Diurnal variation in plasma testosterone.

The plasma testosterone concentrations measured in samples taken at two hourly intervals for a period of 24 h in five animals are shown in Figure 16.

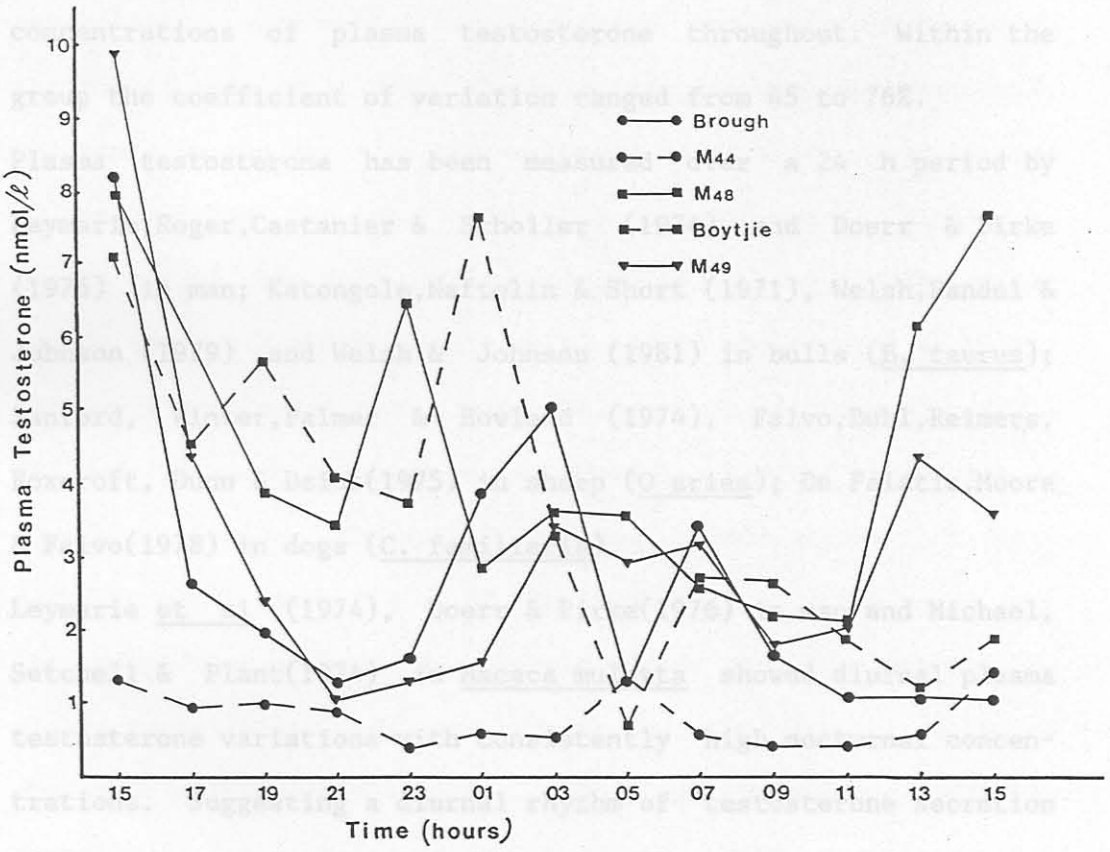

Figure 16: The plasma testosterone concentrations measured in samples taken at two hourly intervals for a period of 24 h. (n = 5).

Testosterone concentrations ranged from 0,4-9,9 nmol/l with a mean 2,92 ±2,23 in all of the samples collected over the 24 h period.

Plasma testosterone peaked 4 to 5 times in each of the animals during the 24 h period. Peaks and troughs in plasma testosterone differed in magnitude,duration and rate of change within and between individual animals. A coefficient of variation of 45% was measured in the plasma testosterone of m44 the animal that showed the lowest peak during the period and had consistently lower concentrations of plasma testosterone throughout. Within the group the coefficient of variation ranged from 45 to 76%.

Plasma testosterone has been measured over a 24 h period by Leymarie,Roger,Castanier & Scholler (1974) and Doerr & Pirke (1976) in man; Katongole,Naftolin & Short (1971), Welsh,Randel & Johnson (1979) and Welsh & Johnson (1981) in bulls (B. taurus); Sanford, Winter,Palmer & Howland (1974), Falvo,Buhl,Reimers, Foxcroft, Dunn & Dziuk(1975) in sheep (O aries); De Palatis,Moore & Falvo(1978) in dogs (C. familiaris).

Leymarie et al (1974), Doerr & Pirke(1976) in man and Michael, Setchell & Plant(1974) in Macaca mulatta showed diurnal plasma testosterone variations with consistently high nocturnal concentrations. Suggesting a diurnal rhythm of testosterone secretion which was not found in other animals. All confirm the unequivocal conclusion of Thomas,Gordon & Smid(1974) and Bartke, Steele, Musto & Caldwell (1973) that a single plasma or serum sample did not have any value in the assessment of plasma testosterone concentration and its functional significance in the animals or men that were examined.

The results of the present study on cheetahs are similar in that plasma testosterone varied considerably over the 24 h period. The cheetah males responded to the two hourly sampling with some distress and it was not possible to bleed the animals more frequently. The use of indwelling catheters proved to be unsuccessful. Animals kept in cages during the study period were nevertheless very mobile and aggressive and therefore catheterization of the relatively immobile jugular was not practical. Catheters in the cephalic and saphenous veins,which could be used by immobilizing the leg, were soon blocked due to kinking or pulled out of the vein. Falvo et al.(1975) who sampled at two hourly intervals and Katongole et al.(1971) who sampled at hourly intervals indicated that plasma testosterone fluctuated rapidly and more frequent sampling was required to show the rapid changes possible. Horton, Shinsako & Forsham (1965) showed that testosterone had a half-life of 34 min in man.

The animal that showed an uncharacteristic and consistently low plasma testosterone, m44, was 3 years old, the same age as two of the other animals in the trial, m48 & m49. He was not available during the later stages of the study when GnRH injections were used. The lower concentrations of plasma testosterone in this animal cannot be explained in the absence of an indication as to LH concentrations that might have influenced the rate of testicular testosterone production.

Table 6: Plasma luteinizing hormone and testosterone concentrations of different species.

ANIMAL	LH µg/ℓ	PLASMA T nmol/ℓ	REFERENCE
Boar (Sus scrofa)	0,8 - 1,8	4,35 - 6,9	Juniewicz & Johnson (1981)
Bull (Bos taurus)	0,1 - 4,4	14,64 - 20,7	Chantaraprateep & Thibier (1978)
	0,5 - 2,5	13,46 - 33,6	Johnson, Welsh & Juniewicz (1982)
	5,0 - 50,0	6,92 - 69,2	Katongole, Naftolin & Short (1971)
	0,3 - 2,4	1,73 - 48,8	Thibier & Rolland (1976)
Sheep (Ovis aries)		5,54 - 90,9	Falvo, Buhl, Reimers, Foxcroft, Dunn & Dziuk(1975)
	0,1 - 1,4		Galloway, Cotta, Pelletier & Terqui (1974)
		2,38 - 14,7	Sanford, Winter, Palmer & Howland (1974)
		3,46	Schanbacher & Echternkamp (1978)
Mice (Mus musculus)		3,1 -132,5	Bartke, Steele,Musto & Caldwell (1973)
Rabbit (Oryctolagus cuniculus)	1,6 - 3,0		Blake, Blake, Thorneycroft & Thorneycroft (1978)
Rat (Rattus norvegicus)		6,23 - 52,9	Bartke, Steele, Musto & Caldwell (1973)
Dog (Canis familiaris)	0,2 - 12,0	1,6 - 21,5	De Palatis, Moore & Falvo (1978)
		3,1 - 14,0	Folman, Haltmeyer & Eik-Nes (1972)
Ferret (Mustela putorius)	0,5 - 2,0	0,4 - 29,4	Donovan & Ter Haar (1977)
Cheetah (Acinonyx Jubatus)	0,45 - 3	0,35 - 7,27	Degenaar (1977)
	2,3 - 5,2	0,40 - 9,9	Present study
Rheses monkey (M mulatta)		27,1 - 61,4	Michael, Setchell & Plant
Man		10,0 - 44,9	Beitins, Bayard, Kowarski & Migeon (1973)
		9,6 - 44,9	Leymarie, Roger,Castanier & Scholler (1974)
		13,6 - 41,5	Thomas, Gordon & Smid (1974)

Plasma testosterone after increasing doses of GnRH

Pre-GnRH stimulation (0-time), maximum plasma testosterone (max. plasma T) and the maximal plasma testosterone response (max. plasma testosterone resp. = maximum plasma testosterone minus 0-time plasma testosterone) were measured together with the mean time taken for the maximum plasma testosterone to be reached (peak time). These results are shown in Figure 17 and summarized in Table 7.

