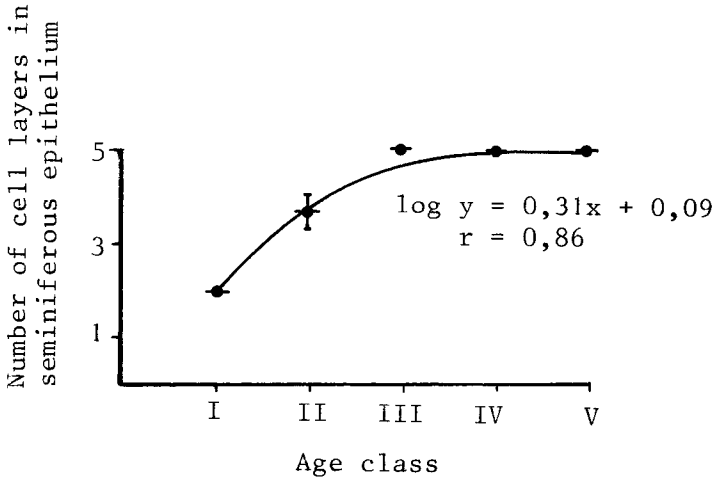


Fig. 34: The relationship between thickness of the seminiferous epithelium and age.

Table 14 presents a summary of the testicular composition analysis, as described by Chalkley (1943), while Figure 34 illustrates an increase in the thickness of the seminiferous epithelium, regardless of variation in sexual activity. From Table 14, it is apparent that a similar trend is shown for the age-related abundance of spermatocytes, spermatids and spermatozoa. It also indicates a difference in the construction of the testes of older animals, as compared to younger individuals, namely the gradual increase in the relative contribution of intra-tubular tissue to the bulk of the testis, as was also seen in Fig. 35.

TABLE 14: TESTICULAR COMPOSITION ANALYSED FOLLOWING CHALKLEY (1943), FOR EACH AGE CLASS

Age Class	% Relative abundance						
	Extra-tubular area	Intra-tubular area	Spermato-gonia	Spermato-cytes	Spermatias & spermato-zoa	Sertoli Cells	Space
I (n=2)	38,5 (35,0-42,0)	61,5 (58,0-65,0)	9,9 (8,5-11,3)	19,8 (18,3-21,3)	17,4 (13,8-21,0)	0,5 ± (0,0-1,0)	12,1 (7,4-16,8)
II (n= 3)	35,1 ± 3,9	64,9 ± 3,9	12,8 ± 1,8	21,4 ± 1,3	13,5 ± 2,7	1,5 ± 0,5	15,8 ± 2,6
III (n=2)	28,0 (20,1-35,9)	72,0 (64,1-79,9)	6,9 (5,2-8,6)	25,0 (24,5-25,5)	27,5 (21,7 - 33,3)	1,0 (0,5-1,5)	11,8 (11,4-12,2)
IV (n=1)	20,4	79,6	11,7	29,4	35,3	2,2	1,1
V (n=1)	20,0	80,0	6,0	16,7	15,2	3,7	17,1

The density of Leydig cells in the testis was determined and the results are expressed in Fig. 35. That the density of Leydig cells ultimately depends on the space available inbetween tubules, is shown by regression C in Fig. 35. A significant correlation was found between the percentage composition as extra-tubular spaces, and densities of Leydig cells (r = 0,98, b = 0,19). In conclusion it seems that there are no age-related differences in the densities of Leydig cells in the testis, although the actual number of Leydig cells would increase with age, since the testis itself increases in size, with age (Fig. 30 & 31).

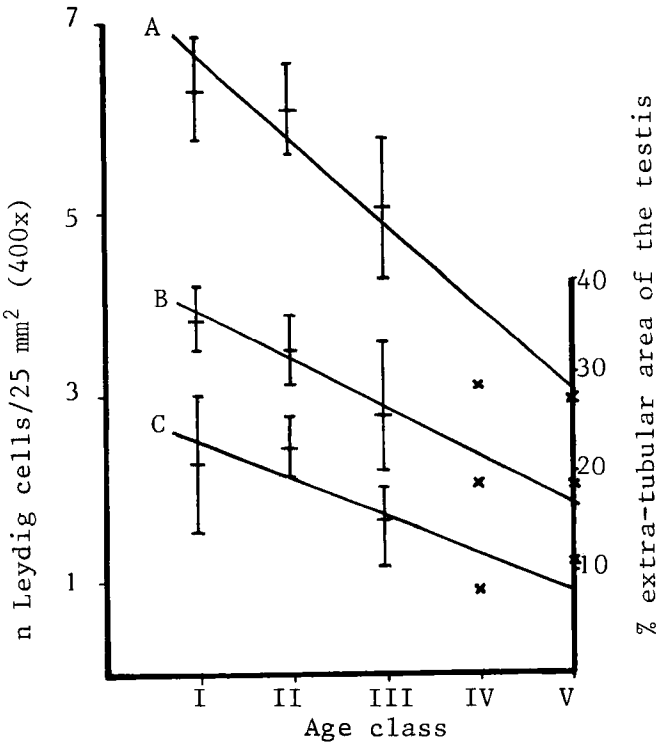


Fig. 35: The relationship between Leydig cell density (A : extra-tubular areas only, B : total area of the testis) with age. Regression C indicates the percentage composition of the testis as extratubular areas, with age. (A : y = 7,62 x -0,97; r = -0,96; B : y = 2,80 x -0,37; r = -0,87; C : y = 43,9 x -5,17; r = -0,97).

Age-related fertility

The presence of spermatozoa, in the lumina of the tubuli seminiferi, and attached to sertoli cells in the wall, was recorded in the youngest male examined (less than one year of age), although another individual of that age was still azoospermic. From Table 14 it is apparent the abundance of the gonocytic elements (spermatogonia, spermatocytes and spermatids) increases with age, possibly due to the overall enlargement of the testis and seminiferous tubules. The same trend was not apparent for spermatozoa as such, and the erratic occurrence of spermatozoa in the different age clas= ses is presumably caused by individual differences in the de= gree of sexual activity and therefore the magnitude of pro= duction and rate of elimination of spermatozoa from the testis.

Figure 36 illustrates the age-related changes in testicular mass, epididymal mass, mean diameter of the seminiferous tubules, and the mean epididymal sperm contents per age class.

Unfortunately, the older age classes are under-represented or absent in the present series, and no conclusions can be made regarding the fertility of older animals, except that all stages in the spermatogenic cycle were evident in hyaenas up to 10-12 years old.

Seasonality

No histological differences were found in the testes of mature males throughout the year, a fact borne out by an analysis of variance in testicular composition per season (Table 15), where no significant relationship between any two parameters could be demonstrated ($F_{(1,n-2)} = p < 0,05$). Seasonal differences did however exist in the average semi= niferous tubule diameter, where this value in August-October was significantly higher than in the other three periods

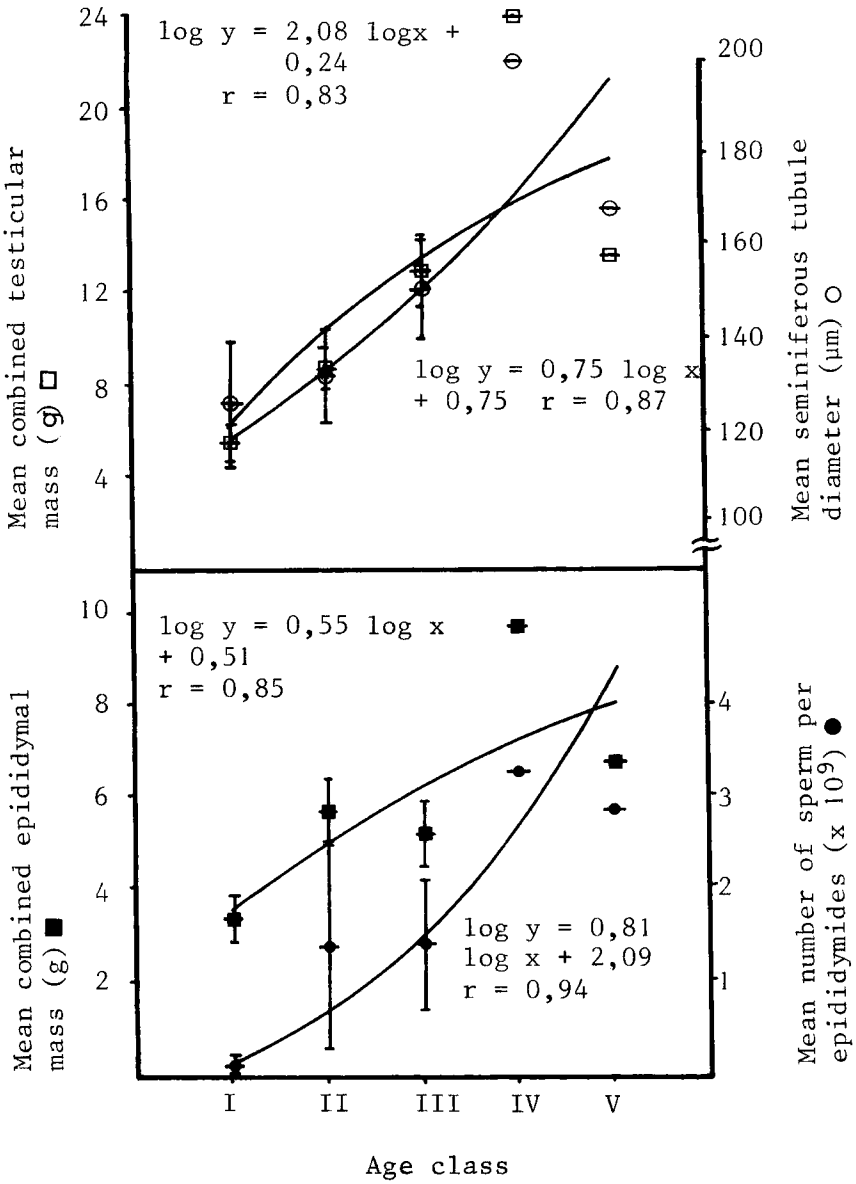


Fig. 36: Age-related changes in combined testicular mass, mean seminiferous tubule diameter, combined epi= didymal mass and sperm content per pair of epidi= dymides in male spotted hyaenas.

TABLE 15: ANALYSIS OF SEASONAL TESTICULAR COMPOSITION OF MATURE SPOTTED HYAENAS

	Combined Test. mass (g)/Body mass (kg)	Av. Sem. Tub. Dia=meter (μm)	% Sperma=tocytes	% Sperma=tids and spermatozoa
Feb-Apr (n=5)	0,164 ± 0,022	126 ± 6	20,1 ± 1,8	13,6 ± 3,2
May-Jul (n=5)	0,185 ± 0,039	141 ± 16	26,9 ± 2,5	12,0 ± 8,1
Aug-Oct (n=5)	0,169 ± 0,043	162 ± 10	24,4 ± 4,6	12,2 ± 1,9
Nov-Jan (n=10)	0,172 ± 0,012	134 ± 17	20,7 ± 1,5	19,5 ± 3,7

($p < 0,01$). The percentage spermatocytes or spermatids and spermatozoa, or testicular mass corrected for body mass, however, did not change significantly from season to season ($p > 0,01$) and the enlargement of the seminiferous tubule diameter during August-October is apparently not related to an increase in the sperm concentration in the testis.

DISCUSSION

The present results confirm the observations of Matthews (1939a), that the male reaches sexual maturity, as indicated by the exocrine function of the testis, a little earlier than the female, at approximately 1-2 years of age. Sexual activity however, has been shown to be dependent on the size of the individual, thus older and larger individuals are inclined to be more sexually active than youngsters.

Although approximately equal numbers of each sex were taken, the samples of females and males differ greatly with respect to age structure. The older age classes were not represented

in the present series of males, the bulk of which are hyaenas from age class II and III. At this stage it is now known whether this situation is a mere artifact of the sampling procedure, or a true representation of the total population, but the predominance of younger males that are generally sexually inactive, lends the overall impression of sexual inactivity as the breeding condition, to the sample of males. Matthews (1939a) came to the same con= clusion, although he had access to more older males. Some 62% of all the males in his sample was considered to be sexually inactive or only intermediately active. A similar estimate for the present series would be 72% inactive and intermediately active males, no doubt in part caused by the over-representation of 1-3 year old individuals in the sample.

Although male hyaenas did show increases in testes and epididymal mass with age, as well as in epididymal sperm reserves, such changes may be not as indicative of changes in fertility, as of general body growth with age. Low numbers of sperm were counted from all males, despite the fact that all were evidently healthy and showing normal spermatogenesis. The fertility level of spotted hyaena males has not been determined yet, but the comparatively low sperm reserves might be sufficient to achieve conception. Sperm reserves of only a few non-domesticated mammals have been determined, but when compared to these, even the highest count of sperm present in the epididymides of a spotted hyaena was still lower than most values obtained during the non-breeding season of various ungulates (Skinner 1971, Skinner & Huntley 1971a,b, Skinner & Van Zyl 1971, Skinner, Scorer & Millar 1975), elephant (Jones, Rowlands & Skinner 1974) and oligospermic men (Speroff, Glass & Kase 1978).

Seasonal differences in the testicular composition of mature males, were not found in this study, as was the case in the study of Matthews (1939a). Spotted hyaena males appear not to exhibit a seasonal sexual cycle, although individual differences in the degree of sexual activity occur at any time. Sexually active and inactive males were collected together at specific sampling sites, indicating that males in breeding condition would always be available to females throughout the year.

No indication of modifications in the breeding status of males, as discussed in the introductory paragraph of this chapter, could be found. There were no instances of delayed puberty, and the relative inactive state of most testes examined could be explained by the effects of age and size on breeding status. Nevertheless, for a population which does not breed in a specific season only, there is a significant proportion of males in the reproductive class, that are apparently incapable of breeding at any time.

CHAPTER 4

PRODUCTION AND SECRETION OF SEX HORMONES

INTRODUCTION

The findings of Racey & Skinner (1979) concerning sex hormone levels in spotted hyaenas, raised a question essential to the understanding of the role of sex hormones in the reproductive process and possibly the social relations of the spotted hyaena. Where exactly are the sex steroids, particularly the androgens, produced? Racey & Skinner (1979) found that plasma levels of testosterone were of the same order for males and females although the testicular concentration of testosterone by far exceeded the ovarian concentration. Androstenedione concentrations were roughly equal in both plasma, gonads and adrenals of males and females.

Once the sources of androgens in the female and male hyaena have been determined, other influences such as peripheral interconversion of steroids, or sex-related differences in production and clearance, could be examined more closely. A first and most important objective should however be to associate endocrine gland structure with function. If androgen production is related to a particular structure, for example the follicle or corpus luteum, then androgen production would rise and wane with the growth and regression of these structures. On the other hand, if the adrenal cortex or ovarian interstitium, which does not show such a marked cyclic change, is involved, then androgen production would perhaps also remain fairly stable.

The nature and circumstances of production and secretion of androgens, which are known to play a role in individual

behaviour and social relationships, would therefore be of great consequence to the understanding of features of hyaena biology such as female dominance, sexual mimicry and females being larger than males.

Different techniques were employed to determine the site and, to a lesser extent, the magnitude of secretion of androgens, testosterone and androstenedione. In the process, additional information on other steroids was also collected. Two techniques were developed to suit the practical limitations imposed in a study of this nature. Using a more docile species one would have been able to embark on a study of productive and metabolic aspects of the sex steroids, but not in the spotted hyaena, for this approach necessitates frequent handling of experimental animals under near-natural conditions. Spotted hyaenas were available only in limited numbers, for limited periods and could only be handled when immobilized.

To suit prevailing field conditions, a rationale was developed which could give a rapid indication of the origin of secretion and the magnitude of production of particular steroids. A base= line level of secretion was established, after which a given target endocrine gland was individually stimulated or suppressed by means of selective exogenous stimulants and suppressants, and the response or lack of it was shown in terms of the relative composition of the circulatory pool of steroid hormones. Experi= ments *in vivo* were repeated *in vitro*, through the modification of an established tissue culturing method as tissues treated in isolation, could not only serve to confirm results obtained from experiments *in vivo*, but could be examined histologically and histochemically to reveal the location and nature of the sites where steroidogenesis occurred.

The purpose of this part of the study was first to determine the sites of androgen production in spotted hyaenas, and second= ly to determine age and sex-related differences in the levels of secretion or secretory pathways.

MATERIALS AND METHODS

Husbandry

After capture, hyaenas were treated with xylazine hydrochloride (1,0 mg/kg) or in combination with ketamine hydrochloride (20,0 mg/kg) for further restraint on muscular spasms and head and limb movement, caused by the drug used in capture, phencyclidine hydrochloride (0,5 mg/kg) (Table 16). Such additional treatment depended on the condition of individual animals and was not necessary in some cases. A short-acting barbiturate, thiopentone sodium (10-50 mg/kg) was used in preference to any morphine derivative, whenever anaesthesia had to be prolonged in order to finish experimentation. Individual responses to all these chemicals mentioned differed greatly, and dosages administered varied according to the level of agitation and the duration of the required response.

TABLE 16: CENTRAL NERVOUS SYSTEM DEPRESSANTS USED IN THIS STUDY FOR IMMOBILIZATION AND GENERAL ANAESTHESIA

Compound	Preparation	Dosage (mg/kg)	Action (hours)
Phencyclidine hydrochloride	Sernylan[1]	0,5-1,0	2-12
Xylazine hydrochloride	Rompun[2]	1,0-5,0 (0,5)*	0,5-2
Ketamine hydrochloride	Ketalar[3]	20-50 (5,0)*	0,5-1
Thiopentone sodium	Intraval[4] Sodium	10-50	0,25-1,5

*When used as synergists to phencyclidine hydrochloride

[1]Bio-ceutic Laboratories, Inc., St. Joseph, Missouri, USA

[2]Bayer, Leverkussen, West Germany

[3]Parke-Davis Laboratories (Pty) Ltd, Cape Town, SA

[4]May & Baker (S.A.)(Pty) Ltd., Port Elizabeth, SA

All supplementary agents were administered intravenously, in small doses, until the required level of anaesthesia had been achieved, and could be maintained by repetitive administration of small doses. One adult brown hyaena (*Hyaena brunnea*) female was also available for experimentation. This hyaena was initially caught in a trap in the vicinity of Brits, and was successfully anaesthetized with 0,5 mg/kg phencyclidine and 0,5 mg/kg xylazine hydrochloride. Com= plete surgical anaesthesia was induced seven minutes after injection via pole-syringe, and lasted more than 24 h.

Additional care in the form of the prevention of disturbance of sedated animals, was provided in the form of cotton wool ear plugs and eye covers. Hyperthermic animals were hosed or splashed down with water, while hypothermia was countered by the use of canvas to cover up animals. All hyaenas due to be released, were given intramuscular injections of long acting penicillin (a combination of procaine and benethamine penicillin, approximately 90 000 iu), and superficial wounds were treated with antibiotic ointments and powders, such as oxytetracycline hydrochloride and sulphanilamide. Before venopuncture, suitable sites were washed and disinfected with 70% ethanol.

Finally, when required, animals were exsanguiniated with a large intravenous dose of suxamethonium chloride (approxi= mately 2-5 mg/kg).

Experimental procedures

Standard diagnostic tests commonly used in human medicine, were adapted to prevailing conditions of available time and effort required in the capture and handling of experimental animals. A summary of the rationale and protocols of standard tests can be found in Zilva & Pannall (1979). The duration of most tests, as well as sampling procedures, had to be

changed, since the standard approach involves the measurement of a response of low intensity over a considerable period, after low-dose treatments with specific drugs.

The stimulants and suppressants used in the present study (Table 17) were of pharmacological grade and are freely available in commerce, except for 'Lutal' which at the time of use was only made available for research purposes.

TABLE 17: STIMULANTS AND SUPPRESSANTS USED IN EXPERIMENTAL PROCEDURES

Action	Compound	Preparation	Dosage	Route	Refractory period (min.)
Pituitary stimulant	Synthetic LRH	Lutal[1]	0,05 mg	iv*	20-30
Gonadal stimulant	Chorionic gonadotrophin	Pregnyl[2]	1500 iu	im**	15-30
Gonadal stimulant	Chorionic gonadotrophin	Chorulon[3]	500 iu	iv	10-20
Adrenal cortex stimulant	Corticotrophin (Tetracosactide peptide)	Synachten Depot[4]	1 mg	im	< 15
	(Tetracosactide peptide)	Cortrosyn Depot[5]	1 mg	im	< 15
	(Porcine pituitary extract	Acorten Gel[6]	100 iu	im	< 15
Pituitary suppressant (Gonadal axis)	Stilboesterol	Stilboesterol[7] dipropionate	20 mg	im	120
(Adrenal axis)	Dexamethazone sodium phosphate	Methason[8]	8-10 mg	iv	60-120
	Dexamethazone sodium phosphate	Decadron[9]	8-10 mg	iv	60-120
(Adrenal axis)	Dexamethazone phenyl propionate	Dexafort[10]	20-25	im	90-120

*intravenous

**intramuscular

[1]Hoechst AG. Frankfurt, West Germany

[2]N.V. Organon. Oss, Holland

[3]Intervet International B.V. Boxmeer, Holland

[4]Ciba-Geigy (Pty.) Ltd. Kempton Park, S.A.

[5]Organon Laboratories Ltd. Surrey, England

[6]Ferring Pharmaceuticals. Malmö, Sweden

[7]May & Baker Ltd. Dagenham, England

[8]Panvet (Pty.) Ltd. Kempton Park, S.A.

[9]MSD (Pty.) Ltd. Halfway House, S.A.

[10]Intervet International B.V. Boxmeer, Holland

Since virtually no published information exists on the application of these chemicals in animals, especially wild species, decisions pertaining to sampling periods, obser= vation periods and dosages of drugs, were based on guess= work and extrapolations of the standard procedures in human medicine.

The vena jugularis was used in preference to all other veins as sampling site, because of its accessability, size and adequate yield. Alternatively, the femoral vein was used with satisfactorily results, although the blood flow rate and therefore yield, was much lower. Because of the long periods that sampling systems were left in these veins, thrombosis inevitably occurred, and alternative sites had to be used. The most effective system proved to be dis= posable 13G cannulae, although satisfactorily results were also achieved with home-made 13G catheters and 19G infusion sets. Direct sampling with syringe and needle or Vacutainer needle and collection tube, was always ineffective and could not be used for repetitive sampling.

