

STUDIES ON EFFECT OF  
GnRH AND LH  
ON  
PLASMA TESTOSTERONE AND  
SEMINAL CHARACTERISTICS  
IN  
HORSE AND DONKEY STALLIONS

BY

*Satish Sharma*

Thesis submitted to the  
Chaudhary Charan Singh Haryana Agricultural University, Hisar  
in partial fulfilment of the requirements for the degree of

MASTERS IN VETERINARY SCIENCES

IN

**Veterinary Gynaecology and Obstetrics**

1997

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**TO  
MY PARENTS**

# Certificate - 1

This is to certify that this thesis entitled "**Studies on effect of GnRH and LH on plasma testosterone and seminal characteristics in horse and donkey stallions**", submitted for the degree of MVSc in the subject of **Veterinary Gynaecology and Obstetrics** to the **Chaudhary Charan Singh Haryana Agricultural University, Hisar**, is a bonafide research work carried out by **Dr Satish Sharma** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.



**(Dr N. S. Bugalia)**

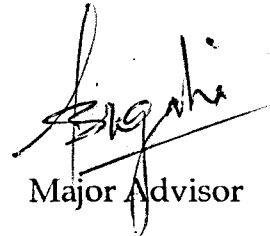
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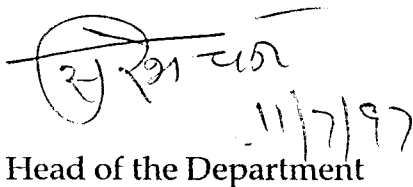
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## Certificate - 2

This is to certify that this thesis entitled "**Studies on effect of GnRH and LH on plasma testosterone and seminal characteristics in horse and donkey stallions**", submitted by **Dr Satish Sharma** to **Chaudhary Charan Singh Haryana Agricultural University, Hisar**, in partial fulfilment of the requirements for the degree of **MVSc** in the subject of **Veterinary Gynaecology and Obstetrics** has been approved by student's advisory committee after an oral examination on the same.



Major Advisor



Head of the Department



Dean, Post Graduate Studies

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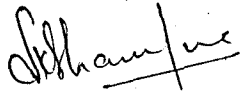
*This acknowledgement will not have any meaning without the mention of Major General RM Kharb, the additional Director General Remount and Veterinary Services, and Brigadier BS Hans the Commandant Equine Breeding Stud, Hisar for granting permission to conduct research work on stallions. Major Virender Kumar deserves thanks for concrete help and personal attention during the investigation. Equally I am grateful to Major JPS Doad, Captain VK Mudgil, Captain CP Churamani and Captain Manoj Batra for their consistent and untiring inspiration during the course of investigation.*

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(Satish Sharma)

Hisar

# STUDIES ON EFFECT OF GnRH AND LH ON PLASMA TESTOSTERONE AND SEMINAL CHARACTERISTICS IN HORSE AND DONKEY STALLIONS

An MVSc Thesis by Satish Sharma  
Department of Veterinary Gynaecology and Obstratics

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# Introduction

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Stallion undergoes annual regression and recrudescence of testicular size (Johnson and Thompson 1983, Clay *et al* 1987). Libido and sperm production is influenced by seasonal changes in luteinizing hormone (LH) secretion (Thompson *et al* 1977) and testicular testosterone production in stallions (Clay *et al* 1988).

During breeding season (from March to October), the sexual activity in horse and donkey stallions is increased (Berndtson *et al* 1974) and is at its peak during mid-summer (from May to August). Maximum sexual activity is manifested by decrease in reaction time and number of mounts required for an ejaculate (Skinner and Bowen 1968, Pickett and Voss 1972).

After ten to twelve years of stud service, stallions usually develop lack of libido or slowness in breeding (Lopes Gusmao 1988). Lack of libido and decreased sperm production reflect testicular dysfunction and warrants premature culling of breeding stallions (Pickett *et al* 1989). Albeit, use of gonadotropic releasing hormone (GnRH) and LH have been reported to be effective in therapeutic management of libido disorders in bulls (Byerley *et al* 1990), their role in stallions with similar disorders is yet to be established. Recommendation of GnRH and LH in slow breeder stallions by Bader *et al* in 1988 has been questioned recently. Blue *et al* (1991) and

Douglas and Umphenour (1992) doubt reliability of these hormones in impotent sub-fertile stallions.