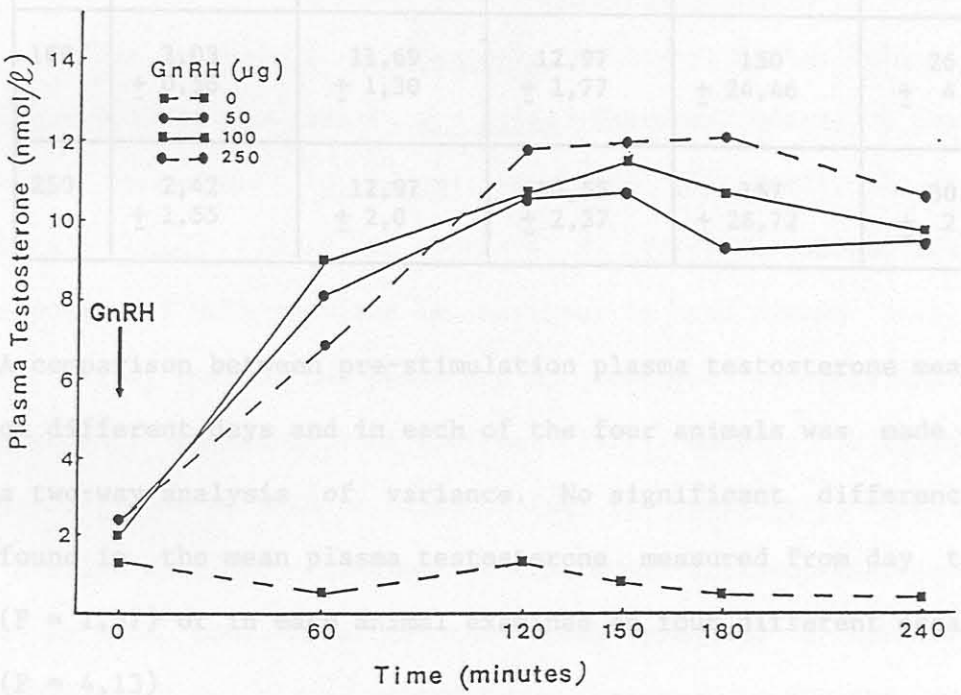

Figure 17. Plasma testosterone after increasing doses of GnRH (n = 4)

Table 7. Plasma testosterone in cheetah males after increasing intramuscular doses of GnRH. Data are presented as mean and standard deviation (±) (n = 4).

GnRH ug	Presti mulation nmol/ℓ	Maximum nmol/ℓ	Maximum response	mean peak minimum	Rate of Production nmol/ℓ/h
0	1,29 ± 0,72				
50	2,31 ± 1,02	10,81 ± 2,60	8,50 ± 3,58	165 ± 51,96	23,07 ± 10,59
100	2,03 ± 0,56	11,69 ± 1,30	12,97 ± 1,77	150 ± 24,46	26,39 ± 4,87
250	2,42 ± 1,55	12,97 ± 2,0	10,55 ± 2,37	157 ± 28,72	30,08 ± 2,77

A comparison between pre-stimulation plasma testosterone measured on different days and in each of the four animals was made using a two-way analysis of variance. No significant difference was found in the mean plasma testosterone measured from day to day ($F = 1,57$) or in each animal examined on four different occasions ($F = 4,13$)

An analysis of variance of the maximum plasma testosterone, the plasma testosterone response in nmol/l and the rate of testosterone production expressed as nmol/l/h indicated that there was no significant difference of the means of each of these parameters as the dose of GnRH was increased ($F = 1,14$, $F = 0,59$ and $F = 1,03$ respectively)

The study of the effect of increasing doses of GnRH on plasma testosterone levels was undertaken to estimate the optimal standard intramuscular dose of the releasing hormone in cheetahs. The amino acid sequence of porcine gonadotrophin releasing hormone (GnRH) was described by Matsuo, Baba, Nair, Arimura & Schally, 1971 in Golter, Reeves, O'Mary, Arimura, & Schally 1973) and synthesized by them (Matsuo et al. 1971b in Golter et al. 1973). Arimura, Matsuo, Baba, Debeljuk, Sandow & Schally (1972) showed that the biological activity of the natural and synthetic hormones were similar in rats (Rattus norvegicus). Golter et al.(1973) in bulls (B. taurus); Galloway, Cotta, Pelletier & Terqui (1974) in rams (O. aries) Hanrahan, Quirke, & Gosling (1981) in lambs (O. aries); Pomerantz, Ellendorff, Elsaesser, König,& Smidt (1974) in boars (S. scrofa); showed that an injection of GnRH resulted in increases in the plasma levels of luteinizing hormone and that the LH response to GnRH was dose dependent. Chakraborty & Fletcher (1977) used a 50 µg intramuscular dose of GnRH in Labrador dogs (C. familiaris) which have a similar mass to cheetahs. The standard intramuscular dose of 50 µg GnRH used later in the study produced a similar and probably maximal plasma testosterone response as did the 100 µg and 250 µg used. These results are similar to those of Galloway et al.(1974) who found that plasma testosterone concentrations did not increase with increasing doses of GnRH in rams.

Plasma LH & Testosterone after 50 µg GnRH

The ability of the LH assay used to measure this hormone in cheetahs was supported by several observations. Parallelism was observed between the dose response curves of the canine pituitary standard and pooled plasma from GnRH treated cheetahs (Fig 18.).

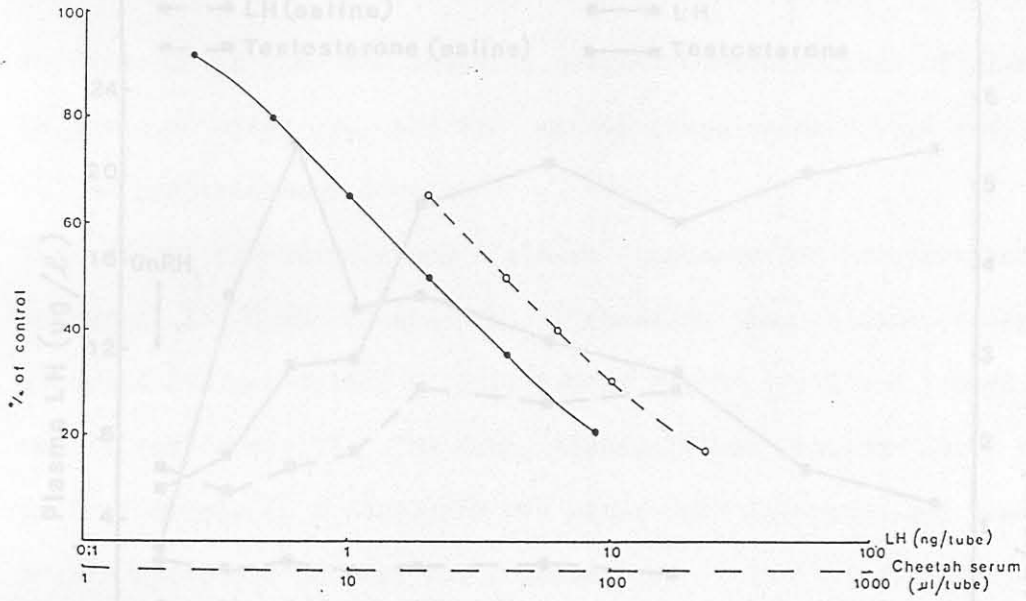

Figure 18. LH inhibition curves of canine pituitary standard (LER-165-1) and pooled cheetah serum

Untreated animals had significantly less detectable LH-like activity in 200 µl samples compared to activity levels measured in 100 - 200 µl of plasma from treated animals. Increased plasma testosterone was measured in GnRH stimulated animals. Because the secretion of testosterone is considered to be controlled by LH

(Smith & Hafs,1973),this observation indicates that the substance detected immunologically with the LH antiserum possessed LH-like biological activity.