Cannulae and catheters were secured at the site of entry with surgical adhesive tape, and after each sample had been delivered, tubing was eluted with a physiological solution of sodium citrate or sodium heparin diluted with physiological saline to a concentration of 100 iu/ml. Blocking of tubes due to clotting hardly ever occurred, but commonly resulted from obstruction and folding against the inside walls of veins, or in the process of venopuncture, as spotted hyaenas have incredibly tough skins. A volume of 10 ml blood was collected per sample, in heparinized vacutainer tubes or test-tubes pre-treated with 2-3 drops of 100 iu/ml heparin solution. Samples were centrifuged immediately after collection, plasma was drawn off and stored in plastic storage ampoules at -18^{o} C until assayed.

Sampling was erratic, but whenever possible, samples were taken at 15 min intervals. The duration of each test varied according to the condition of each individual study subject and observer fatigue, but the preferred period of observation was 120 min. In practice, observation periods lasted from 60 to 360 min.

Tissue cultures

A modification of a culturing technique used in cytogenetic studies (Paul 1975), was used in this study. As soon as possible after death, the gonads and adrenals were removed after which a sample of approximately 2 g was dissected from the host organ, and was cut into pieces, as fine as possible, with scissors, before being placed in McCartney vials containing 10 ml of Hanks-Eagle's culturing medium (T.J. Robinson, pers. comm.), equilibrated at 37^{o} C. Cultures were kept in a thermosflask for 60 min at 37^{o} C, without having to adjust temperatures. Preliminary trials with rat testicular tissue showed that an hour is more than adequate for any tissue reaction to occur.

Incubations were done in duplicate or triplicate, with one or two samples acting as controls. The test incubation was treated with one of the reagents in Table 18. Trophic substances mentioned in Table 18, were of pharmacological grade, as in Table 17. Capsules containing 250 mg of the adrenocorticostatical agent, metopirone (Metyrapone) were dissolved in 100 ml physiological saline, to give a working solution of 2,5 mg/ml. A volume of 0,2 ml containing 0,5 mg metopirone was used in test incubations. After a blood sample was collected by cardiopuncture of foetuses, gonads and adrenals were removed and frozen for tissue extraction of steroids.

TABLE 18: REAGENTS USED IN TISSUE CULTURES

Trophic agents	Trade name	Concentration
Chorionic gonadotrophin	Chorulon	30 iu/10 ml medium
Chorionic gonadotrophin	Pregnyl	40 iu/10 ml medium
Adrenocorticotrophin	Synachten	1 iu/10 ml medium
Suppressant		
11-Hydroxylase inhibitor (Metyrapone)	Metopirone[1]	0,5 mg/10 ml medium

[1]Ciba-Geigy (Pty) Ltd, Kempton Park, SA.

Radioimmunoassay

Plasma samples were assayed for six steroid hormones and one polypeptide hormone, namely testosterone, Δ^4androstenedione, progesterone, oestradiol-17β, oestrone, and the poly-peptide luteinizing hormone (LH). Standard assays in regular use in research and diagnostical services at the Departments of Chemical Pathology, and Obstetrics and Gynaecology, of the Faculty of Medicine, University of Cape Town, were found to be suitable for assaying plasma steroids and LH in spotted hyaenas (Millar & Aehnelt 1977). Detailed information on the reagents used in these assays is provided in Appendix 1. Assay protocols and methodology are described in Abraham (1969), Lindner, Perel, Friedlander & Zeitlin (1972), Millar & Kewley (1976), Carr, Millar & Crowley (1977) and Millar & Aehnelt (1977).

Table 19 presents the calculated intra- and interassay variation encountered in the total number of assays done by myself, not necessarily similar to values obtained by the abovementioned laboratories. The significance of the

results was assessed by means of the separate variance technique utilizing Student's t-test, where the two sample sizes are not equal and sample variances differ significantly (Cornett & Beckner 1975).

TABLE 19: INTRA- AND INTERASSAY VARIATION (%)

	Intra-assay variation	Inter-assay variation	n Assays	n Samples
Testosterone (T)	3,1	19,5	12	402
Δ^4Androstenedione (A)	3,3	6,9	16	388
Progesterone (P)	2,9	19,0	7	256
Oestrone (E_1)	4,1	22,8	8	252
Oestradiol-17β (E_2)	6,1	12,3	7	216
Cortisol (C)	3,7	23,4	5	277
LH	-	14,4*	3	309
		TOT.	58	2 100

*Estimate from standard curve

Histochemistry

Testicular, ovarian and adrenal cortical tissue samples were collected immediately after the death of selected hyaenas, and were stored in liquid nitrogen at approximately -120° C

or on dry ice supercooled with liquid nitrogen to approximately -80° C. Tissue blocks measuring approximately 0,5 x 0,5 x 0,5 cm, were either fixed onto cryostat chucks and frozen, or frozen in separate air-tight plastic storage ampoules. Frozen tissue was sectioned at 5-8 µm at -30° C, dessicated with silica gel, and stored at 0° C.

Sections were pre-treated with acetone at 4° C for 5 min to remove endogenous sterols and steroids (Hoyer & Andersen 1977). Incubation media consisted of 0,5 mg/ml NBT, 2 mg/ml NAD and 20 µg/ml substrate. Concentrations of 20 µg/ml, 100 µg/ml and 200 µg/ml dehydroepiandrosterone (DHEA) and Δ^4androstenedione were used as exogenous steroid substrate, of which the first concentration was the most effective. Each test had duplicate controls, identical to the test incubation except for the absence of exogenous steroids. Incubations were done in microcells for 60 min at 37° C.

During steroid isomerization induced by the steroid isomerase Δ^5-3β hydroxysteroid dehydrogenase, hydrogen is released and this latter aspect of conversion of Δ^5 hydroxysteroids to Δ^4 ketosteroids is utilized in incubations where a hydrogen acceptor such as nitro-blue tetrazolium (NBT) or nicotine amide-adenine dinucleotide is used to indicate the site of hydrogen liberation by the formation of a coloured precipitate. The presence of the diaphorase enzyme system of hydrogen transport (NAD + H $\rightleftharpoons$ NADH and NADP + 2H $\rightleftharpoons$ $NADPH_2$) was demonstrated with incubations as above, except for the absence of exogenous steroid substrate.

Foetal gonads, adrenals and placental tissue were homogenized in phosphate buffered saline (PBS) in a 2 ml homogenizing tube with teflon pestle, and extracted twice in diethyl ether. After evaporation in a nitrogenous atmosphere at 40° C, precipitates were reconstituted in PBS and included in radioimmunoassays for testosterone and Δ^4androstenedione.

RESULTS

By the time hyaenas were available for experimentation, they were generally recumbent and cataleptoid, but in a few cases an additional small amount of xylazine hydro= chloride was necessary to induce a state close to surgical anaesthesia. Since all hyaenas were captured with phency= clidine hydrochloride, either with a fixed dosage of approximately 50 mg per dart, or with bait drugged with phencyclidine hydrochloride, overdosages frequently re= sulted. All captures were effected at night, when circum= stances did not allow either adjustments in the dosage delivered per dart or contained in bait to be made to suit the size of a particular individual, or selective capture of suitable individuals.

Although no side-effects following the use of phencyclidine hydrochloride in spotted hyaenas have been described by Pienaar, Le Riche & Le Roux (1969), Harthoorn (1976), or Whateley (1979), symptoms commonly found to be associated with the use of this drug, such as excess salivation, mus= cular tremors and convulsions, vomiting, hypopnoea and hypo= tension, were exhibited in nearly all subjects. Xylazine hydrochloride was effective in countering most of these symptoms, especially clonic and tonic spasms, but had no effect on respiration and the cardiovascular system. Ketamine hydrochloride did not ease any of these side-effects, in spite of having mild cardiac stimulatory properties (Parke-Davis Laboratories (Pty) Ltd). The latter drug was not as good a synergist with phencyclidine hydrochloride, as xyla= zine hydrochloride, as shown by the inability to procure total surgical anaesthesia with the comparatively large amount of 100 mg in one individual. Excessive salivation, hyperthermia and hypopnoea were the only side-effects evident in the single brown hyaena female anaesthetized with a com= bination of phencyclidine hydrochloride and xylazine hydro=

chloride. The latter agent was in this event efficient in counteracting muscular spasms.

The usefulness of short-acting barbiturates as synergists in carnivore capture and handling is well known (Ebedes 1973, Harthoorn 1973, 1976), but could not be utilized in this study, except in the terminal stage of each experiment, because of the suspected inhibitory effects barbiturates might have on certain endocrine glands.

Difficulties in sampling were attributed to the acute state of hypotension and vasodilatation of peripheral blood vessels caused by phencyclidine hydrochloride, which greatly reduced the blood pressure and resulted in the collapse of blood= vessels during sampling. Respiratory and cardiac stimulants were not used, except in emergencies, due to the possible effects of drugs like atropine and doxapram hydrochloride on the endocrine system, as will be discussed later. In one instance, a juvenile spotted hyaena was injected with thio= pentone sodium too fast which resulted in respiratory arrest. Respiration was artificially reinstated through thoracic massage, and finally remedied with intravenous administration of doxapram hydrochloride (0,5 mg/kg) (Dopram-V, A.H.Robins Co).

This particular individual, the most serious emergency en= countered, suffered no ill effects and was seen feeding at the same bait station during the following night.

Gonadal stimulation

LH-RH administration

The rationale of this test is based on the endogenous release of gonadotrophins from the pituitary, mediated by intravenous administration of luteinizing hormone-releasing hormone

(LH-RH). This test was carried out on two adult male hyaenas (51 kg and 57 kg) and two female hyaenas, one sub-adult (44 kg) and the other adult (56 kg). Figures 37-40 illustrate the pituitary and gonadal responses in terms of the change in the peripheral plasma concentrations of LH, testosterone (T) and androstenedione (A) after the intravenous (jugular vein) or intra-arterial (carotid artery) administration of 50 µg synthetic LH-RH.

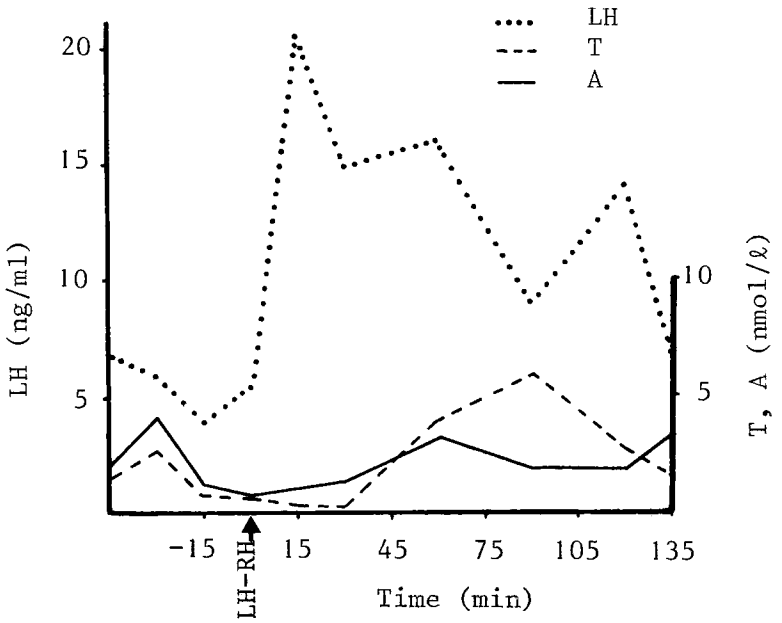


Fig. 37: Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4androstene-dione (A) after LH-RH administration in a 51 kg male spotted hyaena (age class II).

The mean levels of LH, T and A before and after treatment, the mean percentage increase after treatment, the maximum response, and the mean androstenedione : testosterone ratio before and after treatment, are illustrated in Table 20.

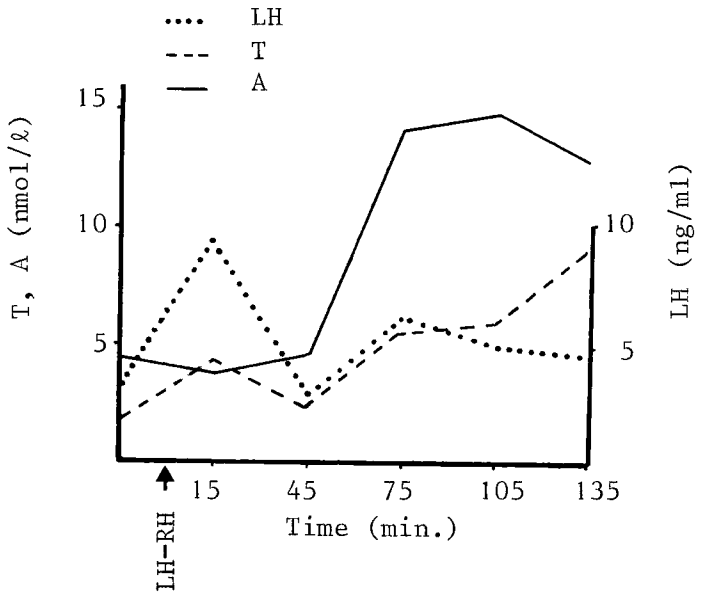


Fig. 38: Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4androstene=dione (A) after LH-RH administration in a 57 kg male spotted hyaena (age class II).

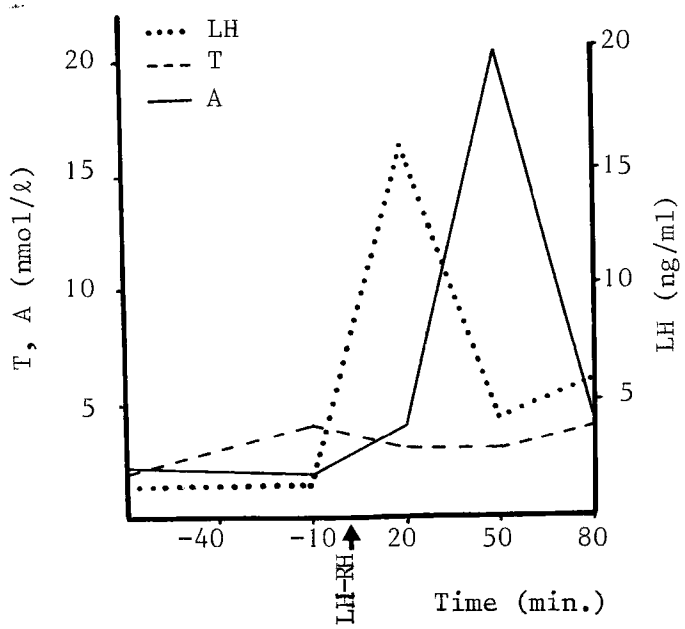


Fig. 39: Peripheral plasma concentrations of luteinizing hormone (LH), testosterone (T) and Δ^4androstene=dione (A) after LH-RH administration in a 44 kg female spotted hyaena (age class II).

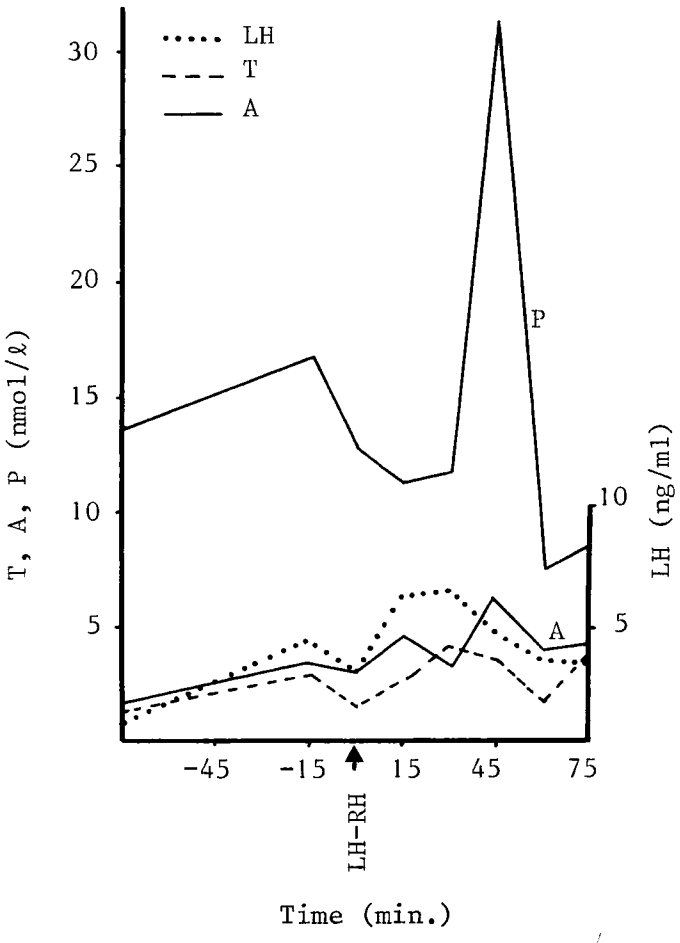


Fig. 40: Peripheral plasma concentrations of progesterone (P) luteinizing hormone (LH), testosterone (T) and Δ^4 androstenedione (A) after LH-RH administration in a 56 kg lactating female spotted hyaena (age class VII).

Latent periods before LH levels were elevated, were short (15-20 min), and the time-lapse between LH peaks and peaks in T and A ranged from 15 min (for T in ♂♂ and ♀♀) to 30-75 min (for A in ♂♂ and ♀♀). Following the episodic release of LH into the circulation, in this case mediated by LH-RH, T was secreted earlier than A from the testis and ovary, although the latter steroid always reached much higher levels eventually (Table 20). In the lactating female, an increase in the peripheral plasma concentration of progesterone (P) occurred 45 min after treatment with LH-RH, but such a response was absent in the other three hyaenas. Peripheral levels of the oestrogens, oestradiol-17β (E_2) and oestrone (E_1), were not affected by LH-RH administration in

TABLE 20: RESPONSES TO LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) ADMINISTRATION

	♂♂		♀♀	
	Adult A II[1]	Adult B II	Sub-adult A II	Adult B VII
$\bar{x}$ pre-v. post treatment level of : LH (ng/ml)	55 ± 6 131 ± 24*	30 ± 0 55 ± 15^{NS}	14 ± 1 88 ± 37^{NS}	261 ± 105 490 ± 63*
: T (nmol/ℓ)	1,6 ± 0,1 2,3 ± 1,1*	1,8 ± 0,0 5,0 ± 0,8*	0,3 ± 0,1 0,3 ± 0,0^{NS}	1,8 ± 0,5 3,1 ± 0,4*
: A (nmol/ℓ)	2,1 ± 0,8 1,9 ± 0,4^{NS}	4,4 ± 0,0 9,3 ± 2,3**	2,0 ± 0,2 10,0 ± 5,8^{NS}	24,9 ± 4,8 44,5 ± 4,5***
$\bar{x}$ % increase : LH	238,2	183,3	628,6	187,7
: T	143,8	277,8	100,0	172,2
: A	90,5	211,4	500,0	178,7
Maximum response[2]: LH (ng/ml)	150	65	148	236
: T (nmol/ℓ)	4,5	5,2	0,1	2,3
: A (nmol/ℓ)	1,1	10,5	19,5	36,0
A/T $\bar{x}$ pre-	1,4 ± 0,5	2,4 ± 0,0	7,5 ± 3,0	14,1 ± 1,6
$\bar{x}$ post-	1,8 ± 0,6^{NS}	1,9 ± 0,3^{NS}	32,0 ± 19,9^{NS}	15,9 ± 2,6^{NS}

[1]Age class

[2]Calculated as : maximum level after treatment - mean level before treatment

Difference between pre- and post-treatment values :

*** : p < 0,001
** : p < 0,01
* : p < 0,05
NS : Not significantly different

the female hyaenas, but a significant increase in E_2 was evident in male B (t_4 = 2,098; $p < 0,10$). The reason for the non-significant responses in LH secretion in two of the hyaenas can be attributed to the small number of samples taken from these two individuals.

The most significant results of this test were that the LH-RH mediated release of gonadotrophins from the pituitary evoked an increase in the peripheral levels of testosterone and androstenedione in male and female spotted hyaenas, indicating a stimulation of production and secretion of both steroids from the testis and ovary. Androstenedione was the principal androgen released from both the testis and ovary.

HCG administration

Human chorionic gonadotrophin is commonly used in the assess= ment of gonadal functioning, often in preference to the native gonadotrophins LH and FSH. The latter two polypeptide hormones are prepared from pituitary extracts, usually con= taminated with other pituitary hormones such as prolactin and ACTH, which might cause a non-specific response to occur in tests *in vivo*. Human chorionic gonadotrophin on the other hand, is freely available as a standard synthesized prepa= ration capable of eliciting a testicular and ovarian response comparable to that of the endogenous gonadotrophins.

Three male hyaenas, one of which was a subadult (41 kg), were treated with intravenous (jugular vein) doses of 500 iu HCG. The responses in the peripheral concentrations of T and A after HCG treatment are illustrated in Fig. 41 & 42. Plasma cortisol (C) was also determined, and was included in Figure 41 and 42 (and Table 21) to give an indication of adrenal secretory activity during the sampling period.