In light of inconsistency in reports on therapeutic efficacy of GnRH and LH in slow breeding stallions *vis-a-vis* effectiveness in bulls, the present investigation was undertaken with following objectives:

- ☞ To study the effect of GnRH and LH treatment on libido and plasma testosterone profiles in horse and donkey stallions.
  
  - ☞ To determine the influence of GnRH and LH treatment on seminal characteristics (semen volume, pH, individual motility, mass activity, sperm concentration, live sperm count) and seminal biochemical constituents (citric acid, glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) in horse and donkey stallions.
-

# Review of Literature

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Libido and fertility in stallions decline with age and overuse (Bielanski 1964, Johnson and Neaves 1981). Frequency of breeding also affects sex desire and seminal characteristics in stallions (Pickett *et al* 1975).

Libido and fertility is usually low in stallions during early and late breeding season (Skinner and Bowen 1968, Pickett *et al* 1970 and 1976). but progressive increase in day light during breeding season activates hypothalamic-pituitary gonadal axis for initiation of testicular endocrine and exocrine functions (Byers *et al* 1983). However, hypothalamic irresponsiveness to increased day light results in cessation of pituitary luteinizing hormone (LH) secretion and testicular androgen production (Joyce *et al* 1983).

Gonadotropic releasing hormone (GnRH) and LH therapy proved effective in slow breeder and sub fertile bulls (Smith *et al* 1973, Byerley *et al* 1990) and stallions (Bader *et al* 1988, Stonina and Pozor 1989).

## 2.1: EFFECT OF GNRH ON LIBIDO IN HORSE AND DONKEY STALLIONS.

Horse and donkey stallions with low libido had low plasma LH concentrations due to deficient GnRH secretion (Hyland and John 1990). Parenteral administration

of GnRH improved the libido in such stallions. (Bader *et al* 1988, Stonina and Pozor 1989).

## 2.2: EFFECT OF LH ON LIBIDO IN HORSE AND DONKEY STALLIONS.

Stallions with low libido had very low circulating LH concentration (Irvine *et al* 1985, Hyland and John 1990). and human chorionic gonadotropin (HCG) injection increased their libido (Cox *et al* 1973, Amann and Ganjam 1981, Setchell and Cox 1982, Irvine *et al* 1985, Bader *et al* 1988). Positive correlation was recorded between plasma LH levels and libido in stallions (Thompson *et al* 1977).

Parenteral administration of HCG (10,000 IU) increased the libido in stallions (Silberzahn *et al* 1988, Okuda *et al* 1991). HCG (500 IU) administration improved the libido in low libido bulls (Katongole *et al* 1971).

## 2.3: EFFECT OF GnRH ON PLASMA TESTOSTERONE PROFILES IN HORSE AND DONKEY STALLIONS

Exogenous GnRH increases peripheral testosterone concentration in rams (Wilson and Lapwood 1978), stallions (Lopes 1988) and in bulls (Kilroy and Dobson 1986). Magnitude and duration of GnRH response was dose dependent in bulls and rams (Wilson and Lapwood 1978).

GnRH treatment (1 mg i.v.) produced three fold increase in plasma testosterone level in horse (from 150 - 225 pg/ml to 480 - 800 pg/ml) and two fold increase in donkey (from 850 - 950 pg/ml to 1140 - 1530 pg/ml) stallions (Minoia *et al* 1983). Parenteral GnRH infusion (2 µg/kg) increased peripheral testosterone concentration in stallions (Clay *et al* 1989, Blue *et al* 1991).

## 2.4: EFFECT OF LH ON PLASMA TESTOSTERONE PROFILES IN HORSE AND DONKEY STALLIONS

HCG administration produces rise in circulatory testosterone levels in rams (Garnier and Saez 1980), bulls (Sundby and Farahat 1978) and stallions (Linder 1961, Cox 1989).