The mean response in plasma LH and testosterone following GnRH is shown in Figure 19.

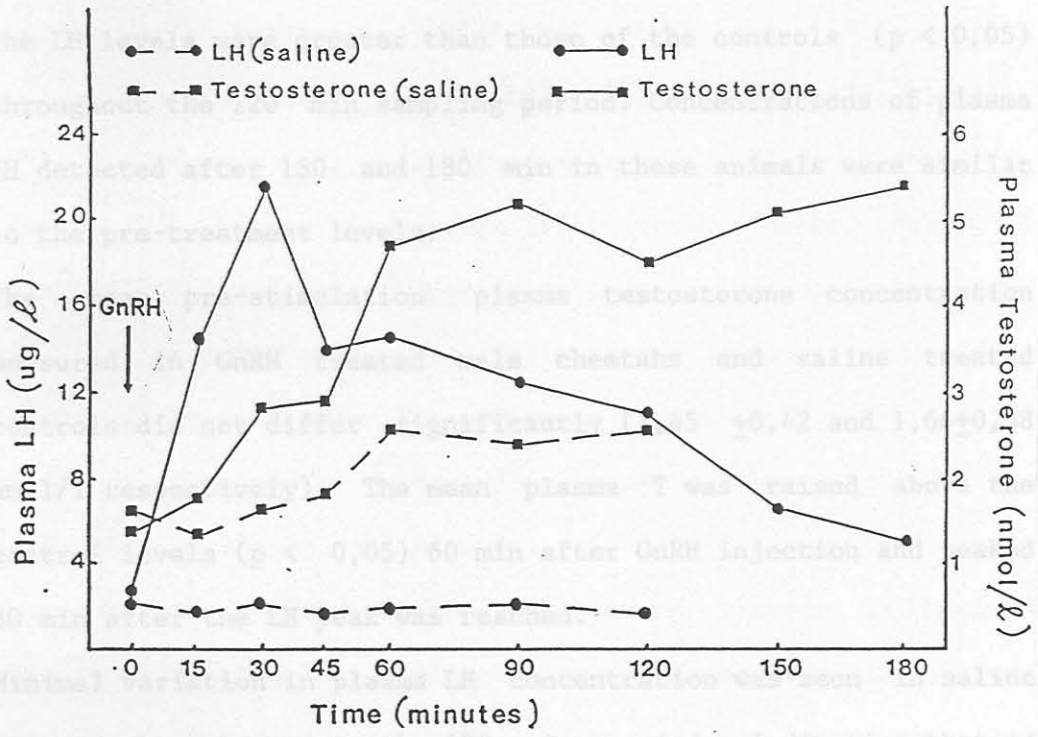

Figure 19. Mean response in plasma LH and testosterone following the administration of 50 µg GnRH.

Neither mean LH nor individual LH patterns fluctuated significantly over the 120 min sampling period in saline treated cheetah males. Mean basal LH concentrations in this group ranged from 2,3 to 5,2 µg/l. GnRH injection stimulated an LH response in all of the cheetahs with considerable variation seen among

individual animals. LH peaks ranged from 4,9 to 50 µg/l and occurred at 15,30,45 and 60 min after the GnRH injection in one,three,two and one animals respectively. After GnRH the mean LH concentration was raised above control levels ($p < 0,05$) at the 15 min sampling interval(14,7 $\pm$ 4,7 µg/l). The mean maximal LH concentration was reached at 30 min after GnRH injection and the LH levels were greater than those of the controls ($p < 0,05$) throughout the 120 min sampling period. Concentrations of plasma LH detected after 150 and 180 min in these animals were similar to the pre-treatment levels.

The mean pre-stimulation plasma testosterone concentration measured in GnRH treated male cheetahs and saline treated controls did not differ significantly (1,45 $\pm$0,42 and 1,66$\pm$0,38 nmol/l respectively). The mean plasma T was raised above the control levels ($p < 0,05$) 60 min after GnRH injection and peaked 30 min after the LH peak was reached.

Minimal variation in plasma LH concentration was seen in saline treated cheetahs over the 120 min period. Indicating that no episodic release of LH occurred during that time. An injection of 5 µg intramuscular GnRH to anoestrous domestic cats (F. catus) stimulated peak LH concentrations after 30 min (Chakraborty, Wildt & Seager,1979). In cheetahs the response was similar, appearing within 10 min of the GnRH injection with the greatest mean LH concentration being reached 30 min later. However the quantitative LH response in cheetahs (21,6$\pm$7,2 µg/l) was

considerably less than that measured in domestic cats (Chakraborty et al. 1979) (114 µg/l) and Labrador dogs (Chakraborty & Fletcher, 1977) (40 µg/l).

Studies in man (Franchimont, Chari & Demoulin, 1975); in bulls (B. taurus) (Mongkonpunya, Hafs, Convey & Tucker, 1975; Thibier, 1976; Kesler & Garverick,1976; Chantaraprateep & Thibier, 1978; Schanbacher & Echternkamp,1978; Malak & Thibier, 1982); in rams (O. aries) (Galloway et al, 1974; Falvo et al. 1975; Lincoln, 1979); in boars (S. scrofa) (Pomerantz et al.1974); in dogs (C. familiaris) (Jones & Boyns, 1974; Jones, Baker, Fahmy, & Boyns,1976); in ferrets (Mustela putorius) (Donovan & ter Haar, 1977) all indicate that plasma testosterone levels increase 10 to 15 min after GnRH injection and reach a peak between 90 and 180 min later. The plasma testosterone peak following 40 to 60 min after that of luteinizing hormone.

The temporal aspects of the testosterone response in cheetahs were within this range although the mean maximum plasma testosterone reached after GnRH 5,6 nmol/l is lower than that measured in most of the animals reported on.

Table 8: Plasma testosterone after GnRH injection in different species.

REFERENCE	ANIMAL	PLASMA TESTOSTERONE nmol/ℓ
Mongkonpunya et al. (1974)	Bulls (Bos taurus)	34,25
Mongkonpunya et al. (1975)	Bull calves (Bos taurus) 2 months	2,42
	4 months	6,92
	6 months	20,1
Thibier (1976)	Bulls (Bos taurus)	39,5
Tannen & Convey (1977)	Bull calves (Bos taurus) 1 month	24,2
Schanbacher & Echternkamp (1978)	Bulls (Bos taurus)	24,2
Falvo et al. (1975)	Sheep (Ovis aries)	62,3 - 103,8
Galloway et al. (1974)	Sheep (Ovis aries)	27,0 - 43,9
Jones et al. (1976)	Dogs (Canis familiaris)	18,8
Donovan & Ter Haar (1977)	Ferrets (Mustela putorius)	27 - 50
Present study	Cheetah (Acinonyx jubatus)	7,86

Plasma testosterone in different age groups after 50 µg GnRH

The effects of a standard dose of 50 µg GnRH by intramuscular injection on the plasma testosterone of animals from different age groups are shown in Figure 20 and summarized in Table 9.