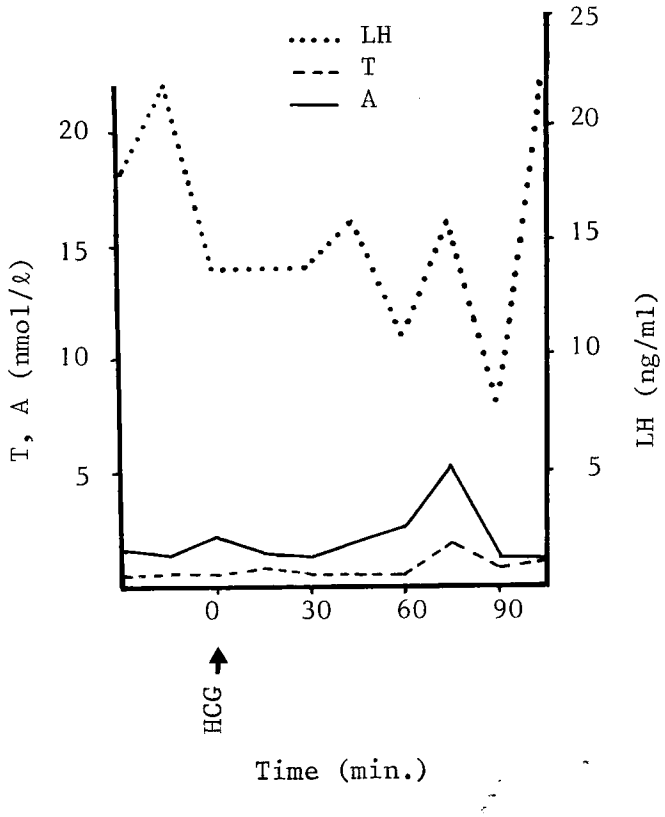


Fig. 41: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and luteinizing hormone (LH) after HCG administration in a 41 kg male spotted hyaena (age class II).

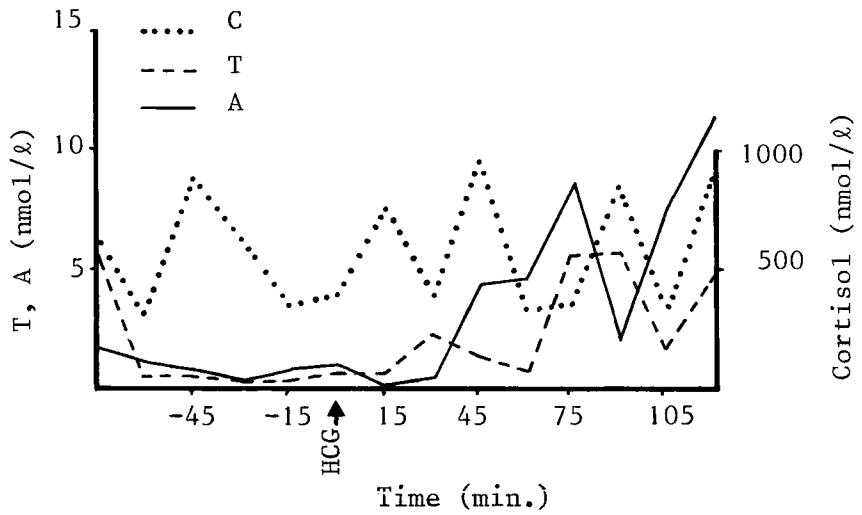


Fig. 42: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after HCG administration in a 76 kg male spotted hyaena (age class IV).

It is presumed that cortisol levels could indicate adrenal contribution to the pool of steroids that would otherwise have been regarded as having originated from the gonads only. Comparative aspects of the changes observed in the peripheral plasma levels of T, A and C are illustrated in Table 21.

TABLE 21: RESPONSES IN MALE SPOTTED HYAENAS TO INJECTIONS OF HUMAN CHORIONIC GONADO=TROPHIN (HCG)

	Subadult II[1]	♂♂ Subadult II	Adult IV
$\bar{x}$ pre- v. post-treatment level of : T (nmol/ℓ)	0,5 ± 0,0 0,8 ± 0,2^{NS}	0,6 ± 0,1 0,7 ± 0,1^{NS}	1,3 ± 0,9 2,7 ± 0,8^{NS}
: A (nmol/ℓ)	1,8 ± 0,2 2,1 ± 0,6^{NS}	1,2 ± 0,1*** 0,7 ± 0,1	1,0 ± 0,2* 5,0 ± 1,4
: C (nmol/ℓ)	- -	115 ± 36 35 ± 0*	533 ± 87 601 ± 91^{NS}
$\bar{x}$ % increase : T	160,0	116,7	207,7
: A	116,7	58,3	500,0
: C	-	30,4	112,8
Maximum response[2]: T (nmol/ℓ)	1,3	0,7	4,3
: A (nmol/ℓ)	3,5	-0,2	10,4
: C (nmol/ℓ)	-	-80	17
A/T $\bar{x}$ pre-	3,5 ± 0,4	2,5 ± 0,3	1,7 ± 0,4
$\bar{x}$ post-	2,7 ± 0,5^{NS}	1,1 ± 0,2**	2,7 ± 1,0^{NS}

[1]Age class

[2]Calculated as the maximum level after treatment minus the mean level before treatment

NS : Not significant

$^{*}p < 0,05$ $^{**}p < 0,01$ $^{***}p < 0,001$

Elevation in the peripheral plasma levels of T and A in the subadult male and adult male B, were evident 15-30 min (T) and 45 min (A) after HCG treatment, with the first peaks occurring 75 min after treatment. Cortisol peaks did not correspond with peaks of T and A, and adrenal secretion of T and A, if any, was regarded as not significant in this test. The peripheral plasma concentration of P in adult male B gradually increased during the experiment, but the response was not significant (t_8 = 0,707, p > 0,10). Adult male A failed to react to HCG and no increase in either T or A was evident after HCG treatment.

Four adult females (two lactating, two pregnant) were injected with HCG. The responses in peripheral plasma concentrations of T, A, P, E_1, E_2 and C after HCG administration are illu= strated in Figures 43-45. Comparative aspects of the responses in the peripheral levels of these steroids are presented in Table 22.

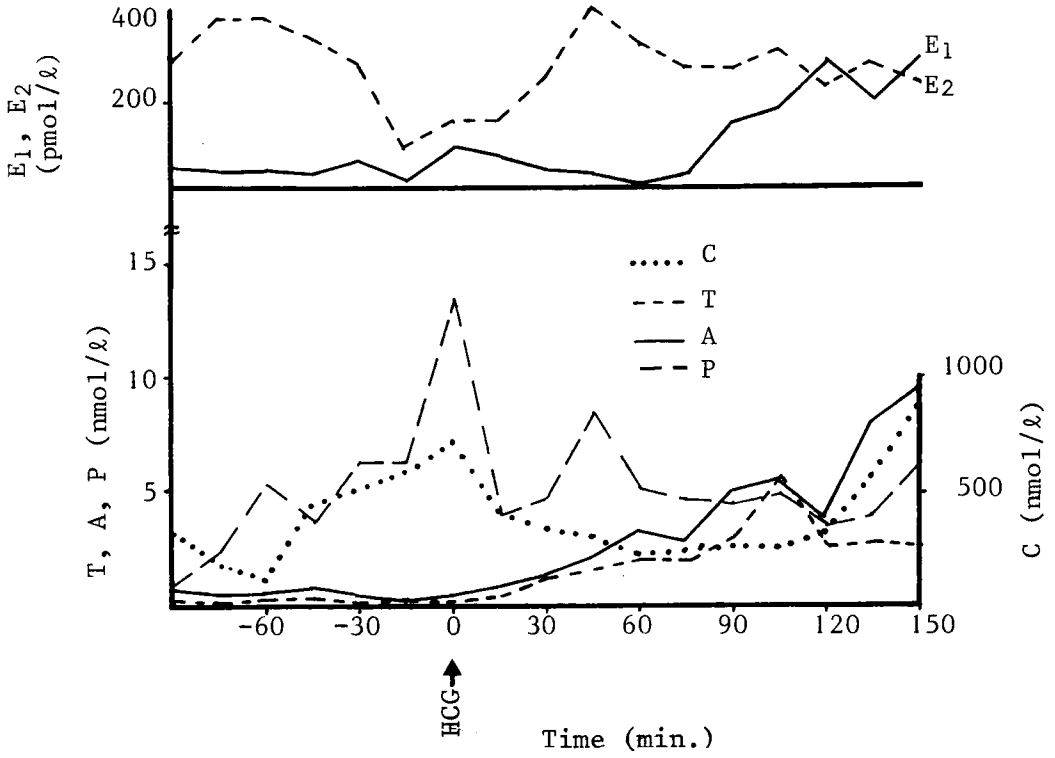


Fig. 43: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after HCG administration in a 68 kg female spotted hyaena (age class VI).

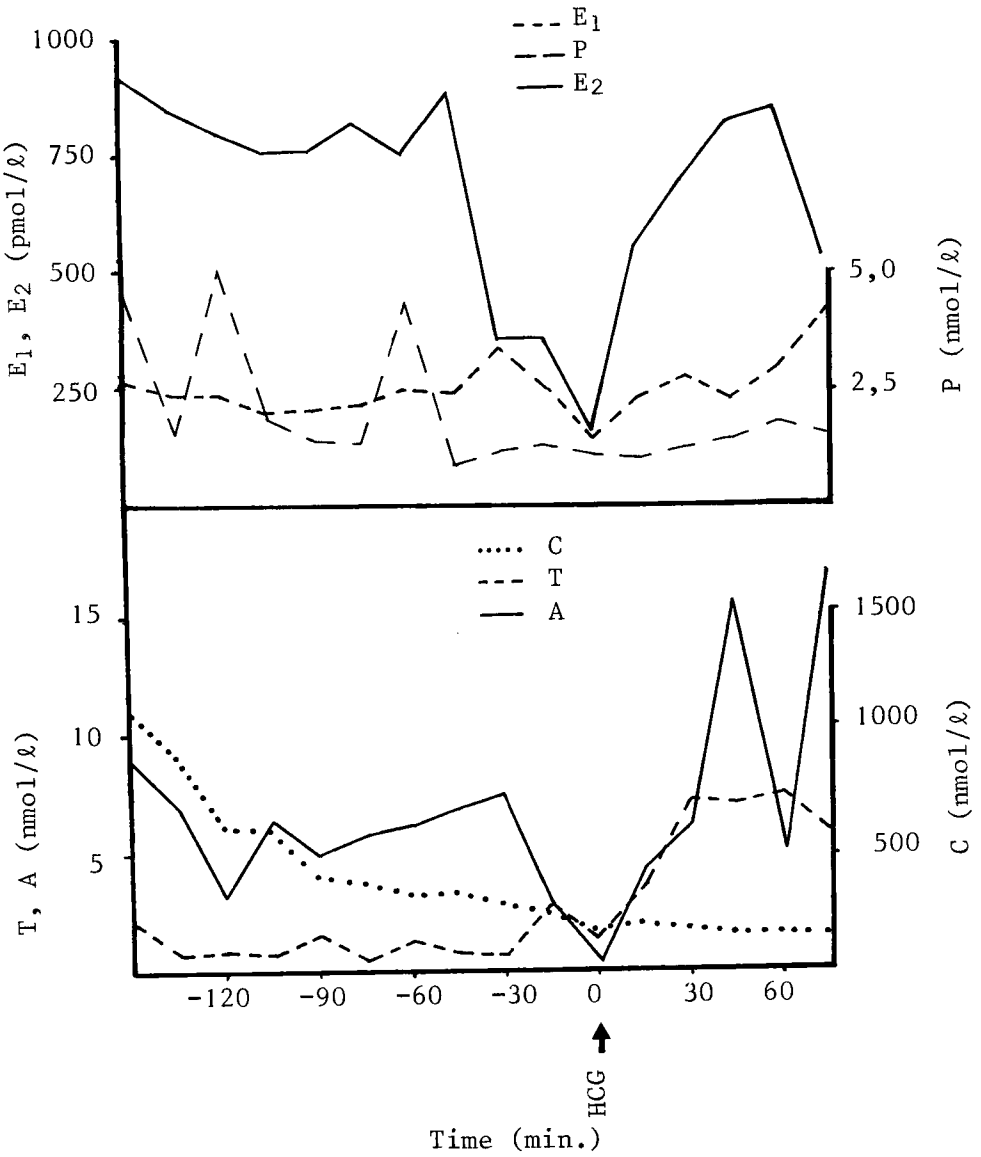


Fig. 44: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after HCG administration in a 79 kg pregnant female spotted hyaena (age class V).

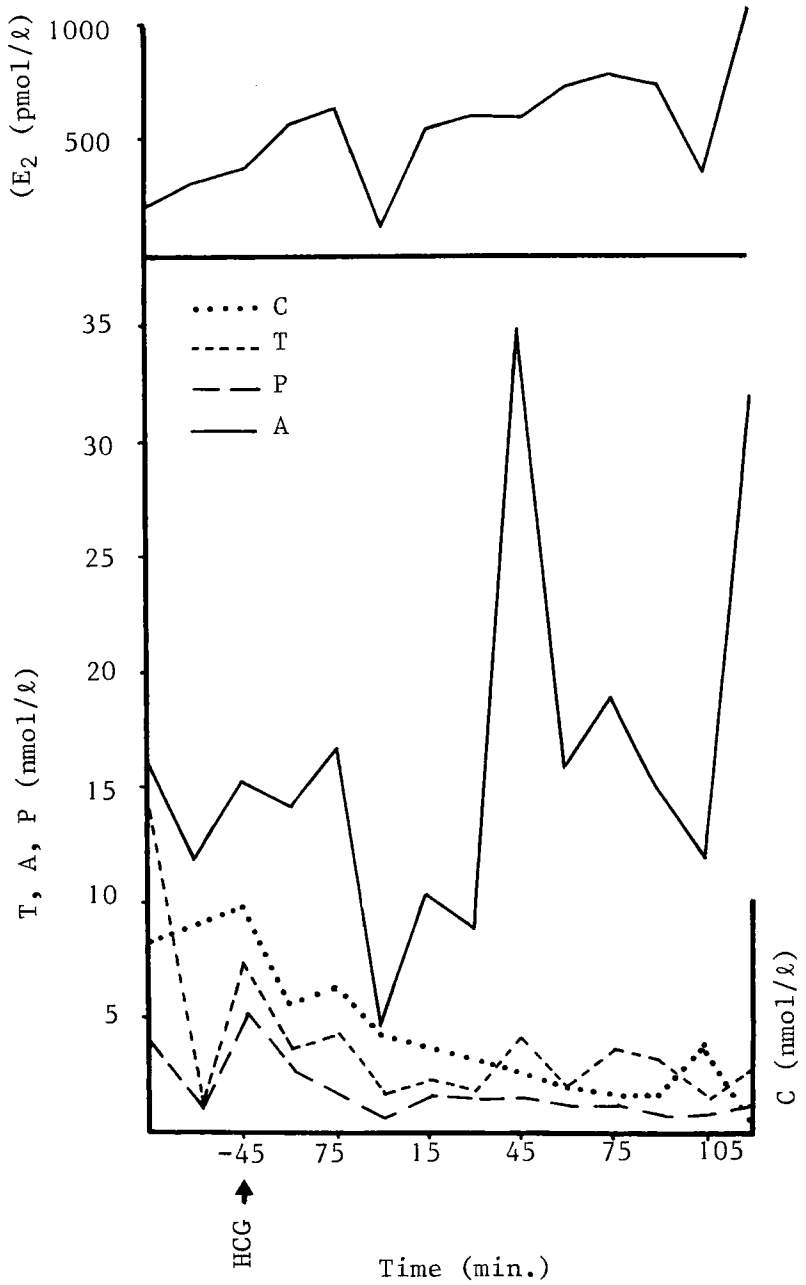


Fig. 45: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A), progesterone (P), oestradiol-17β (E_2) and cortisol (C) after HCG administration in a 75 kg pregnant spotted hyaena (age class V).

TABLE 22: RESPONSES IN FEMALE SPOTTED HYAENAS TO INJECTIONS OF HUMAN CHORIONIC GONADOTROPHIN (HCG)

		Lactating ♀♀		Pregnant ♀♀	
		Adult VI[2]	Adult VII	Adult V	Adult V
$\bar{x}$ pre- v. post treatment level of	: T (nmol/ℓ)	0,2 ± 0,0 2,3 ± 0,5***	0,2 ± 0,1 0,7 ± 0,6^{NS}	5,2 ± 1,7 3,5 ± 0,3^{NS}	1,3 ± 0,2 6,3 ± 0,7***
	: A (nmol/ℓ)	5,0 ± 0,6 41,2 ± 9,9***	6,4 ± 0,6 9,6 ± 1,9^{NS}	13,0 ± 1,8 18,4 ± 3,5^{NS}	51,7 ± 8,8 96,9 ± 27,6*
	: C (nmol/ℓ)	411 ± 84 389 ± 70^{NS}	- -	714 ± 85 208 ± 36***	499 ± 90 170 ± 8**
	: P (nmol/ℓ)	5,5 ± 1,5 5,0 ± 0,5^{NS}	6,6 ± 0,6 2,6 ± 0,8*	244,5 ± 69,8 122,0 ± 12,3*	213,1 ± 45,7 127,8 ± 12,6^{NS}
	: E_1 (pmol/ℓ)	273 ± 41 275 ± 23^{NS}	445 ± 31 489 ± 41^{NS}	- -	22562 ± 1494 27526 ± 3214
	: E_2 (pmol/ℓ)	60 ± 8 132 ± 35*	249 ± 66 339 ± 95^{NS}	683 ± 299 683 ± 84^{NS}	662 ± 78 670 ± 65^{NS}
$\bar{x}$ % increase	: T	1150,0	350,0	67,3	484,6
	: A	824,0	150,0	141,5	187,4
	: C	94,5	-	29,1	34,1
	: P	90,9	39,4	49,9	60,0
	: E_1	100,7	109,9	-	122,0
	: E_2	220,0	136,1	0,0	101,2
Maximum response[1]	: T (nmol/ℓ)	5,4	3,8	-0,2	6,3
	: A (nmol/ℓ)	89,6	11,6	21,6	118,5
	: C (nmol/ℓ)	457	-	-350	-310
	: P (nmol/ℓ)	2,9	0,0	-83,4	-49,6
	: E_1 (pmol/ℓ)	131	134	-	16896
	: E_2 (pmol/ℓ)	232	647	413	165
A/T $\bar{x}$ pre- $\bar{x}$ post-		29,6 ± 6,1 18,4 ± 3,0^{NS}	35,7 ± 12,0 78,8 ± 24,1^{NS}	4,0 ± 1,5 5,2 ± 0,7^{NS}	59,4 ± 11,8 15,5 ± 4,2*

[1]Age class

[2]Calculated as the maximum level after treatment minus the mean level before treatment

***p < 0,001
**p < 0,01
*p < 0,05
NS Not significant

The first signs of elevation in the levels of T and A were evident 15-30 min and 45-60 min after treatment respectively, for three of the females (one pregnant female (C) failed to react to HCG) but peaks varied greatly in the time of occurrence (T : 105 min, A : 90-150 min in the lactating females; T : 30-60 min, A : 45-75 min in pregnant female D). Significant increases in the levels of E_1 and E_2 occurred in one pregnant female (E_1 : 30-75 min)(t_{14}= 1,619; $p < 0,10$) and one lactating female (E_2 : 60-120 min)(t_{14}= 1,760; $p < 0,10$). The latter female had developing follicles in both ovaries, while the other lactating female did not. Progesterone levels were not affected by HCG treatment, in any one of the four female hyaenas.

As in the LH-RH test, testosterone was the first androgen to show an increase in concentration after HCG treatment, followed by androstenedione which ultimately surpassed the highest level of testosterone. The difference between the maximum responses in testosterone and androsteredione was less in male hyaenas treated with HCG than in females, but androstenedione was nevertheless always secreted in greater quantities than testosterone. Both the maximum responses in T and A, and the mean percentage increase in T and A after HCG treatment were generally greater in the female than similar values in the male.

This test confirmed the results obtained from the LH-RH test, that testosterone and androstenedione were produced and secreted in both the testis and ovary during acute stimulation with a gonadotrophin analogue. It does, however, seem that the spotted hyaena testis is not capable of producing androgens in similar quantities to the ovary, when both these organs are acutely stimulated.

Adrenal stimulation

ACTH administration

Assessment of adrenocortical steroidogenic efficiency through ACTH administration, is standard practice in the diagnosis of disorders of the pituitary-adrenal axis, such as Cushing's syndrome, Addison's disease and congenital adrenal hyper= plasia (Zilva & Pannall 1979). The adrenal cortex is known to produce a great variety of steroids, some of which are only present in minute quantities. Different pathways involving different enzyme systems and precursors produce these steroids in the adrenal, and sometimes in other organs of the body as well. Because of the multiple origins of specific steroids, the only feasible way of assessing the adrenal contribution of sex steroids, was by stimulating the adrenal with ACTH, in the absence of gonadal stimulation.

Four male hyaenas, one of which was a juvenile, two were subadults and one adult, were treated with intramuscularly administered dosages of 100 iu ACTH, either as the synthetic corticotrophin-(1-24)-tetracosapeptide or purified porcine ACTH (from pituitary extracts). Figures 46-48 illustrate responses in the peripheral plasma levels of T, A and C after ACTH administration, while comparative aspects of the responses encountered are illustrated in Table 23.

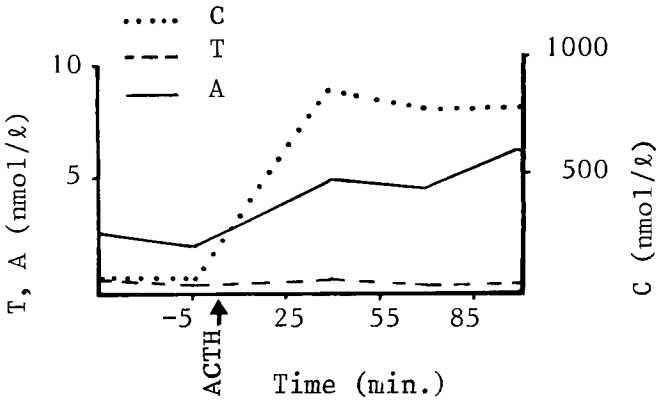


Fig. 46: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after ACTH administration in a 17 kg male spotted hyaena (age class I).