Significant rise in plasma testosterone occurred at 24 hours (1.5 - 2.5 ng/ml vs. 0.1 - 0.5 ng/ml) and 36 hours (5 - 6 ng/ml vs. 0.1 - 0.5 ng/ml) after intravenous administration of HCG (10,000 IU) in stallions. (Silberzahn *et al* 1988). Four to five

folds increase in peripheral testosterone levels was observed within 60 minutes of HCG infusion (10,000 IU, i.v.) in stallions (Hyland 1990).

HCG infusion in two stallions (0.5 IU/kg, 1.5 IU/kg) produced rise in peripheral testosterone concentration in spermatic vein within 30 minutes (Linder 1961).

## 2.5: EFFECT OF GnRH ON SEMINAL CHARACTERISTICS IN HORSE AND DONKEY STALLIONS

GnRH injection increases semen volume and sperm output in bulls (Abdelmalak and Thibier 1982) and stallions (Byers *et al* 1983, Courot 1988).

Seminal characteristics are influenced by circulatory testosterone profiles and frequency of ejaculation in stallions (Bielanski 1964, Pickett *et al* 1970).

Circulatory testosterone influences seminal characteristics in stallions (Berndtson *et al* 1974). Peak plasma testosterone concentration was positively correlated with number of spermatozoa per ejaculate in bulls (Wildeus and Holroyd 1984). Distinct increase in semen volume and sperm output was associated with rise in peripheral testosterone profiles during mid summer in stallions (Byres *et al* 1983, Berndtson and Jones 1989).

## 2.6: EFFECT OF LH ON SEMINAL CHARACTERISTICS IN HORSE AND DONKEY STALLIONS

Exogenous HCG administration increased semen volume and sperm out put in stallions (Silberzahn *et al* 1988). Positive correlation between plasma LH concentration and seminal characteristics was reported in stallions (Thompson *et al* 1977).

## 2.7: EFFECT OF GnRH ON SEMINAL BIOCHEMICAL CONSTITUENTS IN HORSE AND DONKEY STALLIONS

Biochemical constituents of stallions' ejaculate reflect functional status of ampullae, seminal vesicles and epididymis (Mann 1975). Exogenous GnRH increases plasma testosterone profile and testosterone promotes secretion of accessory sex glands in stallions (Byres *et al* 1983). Literature lacks reports on efficacy of GnRH on seminal biochemical constituents in horse and donkey stallions.

## 2.8: EFFECT OF LH ON SEMINAL BIOCHEMICAL CONSTITUENTS IN HORSE AND DONKEY STALLIONS

Concentrations of citric acid and transaminases in seminal plasma of equine semen are influenced by season and androgen production (Mann *et al* 1956, Skinner and Bowen 1968, Harris *et al* 1983). HCG stimulates gonadal-androgen activity (Cox and Williams 1975) and increases secretion of accessory sex glands in stallions (Byres *et al* 1983). Positive correlation between seminal biochemical constituents and androgen activity has been reported in stallions (Humphery and Mann 1948, 1949). literature lacks reports on efficacy of LH on seminal biochemical constituents in horse and donkey stallions.

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# Materials and Methods

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## 3.1: STALLIONS AND THEIR MANAGEMENT

Present study was conducted on 8 horse and 8 donkey stallions (*total = 16*) of 5 to 12 years during the breeding season (July 1995 to October 1995).