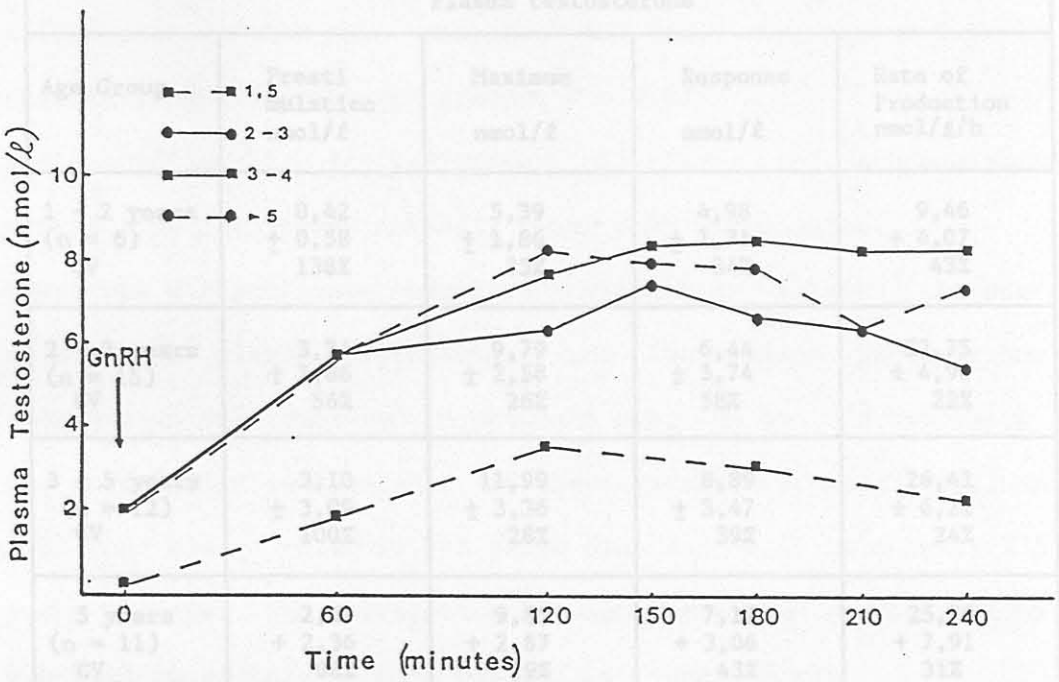

Figure 20. Plasma testosterone following 50 µg GnRH in animals from different age groups

Table 9: Plasma testosterone following a standard dose of 50 µg GnRH in cheetahs of different ages.

Data are presented as mean, standard deviation (±) and coefficient of variation(%).

	Plasma testosterone			
Age Group	Presti mulation nmol/ℓ	Maximum nmol/ℓ	Response nmol/ℓ	Rate of Production nmol/ℓ/h
1 - 2 years (n = 6) CV	0,42 ± 0,58 138%	5,39 ± 1,86 35%	4,98 ± 1,71 34%	9,46 ± 4,07 43%
2 - 3 years (n = 15) CV	3,34 ± 1,86 56%	9,79 ± 2,58 26%	6,44 ± 3,74 58%	22,75 ± 4,90 22%
3 - 5 years (n = 12) CV	3,10 ± 3,09 100%	11,99 ± 3,36 28%	8,89 ± 3,47 39%	26,41 ± 6,21 24%
5 years (n = 11) CV	2,69 + 2,36 88%	9,85 + 2,87 29%	7,17 + 3,06 43%	25,79 + 7,91 31%

The mean pre-stimulation and maximum plasma testosterone concentrations of animals less than 2 years of age were significantly lower than those of the older animals ($F = 4,05$, $p < 0,05$ and $F = 8,40$ $p < 0,01$ respectively) as was their mean rate of production of testosterone expressed as plasma testosterone nmol/l/h ($F = 11,76$ $p < 0,01$). There was no significant difference in the mean response to GnRH stimulation between animals from different age groups($F = 2,07$).

The coefficient of variation of plasma testosterone concentrations decreased markedly after GnRH stimulation.

There was a significant negative correlation between the pre-stimulation plasma testosterone and the plasma testosterone response to GnRH measured in cheetahs over the age of two years, the correlation coefficients of these parameters being -0,72 in 2-3 year olds, -0,48 in 3-5 years olds and -0,46 in animals > 5 years of age. The correlation between these two concentrations was 0,13 in animals between one and two years of age.

The pre-stimulation plasma testosterone concentration in all of the animals over the age of two years ranged from 0,03 - 6,81 with a mean of 1,765 $\pm$1,63 nmol/l (n = 44). This was significantly lower than that of the mean plasma testosterone measured in the animals sampled over 24 h (2,924 $\pm$2,23 n = 64)

The mean time taken to reach maximum plasma testosterone in animals from each of the age groups is summarized in Table 10.

Table 10. Mean time taken to reach maximum plasma testosterone after GnRH stimulation.

Age group	Mean peak time (minutes)	Standard deviation
1 - 2 years	130	24,49
2 - 3 years	148	38,40
3 - 5 years	185	51,65
5 years	167	41,37

The maximum plasma testosterone after GnRH of cheetah males aged between 1- 3 years was reached significantly sooner than that of older animals ($F = 2,96$, $p < 0,05$)

The plasma testosterone concentrations measured in cheetah males from different age groups indicated that the response to GnRH stimulation was attenuated in the younger groups of animals. Mongkonpunya et al.(1975) found that the plasma testosterone concentration of bulls (B. taurus) aged between 2 - 4 months was lower than that of animals aged six months and that the testicular response to GnRH injection was insignificant in the

younger animals. Kesler & Garverick(1976) measured both the LH and testosterone response to GnRH in two groups of calves aged 3 and 17 days younger animals showed a similar LH response to GnRH but their T response was insignificant (1,5±0,4 nmol/l peak). The 17 day old calves showed a plasma testosterone response to GnRH with a mean peak of 3,73 ±0,9 nmol/l. Monkongpunya et al. (1975) measured peaks of 2,1 & 4,2 nmol/l in bull calves two and four months of age respectively and a peak of 18,3 nmol/l in calves aged six months after GnRH. Jacacki, Kelch, Sauder ,LLoyd, Hopwood & Marshall (1982) reported plasma testosterone concentrations that ranged from 0,2 to 1,5 nmol/l in pre-pubertal boys. This being considerably lower than the reported range of 9,6 to 13,9 nmol/l in adult men (Leymarie et al. 1974). The onset of episodic secretion of LH has been proposed as a biological marker for the onset of puberty in man (Boyar, Finkelstein, Roffwarg, Kapen, Weitzman & Hellman,1972, in Jacacki et al. 1982). However this was contradicted by Jacacki et al.(1982) who, with others, showed that pulsatile LH occurred in pre-pubertal children as well.

The significant negative correlation between the pre-stimulation plasma testosterone and the plasma testosterone response to GnRH measured in cheetahs over the age of two years indicates that the 50 µg dose of GnRH was followed by maximal plasma testosterone concentrations. This was not the case in the group of cheetah males under the age of two years.

The maximum plasma testosterone after GnRH of cheetah males aged between 1 - 3 years was reached significantly sooner than that of older animals ($F = 2,96$, $p < 0,05$).

Plasma testosterone in July & November

Plasma testosterone (Plasma T) measured after 50 µg in July and November 1982 in 5 two year old cheetah males are summarized in Table 11.

Table 11: Plasma testosterone measured in cheetah males after 50 µg GnRH in July and in November. Data are presented as mean and standard deviation (±).

Plasma testosterone				
Month	Presti-mulation nmol/ℓ	Maximum nmol/ℓ	Response nmol/ℓ	Rate of Production nmol/ℓ/h
July	2,85 ± 2,40	9,28 ± 1,44	6,43 ± 2,94	12,30 ± 5,84
November	3,60 ± 2,05	9,88 ± 1,99	6,28 ± 3,53	17,34 ± 5,16

The plasma testosterone levels measured in cheetah males examined in July and November were not significantly different.