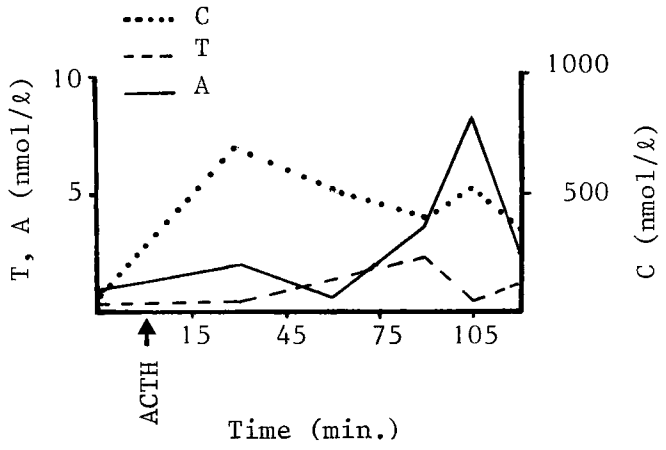


Fig. 47: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after ACTH administration in a 51 kg male spotted hyaena (age class II).

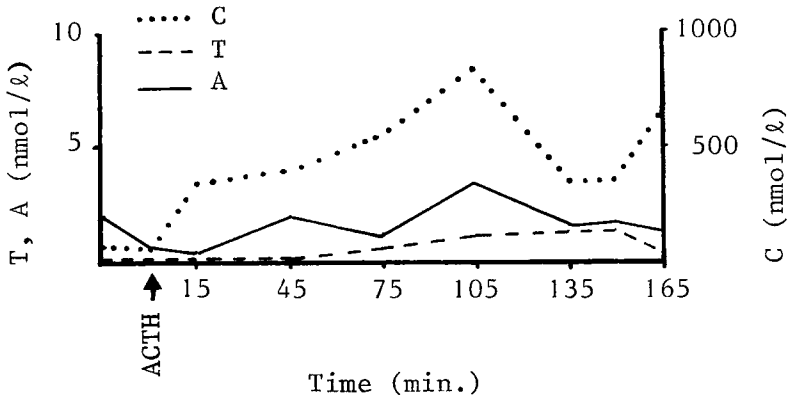


Fig. 48: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after ACTH administration in a 43 kg male spotted hyaena (age class II).

Increased levels of T and A were evident 45-90 min (T) and 40-130 min (A) after treatment with ACTH, and peaks occurred at 90-150 min (T) and 90-105 min (A). The intramuscular route of administration used, could however be responsible for some of the variation in time of occurrence of peaks and the magnitude of responses in general, due to slower and variable absorption. The half-life of ACTH is approximately one minute (Hudson & McMartin 1980), therefore necessitating the use of a dosage as large as 100 iu (approximately 100 mg Ciba-Geigey Pty, Ltd).

TABLE 23: RESPONSES IN MALE SPOTTED HYAENAS TO INJECTIONS OF ADRENOCORTICO HORMONE (ACTH)

♂♂

		Juvenile I[1]	Sub-adult II	Sub-adult II	Adult IV
$\bar{x}$ pre- v. post treatment level of	: T (nmol/ℓ)	0,5 ± 0,2 0,4 ± 0,1^{NS}	0,1 ± 0,0 0,7 ± 0,2*	0,2 ± 0,0 1,0 ± 0,4^{NS}	2,1 ± 0,6 4,9 ± 1,5^{NS}
	: A (nmol/ℓ)	2,4 ± 0,3 5,4 ± 0,8*	1,3 ± 0,5 1,8 ± 0,3^{NS}	0,9 ± 0,0 3,4 ± 1,3*	3,3 ± 1,0 8,1 ± 1,2***
	: C (nmol/ℓ)	64 ± 8 699 ± 2***	80 ± 6 568 ± 61***	65 ± 0,0 496 ± 63***	572 ± 62 848 ± 108*
	: P (nmol/ℓ)	0,8 ± 0,1 0,7 ± 0,2^{NS}	3,3 ± 0,0 2,8 ± 0,4^{NS}	- -	7,7 ± 4,2 9,0 ± 1,2^{NS}
	: E_1 (pmol/ℓ)	112 ± 7 124 ± 4^{NS}	112 ± 8 109 ± 4^{NS}	105 ± 0,0 148 ± 26***	- -
$\bar{x}$ % increase	: T	80,0	700,0	500,0	233,3
	: A	225,0	138,5	377,8	245,5
	: C	1092,2	710,0	763,1	148,3
	: P	87,5	84,9	-	116,9
	: E_1	110,7	97,3	141,0	-
Maximum response[2]	: T (nmol/ℓ)	-0,1	1,2	2,0	14,5
	: A (nmol/ℓ)	3,7	2,2	7,3	13,5
	: C (nmol/ℓ)	633	771	635	653
	: P (nmol/ℓ)	0,0	0,4	-	4,5
	: E_1 (nmol/ℓ)	16	64	131	-
A/T $\bar{x}$ pre- $\bar{x}$ post-		5,8 ± 1,3 15,3 ± 0,0**	13,0 ± 4,5 5,7 ± 2,6*	4,5 ± 0,0 7,7 ± 5,0*	2,3 ± 0,6 4,1 ± 1,3^{NS}

[1]Age class

[2]Calculated as the maximum level after treatment minus the mean level before treatment

NS Not significant
*p < 0,05
**p < 0,01
***p < 0,001

Cortisol levels, indicative of adrenal stimulation, reached peaks 45-105 min after stimulation, and post-treatment levels were significantly higher than pre-treatment levels in all four hyaenas (Table 23). The mean peripheral plasma con= centration of testosterone after ACTH treatment was slightly higher in all four males, but only significantly so in one subadult male (t_8 = 1,880; $p < 0,10$). This individual was the only one not to show significantly (t_8 = 0,833; $p > 0,10$) higher plasma concentrations of androstenedione after ACTH treatment indicating perhaps that conversion of androstenedione to testosterone had taken place. The maximum response in testosterone production by the adrenals of the adult male exceeded that of androstenedione, but otherwise the responses in the two androgens were fairly similar. Peripheral plasma levels of androstenedione were nevertheless always higher than those of testosterone, and androstenedione was considered to be the principal androgen produced by the adrenal cortex, although only marginally so.

The peripheral plasma levels of progesterone were not sig= nificantly (Table 23) altered as the result of ACTH admini= stration, neither were the oestrone levels, except for a highly significant increase in one of the subadult males (t_4 = 39,793; $p < 0,001$).

Four female hyaenas, two of which were juveniles, one was subadult and one adult (pregnant) were treated with ACTH as described for males. The results of these experiments are illustrated in Figures 49-51, and analysed in Table 24. Cortisol levels in the peripheral circulation were signifi= cantly higher ($p < 0,001$) in three of the four females used. The remaining one (subadult) also secreted cortisol in higher concentrations after treatment, but not significantly so due to the small number of samples (n = 5) collected from this particular individual. Testosterone levels were not sig= nificantly higher after ACTH administration than before,

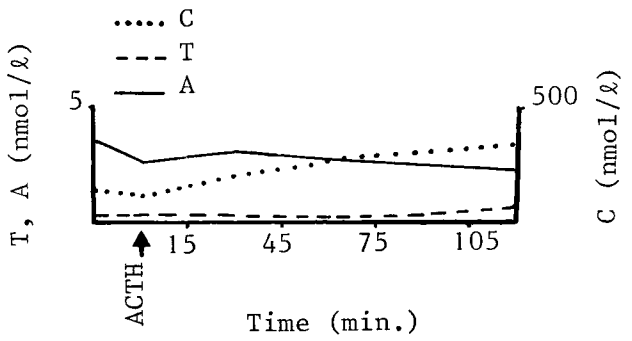


Fig. 49: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after ACTH administration in a 18 kg female spotted hyaena (age class I).

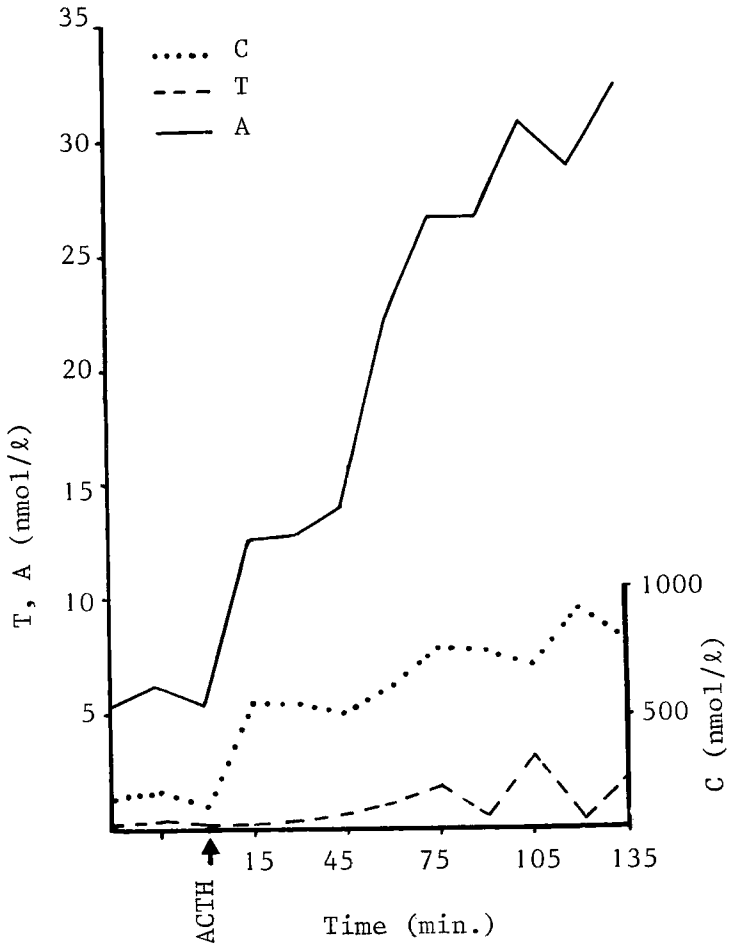


Fig. 50: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after ACTH administration in a 31 kg female spotted hyaena (age class I).

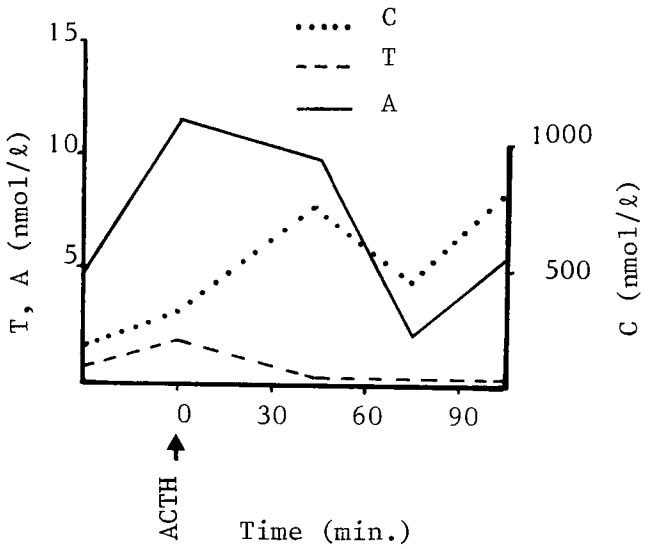


Fig. 51: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A) and cortisol (C) after ACTH administration in a 56 kg female spotted hyaena (age class II).

in contrast with androstenedione levels which were significantly higher ($p < 0,01$ — $p < 0,001$) after treatment in three out of four females (a non-significant response in the subadult female ($t_3 = 0,592$; $p > 0,10$). Both the peripheral levels and the maximum response in androstenedione production were always markedly higher than that of testosterone. The peripheral plasma concentrations of neither progesterone nor oestrone were increased by ACTH administration.

Testosterone production, as reflected by the peripheral plasma concentration of this steroid, was not substantially altered by ACTH administration, in both male and female hyaenas, although the opposite was true for androstenedione production. The adrenals of the female spotted hyaena are seemingly capable of producing more androstenedione under acute ACTH stimulation, than those of the male hyaena, as could be seen from the greater differences in the peripheral levels and maximum response in androstenedione and testosterone respectively, than was evident in the male. It was further evident that some degree

TABLE 24: RESPONSES IN FEMALE SPOTTED HYAENAS TO INJECTIONS OF ADRENO-CORTICOTROPHIC HORMONE (ACTH)

♀♀

		Juvenile I[1]	Juvenile I	Sub-adult II	Adult (pregnant) VI
$\bar{x}$ pre- v. post treatment level of	: T (nmol/ℓ)	0,2 ± 0,1 0,3 ± 0,2^{NS}	0,2 ± 0,1 0,9 ± 0,4^{NS}	0,6 ± 0,3 0,1 ± 0,0^{NS}	4,2 ± 0,8 4,1 ± 0,4^{NS}
	: A (nmol/ℓ)	1,5 ± 0,2 2,4 ± 0,1*	5,6 ± 0,2 24,0 ± 2,7***	8,1 ± 3,6 5,8 ± 2,2^{NS}	16,1 ± 2,2 30,6 ± 4,2***
	: C (nmol/ℓ)	104 ± 14 292 ± 19***	113 ± 23 715 ± 50***	240 ± 75 692 ± 117^{NS}	424 ± 80 1211 ± 133***
	: P (nmol/ℓ)	3,6 ± 2,7 0,9 ± 0,1^{NS}	1,9 ± 0,9 2,2 ± 0,3^{NS}	1,3 ± 0,5 5,2 ± 3,9^{NS}	178,6 ± 35,9 82,5 ± 13,8*
	: E_1 (pmol/ℓ)	123 ± 12 153 ± 43^{NS}	121 ± 5 153 ± 17^{NS}	309 ± 39 116 ± 10***	- -
$\bar{x}$ % increase	: T	150,0	450,0	16,7	97,6
	: A	160,0	428,6	71,6	190,1
	: C	280,8	632,7	288,3	285,6
	: P	25,0	115,8	400,0	46,2
	: E_1	124,4	126,5	37,5	-
Maximum response[2]	: T (nmol/ℓ)	0,4	2,8	-0,5	4,0
	: A (nmol/ℓ)	1,1	26,5	1,7	50,3
	: C (nmol/ℓ)	222	839	590	1361
	: P (nmol/ℓ)	-2,6	1,3	11,6	-35,6
	: E_1 (pmol/ℓ)	117	145	-174	-
A/T $\bar{x}$ pre- $\bar{x}$ post-		11,8 ± 5,3 14,3 ± 6,1^{NS}	42,8 ± 11,2 60,1 ± 17,9^{NS}	14,0 ± 1,1 58,3 ± 22,0^{NS}	4,7 ± 0,7 8,2 ± 1,1**

[1]Age class

[2]Calculated as the maximum level after treatment minus the mean level before treatment

NS Not significant
*p < 0,05
**p < 0,01
***p < 0,001

of individual difference in response does occur, as could be seen from significantly higher levels of oestrone and progesterone in some individuals only. At this stage, these differences are apparently not related to age, sex or reproductive status.

Before it was established that pituitary secretion was inhibited by phencyclidine hydrochloride anaesthesia, a number of hyaenas used in the HCG and ACTH tests, were pre-treated with dexamethazone and/or stilboestrol. Both these synthetic steroids are potent inhibitors of corticotrophin and gonadotrophin secretion by the pituitary, acting through the negative feedback system regulating the secretion of the two trophic hormones.

Dexamethazone is commonly used in the diagnosis and treatment of adrenal hyperplasia (including female hirsutism) and the diagnosis of ACTH-secreting tumours, while stilboestrol is used in the hormonal control of breeding in farm animals through the induction of oestrus, as a remedy for cystic follicles and as an oestrogen supplement. The effects of both dexamethazone and stilboestrol were usually apparent only after a considerable time in the many studies in this field, and the sampling periods followed in this study were therefore usually of too short a duration in this instance.

Two female hyaenas (one juvenile, one adult) and one subadult male were treated with dexamethazone (10 mg iv) only. All steroids measured decreased throughout the observation period (Fig. 52), although the pattern of change was masked by the fluctuating levels of the various steroids in the peripheral circulation. At this stage, it seems that unless treatment with dexamethazone or stilboestrol can be conducted in the absence of surgical anaesthesia and sampling periods can be extended to at least 24 h, suppression of either adrenal or gonad cannot be successfully demonstrated.

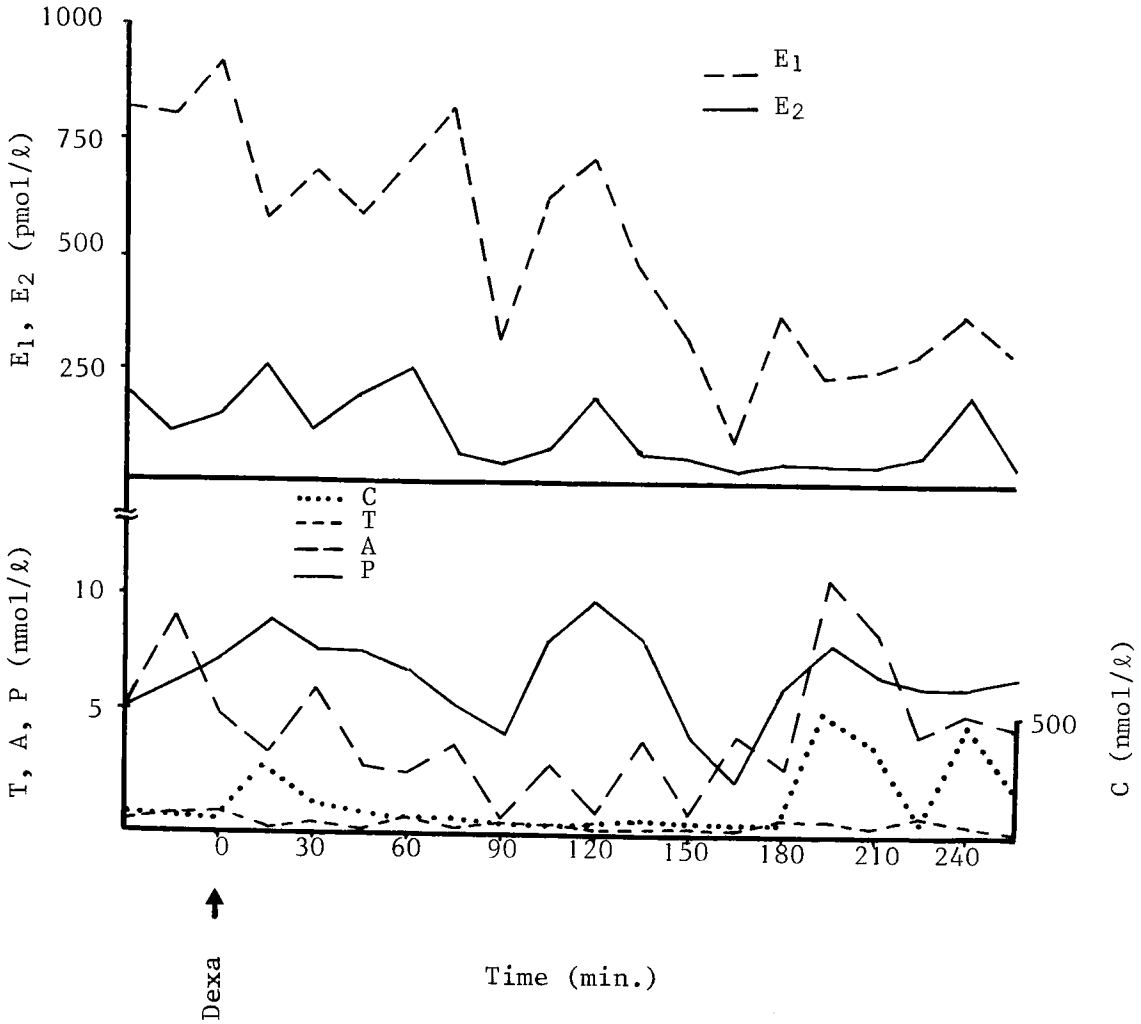


Fig. 52: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after dexametha=zone administration in a 67 kg female spotted hyaena (age class V).

The effects of dexamethazone and HCG on the peripheral plasma steroid concentrations in a single brown hyaena female

The first hyaena on which the *in vivo* tests were tried out, was an adult brown hyaena (*Hyaena brunnea*) female (37 kg, head-body length 116 cm) caught in a trap on a farm near Brits, Transvaal, that was subsequently released in a protected area.

The peripheral plasma concentrations of T, A, E_1, E_2 and C, are illustrated in Fig. 53, while an analysis of the effects of dexamethazone and HCG on steroid production is presented in Table 25. No significant alteration in the levels of steroids other than cortisol and oestrone occurred after dexamethazone administration, but whether this was the result of the treatment only, is not known.