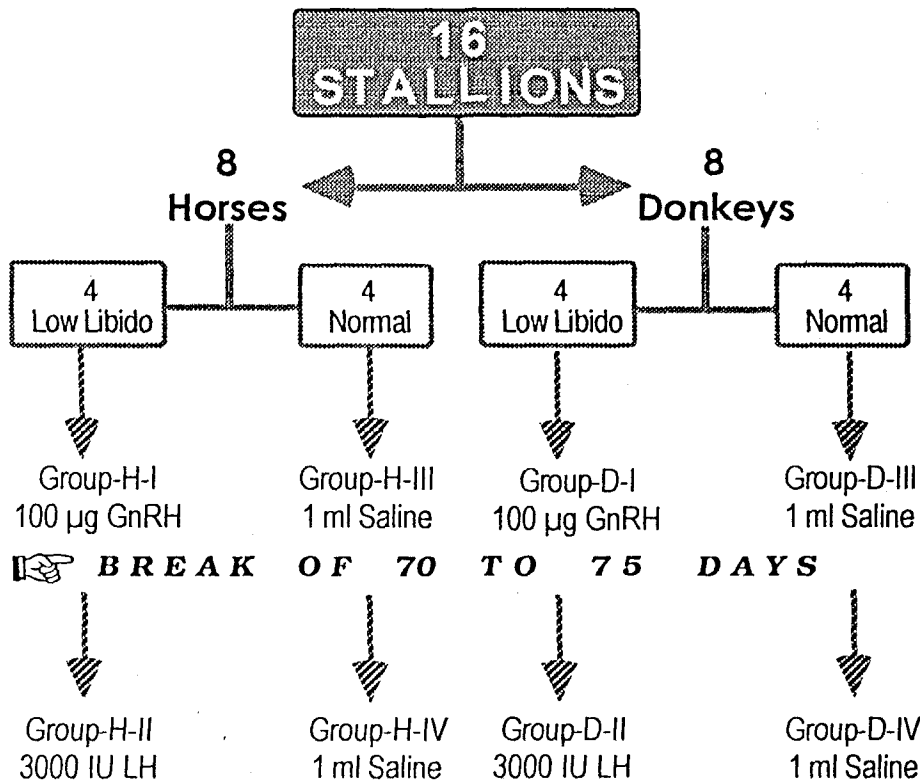
These stallions belonged to Equine Breeding Stud of Remount Veterinary Corps, Hisar. Stallions were housed in pucca ventilated sheds covered from three sides. Their feed comprised of green maize, green barseem, oat hay, dry lucerne and concentrate mixture. They had free access to drinking water.

## 3.2: EXPERIMENTAL DESIGN

Effectiveness of luteinizing hormone (LH) and gonadotrophic releasing hormone (GnRH) was investigated on 4 low libido horse and 4 low libido donkey stallions (*total = 8*). Low libido was defined by reaction time of above 15 minutes in all stallions. Remaining 8 stallions (4 horses and 4 donkeys) had normal sexual behaviour characterised by reaction time of less than 4 minutes, and they served as control for the hormonal treatment.

The treatment regimen of each group is depicted in a flow diagram. Since, GnRH and LH was evaluated on same stallion, a gap of 70 to 75 days was allowed

between their administration. Four horse and four donkey stallions were treated with 100 µg of GnRH (Fertagyl™, Intervet - Holland) intravenously (Group-H-I and Group-D-I respectively). Same stallions after a gap of 70 to 75 days were treated with 3,000 IU of LH (Chorulon™, Intervet, Holland) intravenously (Group-H-II and Group-D-II respectively). Groups H-III, H-IV, D-III and D-IV were treated with 1 ml of saline intravenously and served as normal control for their respective hormonal treatment stallions.



### 3.3: SEMEN COLLECTION

Colorado mode of artificial vagina was used for semen collection from horse stallions (Singhvi 1980) while Nishikawa model of artificial vagina was employed for semen collection from donkey stallions (Nishikawa 1959). Semen was collected from horse and donkey stallions at -144 and -72 hours before GnRH and LH treatments and subsequently at 4 and 72 hours after their administration. Similarly semen was collected from control horse and donkey stallions at 144 and 72 hours before, and at 4 and 72 hours after saline administration.

### 3.3.1: PREPARATION AND RESTRAINT OF MARES AND STALLIONS

Pre-treatment and post-treatment semen collections from the horse and donkey stallions was done using mares in oestrus. Mares were usually restrained with a head halter, service hobbles and a nose twitch. Tail of the mare was wrapped with a bandage, perineal region was washed with 0.1% potassium permanganate solution followed by scrubbing with povidone iodine (Wokardine™, Wockhardt, Bombay) and rectified spirit before exposure to the stallion.

Horse and donkey stallions were restrained with head stall and lead for collection of semen. Penis was dipped in the potassium permanganate solution (0.1%) and diluted povidone iodine solution (Wokardine™, Wockhardt, Bombay) after semen collection as routine antiseptic preventive measure.