Results of paired t tests were t = 0,86 for pre-stimulation plasma T, t = 2,36 Maximum plasma T, t = 0,14 for response plasma T and t = 1,79 for plasma T nmol/l/h.

Plasma testosterone levels in rams (O. aries) in,the northern hemisphere,following GnRH were significantly higher in January than in May and September (Sanford et al,1974). Indicating clearly the influence of seasonality on testicular function in these animals. Malak & Thibier(1982) found that there were significant individual differences in the LH response to GnRH in bulls (B. taurus). The testosterone response however showed no significant differences and repeatable results were obtained in the same animals examined one month apart.

The effect of anaesthesia on plasma testosterone response

Plasma testosterone concentrations after intravenous GnRH in animals anaesthetized with thiopentone sodium or CT1341 are compared in Figure 21.

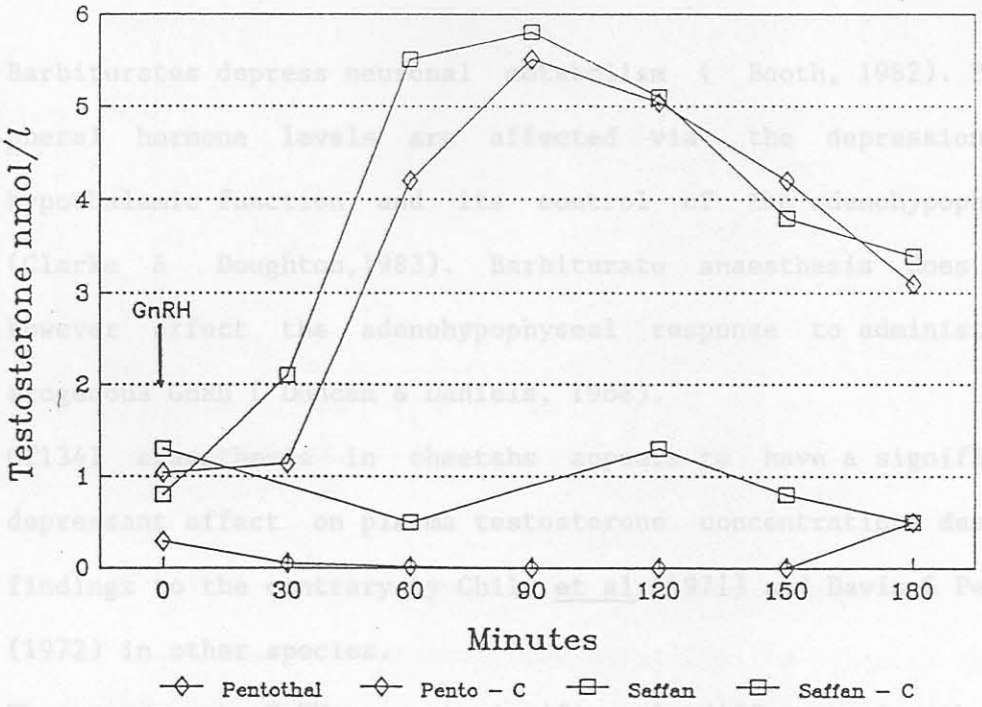

Figure 21. A comparison between the response to GnRH in cheetah males anaesthetized with thiopentone sodium or CT1341.

The response after GnRH was remarkably similar in each of the two groups. plasma testosterone concentrations declined to undetectable levels after 60 min and remained depressed throughout in two control animals anaesthetized with thiopentone sodium. The plasma T levels after CT1341 were not as low but nevertheless appear to be depressed below levels in un-anaesthetized animals.

Pre-stimulation plasma testosterone concentrations measured in the present study in all males over the age of two years, anaesthetized with CT1341, ranged from 0,03 - 6,81 with a mean of 1,765 +1,63 nmol/l (n = 44). This was significantly lower than the mean plasma testosterone, 2,924 +2,22 nmol/l (n = 60), measured in the animals sampled over 24 h.

Barbiturates depress neuronal metabolism (Booth, 1982). Peripheral hormone levels are affected via the depression of hypothalamic function and its control of the adenohypophysis (Clarke & Doughton,1983). Barbiturate anaesthesia does not however affect the adenohypophyseal response to administered exogenous GnRH (Duncan & Daniels, 1968).

CT1341 anaesthesia in cheetahs appears to have a significant depressant effect on plasma testosterone concentrations despite findings to the contrary by Child et al.(1971) and Davis & Pearce (1972) in other species.

The response to GnRH was not significantly different in the two groups of cheetahs anaesthetized with thiopentone sodium and CT1341 respectively.

Hormone studies in anaesthetized & electroejaculated males

Profiles of mean plasma concentrations of cortisol,testosterone and LH before and during CT1341 anaesthesia and before and during electroejaculation are presented in Fig 22, 23 & 24.

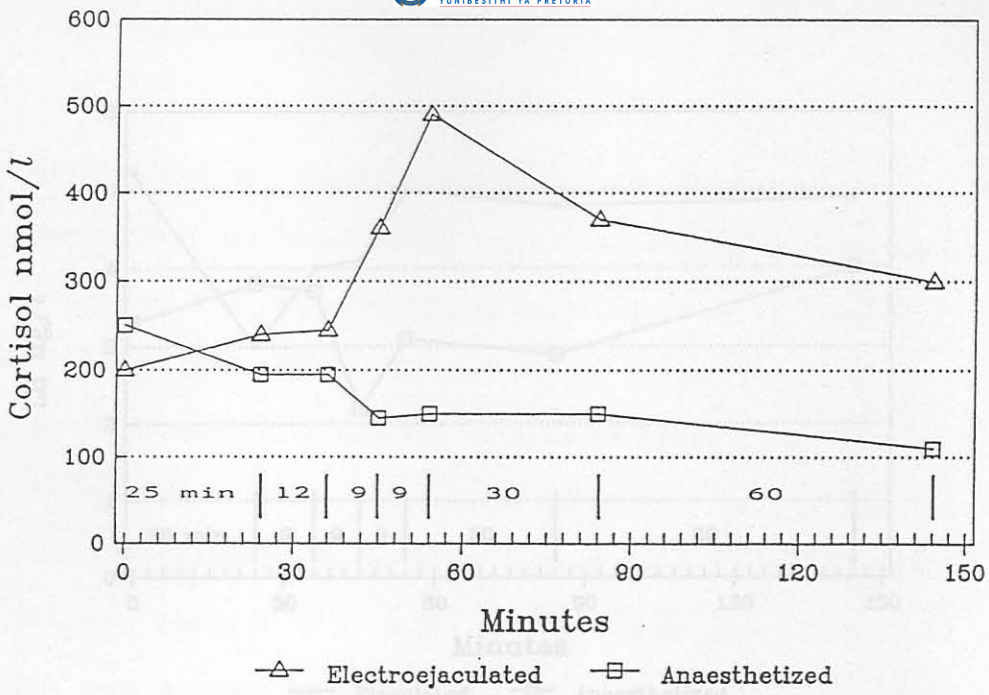

Figure 22: Plasma cortisol concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.