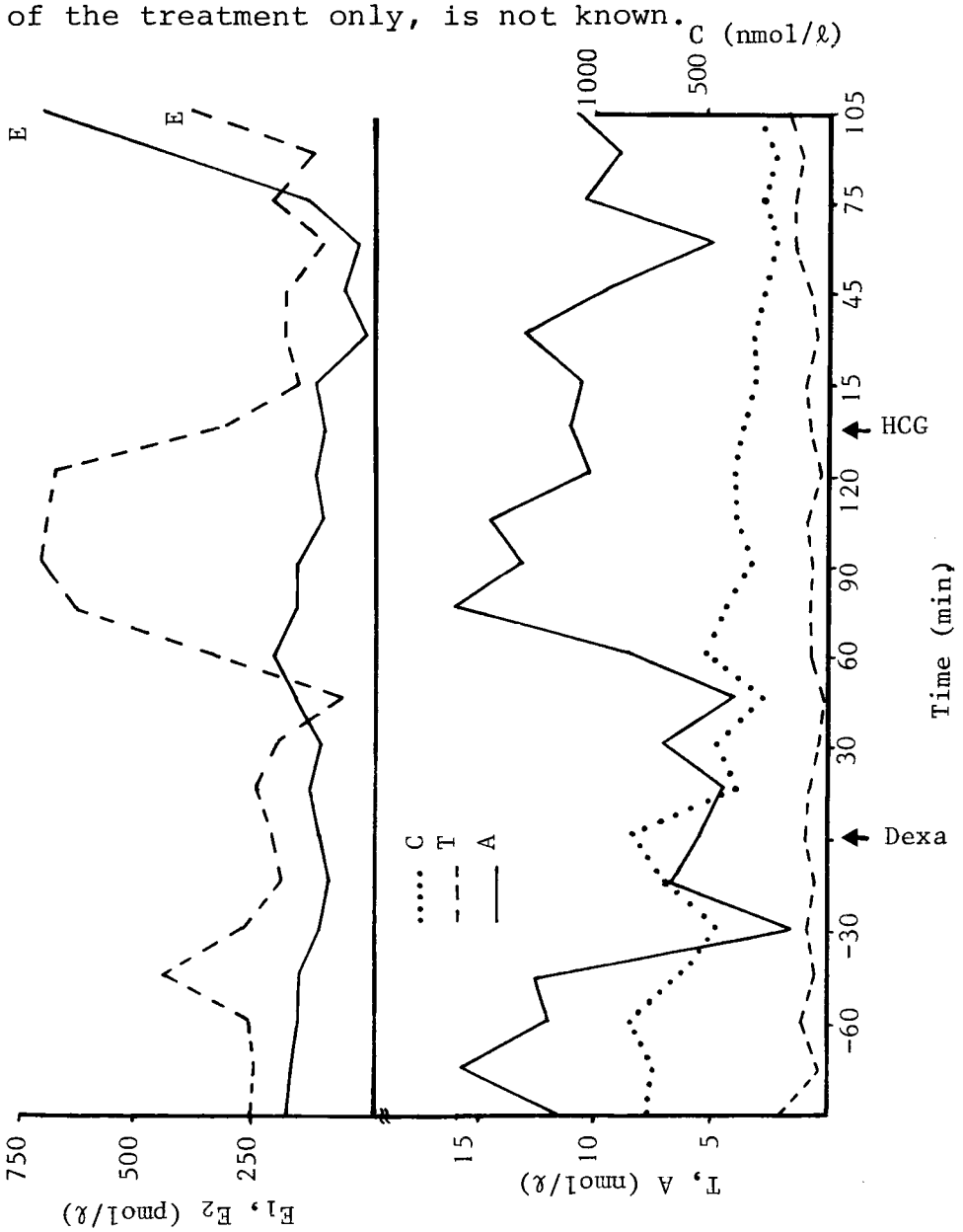


Fig. 53: Peripheral plasma concentrations of testosterone (T), Δ^4androstenedione (A), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) after dexamethazone and HCG administration in a 37 kg female brown hyaena.

TABLE 25: RESPONSES IN AN ADULT BROWN HYAENA (*Hyaena brunnea*) FEMALE TO INJECTIONS OF HUMAN CHORIONIC GONADOTROPHIN (HCG) AND DEXAMETHAZONE

		HCG	Dexamethazone
$\bar{x}$ Pre- v. post treatment level of	: T (nmol/ℓ)	0,7 ± 0,1 1,1 ± 0,1*	0,9 ± 0,2 0,6 ± 0,1^{NS}
	: A (nmol/ℓ)	9,5 ± 1,2 10,0 ± 0,8^{NS}	9,3 ± 1,9 9,7 ± 1,6^{NS}
	: C (nmol/ℓ)	534 ± 48 280 ± 17***	698 ± 48 390 ± 28***
	: E_1 (pmol/ℓ)	344 ± 58 178 ± 31*	228 ± 12 445 ± 98*
	: E_2 (pmol/ℓ)	131 ± 12 158 ± 92^{NS}	126 ± 18 135 ± 17^{NS}
$\bar{x}$ % increase	: T	157,1	66,7
	: A	105,3	104,3
	: C	52,4	55,9
	: E_1	51,7	195,2
	: E_2	120,6	107,1
Maximum response[1]	: T (nmol/ℓ)	1,0	-0,8[1] 0,0[2]
	: A (nmol/ℓ)	3,5	-5,3 6,7
	: C (nmol/ℓ)	-184	-435 -187
	: E_1 (pmol/ℓ)	31	-173 466
	: E_2 (pmol/ℓ)	568	-33 77
A/T $\bar{x}$ pre- $\bar{x}$ post-		18,0 ± 3,5 11,1 ± 2,4*	16,4 ± 6,6 19,5 ± 3,6^{NS}

[1]Calculated as the maximum value after treatment minus the mean value before treatment

[2]Calculated as the minimum value after treatment minus the mean value before treatment

NS Not significant

*p < 0,05
**p < 0,01
***p < 0,001

Following treatment with HCG the peripheral plasma levels of testosterone only, were significantly higher than before treatment (t_{21} = 2,141; $p < 0,05$) although a sharp increase in the peripheral concentration of oestradiol did occur in the terminal stage of the sampling period. No explanation for the wildly fluctuating levels of oestrone and androstene=dione can be given at this stage.

Summary of results

Tables 26-28 provide a summary of the various aspects of responses in the peripheral plasma concentrations of tes=tosterone and androstenedione obtained after administration of LH-RH, HCG and ACTH. From Table 26 it is apparent that the pre-treatment ratio of androstenedione : testosterone was similar for all males and all females, although differing between the two sexes. None of the treatments resulted in a significant change in this ratio after treatment, thereby indicating that a non-selective stimulation of both adrenal and gonads had been achieved. Furthermore, it indicates that testosterone and androstenedione are in fact secreted from both the adrenal and gonad, and are not only derived from peripheral steroid interconversion. This latter phenomenon would have caused delays in the elevation of either steroid which would have altered the ratio of androstenedione : testosterone.

It is not easy to compare the responses in testosterone and androstenedione secretion obtained in males and females, in terms of the magnitude of the response, since the exact rate of secretion and metabolism of these steroids are unknown. An approximate comparison is presented in Table 27 where the maximum responses in secretion of each hormone are compared between males and females. The only significant differences between the responses of males and females, were in andro=

TABLE 26: A COMPARISON BETWEEN THE RATIO ANDRO=STENEDIONE (nmol/ℓ) : TESTOSTERONE (nmol/ℓ), IN THE PERIPHERAL CIRCULATION OF MALE AND FEMALE SPOTTED HYAENAS BEFORE AND AFTER TREATMENT WITH LH-RH, HCG AND ACTH (JUVENILES EXCLUDED)

Treat=ment	♂♂ n	♂♂ $\bar{x}$ A/T Pre-*	♂♂ $\bar{x}$ A/T Post-*	♀♀ n	♀♀ $\bar{x}$ A/T Pre-*	♀♀ $\bar{x}$ A/T Post-*
LH-RH	2	1,9 (1,4-2,4)	1,9 (1,8-2,0) $t_2 = 0,000^{NS}$ [3]	2	10,8 (7,5-14,1)	24,0 (15,9-32,1) $t_2 = 4,649^{NS}$
HCG	3	2,6 ± 0,5 [1]	2,2 ± 0,5 $t_4 = 0,604^{NS}$	4	32,2 ± 11,3	29,5 ± 16,7 $t_6 = 0,722^{NS}$
LH-RH [2] +HCG	5	2,3 ± 0,4	2,0 ± 0,3 $t_8 = 0,536^{NS}$	6	25,1 ± 8,5	27,6 ± 10,8 $t_{10} = 0,891^{NS}$
ACTH	3	6,6 ± 3,3	5,8 ± 1,8 $t_4 = 0,466^{NS}$	2	9,4 (4,7 - 14,1)	33,3 (8,2 - 58,4) $t_2 = 5,207^{NS}$

*Refers to : before or after treatment

[1]Standard error

[2]Combined results from LH-RH tests and HCG tests

[3]Difference between pre- and post-treatment values

NS : Not significant ($p > 0,01$)

stenedione production during gonadal stimulation, where the female gonad appears to be capable of a far greater response in androstenedione production ($t_5 = 12,629$; $p < 0,001$). Responses in both testosterone and androstenedione production

in male and female hyaenas did not differ significantly between the three treatments. It is remarkable that adrenal responses were so similar to gonadal responses, although the latter differed between males and females.

TABLE 27: A COMPARISON BETWEEN THE MAXIMUM RESPONSE IN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T) AND Δ^4ANDROSTENEDIONE (A) IN MALE AND FEMALE SPOTTED HYAENAS (JUVENILES EXCLUDED) FOLLOWING TREATMENT WITH LH-RH, HCG AND ACTH

Treatment	Maximum response in T (nmol/ℓ)				Maximum response in A (nmol/ℓ)			
	n	♂♂	n	♀♀	n	♂♂	n	♀♀
LH-RH	2	4,9 (4,5 - 5,3)	2	1,2 (0,1 - 2,3) $t_2 = 3,593^{NS}$[2]	2	5,8 (1,1 -10,5)	2	27,8 (19,5-36,1) $t_2 = 7,256^{NS}$
HCG	3	2,1 ± 1,1[1]	4	3,8 ± 1,4 $t_5 = 1,424^{NS}$	3	4,6 ± 3,1	4	60,3 ± 26,0 $t_5 = 12,629^{**}$
LH-RH[3] + HCG	5	3,2 ± 0,9	6	3,0 ± 1,1 $t_9 = 0,214^{NS}$	5	4,7 ± 1,7	6	49,5 ± 18,0 $t_9 = 14,458^{**}$
ACTH	3	5,9 ± 4,3	2	1,8 (1,3 - 2,3) $t_3 = 1,826^{NS}$	3	7,7 ± 3,3	2	26,0 (1,7 - 50,3) $t_3 = 5,131^{NS}$

[1]Standard error

[2]Difference between max. response in ♂♂ and ♀♀

[3]Combined results from LH-RH tests and HCG tests

NS : Not significant ($p > 0,01$)

$^{**}p < 0,001$

An analysis of the mean percentage increase in the peripheral plasma levels of testosterone and androstenedione after treatment with LH-RH, HCG and ACTH is presented in Table 28.

TABLE 28: A COMPARISON BETWEEN THE MEAN PERCENTAGE INCREASE IN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T) AND Δ^4ANDROSTENEDIONE (A) IN MALE AND FEMALE SPOTTED HYAENAS (JUVENILES EXCLUDED) FOLLOWING TREATMENT WITH LH-RH, HCG AND ACTH

Treatment	$\bar{x}$ % increase in T				$\bar{x}$ % increase in A			
	n	♂♂	n	♀♀	n	♂♂	n	♀♀
LH-RH	2	210,8 (143,8-277,8)	2	136,1 (100,0-172,2)[2] $t_2 = 8,749^{NS}$	2	151,0 (90,5-211,5)	2	339,4 (178,7-500,1) $t_2 = 15,004^{*}$
HCG	3	161,5 ± 26,3[1]	4	513,0 ± 229,5 $t_5 = 26,858^{**}$	3	225,0 ± 138,5	4	325,7 ± 166,4 $t_5 = 7,668^{**}$
LH-RH[3] + HCG	5	181,2 ± 20,7	6	387,4 ± 165,7 $t_9 = 21,719^{**}$	5	195,4 ± 80,3	6	330,3 ± 113,2 $t_9 = 14,568^{**}$
ACTH	3	477,8 ± 135,2	3	57,2 ± 40,5 $t_4 = 41,760^{**}$	3	253,9 ± 69,2	3	130,9 ± 59,3 $t_4 = 14,280^{**}$

[1]Standard error
[2]Difference between $\bar{x}$ % increase in ♂♂ and ♀♀
[3]Combined results from LH-RH tests and HCG tests
NS: Not significant ($p > 0,01$)
*$p < 0,01$
**$p < 0,001$

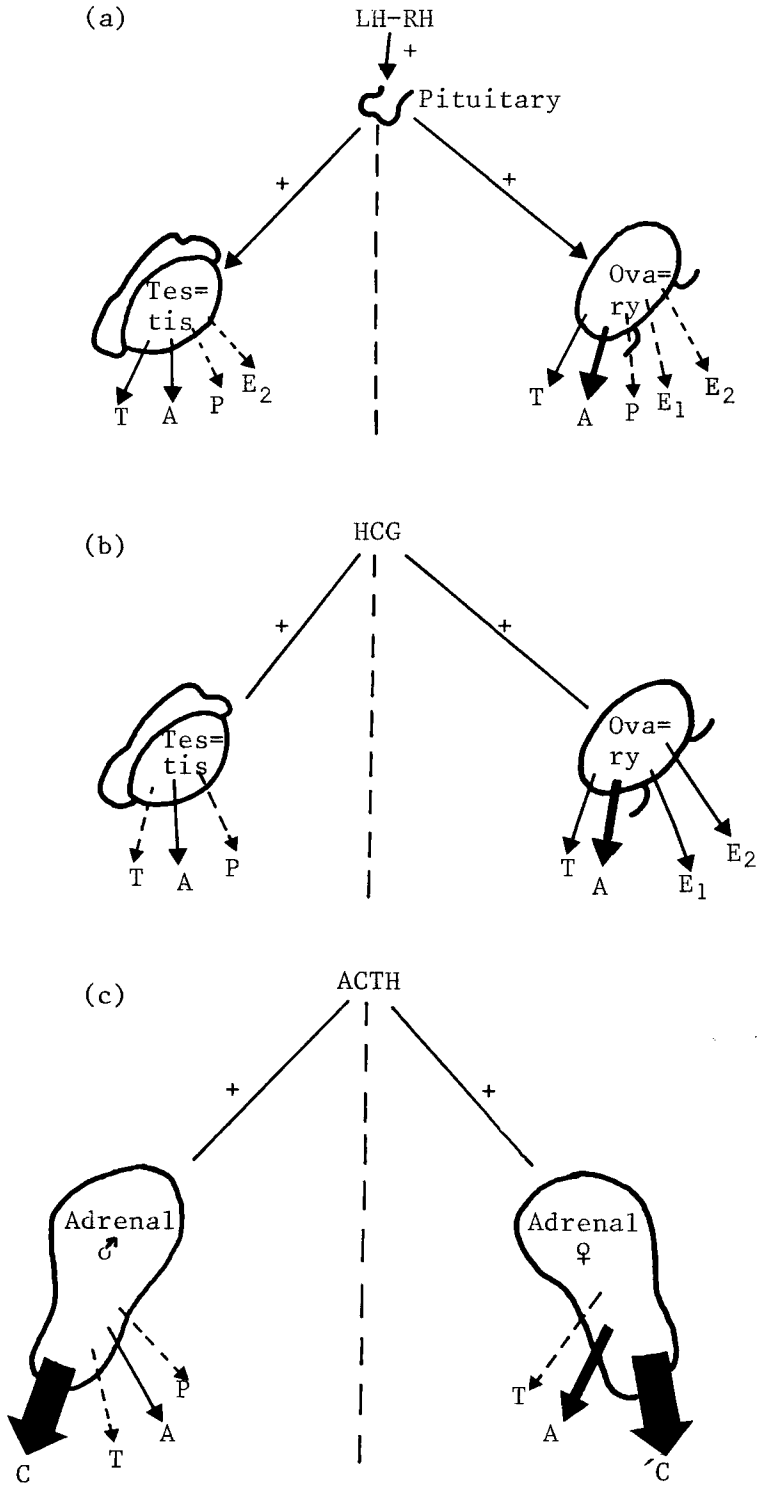


Fig. 54: A schematic representation of the LH-RH-mediated, LH-induced (a), HCG-induced (b) and ACTH-induced (c) gonadal and adrenal secretion of testosterone (T), Δ^4androstenedione (A), progesterone (P), oestrone (E_1), oestradiol-17β (E_2) and cortisol (C) during acute stimulation in male and female spotted hyaenas.

Significant differences in the mean percentage increase, were evident between the responses in testosterone and androstenedione production in both males and females, where adrenal stimulation resulted in a higher mean percentage increase in both testosterone and androstenedione production than during testicular stimulation, but the reverse occurred in female hyaenas. This does not necessarily imply that the male and female adrenals were responding in a different way, as seen from Table 27, but indicates perhaps that the testis of the male spotted hyaena is not capable of as great an increase in testosterone production as the ovary.

A summary of the origins of the major sex steroids in the spotted hyaena, as seen from the experimental stimulation of the adrenal and gonads with ACTH, HCG and LH-RH, is presented in Figure 54.

Short term incubations

In order to establish a protocol for the incubation of hyaena testes, ovaries and adrenals, a trial experiment was conducted in the laboratory under the same conditions as foreseen in the field. The results of this experiment are presented in Table 29. Incubations of hyaena testicular tissue in the presence of HCG (Table 30), resulted in testosterone levels which were in general similar or slightly higher than in control incubations. Tissue pieces were not of exactly the same weight, and results could not be statistically qualified and are best regarded as indications of possible responses. Testicular tissue from either male showed an increased produc= tion of A or P, but not of both.

Ovarian cortical tissue showed increased production of testosterone, androstenedione and oestradiol-17β in the presence of HCG, but the levels of progesterone were virtual= ly unaffected (Table 31). Incubations of the adrenal cortex

TABLE 29: TRIAL INCUBATION EXPERIMENT WITH RAT TESTICULAR TISSUE

Time	Control	T[1] (nmol/ℓ)	Test*	T (nmol/ℓ)
1 hour	Hanks-Eagle's Medium (HE)	3,1	HE	> 20
	HE + Bovine Serum (BS)	5,6	HE + BS	> 20
	BS[2]	0,7	-	-
5 hours	HE	3,4	HE	> 20
	HE + BS	7,5	HE + BS	12,4
	BS	0,8	-	-
8 hours	HE	2,7	HE	> 20
	HE + BS	4,5	HE + BS	> 20
	BS	0,6	-	-

*30 iu Chorulon (Intervet) Chorionic Gonadotrophin

[1]Testosterone

[2]Pre-incubation : 0,7 nmol/ℓ T

TABLE 30: SHORT TERM INCUBATIONS OF TESTICULAR TISSUE

	T (nmol/ℓ)		A (nmol/ℓ)		P (nmol/ℓ)	
	♂A	♂B	♂A	♂B	♂A	♂B
Control Incubations	32,8 ± 1,8	15,6 ± 1,1	2,5 ± 0,2	3,3 ± 0,2	1,0 ± 0,1	0,9 ± 0,1
+HCG*	17,5 ± 2,3	34,5 ± 8,2	2,3 ± 1,1	30,9 ± 4,4	329,6 ± 90,3	1,5 ± 0,3
+HCG**	39,1 ± 4,7	26,6 ± 5,1	1,7 ± 0,7	15,8 ± 3,7	115,0 ± 37,6	1,3 ± 0,4

*Pregnyl
**Chorulon

of male and female adrenals did not show any differences related to sex, and the observed increases in the production of T, A and P in the presence of ACTH were of the same magnitude (Table 32). In this experiment metopirone failed to cause higher levels in either steroid, except possibly in A (in the incubation of a female hyaena adrenal).

Histochemistry

Table 33 provides a summary of the distribution of the steroid isomerase $\Delta^5 3\beta$ hydroxysteroid dehydrogenase and the diaphorase system of hydrogen transport, as demonstrated in the testis, ovary, adrenal and placenta (32 days pregnant) of the spotted hyaena. A typical precursor steroid, dehydroepiandrosterone (DHEA) of the Δ^5-3β hd enzyme was used, as well as an atypical precursor, namely Δ^4-androstenedione (A). The intensity of reactions were determined by comparison with control incuba= tions, and only structures that showed positive reactions are included in Table 18. The intensity of reactions was clas= sified according to a 1-3 scale. The notation "+" signifies a positive but non-specific reaction (a diffuse positive reaction), while "++" indicates a positive specific reaction of low intensity, and "+++" a positive specific reaction of high intensity.

Δ^5-3β Hydroxysteroid dehydrogenase was present in both gonads and adrenals, but was notably absent in the placenta and ovarian follicles. No differences could be found between the male and female adrenal, and the capsular nodules of the male adrenal, which are not a characteristic of all male adrenals, lacked the diaphorase system although showing the presence of the Δ^5-3β hd enzyme.

Plasma, gonadal and adrenal steroids in the foetus

Tissue concentrations of testosterone and Δ^4androstenedione in the testes, ovaries and adrenals of two male and two

TABLE 31: SHORT TERM INCUBATIONS OF THE OVARIAN CORTEX

	T (nmol/ℓ)		A (nmol/ℓ)		P (nmol/ℓ)		E_2 (pmol/ℓ)	
	♀ A	♀ B	♀ A	♀ B	♀ A	♀ B	♀ A	♀ B
Control Incubations	20,7 ± 2,0	0,4 ± 0,0	50,0 ± 5,4	115,4 ± 14,4	1,4 ± 0,3	3,5 ± 0,6	155 ± 33	114 ± 28
+HCG*	30,1 ± 2,7	2,9 ± 0,3	63,3 ± 7,3	189,0 ± 19,1	1,5 ± 0,2	2,5 ± 0,1	282 ± 49	796 ± 91
+HCG**	11,2 ± 8,4	1,0 ± 0,1	20,9 ± 11,4	211,4 ± 21,2	1,3 ± 0,4	3,3 ± 0,2	186 ± 11	342 ± 76

*Pregnyl

**Chorulon

TABLE 32: SHORT TERM INCUBATIONS OF THE ADRENAL CORTEX

	T (nmol/ℓ)		A (nmol/ℓ)		P (nmol/ℓ)	
	♂	♀	♂	♀	♂	♀
Control Incubations	2,0 ± 0,2	1,7 ± 0,2	9,9 ± 1,3	5,4 ± 0,9	18,7 ± 3,3	5,8 ± 1,7
+ACTH	3,7 ± 0,6	3,2 ± 0,5	26,6 ± 3,3	19,8 ± 2,6	21,3 ± 3,5	7,8 ± 1,0
+HCG*	1,3 ± 0,1	0,8 ± 0,1	-	-	-	-
+Metopirone	1,3 ± 0,2	0,7 ± 0,0	9,6 ± 1,1	8,1 ± 2,1	16,2 ± 5,3	5,7 ± 1,5

*Chorulon

TABLE 33: THE PRESENCE OF ENZYME SYSTEMS INVOLVED IN STEROID CONVERSION

	Diaphorase		$\Delta^5 3\beta$HD	
	NAD	NADP	DHEA	A
Testis : Seminiferous tubuli	+	+	+	
: Interstitial cells	+	+	+	
Ovaries : Corpus luteum		+++	+++	+
: Cortex[1]			+++	+
: Tertiary follicles[2]	+	++		
: Atretic follicles[2]	+	+		
: Interstitium	+	+++	+	+++
Adrenal (♂): Z.glomerulosa	+	+		
: Z.fasciculata	++	++	+	
: Z.reticularis	++	++	++	
: Capsular nodules[3]			+++	++
Adrenal (♀) : Z.glomerulosa	+	+	+	+
: Z.fasciculata	++	++	++	
: Z. reticularis	++	++	+++	
Placenta (32 days old)	+	+		

[1]Arca adjacent to ovarian capsule

[2]Zona granulosa

[3]Isolated nodules of cortical tissue contained in capsule

female foetuses were determined by tissue extraction and subsequent radioimmunoassay, and are presented in Table 34. Both the gonadal and adrenal concentrations of testosterone were higher in the male foetus than the female foetus of the same pregnancy, but the reverse was true for androstene=dione. Testosterone and androstenedione concentrations were similar in the testes of the two male foetuses, and in the adrenals of the two male foetuses. In the male foetuses, testosterone was always present in higher concentrations than Δ^4androstenedione in either testis or adrenal. Andro=stenedione was present in higher concentrations in the foetal ovary and female foetal adrenal than testosterone, although the latter was nevertheless present in detectable quantities.