### 3.3.2: PREPARATION OF ARTIFICIAL VAGINA

#### 3.3.2.1: Colorado Model.

Components of the Colorado model of artificial vagina and assembled AV (Singhvi 1980) are shown in figures 1 and 2.

To assemble artificial vagina, inner liner is inserted into heavy plastic cylinder and turned back over front and rear end of the cylinder, avoiding folds in the liner and rubber bands are applied over the liner on both the ends of cylinder. Liner-cum-cone is inserted through the inner liner and turned back over the front end of the cylinder to fix with rubber bands and the free rear end of the liner-cum-cone is stretched to attach with filter assembly. Filter was placed inside the collection bottle and its free top end was fixed in the mouth of collection bottle with a plastic ring. Insulating bag covering the filter assembly was tied on the rear end of the cylinder. Three and half to four litre of warm water (55 to 60 °C — to equilibrate at 45 °C) was poured in the artificial vagina. The artificial vagina liner was lubricated with K-Y Jelly™ (Johnson and Johnson, USA) just prior to semen collection.

#### 3.3.2.2: Nishikawa Model.

Components of the Nishikawa model of artificial vagina and assembled AV (Nishikawa 1959) are shown in figures 3 and 4.

To assemble artificial vagina, two cone shaped liners are inserted in to outer aluminium case and turned back over front and back end of the aluminium case, avoiding folds and rubber bands are applied over both the ends of liners on outer case.

Figure 1: Components of the Colorado model of artificial vagina.

- (a) - Heavy plastic cylinder with built-in valve, leather covering and handle.
- (b) - Inner liner — 30 inches in length and 7 inches in width. It forms the water jacket in heavy plastic cylinder.
- (c) - Liner-cum-cone — 28 inches long, 7 inches wide at anterior end and 3 inches wide at posterior end.
- (d) - Filter assembly — Includes collection bottle, filter and filter ring.
- (e) - Insulating cover: Quilted cover to protect the collection bottle from environmental temperature and sunlight.

Figure 2: Assembled artificial vagina - COLORADO MODEL.