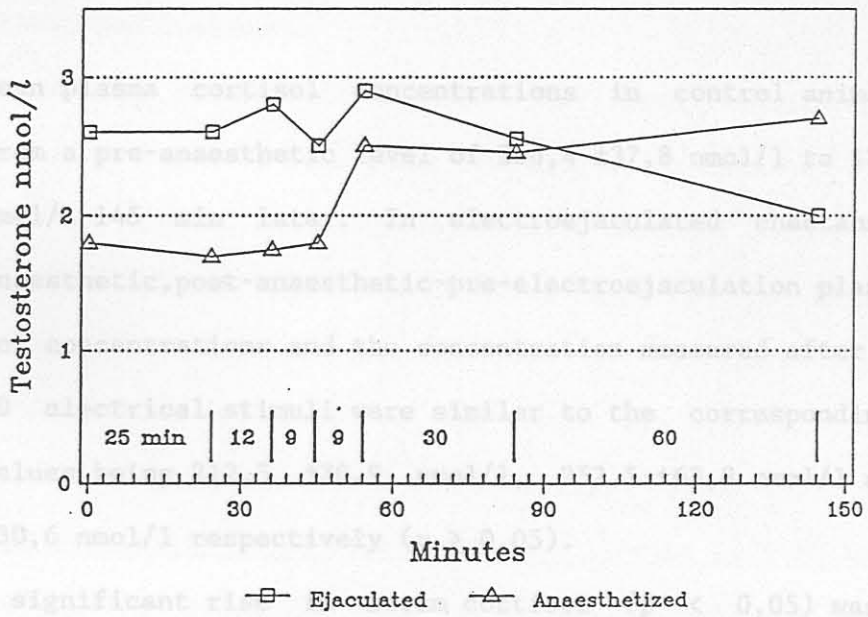

Figure 23: Plasma testosterone concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.

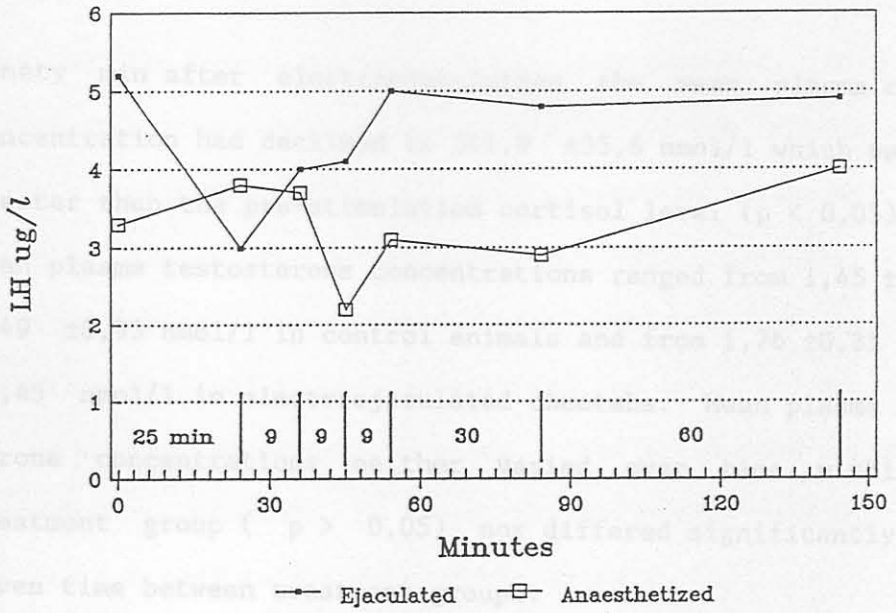

Figure 24: Plasma luteinizing hormone concentrations in cheetah males subjected to serial bleeding and regimented electroejaculation under CT1341 anaesthesia.

Mean plasma cortisol concentrations in control animals ranged from a pre-anaesthetic level of 256,4 ±37,8 nmol/l to 121,7 ±21,3 nmol/l 145 min later. In electroejaculated cheetahs the pre-anaesthetic,post-anaesthetic-pre-electroejaculation plasma cortisol concentrations and the concentration measured after the first 30 electrical stimuli were similar to the corresponding control values being 212,5 ±30,9 nmol/l, 252,5 ±63,8 nmol/l and 256,1 ±30,6 nmol/l respectively ($p > 0,05$).

A significant rise in serum cortisol ($p < 0,05$) was measured following the second series of electroejaculatory stimuli (374,5 ±26,5 nmol/l) with a further rise to a peak of 508 ±26,5 nmol/l detected immediately after the end of the ejaculating stimuli.

Ninety min after electroejaculation the mean plasma cortisol concentration had declined to 311,9 ±35,6 nmol/l which was still greater than the pre-stimulation cortisol level ($p < 0,05$)

Mean plasma testosterone concentrations ranged from 1,45 ±0,42 to 2,49 ±0,93 nmol/l in control animals and from 1,76 ±0,35 to 2,73 ±0,45 nmol/l in electroejaculated cheetahs. Mean plasma testosterone concentrations neither varied over time within each treatment group ($p > 0,05$) nor differed significantly at any given time between treatment groups.

LH titres ranged from 2,3 ±0,4 to 3,9 ±1,3 µg/l in the control group of cheetahs and from 3,2 ±0,4 to 5,2 ±1,1 µg/l in the electroejaculated animals. Not significantly different over time or between treatments.

Plasma LH concentrations within individual animals appeared to fluctuate randomly over time, varying by as much as 9,0 µg/l in both the control and electroejaculated animals. Within individual cheetahs plasma LH concentrations appeared to be related to plasma testosterone concentrations. The correlation coefficient between these two hormones for both the control and electroejaculated groups combined was 0,77 ($p < 0,01$).

Cortisol-testosterone relationships following an ACTH injection

Immediately prior to ACTH injection, plasma cortisol concentrations were 168,3 and 179,4 nmol/l respectively, in the two anaesthetized cheetahs and 309,0 and 383,6 nmol/l in the two un-anaesthetized animals. ACTH caused a rapid rise in the plasma cortisol concentration which peaked at 602,0 and 1049 nmol/l within 30 to 60 min after the injection and then gradually

declined. The use of anaesthesia had no discernible effect on the magnitude or the temporal characteristics of the plasma cortisol profile following ACTH. Neither plasma testosterone nor LH profiles appear to be affected by ACTH injection and the subsequent rise in cortisol concentration. Testosterone levels gradually declined in one awake animal and increased and declined in one of the anaesthetized males, levels remained unchanged in the other two cheetahs. LH concentrations varied randomly between 2,0 and 8,0 µg/l in all four of the animals.

Electroejaculation under general anaesthesia was followed by an acute rise in plasma cortisol levels which declined immediately following the termination of the electrical stimulus. While it was evident that anaesthesia did not prevent an adrenal response to the electrical stimulus the plasma cortisol concentrations measured in these animals were lower than those in ACTH-treated cheetahs. There was no evidence that cortisol impaired or modulated tonic release of LH or testosterone.

Comparative data on the effects of electroejaculation in domestic animals are limited. In un-anaesthetized bulls (B. taurus), corticosteroids rise within 5 min of the beginning of electroejaculation and reach a peak 15 min thereafter. The hormone levels remain significantly greater than pre-electro-ejaculation values until 2 h post-stimulation (Welsh & Johnson, 1981). The adrenocortical response in anaesthetized cheetahs was similar to that of bulls (Welsh & Johnson, 1981). Plasma cortisol concentrations rose gradually with peak levels measured after electroejaculation. A marked variation in basal and

post-ejaculation, peak, cortisol concentrations was measured in individual cheetahs. Indicating that individual animals varied in the degree of susceptibility and combativeness to stress.

Information on testosterone levels in carnivores is uncommon. In the domestic dog the circulating concentrations range from 7-20 nmol/l (Jones et al. 1976; De Palatis et al. 1979). No comparative data are available on values in male Felidae , including the domestic cat (F. catus). In the bull electroejaculation induced elevations in the plasma cortisol concentrations which were correlated with decreasing levels of circulating testosterone and LH (Welsh & Johnson, 1981). Data from a variety of species suggest that corticosteroid hormones can alter testicular function by affecting either the hypothalamus (Collu, Tache & Ducharme, 1979) the adenohypophysis (Chantaratreep & Thibier, 1978) or gonads (Beitins et al. 1973; Doerr & Pirke, 1976; Saez, Morera, Haour & Evain,1977; Bambino & Hseuh,1981). The administration of ACTH is negatively correlated to subsequent plasma testosterone levels in man (Doerr & Pirke,1976) and bulls (B.taurus) (Welsh & Johnson ,1981 ; Johnson et al. 1982; Barnes, Kazmer, Birrenkott & Grimes, 1983). The administration of cortisol or dexamethasone also eliminates the nocturnal rise of plasma testosterone in man (Doerr & Pirke,1976) and decreases LH synthesis in bulls (B. taurus) (Thibier & Rolland,1976; Chantaratreep & Thibier, 1978). However it is also evident that the stress associated adrenal-testicular-adenohypophyseal relationship is species-specific, not always directly linked to cortisol and not always easily explained.