TABLE 34: GONADAL AND ADRENAL CONCENTRATIONS OF TESTOSTERONE (T) AND Δ^4ANDROSTENEDIONE (A) IN THE SPOTTED HYAENA FOETUS

	T (ng/mg tissue)	T (ng/total organ)	A (ng/mg tissue)	A (ng/total organ)
♂ (65 days) Testis	1,16	19,1	0,08	1,3
Adrenal	0,03	2,9	0,02	2,2
♀ (65 days) Ovary	0,19	1,0	0,20	7,5
Adrenal	0,01	1,7	0,01	5,6
♂ (80 days) Testis	0,91	31,1	0,04	1,3
Adrenal	0,01	5,8	0,01	3,7
♀ (80 days) Ovary	0,61	23,7	0,03	1,3
Adrenal	0,01	3,8	0,00	2,1

Plasma concentrations of the major steroids and LH in seven foetuses are presented in Table 35. Plasma testosterone concentrations were consistently higher in male foetuses than in female foetuses, and it would seem that the same might be true for oestrone and oestradiol-17β. None of the other steroids or LH seems to differ in concentration between male and female foetuses. Apart from higher concentrations of testosterone in both the male and female foetus during the first trimester of pregnancy, and the subsequent decrease in the second trimester, none of the other hormones changed in plasma concentration with progressive gestation.

TABLE 35: PLASMA CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDROSTENEDIONE (A), PROGESTERONE (P), OESTRONE (E_1), OESTRADIOL-17β (E_2), CORTISOL (C) AND LUTEINIZING HORMONE (LH) IN SPOTTED HYAENA FOETUSES

Foetal age	T (nmol/ℓ)	A (nmol/ℓ)	P (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	LH (ng/ml)	C (nmol/ℓ)
31 days ♂	25,4	-	-	-	-	-	-
31 days ♀	22,5	-	-	-	-	-	-
65 days ♂	7,4	2,0	429	4709	1082	27	1505
65 days ♀	4,2	2,2	418	1395	154	44	1400
75 days ♂	12,7	3,8	453	1663	1992	38	805
80 days ♂	6,9 (3,1*)	2,5	476	1613	444	41	2450
80 days ♀	12,4 (3,5*)	2,5	445	1302	437	47	1995

*Plasma testosterone concentrations in two female foetuses (78 days) given by Racey & Skinner (1979).

Amniotic fluid concentrations of testosterone were similar for male and female foetuses (1,7 nmol/ℓ at 31 days, 0,3 nmol/ℓ at 65-75 days, and 0,15 nmol/ℓ at 80 days). Placental concentrations of testosterone were 4 pg T/mg tissue at 14 days and 80 days pregnant. No indication of abnormal placental transfer of testosterone or androstenedione could be found, since the levels of these steroids in the umbilical vein blood (placental effluent) were less than in the maternal circulation (2,4 nmol/ℓ T : 12,6 nmol/ℓ and 2,0 nmol/ℓ A : 16,0 nmol/ℓ A), at least at 80 days pregnant.

DISCUSSION

Due to the fact that hyaenas could only be handled when immobilized, inevitable complications brought about by the use of chemicals to effect anaesthesia, were encountered. The safety margins of both immobilizing agent and synergists used, were great enough to allow for accidental overdosages without any casualties, but the preferred state of anaesthesia was when the animal was calmly asleep and insensitive to pain or any sensory stimulus. To maintain this level of anaesthe=sia, constant adjustments through additional amounts of syner=gistic drugs given, were essential for successful experiment=ation, since muscular spasms lead to the dislodging of sampling systems and intravenous irritation which caused the dreaded venous thrombosis.

With progressive anaesthesia, sampling inevitably became more and more cumbersome, mainly as the result of venous thrombosis, oedema around the site of venopuncture, and decreased blood pressure caused by peripheral vasodilatation, bradycardia and hypopnoea. The effects of the anaesthetic agents used in this study on endocrine systems are still largely unknown, except where the central nervous system is directly involved. All the drugs used were central nervous system depressants, and all would have resulted in the disfunction of the

hypothalmic-pituitary axis, and therefore production and release of gonadotrophins and corticotrophin would have been inhibited to some extent. Barbiturates have been reported to affect gonadal steroidogenesis (Carstensen, Amér, Amér & Wide 1973, Nakashima, Koshiyama, Uozumi, Monden, Hamanaka, Kurachi, Aono, Mizutani & Matsumoto 1975) and were not used to produce general anaesthesia, for this reason. Doxapram hydrochloride, which is an effective respiratory stimulant, could not be used at liberty during experimentation, and was only used in emergencies, because it stimulates the production of adrenal 17-hydroxycorticosteroids (A.H. Robins Co 1978, Sales pamphlet). Atropine was not used as a cardiac stimulant, since it does little to relieve hypotension and is a further cause of vasodilation (Harthoorn 1976).

The results obtained from the *in vivo* experiments generally indicate a successful application of the technique as described. Dosages of stimulants and suppressants used were usually effective in stimulating or suppressing the target organs, seen in terms of the sampling periods in this study. Although responses could seldom be followed throughout until the return to the baseline level, that portion of the response that could be demonstrated was usually adequate for diagnostic purposes, except in the case of suppressions. Much longer periods of observation and sampling, preferably more than 24 h, will certainly yield better results, especially in the case of intramuscularly administered preparations. For the purpose of this study, the sampling periods followed may be considered adequate, but optimal results would perhaps have been achieved through an increase in the period allowed before treatment, for the establishment of a better base-line level of secretion. The best results were obtained with a pre-treatment period of > 60 min (5 blood samples), and a post-treatment sampling period of approximately 120 min or more.

This study is, to my knowledge, one of the very few in which the effects of exogenous gonadal and adrenal stimulants on the peripheral levels of the major sex steroids, were de= termined in a non-domesticated mammal. Most studies in this field were concerned with the localization of the origin of secretion of various steroids found in the peripheral circulation, or with the clinical chemistry of endocrine disorders. The findings of both types of studies previously conducted, are still controversial, especially in the few cases where the classical concepts of a pituitary-adrenal axis and a pituitary gonadal axis, were shown to be inadequate to explain results obtained from recent tests conducted *in vivo*. New concepts, like intra-gonadal regulation of secretion, and an adrenal-gonadal axis of the regulation of steroid synthesis, are not fully understood at present, nor readily accepted by all concerned, but are potentially important enough to warrant consideration.

The present study showed that LH-RH mediated release of LH evoked an increased production and secretion of testosterone and androstenedione by both the ovary and testis. This is in accordance with published findings of the effect of LH-RH in rats, cattle, sheep and man (Reeves, Arimura & Schally 1970, Kaltenbach, Dunn, Kiser, Corah, Akbar & Niswender 1974, Falvo, Buhl, Reimers, Foxcroft, Hunzicker Dunn & Dziuk 1975, Katz & Carr 1976, Tannen & Convey 1977, Schanbacher & Echtern= kamp 1978, Barnes, Bierley, Halman & Henricks 1980, Madej, Barcikowski, Stupnicki, Kula & Binienda 1980). In hyaenas, however, the testicular response in testosterone production exceeded that of the ovary, but not to the same extent as reported for other species.

Human chorionic gonadotrophin has been shown to induce sig= nificant increases in plasma testosterone levels of rats (Moger & Armstrong 1974), rams (Falvo *et al* 1975, Garnier & Saez 1980, Sundby & Velle 1980), dogs (Brinck-Johnsen

& Eik-Nes 1957, Eik-Nes 1964, 1971) and men (Rivarola, Saez, Meyer, Jenkins & Migeon 1966, Saez & Forest 1979). Unfortunately, little is known about the fate of other steroids during acute HCG stimulation in these species.

Such a sharp increase in testosterone levels was not evident in the spotted hyaena males treated with HCG. Mean levels of this steroid before and after treatment were not significantly different ($p > 0,01$), although the levels of androstenedione were significantly higher ($p < 0,01$) in two of the three males treated. Responses in both the level of testosterone and androstenedione after HCG treatment, were slightly lower than those obtained after LH-RH mediated-LH stimulation. The overall impression derived from both this and the LH-RH test suggests that the testis of the spotted hyaena is incapable of producing high levels of testosterone during acute stimulation with either LH-RH-mediated LH or HCG.

On the other hand, the situation differed somewhat in the females treated with HCG. Testosterone and androstenedione levels were significantly higher after treatment with HCG, in two of the four adult females used in this test. This apparently bears no relationship to reproductive status, since one of the females was lactating and the other pregnant, the same as the two that failed to show a response. It is not easy to compare steroid production between males and females, due to the difference in secretory pathways in the ovary and testis, but it seems that the ovary is capable of a greater response in both testosterone and androstenedione secretion during acute HCG stimulation. As in the LH-RH test, the levels of androstenedione obtained after HCG stimulation were considerably higher than testosterone, suggesting that androstenedione is indeed the major androgenic secretory product of the ovary during acute stimulation, as has been suggested by Eckstein (1977), and is indeed the principal steroid secreted by the ovary, except for progesterone during the luteal phase.

In agreement with the results of Short, McDonald & Rowson (1963) HCG treatment did not result in increased production of progesterone, confirming, as is now accepted, that progesterone production from the corpus luteum in particular, is not as dependent on gonadotrophic stimulation, as on prolactin (Hutchinson & Sharp 1977). Species differences are never= theless likely to mask results. One of the lactating females treated with HCG, had developing follicles in both ovaries, and was incidentally also the only female in the group which showed a significant increase in the peripheral plasma con= centration of oestradiol-17β, a steroid almost exclusively produced by the follicle in female mammals (Eckstein 1977).

The results from the ACTH administration tests could fortunate= ly be interpreted knowing the degree of stimulation of the adrenal cortex achieved by treatment with ACTH, as shown by the secretory pattern of cortisol, which is not only the major secretory product of the adrenal cortex, but also one of the few steroids not produced by the ovary and testis. When adrenal cortex stimulation had been achieved, peripheral testosterone levels were significantly elevated in two of the four males treated. This is possibly the first record of increased testosterone levels after short-term ACTH ad= ministration.

Many scientists have demonstrated dramatic decreases in the peripheral levels of testosterone after ACTH treatment, and much controversy still exists as far as the respective influences of circadian rhythms of testosterone secretion, inhibition of gonadotrophin secretion by either cortisol or ACTH, or even the direct action of ACTH and cortisol on the Leydig cell, are concerned (Sorcini, Sciarra, Concolino & Rascio 1963, Smals, Kloppenborg & Benraad 1972, Beitins, Bayard, Kowarski & Migeon 1973, Irvine, Toft, Wilson, Frazer, Wilson, Young, Hunter, Ismail & Burger 1974, Doerr & Pirke

1975, 1976, Verjans & Eik-Nes 1975, Vinson, Bell & Whitehouse 1976, Welsh, McGraw & Johnson 1979). Baird, Uno & Melby (1969) and Vinson *et al.* (1976) nevertheless showed that testosterone is produced in small quantities by the rat and human adrenal respectively.

No significant increase in the plasma testosterone levels of female hyaenas treated with ACTH, occurred. Three of the four females showed an adequate response in cortisol production, and all three had significantly higher levels of androstenedione after treatment, the same as in the majority of the males. None of the other steroids were significantly higher in peripheral plasma concentration after ACTH treatment, except for a significant rise in oestrone production in one subadult male. This is in keeping with the findings of Baird *et al.* (1969), Doerr & Pirke (1975), Eik-Nes & Verjans (1975) and Vinson *et al.* (1976), that oestrone is the only oestrogen secreted by the adrenal cortex.

The results from short term incubations of hyaena testicular, ovarian and adrenal cortical tissues were in agreement with those obtained from *in vivo* stimulations. Testis cultures treated with HCG did not show as great an increase in testosterone concentration as in androstenedione and progesterone, supporting perhaps earlier indications of androstenedione as the principal androgen produced by the testis. The great increase in the concentration of progesterone in the HCG treated cultures, representing the accumulation of endogenously produced precursor, is perhaps best explained by the absence of conversion to a further metabolite such as DHEA or androstenedione, induced by the culturing technique.

Ovarian cultures treated with HCG had concentrations of testosterone generally higher than the controls, but not as high as the concentrations of androstenedione. The oestradiol-17β concentrations of tests and controls indicate that HCG treatment produces a variable response in oestrogen

production, ranging from slightly higher to substantially higher than the controls.

From the adrenal cultures, only ACTH was capable of producing slight increases in the concentration of testosterone and progesterone, with a greater increase in androstenedione concentrations.

Although the histochemical demonstration of enzyme systems involved in steroidogenesis has been superceded by the more accurate demonstration of steroid biosynthesis in highly specific cell culture studies, micropuncture and radiolabel studies (*in vivo*), it nevertheless remains useful insofar that possible sites of steroidogenic activity can be rapidly located and identified. The Δ^5-3β hydroxysteroid dehydrogenase enzyme is essential in the biosynthesis of androgens (Deane, Lobel & Romney 1962, Goldberg, Jones, Turner, Sarlos & Horton 1963, Goldberg, Jones & Borkowf 1964), and is the catalyst for conversion of pregnenelone to progesterone, and dehydroepiandrosterone to Δ^4androstenedione. This enzyme is, however, not involved in the direct formation of testosterone, and is therefore not an indication of androgen biosynthesis only. The demonstration of $\Delta^5$3β hd, according to the original method of Wattenberg (1958), lead to numerous publications on the localization of this enzyme in the endocrine glands of many species.

This enzyme as well as the diaphorase enzyme system has been successfully demonstrated in the Leydig cells and the basal layer of the seminiferous tubule in the spotted hyaena testis, as in fact in all other species examined previously (Bailey, Calman, Ferguson & Hart 1966, Davies, Davenport, Norris & Rennie 1966, Galil & Deane 1966, Hay & Deane 1966, Dorrington & Fritz 1975, Osman, Moniem & Tingari 1976, Täkhä 1979). The presence of this enzyme in the corpus luteum and ovarian stroma of the spotted hyaena ovary, has been demonstrated

likewise in the corpora lutea and ovarian stromal compart= ments of many other species (Deane *et al.* 1962, Goldberg *et al.* 1963, Rubin, Deane & Hamilton 1963, Davies *et al.* 1966, Galil & Deane 1966, Blaha & Leavitt 1970, Motta & Bourneva 1970, Zoller & Weisz 1979), although the absence of Δ^5-3β hydroxy= steroid dehydrogenase in any part of the follicle in the spotted hyaena ovary, seems to be somewhat rare.

There seems to be interspecific variation in the distribution of this enzyme in the adrenal cortex, but the presence of this enzyme in all three zones of the adrenal cortex, and especially in the inner cortex, is in agreement with the findings for other species (Goldberg *et al.* 1963, Rubin *et al.* 1963, Galil & Deane 1966, and Täkhä 1979). This enzyme was not located in the placenta (32 days pregnant) of the spotted hyaena, as was the case in the ferret (*Mustela putorius*) (Galil & Deane 1966), although it has been demonstrated in the trophoblastic tissue of the placentas of rodents (Davies *et al.* 1966) the cat (Malassiné & Ferré 1979), pig (Christie 1968), and the man (Goldberg *et al.* 1963, Bailie *et al.* 1966, Hart 1966). It appears that the distribution of the Δ^5-3β hydroxysteroid dehydrogenase and diaphorase system of androgen biosynthesis in the testis, ovary and adrenal of the spotted hyaena, con= forms to the general mammalian pattern, except perhaps for the absence of this system in the follicle.

With the limited sample of foetuses from which blood could be collected, available at present, it seems that hormone concentrations in the spotted hyaena foetus conforms to the findings in other species. Plasma testosterone was higher in male than in female foetuses, as in the rabbit (Lipsett & Tullner 1965, Veyssiere, Berger, Jean-Faucher, De Turckheim & Jean 1976), rhesus monkey (Resko 1970), rat (Buhl, Pasztor & Resko 1979), pig (Ford, Christensen & Maurer 1980) and man (Belisle & Tulchinsky 1980). Most studies in this field were, however, concerned with androgen production by the foetal testis and the resulting effects on organogenesis,

and little is known about the other steroids in the male or female foetus. Comparisons between male and female foetuses with regard to a specific steroid are equally rare.

Compared to recent findings, testosterone levels in male hyaena foetuses are not out of the ordinary, with the typically higher concentrations during that crucial period of sexual differentiation in early gestation, and a gradual decrease through late gestation. What is truly remarkable, is a similar rise in testosterone content during the period of gonadal differentiation, in the female foetus (31 days, Table 35) than in the male foetus. This situation is regarded as the cause of masculinization of the external genitalia of the female spotted hyaena foetus, but the reason why the foetal ovary is left intact is still an enigma. It does seem that testosterone in the female foetus, as in the male, decreases with progressive gestation, seen in the light of the testosterone concentrations in two female foetuses in the second trimester of gestation in Racey & Skinner (1979) (Table 35).

At present it seems that the foetal adrenal is not involved in testosterone secretion in either the male or female spotted hyaena foetus, and the concentrations of testosterone found in foetal adrenals in the present study point towards the perfusion of the adrenal with blood containing androgens of gonadal origin, rather than production within the adrenal itself. Maternal contribution of androgens to the foetal circulation cannot be ruled out completely as a cause of masculinization of the female foetal external genitalia, but it is unlikely that the placental barrier, operational in other species, is not functioning in the spotted hyaena. At 80 days of gestation, foetal plasma concentrations of testosterone and androstenedione were higher than in the placental effluent, thereby confirming endogenous production of these steroids in the foetus, and most likely the foetal gonads.

In the light of the results of the stimulation *in vivo* of steroid secretion by the adrenal and gonads, as well as the stimulation *in vitro* of steroidogenesis in short-term tissue cultures of the adrenals and gonads, and the demonstration of the Δ^5-3β hydroxysteroid dehydrogenase enzyme in the testis, ovary and adrenal, the following conclusions can be made regarding the origin of secretion of steroid hormones present in the peripheral circulation.

Testosterone and androstenedione are both produced and secreted by the interstitial cells of Leydig in the spotted hyaena testis. These hormones are nevertheless produced in signi= ficant quantities by the adrenal cortex, and the inner cortex comprising the zona fasciculatis and zona reticularis, in particular. Oestrone and progesterone present in the peripheral circulation originated in part from the same sites as testos= terone and androstenedione, while circulatory oestradiol-17β originated in part from the interstitial cells of the testis only. Interconversion of steroids has been shown to play a major role in the establishment of the final circulatory profiles of the various steroids, especially oestrogens which are largely derived from peripheral conversion of androgens to oestrone and oestradiol (Mahesh & Greenblatt 1962, Horton & Tait 1966, 1967, MaDonald, Rombaut & Siiteri 1967, Abraham, Lobotsky & Lloyd 1969, Longcope, Kato & Horton 1969, Schindler, Ebert & Friedrich 1972). It would therefore be incorrect to assume that all steroids present in the peripheral cir= culation were secreted as such. The effects of intercon= version on the steroid pool had not been determined for the spotted hyaena, although only minor changes were likely to occur during the relatively short sampling periods in this study.

In the female, the situation is much more complex, in part due to the nature of steroid synthesis in the ovary. Specific structures in the ovary are responsible for the

production of the bulk of specific steroids, and the con= figuration of steroid producing structures, such as the follicle, corpus luteum and insterstitium will vary because of the cyclicity of ovarian function (Eckstein 1977). One would therefore expect differences in the steroid output, in quantity and composition, during the oestrus cycle and between females in different phases of the reproductive cycle. The small sample size in the present study did not allow meaningful comparisons between the responses obtained from individual hyaenas of different ages or reproductive status. Such differences in the temporal circulatory levels of plasma steroids would however be discussed in the following chapter.

In the female spotted hyaena, the androgens testosterone and androsteredione were secreted by the ovary in significant amounts. The ovarian interstitium, or interstitial gland in the ovarian stroma, is the principal site of production and secretion of these steroids from the ovary although, as in the male, significant quantities of both hormones are produced by the inner region of the adrenal cortex. Oestradiol-17β was the only oestrogen that was produced by the ovary, and although the site of secretion could not be demonstrated histochemically, probably originated from developing follicles only, as in many other species (Eckstein 1977). Oestradiol-17β was not produced by the adrenal cortex as such, but could perhaps also originate from the interstitial gland or corpus luteum, as in most primates (Eckstein 1977). Oestrone production and secretion from the ovary has also been demonstra= ted in the present study, although the exact site of production is like that of oestradiol-17β, still unknown. Oestrone was also produced by the adrenal cortex, probably the same region where androgen and cortisol biosynthesis take place. Progesterone was secreted from both the ovary and adrenal, most likely largely from the corpus luteum and the inner cortex of the adrenal. All females treated with HCG or LH-RH unfortunately had one or more corpora lutea, but these treat=

ments never resulted in an increased production of pro= gesterone. Progesterone was secreted by the adrenal during acute stimulation.

A feature of spotted hyaena endocrinology is, as Racey & Skinner (1979) originally suggested, the similarity in the hormonal milieu of males and females, and a further feature certainly is the close similarity between the steroid products of the gonad and adrenal. Androstenedione is the principal androgen produced by both testis, ovary and adrenal, although the smaller quantities of testosterone produced by each of these organs could have a marked biological significance, since the latter hormone is far more androgenic than andro= stenedione.