Although dexamethasone or elevated cortisol decrease plasma testosterone concentrations in men, LH concentrations are not generally affected (Schaison, Durand & Mowszowicz,1978; Rose & Sachar,1981). ACTH induces cortisol elevations in dogs (C. familiaris) and boars (S. scrofa), however, testosterone levels are unaffected in the former (Eik- Nes,1962; Hagan & Andersen, 1981) and even increased in the latter species (Juniewcz & Johnson, 1981). The increased plasma testosterone concentrations in boars were seen in the absence of any detectable rise in LH (Juniewicz & Johnson, 1981). ACTH also has no influence on testosterone levels in rabbits (Oryctolagus cuniculus) (Haltmeyer & Eik-Nes, 1969) or the rhesus monkey (M. mulatta) (Michael et al. 1974). The cheetah can be classified with these species as there was no evidence that the elevations in plasma cortisol, after ACTH or as a result of electroejaculation, modulated acute secretory patterns of either testosterone or LH.

Plasma testosterone and semen quality

Plasma testosterone concentrations measured in these animals are summarized in Table 12.

Table 12. The relationship of plasma testosterone concentrations and semen quality in 18 cheetah males. Data are presented as mean (±) standard deviation.

Semen quality	Plasma testosterone			
	Pre-stimulation nmol/ℓ	Maximum nmol/ℓ	Response nmol/ℓ	Rate of Production nmol/ℓ/h
Good (n = 5)	2,17 ± 2,15	11,31 ± 5,00	9,14 ± 3,92	20,59 ± 9,27
Fair (n = 6)	3,02 ± 4,03	11,60 ± 3,76	8,58 ± 3,14	23,87 ± 4,31
Poor (n = 7)	3,40 ± 2,72	10,21 ± 2,91	6,80 ± 3,35	23,89 ±6,41

An analysis of variance of the data shows that mean plasma testosterone measured did not differ significantly in each of the three groups of cheetah males examined. (F = 0,91 for Pre-stimulation, F = 0,20 for maximum, F = 0,72 for response and F = 0,44 for plasma T in nmol/l/h).

Plasma testosterone concentrations are influenced by the age of the animal (Winters & Troen, 1982; Malak & Thibier, 1982), the season(Haigh,Cates & Glover 1982) and by testicular pathology (Christiansen 1975, Franchimont, Chari & Demoulin 1975; Fossati Asfour, Blacker, Boutemy & Hermand, 1979)

Increased plasma gonadotrophins have been reported in men with azoospermia and oligozoospermia (Christiansen 1975; Franchimont et al. 1975; Fossati et al. 1979; Batrinos et al. 1982). While Batrinos et al. (1982) reported lower plasma testosterone concentrations in men with azoospermia, the others found plasma testosterone levels no different from those of fertile males.

The measurement of plasma testosterone was undertaken to establish an understanding of the hormonal relationships in male cheetah which might be of value in the assessment of an animals breeding potential. Measurement of gonadotrophins was not always possible during this study.

Plasma testosterone did not differ significantly in the groups of animals examined despite differences in semen quality.

diZerega & Sherins(1981) reviewed the endocrine control of testicular function.

Steinberger (1971, in diZerega & Sherins,1981) and Steinberger, Root, Ficher & Smith (1973, in diZerega & Sherins,1981) have shown that, in immature hypophysectomized rats, testosterone alone could account for the initiation of spermatogenesis and that FSH was required only for its completion. The administration of testosterone in hypogonadotrophic men, however, does not induce spermatogenesis (MacLeod 1970, in diZerega & Sherins,

1981). HCG injection stimulates increased intratesticular testosterone concentrations and promptly initiates spermatogenesis (Sherins,Winters & Wachslicht 1970, in diZerega & Sherins,1981).

Plasma testosterone after intravenous & intramuscular GnRH

The effect of 50 µg GnRH given intravenously and intramuscularly in four cheetah males is compared in Fig. 25 and the results are summarized in Table 13.

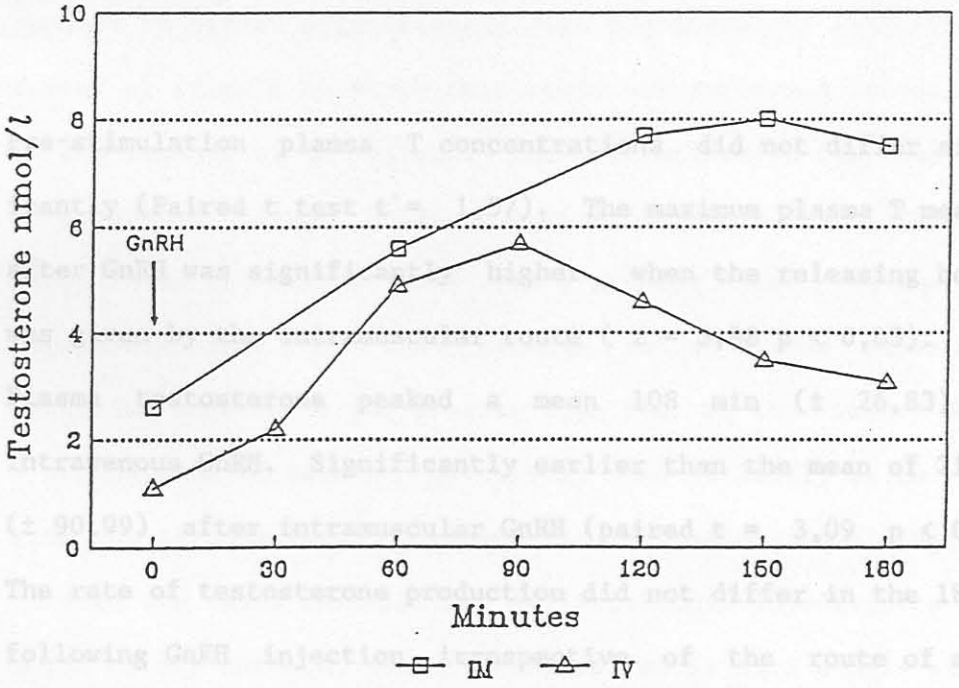

Figure 25: Plasma testosterone after GnRH by intravenous and intramuscular injection (n = 4).

Table 13: Plasma testosterone after GnRH by intravenous and intramuscular injection. Data are presented as mean and standard deviation (±), (n = 4).

Plasma testosterone			
Route of injection	Presti-mulation nmol/ℓ	Maximum nmol/ℓ	Rate of production nmol/ℓ/h
IV	0,92 ± 0,46	5,66 ± 1,79	11,32 ± 4,79
IM	2,45 ± 2,23	11,39 ± 4,66	17,91 ± 7,65

Pre-stimulation plamsa T concentrations did not differ significantly (Paired t test t = 1,57). The maximum plasma T measured after GnRH was significantly higher when the releasing hormone was given by the intramuscular route (t = 3,88 $p < 0,05$).

Plasma testosterone peaked a mean 108 min (± 26,83) after intravenous GnRH. Significantly earlier than the mean of 216 min (± 90,99) after intramuscular GnRH (paired t = 3,09 $p < 0,05$). The rate of testosterone production did not differ in the 180 min following GnRH injection irrespective of the route of administration (t = 0,79)

Gonadotrophin releasing hormone has been administered intravenously by Schanbacher & Echternkamp (1978) and Johnson, Welsh, & Juniewicz (1982) in cattle (B. taurus); Bremner, Findlay, Lee,

de Kretser & Cumming (1980) in sheep (O. aries) Pomerantz et al.(1974) in boars (S. scrofa); Jones & Boyns (1974) and Jones,Baker, Fahmy & Boyns (1976) in dogs (C. familiaris) Haltmeyer & Eik-Nes (1969) in rabbits (Oryctolagus cuniculus). Comparisons were not made between the intravenous and intramuscular routes of administration.