The high levels of circulatory androgens in the female spotted hyaena, reported by Racey & Skinner (1979), are strongly con= firmed in the present study. The spotted hyaena ovary and adrenal are capable of secreting significant amounts of andro= stenedione, but furthermore also significant amounts of tes= tosterone. In the typical female mammal, testosterone is mostly derived from peripheral conversion of precursors produced by the ovary and adrenal, such as androstenedione, but it seems that testosterone in the circulation of female spotted hyaenas originated only partly from the peripheral conversion of precursors, as testosterone is secreted by the ovary as such.

The male spotted hyaena appears to be incapable of as great an output of androgens during acute stimulation of the testis, as in female spotted hyaenas. The similarity in circulatory levels of androgens in male and female hyaenas therefore appears to be the result of the rather high levels of androgens in the female, and a comparatively low testicular output of androgens in the male.

CHAPTER 5

ENDOCRINE CORRELATES OF REPRODUCTION

INTRODUCTION

Changes in the pattern of hormonal secretion during the reproductive cycle are one of the cornerstones of modern reproductive physiology, and such changes in especially the sex hormones have been determined for many wild, laboratory and domesticated mammals. The advent of competitive steroid binding or radioimmunoassay, as a highly specific and highly sensitive method of determination of hormonal factors in biological fluids, greatly facilitated determination of the exact concentrations of circulating hormones. Each phase of the reproductive cycle has been shown to have a typical complement of sex hormones, since these are inseparable from gonadal function and the reproductive process. If the typical hormonal environment associated with a particular phase of the reproductive cycle is known, it can be used to diagnose the reproductive status of an individual of unknown reproductive status, as long as the degree of variation in the hormonal environment during the reproductive cycle is adequate for discrimination.

Racey & Skinner (1979) presented such a correlation between hormonal environment and reproductive status, and established that pregnant hyaenas could be identified as such because of their high concentrations of progesterone. No other phase could be identified, but this could be attributed to the relatively small sample at their disposal. Their results, as well as those presented in this chapter, were based on the circulatory concentration of plasma hormones as reflected by a single blood sample obtained from culled or immobilized hyaenas. This method is not beyond suspicion, for it has been shown that rapid decay of plasma steroids and polypeptides may result from bacterial activity and enzymal degeneration, occurring in blood samples incubated *in vitro* (Owens, Atkins, Rahe, Fleeger & Harms 1980), but the same situation could conceivably occur in culled animals. Blood samples should therefore be taken reasonably soon after death.

As discussed previously, time-dependent losses of circulatory hormones are likely to occur in immobilized hyaenas, and again samples should be taken reasonably soon after capture. The rate of decline has not been determined yet, and no correction factor could be introduced in the analysis. The use of a single blood sample to describe the time-specific hormonal environment of an individual is valid, but cannot be considered representative of the true pattern of secretion associated with each phase of the reproductive cycle, mainly because of the pulsatile pattern of secretion followed by most gonadal steroids, and possible circadian fluctuations, as have been demonstrated by Rivarola *et al.* (1966), Eik-Nes (1971), Falvo *et al.* (1975), Verjans & Eik-Nes (1975), and Speroff *et al.* (1978). Although it was practically impossible to collect samples at the same time of night or at a fixed time after capture, most samples were taken approximately 2-3 h after death or immobilization of hyaenas.

Plasma concentrations of the major sex steroids were determined by radioimmunoassay and correlated with reproductive status to provide some of the basic endocrine correlates of reproduction, and to ascertain the diagnostic value of the steroid concen= trations in a single blood sample in the diagnosis of reproductive status.

MATERIALS AND METHODS

A single blood sample (10-20 ml) was collected with hypodermic needle and syringe from the jugular vein or heart (in culled hyaenas), of 36 female and 25 male spotted hyaenas in the KNP and Umfolozi Game Reserve. Blood was collected in heparinized tubes and centrifuged at 2000 rpm within 2 h after collection. Plasma was stored in airtight plastic storage ampoules at -18^{o} C until assayed for testosterone, Δ^{4}androstenedione, progesterone, oestrone, oestradiol-17β, cortisol and luteinizing hormone (LH).

RESULTS

Table 36 provides the observational ranges of the major sex steroids, cortisol and LH in the peripheral circulation of 61 spotted hyaenas.

TABLE 36: RANGES OF TESTOSTERONE, Δ^4ANDROSTENEDIONE, PROGESTERONE, OESTRONE, OESTRADIOL-17β, CORTISOL AND LUTEINIZING HORMONE (LH) IN THE PERIPHERAL CIRCULATION OF SPOTTED HYAENAS

	♂♂	♀♀ (non-pregnant)	♀♀ (pregnant)
Testosterone (nmol/ℓ)	0,1 - 5,5	0,1 - 4,3	0,1 - 12,6
Androstenedione (nmol/ℓ)	0,6 - 8,5	0,7 - 16,2	6,7 - 91,6
Progesterone (nmol/ℓ)	0,7 - 23,8	1,0 - 64,3	77,1 - > 440
Oestrone (pmol/ℓ)	105 - 748	109 - 1924	2200 - > 69124
Oestradiol-17β (pmol/ℓ)	6 - 224	7 - 462	66 - 1990
Cortisol (nmol/ℓ)	35 - 637	35 - 426	67 - 1120
LH (ng/ml)	11 - 86	10 - 80	10 - 33

The only meaningful relationship between the circulatory level of a steroid and a non-hormonal parameter of reproduction is presented in Figure 55. Although a limited sample of pregnant females was available, it seems that progesterone production increases with progressive gestation, at least until the eleventh week of gestation (end of second trimester). Apart from this

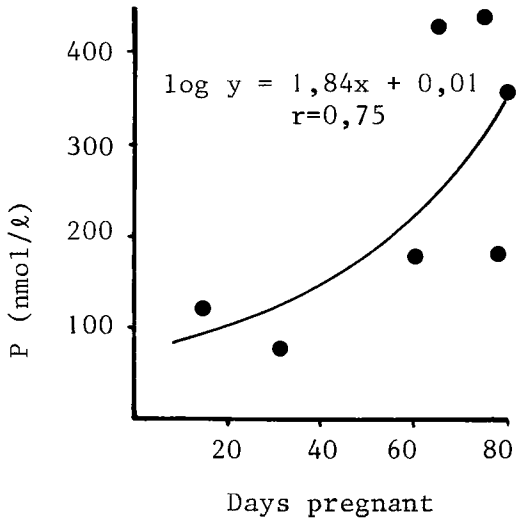


Fig. 55: The relationship between peripheral plasma concentration of progesterone (P) and days pregnant in spotted hyaenas.

relationship between progesterone concentration and age of the corpus luteum (gestational age), none of the other parameters of growth and regression of the corpus luteum could be shown to be related to the secretory activity of the corpus luteum.

In male spotted hyaenas, no meaningful relationship between androgen concentration in the peripheral circulation, and the abundance of Leydig cells in the testis could be demonstrated. The best correllation coefficient between testosterone con= centration (y) and Leydig Cell density (x) was -0,23 (logy = 0,40 x -0,11) and for androstenedione 0,02 (logy = 0,28 x + 0,01). The relationships between seminiferous tubuli diameter and testosterone or androstenedione concentrations were similar= ly weak (r = 0,52 and r = 0,15 respectively). Circulatory levels of either androgen can therefore not be related to the histological picture in the testis.

Age-related changes in the steroid pool

Table 37 presents the peripheral plasma concentrations of testosterone, androstenedione, progesterone, oestrone and oestradiol-17β of male and female spotted hyaenas in each age class. The only significant difference between males and females of age class I was the higher concentration of oestrone in the former (t_6 = 20,600; $p < 0,001$). Both oestrone, oestradiol-17β and androstenedione were significantly higher in females than in males of age class II (t_{25} = 33,268; $p < 0,001$; t_{25} = 8,982; $p < 0,001$ and t_{25} = 4,890; $p < 0,001$ respectively). The same was apparent for oestrone and oestradiol-17β between the males and females of age class III (t_4 = 11,200; $p < 0,001$ and t_4 = 7,370; $p < 0,01$).

Male hyaenas of age class I and II differred significantly in oestradiol-17β concentrations, which was higher in the latter age class (t_{18} = 5,761; $p < 0,001$). Oestrone and oestradiol-17β were both significantly higher in females of age class II, than those of age class I (t_{13} = 125,128; $p < 0,001$ and t_{13} = 10,638; $p < 0,001$ respectively). Progesterone and oestrone were sig= nificantly higher in males of age class III than in those of age class II (t_{17} = 12,777; $p < 0,001$ and t_{17} = 7,493; $p < 0,001$) but the opposite was true for oestradiol-17β (t_{17} = 6,995;

$p < 0,001$). The only significant difference between females of age class II and III was a significantly higher concentration of oestradiol-17β in the latter ($t_{12} = 3,400$; $p < 0,01$).

TABLE 37: MEAN (+ SE) PERIPHERAL CIRCULATORY CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDROSTENEDIONE (A), PROGESTERONE (P), OESTRONE (E_1) AND OESTRADIOL-17β (E_2) OF MALE AND NON-PREGNANT FEMALE HYAENAS PER AGE CLASS

	♂♂						♀♀					
	n	T (nmol/ℓ)	A (nmol/ℓ)	P (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	n	T (nmol/ℓ)	A (nmol/ℓ)	P (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)
I	4	1,2 ± 0,8	2,6 ± 0,8	5,1 ± 1,9	408 ± 187	49 ± 13	4	0,9 ± 0,5	4,5 ± 1,2	3,3 ± 1,1	180 ± 58	39 ± 18
II	16	2,2 ± 0,6	2,3 ± 0,5	6,3 ± 1,5	338 ± 53	72 ± 14	11	1,6 ± 0,5	5,5 ± 1,2	9,1 ± 5,6	558 ± 119	105 ± 41
III	3	1,7 ± 0,1	3,9 ± 0,9	14,6 ± 3,4	408 ± 164	40 ± 17	3	2,0 ± 0,3	2,2 ± 0,5	9,7 ± 7,4	525 ± 25	130 ± 49
IV	1	5,5	1,8	3,8	166	6	2	2,4 ± 1,7	6,4 ± 1,1	5,1 ± 1,0	949 ± 611	-
V	-	-	-	-	-	-	2	0,6 ± 0,5	5,0 ± 0,6	4,0 ± 1,6	943 ± 134	137 ± 66
VI	-	-	-	-	-	-	4	1,1 ± 0,4	7,4 ± 1,9	3,3 ± 1,3	833 ± 343	40 ± 15
VII	-	-	-	-	-	-	2	0,7 ± 0,5	11,0 ± 5,2	10,7 ± 3,0	1187 ± 738	115 ± 6

Between females of age class III and IV, the only significant difference was the higher levels of oestrone in the latter (t_3 = 20,799; $p < 0,001$), while no significant difference could be demonstrated between females of age class IV and V. Both oestrone and oestradiol-17β were significantly lower in age class VI females than those in age class V (t_4 = 5,359; $p < 0,01$ and t_4 = 16,544; $p < 0,001$), but also significantly lower than that in females from age class VII (t_4 = 14,679; $p < 0,001$ and t_4 = 17,453; $p < 0,001$).

A more robust classification is presented in Table 38, where juveniles represent hyaenas in age class I, subadults age class II and adults age classes III-VII. Juveniles and subadults have already been compared above, but in the adult group, it appears that oestrone and oestradiol-17β were always present in significantly greater quantities in females than in males (t_{15} = 40,594; $p < 0,001$ and t_{15} = 15,432; $p < 0,001$ respectively).

TABLE 38: MEAN (± SE) PERIPHERAL CIRCULATORY CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDROSTENEDIONE (A), OESTRONE (E_1), OESTRADIOL-17β (E_2) AND PROGESTERONE (P) IN MALE AND NON-PREGNANT FEMALE SPOTTED HYAENAS

	T (nmol/ℓ)	A (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	P (nmol/ℓ)
Juvenile ♂♂ (n=4)	1,2 ± 0,8	2,6 ± 0,8	408 ± 187	49 ± 13	5,1 ± 1,9
Juvenile ♀♀ (n=4)	0,9 ± 0,5	4,5 ± 1,2	180 ± 58	39 ± 18	3,3 ± 1,1
Subadult ♂♂ (n=16)	2,2 ± 0,6	2,3 ± 0,5	338 ± 53	72 ± 14	6,3 ± 1,5
Subadult ♀♀ (n=11)	1,6 ± 0,5	5,5 ± 1,2	558 ± 119	105 ± 41	9,1 ± 5,6
Adult ♂♂ (n=4)	2,9 ± 1,3	3,4 ± 0,8	347 ± 131	29 ± 15	11,9 ± 3,6
Adult ♀♀ (n=13)	1,4 ± 0,3	5,4 ± 0,9	882 ± 166	101 ± 21	6,3 ± 1,8

No significant difference in the concentration of testosterone in males and females of the same age group, nor between age groups, could be demonstrated. Apart from the higher levels of androstenedione in juvenile females than in juvenile males, as mentioned, there was no other difference in the level of androstenedione between males and females per age class, age group, or between age classes and age groups.

Amounts of progesterone did not differ significantly between any age group of females, while oestrone levels did not differ sig= nificantly between any age group of males. In males however, progesterone levels were significantly higher in adult males than in subadult males ($t_{18} = 4,023$; $p < 0,001$) and in juvenile males ($t_6 = 4,101$; $p < 0,01$). Oestrone levels in female hyaenas were significantly higher in adults than in subadults ($t_{22} = 35,163$; $p < 0,001$) and juveniles ($t_{15} = 14,149$; $p < 0,001$). Oestrone levels in subadult females were significant= ly higher than in juvenile females ($t_{13} = 35,618$; $p < 0,001$).

Oestradiol-17β levels in adult female hyaenas were significantly higher than in juvenile females ($t_{15} = 8,216$; $p < 0,001$), but not significantly different from subadults ($t_{22} = 0,962$; $p < 0,01$). Subadult females had nevertheless significantly higher plasma concentrations of this steroid than juvenile females ($t_{13} = 10,638$; $p < 0,001$). Adult males also had significantly higher plasma levels of oestradiol-17β than subadult ($t_{18} = 4,023$; $p < 0,001$) and juvenile males ($t_6 = 4,101$; $p < 0,01$).

Interspecific sex steroid relationships

Tables 39 and 40 illustrate age-specific ratios of testosterone : androstenedione and oestrone : oestradiol-17β. Although not statistically significant, a trend is apparent in Table 39, where the T/A ratio increases with age in males. In age class II, representing the attainment of sexual maturity in the male,

TABLE 39: THE RATIOS TESTOSTERONE (T) : Δ^4ANDROSTENEDIONE (A), AND OESTRONE (E_1) : OESTRADIOL-17β (E_2) IN MALE AND NON-PREGNANT FEMALE SPOTTED HYAENAS PER AGE CLASS

Age class	T(nmol/ℓ)/A(nmol/ℓ)	E_1(pmol/ℓ)/E_2(pmol/ℓ)
I ♂♂ (n=4)	0,328 ± 0,138	9,357 ± 1,664
♀♀ (n=4)	0,209 ± 0,138	11,790 ± 8,157
II ♂♂ (n=16)	1,111 ± 0,230	7,394 ± 2,360
♀♀ (n=11)	0,330 ± 0,099	15,261 ± 4,638
III ♂♂ (n=3)	1,573 ± 1,030	10,074 ± 7,969
♀♀ (n=3)	0,960 ± 0,176	3,582 ± 1,330
IV ♂♂ (n=1)	3,056	27,213
♀♀ (n=2)	0,340 (0,132 - 0,548)	-
V ♂♂ -	-	-
♀♀ (n=2)	0,129 (0,019 - 0,239)	9,548 (4,005 - 15,091)
VI ♂♂ -	-	-
♀♀ (n=4)	0,156 ± 0,049	32,545 ± 16,396
VII ♂♂ -	-	-
♀♀ (n=2)	0,055 (0,035 - 0,075)	10,697 (3,742 - 17,652)

more testosterone than androstenedione is produced, and this continues throughout adult life. The same trend was apparent for female hyaenas until in the fourth age class, where this ratio decreases again, and in fact throughout adult life. Androstenedione was nevertheless always produced in greater quantities than testosterone, in female spotted hyaenas. This ratio did not differ significantly between males and females of the first three age classes.

TABLE 40: THE RATIO TESTOSTERONE (T) : Δ^4ANDROSTENEDIONE (A), AND OESTRONE (E_1) : OESTRADIOL-17β (E_2) IN MALE AND NON-PREGNANT FEMALE SPOTTED HYAENAS

	T(nmol/ℓ)/A(nmol/ℓ)	E_1(pmol/ℓ)/E_2(pmol/ℓ)
Juvenile ♂♂ (n=4)	0,33 ± 0,14	9,36 ± 1,66
Juvenile ♀♀ (n=4)	0,21 ± 0,14	11,79 ± 8,16
Subadult ♂♂ (n=16)	1,11 ± 0,23	7,39 ± 2,36
Subadult ♀♀ (n=11)	0,96 ± 0,18	15,26 ± 4,64
Adult ♂♂ (n=4)	1,94 ± 0,82	15,79 ± 7,34
Adult ♀♀ (n=13)	0,35 ± 0,11	16,14 ± 6,50

The oestrone : oestradiol 17β ratio did not show any tendency to increase or decrease with age, and there was no significant difference in this ratio between males of the first three age classes. A significant difference between the E_1/E_2 ratio of males and females of age class II could be demonstrated, with a higher ratio in females ($t_{25} = 5,845$; $p < 0,001$). Higher concentrations of oestradiol-17β, coincidental with reproductive parity in age class III, significantly reduced the ratio, as compared to age class II ($t_{12} = 4,935$; $p < 0,001$). The E_1/E_2 ratio was significantly different among all of the last three age classes, but no consistent pattern of change was evident.

From Table 40, it appears that the only significant difference in T/A was between adult males and females ($t_{15} = 3,462$; $p < 0,01$). The E_1/E_2ratio did not change significantly with age in females, but was significantly higher in adult males than in subadults ($t_{18} = 4,679$; $p < 0,001$). Subadult females had a significantly higher E_1/E_2 ratio than subadult males ($t_{25} = 5,845$; $p < 0,001$).

Predicting reproductive status from circulatory levels of sex steroids

Table 41 presents a comparison between the five major sex steroids in nulliparous and parous females, and parous non-pregnant females. Nulliparous females had significantly lower levels of the two oestrogens and progesterone than both parous and parous non-pregnant female hyaenas. Androstenedione in nulliparous females was significantly lower than in parous females, but not when pregnant hyaenas were excluded. There seems to be no difference in testosterone concentration between nulliparous and parous or parous non-pregnant females.

TABLE 41: A COMPARISON BETWEEN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDROSTENEDIONE (A), OESTRONE (E_1), OESTRADIOL-17β (E_2) AND PROGESTERONE (P) OF NULLIPAROUS AND PAROUS FEMALE SPOTTED HYAENAS

	T (nmol/ℓ)	A (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	P (nmol/ℓ)
Nulliparous (n=12)	1,1 ± 0,3	4,5 ± 1,8	338 ± 67	78 ± 36	2,9 ± 0,6
Parous (n=24)	2,5 ± 0,6	11,8 ± 3,8	6106 ± 3542	469 ± 154	84,6 ± 29,2
t_{34}	2,597^{NS}	5,402**	150,101**	47,126**	23,410**
Nulliparous (n=12)	1,1 ± 0,3	4,5 ± 1,8	338 ± 67	78 ± 36	2,9 ± 0,6
Parous non-pregnant (n=16)	1,6 ± 0,3	6,7 ± 0,9	919 ± 144	107 ± 20	10,0 ± 3,9
t_{26}	1,231^{NS}	1,824^{NS}	73,326**	7,636**	5,915**

NS : Not significant
** : $p < 0,001$

From Table 42, it is clear that discrimination between parous pregnant and parous non-pregnant females is relatively easy, due to the far greater concentrations of all five steroids in the peripheral circulation of pregnant females, than in those not pregnant. A comparison between parous lactating females and parous anoestrus (non-lactating) to further divide the parous non-pregnant groups, is presented in Table 43. Oestrone levels in the anoestrous group were significantly higher than in lactating females, but none of the other steroids showed sig=nificant differences.

TABLE 42: A COMPARISON BETWEEN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDRO=STENEDIONE (A), OESTRONE (E_1), OESTRADIOL-17β (E_2) AND PROGESTERONE (P) OF PAROUS NON-PREGNANT AND PAROUS PREGNANT SPOTTED HYAENAS

	T (nmol/ℓ)	A (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	P (nmol/ℓ)
Parous non-pregnant (n=16)	1,6 ± 0,3	6,7 ± 0,9	919 ± 144	107 ± 20	10,0 ± 3,9
Parous pregnant (n=8)	4,3 ± 1,4	23,7 ± 11,6	15738 ± 9468	1142 ± 309	254,9 ± 56,6
t_{22}	4,325**	10,933**	362,489**	131,056**	72,076**

** $p < 0,001$

It is therefore possible to ascertain the reproductive status of a female spotted hyaena, once determined as being a female as such, by relative differences in the peripheral plasma con=centrations of one or more of the major sex steroids. Further distinction, pertaining to the progressive state of a particular phase is not possible as yet, except perhaps in the case of nulli=parous females, which could be further subdivided into those exhibiting total ovarian inactivity (Nulliparous A), and those

TABLE 43: A COMPARISON BETWEEN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDROSTENEDIONE (A), OESTRONE (E_1), OESTRADIOL-17β (E_2) AND PROGESTERONE (P) OF PAROUS LACTATING AND PAROUS ANOESTRUS SPOTTED HYAENAS

	T (nmol/ℓ)	A (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	P (nmol/ℓ)
Parous lactating (n=14)	1,5 ± 0,3	6,7 ± 1,1	847 ± 157	110 ± 22	10,9 ± 4,4
Parous anoestrus (n=2)	2,6 (1,1 - 4,1)	6,1 (4,6 - 7,6)	1368 (1076 - 1660)	71	4,2 (2,4 - 6,0)
t_{14}	$1,332^{NS}$	$0,398^{NS}$	28,743**	-	$2,254^{NS}$

NS : Not significant
** : $p < 0,001$

with developing follicles which are considered as approaching puberty (Nulliparous B) (Table 44). Significantly higher levels of oestrogens in the pubertal group, testifying to increased ovarian activity in this group, were sufficient to discriminate between the two groups.

Earlier in this study, a further distinction has been made between lactating females, namely those with developing follicles (D), and those without (A). A comparison between the two groups, is presented in Table 45 with progesterone perhaps the only steroid not present in similar concentrations in both groups. Lactating females A had significantly higher concentrations of progesterone than in lactating females D.

From Table 46 it is apparent that neither the ratio of testosterone : androstenedione, nor oestrone : oestradiol-17β is related to reproductive status in as much as to be of diagnostic

TABLE 44: A COMPARISON BETWEEN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDRO=STENEDIONE (A), OESTRONE (E_1), OESTRADIOL-17β (E_2) AND PROGESTERONE (P) OF NULLIPAROUS A AND NULLIPAROUS B FEMALE SPOTTED HYAENAS

	T (nmol/ℓ)	A (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	P (nmol/ℓ)
A (n=4)	0,9 ± 0,5	4,5 ± 1,2	180 ± 58	39 ± 18	3,3 ± 1,1
B (n=8)	1,2 ± 0,4	4,4 ± 1,5	417 ± 84	98 ± 54	2,7 ± 0,8
t_{10}	$0,469^{NS}$	$0,085^{NS}$	27,291**	8,880**	$0,654^{NS}$

NS : Not significant
** : $p < 0,001$

TABLE 45: A COMPARISON BETWEEN THE PERIPHERAL PLASMA CONCENTRATIONS OF TESTOSTERONE (T), Δ^4ANDRO=STENEDIONE (A), OESTRONE (E_1), OESTRADIOL-17β (E_2) AND PROGESTERONE (P) OF LACTATING SPOTTED HYAENAS WITH (D) OR WITHOUT (A) DEVELOPING FOLLICLES

	T (nmol/ℓ)	A (nmol/ℓ)	E_1 (pmol/ℓ)	E_2 (pmol/ℓ)	P (nmol/ℓ)
Lactating A (n=7)	1,2 ± 0,3	6,3 ± 1,1	901 ± 295	94 ± 27	13,3 ± 8,6
Lactating D (n=6)	1,7 ± 0,6	8,2 ± 1,9	869 ± 227	103 ± 34	5,8 ± 1,8
t_{11}	$0,857^{NS}$	$1,775^{NS}$	$2,208^{NS}$	$1,846^{NS}$	3,551*

NS : Not significant
* : $p < 0,01$

value. Figure 57 presents a flow diagram for the determination of female reproductive status based on plasma steroid concentration in a single blood sample.

TABLE 46: THE RATIOS TESTOSTERONE (T) : Δ^4ANDROSTENEDIONE (A) AND OESTRONE (E_1) : OESTRADIOL-17 (E_2), IN EACH REPRODUCTIVE CATEGORY OF FEMALE, AS WELL AS IN MALE SPOTTED HYAENAS

	T(nmol/ℓ)/A(nmol/ℓ)	E_1(pmol/ℓ)/E_2(pmol/ℓ)
Nulliparous (n=12)	0,29 ± 0,10	14,15 ± 4,41
Nulliparous A (n=4)	0,21 ± 0,14	11,79 ± 8,16
Nulliparous B (n=8)	0,33 ± 0,13	15,33 ± 5,57
Parous (n=24)	0,35 ± 0,01	17,49 ± 4,31
Parous non-pregnant (n=16)	0,35 ± 0,09	15,93 ± 5,43
Parous pregnant (n=8)	0,35 ± 0,10	19,94 ± 7,53
Parous lactating (n=14)	0,27 ± 0,07	16,02 ± 6,00
Parous lactating A (n=7)	0,25 ± 0,10	14,18 ± 8,79
Parous lactating D (n=6)	0,28 ± 0,13	19,23 ± 9,17
Parous anoestrus (n=2)	0,39 (0,24 - 0,54)	15,09
Males (n=25)	1,11 ± 0,21	9,44 ± 1,86

DISCUSSION

Adrenal involvement in androgen production in the spotted hyaena has been described in the previous chapter. It is therefore not totally surprising that testosterone and androstenedione in the peripheral circulation of males are not related to testicular morphology only. Furthermore, it has been shown that androgen production bears no relationship to age or reproductive

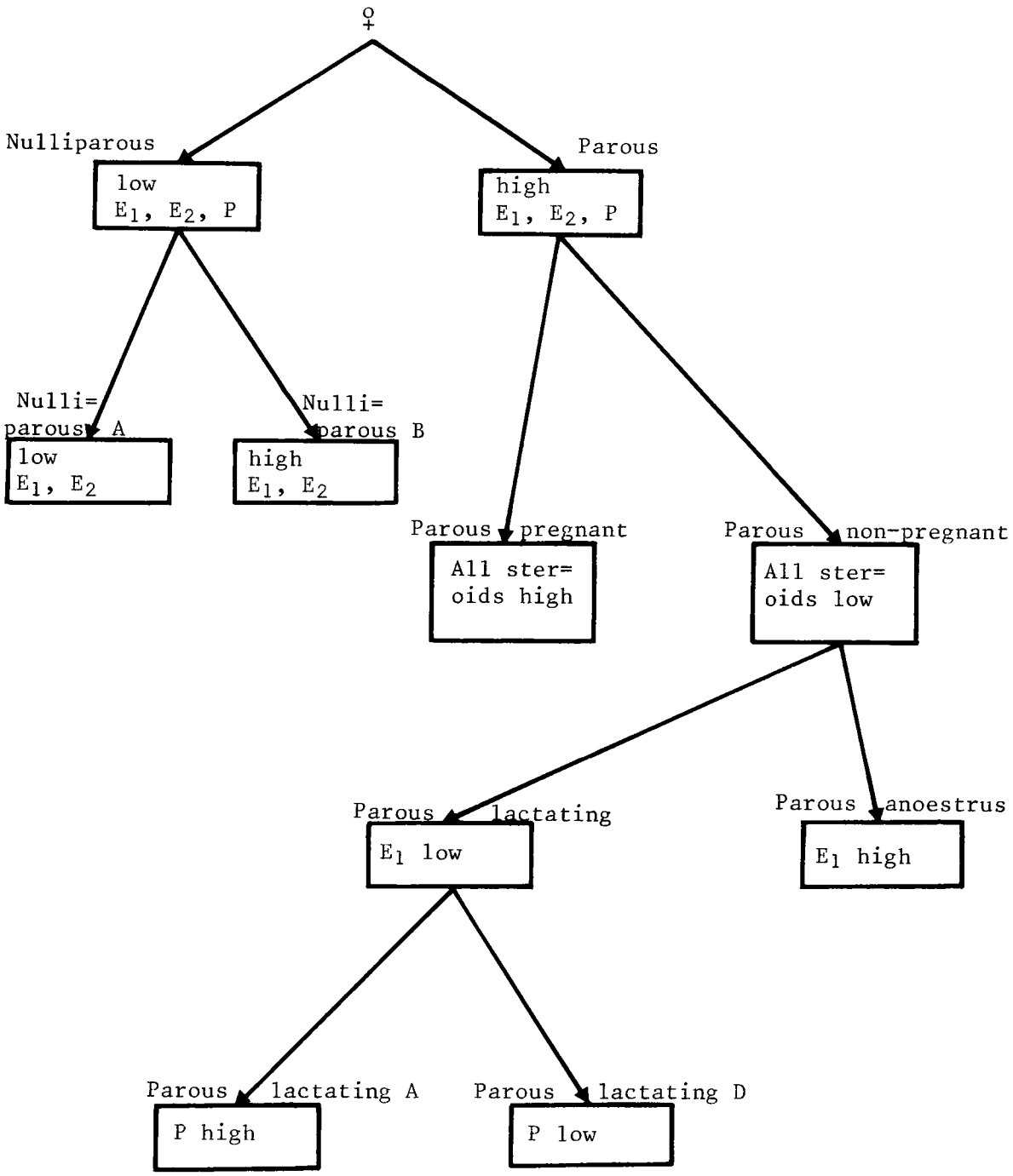


Fig. 56: A flow diagram for the preliminary determination of reproductive status of female spotted hyaenas.

status, and that testosterone did not differ significantly between males and females, regardless of age or sex, confirming what Racey & Skinner (1979) originally suggested.

At present, age-related changes in the sex steroids in male hyaenas have not been proven to exist beyond all doubt, except the ratio of testosterone : androstenedione. Androstenedione in the male hyaena, is the dominant androgen in the circulation in juveniles, but with advancing age, the ratio of testosterone : androstenedione became greater than 1 in the second age class (1-3 years old) and shows a gradual increase until at least in age class IV (6-10 years old), as was originally described for bulls (Skinner, Mann & Rowson 1968, Bedair & Thibier 1979, Chantaraprateep & Thibier 1979). In female hyaenas, androstenedione was always the principal circulatory androgen, although the ratio of A:T approached unity in age class III, which roughly coincides with the attainment of sexual maturity in the female spotted hyaena. With advancing age, this ratio gradually decreased.

Similar trends in the ratio of oestrone : oestradiol-17β in males and females were not found in this study, as both these oestrogens seem to increase in peripheral concentrations with age. It is known that oestrone is the only oestrogen produced by the adrenal (Baird *et al*. 1969, Doerr & Pirke 1975, Vinson *et al*.1976), although both oestrone and oestradiol-17β may be derived from oestrogen precursors such as androstenedione, testosterone and dehydroepiandrosterone in the peripheral circulation (MacDonald *et al*. 1967, Abraham *et al*. 1969, Longcope *et al*. 1969). Higher levels of oestradiol-17β in hyaenas with follicular secretion of steroids than in those with quiescent ovaries, did not result in a significant change in the ratio oestrone:oestradiol-17β with age, probably because of the masking effect of peripheral conversion of precursors to oestradiol-17β even in hyaenas where oestradiol-17β was not secreted as such. Juvenile males had higher concentrations

of both oestrogens than juvenile females, but the opposite was true for the older age groups, as could be expected. Proges= terone levels did not change consistently with age in either males or non-pregnant female hyaenas. The very high levels of this hormone found in pregnant hyaenas, and in fact of every other steroid too, can be ascribed to the increased secretion of steroids from the ovary during pregnancy.

Changes in the metabolic clearance rate of plasma steroids during pregnancy, as well as an increased concentration of steroid-binding plasma globulins could probably account for the higher concentrations of all steroids in the peripheral cir= culation of pregnant hyaenas, as in other species (Corvol & Bardin 1973, Heap & Illingworth 1974).

The high levels of all steroids in pregnant hyaenas greatly facilitates pregnancy diagnosis in females of uncertain re= productive status, as has been successfully implemented in livestock management (Tyrrell, Gleeson, Peter & Connell 1980). Diagnosis of the other phases of the reproductive cycle in female hyaenas is not as simple as for pregnant hyaenas, but could possibly be successfully conducted according to the characteristic hormone profiles of each phase, as presented in the present study, and especially if additional information is available. This would include age, the state of the external genitalia (to indicate reproductive parity) and the presence of milk in the mammae.

Another important consideration is that individuals should be correctly sexed, especially young individuals. It is no easy matter to sex hyaenas in the wild, but is nevertheless possible when subjects are immobilized, as mentioned earlier in this study. Careful palpation of the scrotum/scrotal swellings of young spotted hyaenas is necessary to reveal the presence or absence of testes, and therefore the sex of the subject.

It is not known when the process of testicular descent into the scrotum is completed, but a male of approximately 6 months of age (A. Whateley, pers. comm.) had testes that were fully descended into the scrotum. Circulatory levels of the sex steroids cannot be relied on to distinguish between males and females at an early age, but higher levels of androstenedione and lower levels of oestrone could perhaps indicate the female sex when individuals of the same age are compared. It seems that as soon as the age and sex of a spotted hyaena can be ascertained, sufficient discrimination between steroid profiles characteristic of each phase of the reproductive cycle would also enable the diagnosis of the reproductive state of that particular individual.

SUMMARY

Thirty-five skulls of spotted hyaenas were used to develop a technique for age estimation in this species. The occlusal surfaces of second mandibular premolars were measured and these values were arranged in a standard size-frequency analysis to construct an age class classification based on relative tooth attrition. Chronological age limits to these age classes were assigned according to a modification, based on regression analyses, of the age class schedule of Kruuk (1972). Only four age classes were defined previously, but seven age classes were developed in the present study. Body morphometrical data and skull growth parameters were used to confirm the validity of the above classification.

Aspects of the reproductive biology of female hyaenas were described based on material collected from 39 females from 1974 to 1979. Hyaenas were culled in the Kruger National Park (Central District) for considerations other than the requirements of this study, with the result that samples were not always adequate. Smaller numbers of hyaenas were also collected specifically for this study, in the Southern District of the KNP and in the Umfolozi Game Reserve, Natal.

Ovarian morphology was studied by measuring various histological parameters of growth and regression of the corpus luteum, and by counting and classifying the various structures in the ovary. The present study confirms earlier observations concerning the age of first reproduction (approximately 3 years) of females, an average litter size of two, and the persistent nature of corpora lutea. It was also established that oestrous periods regularly occur in female hyaenas that were lactating, probably until the weaning of cubs when conception will occur. Female hyaenas are apparently still capable of breeding when at the advanced age of 16 years and more. No apparent effect of the

population control programme in the KNP, on female reproduction could be demonstrated. Breeding occurs throughout the year, although a slight peak in conceptions was observed to follow annual periods of abundant food, such as during the birth seasons of impala and other ungulates.

No seasonal variation in the fertility level of male hyaenas could be demonstrated, although not all hyaenas were considered sexually active at any time. Spermatogenesis in 29 male hyaenas conformed to the general mammalian pattern, although epididymal sperm reserves were considerably lower than those of other African mammals or domesticated mammals. The sample of males consisted mostly of young individuals, probably the result of incidental sampling bias, but males, after attaining sexual maturity at approximately two years of age, continued to be sexually active until at least 12 years of age.

The peripheral plasma concentrations of the six major sex steroids and LH during acute stimulation of the gonad or adrenal, induced by exogenous stimulants, were measured by radioimmuno= assay. The secretory products of the ovary, testis and adrenal under these circumstances were remarkably similar in composition with regard to the sex steroids, with testosterone, androstenedione, oestrone, oestradiol-17β and progesterone being produced by both the testis and ovary, and the adrenal with the exception of oestradiol-17β. The spotted hyaena ovary seems to be capable of a far greater response in androgen secretion than the testis. Adrenal contribution to the circulatory pool of androgens is considerable, with the result that circulatory levels of androgens in both male and female spotted hyaenas were similar, both in order of magnitude and multiplicity of origin. The secretory origins of androgens in the male and female were further sub= stantiated by tissue cultures and histochemical observations. The foetal ovary, like the adult ovary, appears to secrete androgens throughout foetal life, with a peak during the period

of sexual differention. This is thought to be the cause of masculinization of the external genitalia of the female foetus.

The concentrations of sex steroids in single blood samples were correlated with reproductive status, and a method for the de= termination of reproductive status, based on steroid concen= trations in a single blood sample, is presented in this study. Previous observations on the similarity between hormone levels in male and female spotted hyaenas, were supported by the present study.

OPSOMMING

Vyf en dertig skedels van gevlekte hiënas is gebruik in die daarstelling van 'n ouderdomsbepalingsmetode. Slytasie-opper= vlaktes van tweede mandibulêre premolaartande is bepaal en hierdie waardes is verwerk in 'n standaard tipe grootte-frekwensie-analise tot 'n ouderdomsklassifikasie gebaseer op relatiewe tand= slytasie. Chronologiese ouderdomsperke is aan ouderdomsklasse toegeken aan die hand van 'n modifikasie, gebaseer op regressie-analises, van die ouderdomsklassifikasie van Kruuk (1972). Slegs vier ouderdomsklasse kon vroeër gedefinieer word, maar sewe ouderdomsklasse is beskryf in die huidige studie. Lig= gaamsmorfometriese gegewens en parameters van skedelgroei is gebruik om die akkuraatheid van bogenoemde klassifikasie te bevestig.

Aspekte van die voortplantingsbiologie van wyfiehiënas is beskryf aan die hand van materiaal versamel van 39 wyfies vanaf 1974 tot 1979. Hiënas is uitgedun in die Sentrale Distrik van die Nasionale Kruger Wildtuin om ander redes as net die doelwitte van die huidige studie, met die gevolg dat monstergroottes nie altyd toereikend was nie. Bykomende aantalle hiënas is ook spesifiek vir hierdie studie verkry vanuit die Suidelike Distrik van die NKW en die Umfolozi Wildtuin te Natal.

Morfologiese aspekte van die ovarium is beskryf aan die hand van verskeie parameters van groei en ontwikkeling van die corpus luteum, sowel as getalle en klassifisering van die verskillende strukture in die ovarium. Die huidige studie bevestig vroeëre waarnemings aangaande die ouderdom waarby voortplanting die eerste keer voorkom in wyfies (nagenoeg 3 jaar), 'n gemiddelde werpselgrootte van twee, en die behoud van corpora lutea na die beëindiging van dragtigheid. Daar was verder vasgestel dat oestrus periodes gereeld voorkom in wyfiehiënas tydens laktasie, vermoedelik tot en met die speen van welpies waarna bevrugting

weer sal plaasvind. Wyfies is klaarblyklik in staat tot voortplanting tot ten minste 16 jaar oud. Geen opvallende gevolge van die kontroleringsprogram in die NKW op vroulike voortplanting kon bespeur word nie. Voortplanting vind reg= deur die jaar plaas, alhoewel 'n effense piek in bevrugtings voorgekom het na 'n tydsvak van oorvloedige voedselbronne, soos tydens die aanteelseisoene van die rooibok en ander hoef= diere.

Geen seisoenale verskille in die vrugbaarheidsvlakke van mannetjie-hiënas is gevind nie, alhoewel alle mannetjies nie op een tydstip ewe aktief was nie. Spermatogenese in die 29 mannetjies beskikbaar, verloop klaarblyklik volgens die tipiese patroon in soogdiere, maar spermreserwes in die epididymides was aansienlik laer as in ander Afrikaanse of gedomestikeerde soogdiere. Mannetjies in die monster was hoofsaaklik van 'n lae ouderdom, moontlik as gevolg van nie-ewekansige monster= neming, maar blyk seksueel aktief te bly vanaf geslagsrypwording op ongeveer 2 jaar, tot ten minste 12 jaar.

Konsentrasies van die ses belangrikste voortplantingsteroiedes, sowel as LH, in die perifere sirkulasie gedurende akute stimu= lering van die gonade of bynier, is bepaal deur radio-immuno= essaiering. Die afskeidings van die ovarium, testis en bynier was merkwaardig ooreenstemmend met betrekking tot die geslag= steroiedes, want testosteroon, androstenedioon, oestroon, oestradiol-17β en progesteroon is deur beide die ovarium en die testis geproduseer, sowel as die bynier behalwe in die geval van oestradiol-17β. Die eierstok van die gevlekte hiëna blyk aansienlik groter hoeveelhede androgene te produseer as die testis. Byniersekresie van androgene is aansienlik, met die gevolg dat sirkulerende androgeenvlakke in die mannetjie- en wyfiehiëna grootliks ooreenstem in konsentrasie sowel as in veelvuldigheid van oorsprong. Oorsprong van sekresie van die androgene is verder bevestig met behulp van weefselkulture en histochemiese

metodes. Die fetale ovarium produseer klaarblyklik, net soos die volwasse ovarium, androgene tydens dragtigheid, met 'n piek tydens die periode van geslagtelike differensiasie. Hierdie situasie is vermoedelik verantwoordelik vir die virilisering van die eksterne genitalieë van die wyfiefetus.

Die peile van geslagsteroiedes in 'n enkele bloedmonster is in verband gebring met voortplantingstatus, en 'n metode vir die bepaling van voortplantingstatus aan die hand van hormoon= peile in 'n enkele bloedmonster word voorgestel. Vorige be= vindinge aangaande die noue ooreenkoms tussen die hormoonpeile in manlike en vroulike gevlekte hiënas word bevestig in die huidige studie.

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APPENDIX I

Reagents used in radioimmunoassay

1. Antisera (steroids)

Testosterone antiserum : produced by Prof R.P. Millar, Dept. of Chemical Pathology, University of Cape Town

Δ^4Androstenedione antiserum : Rabbit-Anti-Androstenedione -7α-BSA Serum*

Progesterone antiserum : Rabbit-Anti-Progesterone 11αBSA Serum*

Oestrone antiserum : Rabbit-Anti-Estrone-6-Thyroglobulin Serum*

Oestradiol-17β antiserum : Rabbit-Anti-17β-Estradiol-6-BSA Serum

Cortisol antiserum : Rabbit-Anti-Cortisol-21-Thyroglobulin Serum

*Miles-Yeda Ltd., Kiryat Weizmann, Rehovot, Israel.

2. Tritiated steroids

(1,2,6,7(n)-^{3}H)-Testosterone TRK402*

(1,2,6,7-^{3}H)-Androstenedione TRK454*

(1,2,6,7(n)-^{3}H)-Progesterone TRK413*

(1,2,6,7(n)-^{3}H)-Estradiol TRK322*

(2,4,6,7-^{3}H)-Estrone TRK321*

(1,2,6,7(n)-^{3}H)-Cortisol TRK609*

*Radiochemical Centre, Amersham, England.

3. Luteinizing hormone radioimmunoassay

Purified LH for iodination : Ovine LH LER-1056-C2*

Purified LH for standards : Ovine LH NIH-LH-S18**

LH antiserum : Anti-Ovine-LH-Serum GDN15***

Second antiserum : Anti-Rabbit-Gamma-Globulin (ARGG)****

*Dr. L.E. Reichert

**Hormone Distribution Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland

***Dr. G.D. Niswender

****Wellcome Laboratories, London.

4. Control Sera

Ortho RIA control serum (Human clinical chemistry control serum) II, III and IV (Ortho Diagnostics Inc., Raritan, New Jersey.

5. Scintillation Media

Insta-Gel Liquid Scintillation cocktail*

Dimilume Liquid Scintillation cocktail (with chemi-luminescence inhibitor)*

*Packard Instrument Co., Cape Town.