The intravenous administration of GnRH as a standardised stimulus has advantages in that the peak of plasma testosterone is reached earlier. Serial blood collection takes place over a shorter period of time and the number of hormone assays required is decreased.

The rate of response and decline in the plasma testosterone appears to differ significantly with the route of injection. The number of animals on which this study was performed is small and, in the absence of LH values, this difference in response cannot be explained. Aiyer, Chiappa, & Fink (1973, in Fink & Pickering, 1980) have shown that GnRH has a priming effect on the adenohypophysis and that the response to a second exposure to releasing hormone is far greater than that of the first. GnRH is a decapeptide (Matsuo et al. 1971) and is likely to be removed from the circulation, by glomerular filtration, in a single pass through the renal circulation. An intravenous injection, a single bolus of the hormone, would therefore result in a single stimulus to the adenohypophysis. However intramuscularly injected hormone, with a slower rate of absorption into the circulation, could conceivably have a more prolonged effect. Added to this is the

possibility of a partial subcutaneous or intraseptal placement of the hormone which would reduce its rate of absorption and extend its effect.

Plasma testosterone after HCG injection

The effect of increasing doses of HCG on plasma testosterone was measured in four cheetah males and the results are summarized in Table 14.

Table 14: The effect of increasing doses of HCG on plasma testosterone in cheetah males. Data are presented as mean and standard deviation(±), n = 4.

Plasma testosterone			
HCG mg	Presti-mulation nmol/ℓ	Maximum nmol/ℓ	Rate of Production nmol/ℓ/h
2,5	2,0 ± 1,21	10,84 ± 0,70	28,49 ± 2,33
5	1,16 ± 1,69	15,40 ± 5,33	35,24 ± 10,58
10	2,48 ± 1,54	11,84 ± 3,56	31,72 ± 8,41
20	0,71 ± 0,26	12,75 ± 3,01	34,20 ± 4,45

An analysis of variance of the above data shows that there was no significant difference of the mean pre-stimulation, mean maximum or the rate of testosterone production in nmol/l/h ($F = 1,14$, $F = 0,91$ and $F = 0,52$ respectively). Plasma testosterone response and peak time were not included as Figure 26 shows that increases in plasma testosterone in animals after HCG were more sustained

and that the maximum plasma testosterone concentrations were probably reached after the sampling period. Plasma testosterone measured in the same animals after 50 µg GnRH are included in Figure 27.

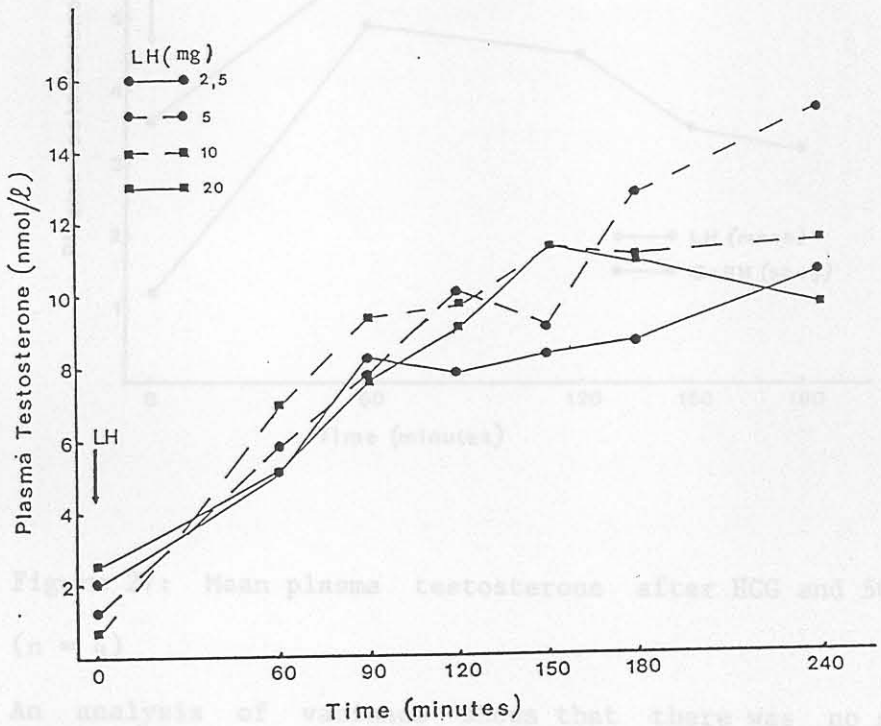

Figure 26: Plasma testosterone after increasing doses of HCG. (Dosage is presented as the equivalent dose of LH in mg).

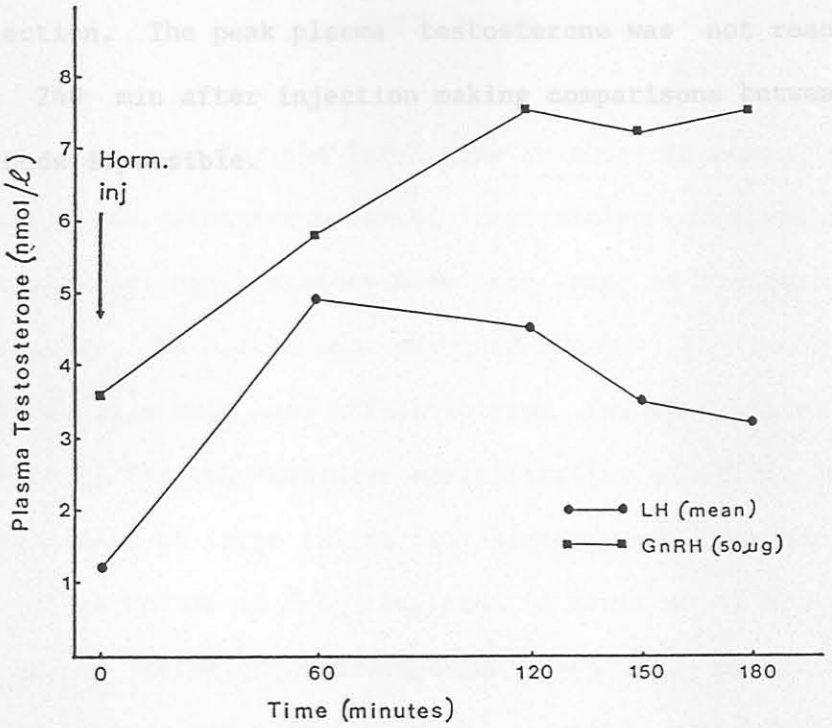

Figure 27: Mean plasma testosterone after HCG and 50 µg GnRH. (n = 4)

An analysis of variance shows that there was no significant difference in the mean rate of testosterone production,measured in nmol/l/h, after HCG and GnRH when measured over a period of 240 minutes.

The plasma T response following HCG injection is used in the clinical assessment of testicular interstitial function in man (Anderson, Marshall, Young & Russell Fraser, 1974 in Anderson, 1984) and has been used by Falvo et al.(1975) in rams (O. aries); Haltmeyer & Eik-Nes (1969) in rabbits (O. cuniculus) ; Bambino & Hsueh (1981) in rats (R. norvegicus). The response measured in cheetahs was prolonged in comparison to that seen after GnRH

The study of the effects of ACTH on plasma hormones and their relationship to one another reported on below shows that stress responses with raised plasma cortisol concentrations which undoubtedly were present in this group of animals probably did not influence testosterone concentrations significantly.

Plasma testosterone concentrations measured in other animals are summarized in Table 6.

injection. The peak plasma testosterone was not reached during the 240 min after injection making comparisons between the two methods impossible.