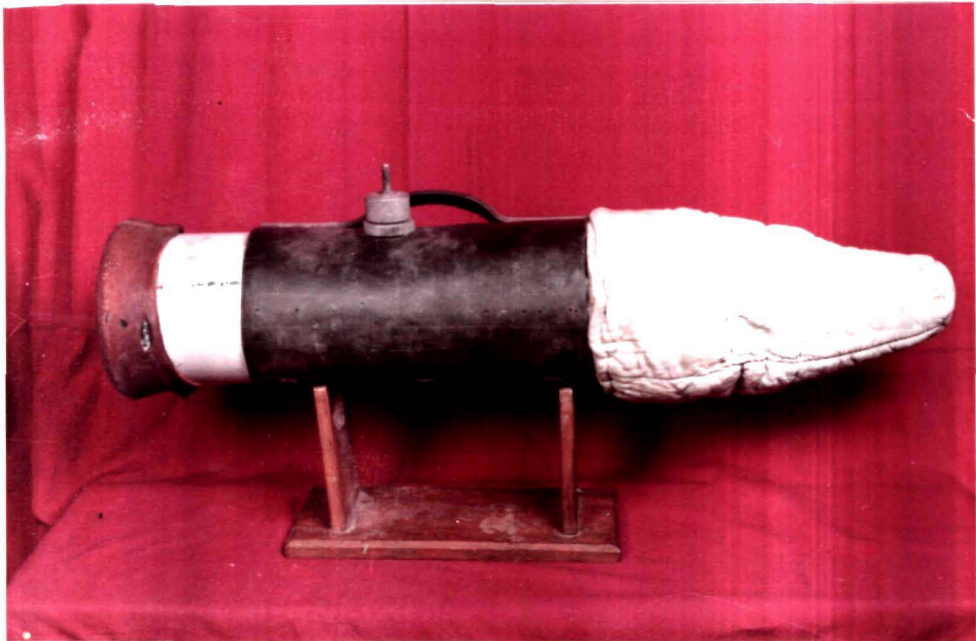
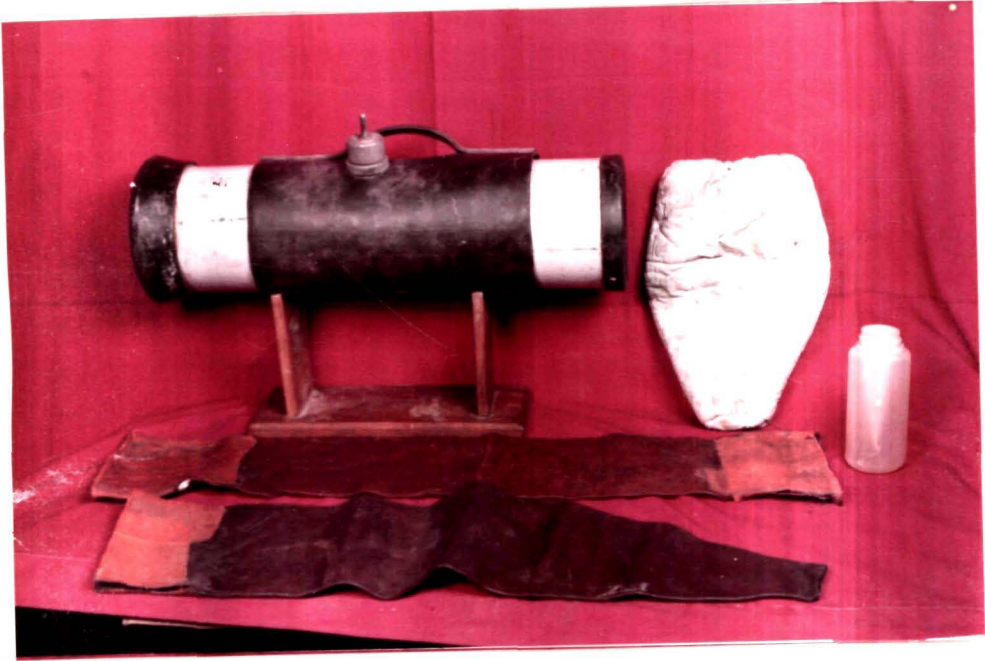
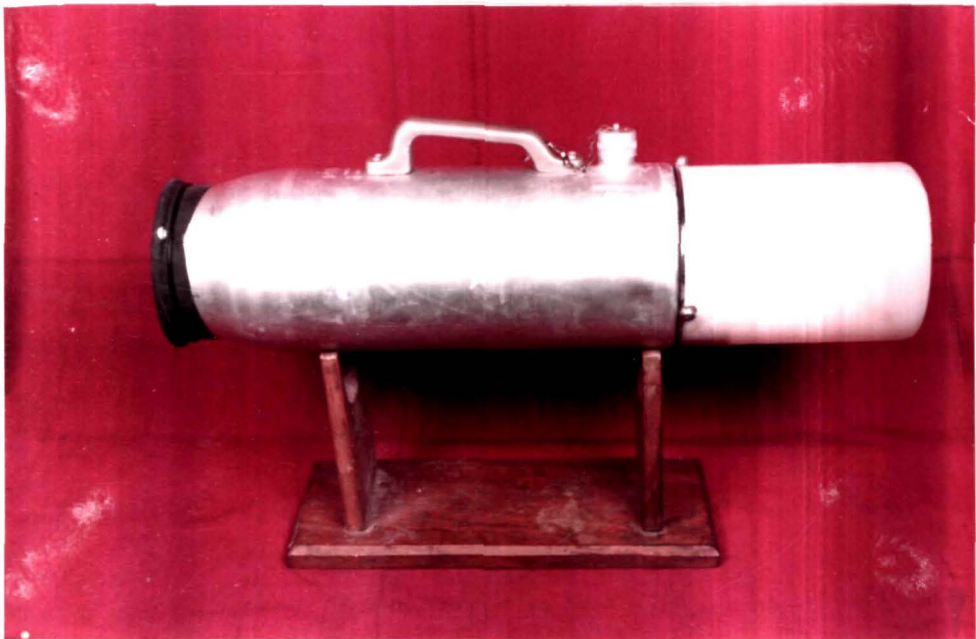


Figure 3: Components of the Nishikawa model of artificial vagina.

- (a) - Aluminium case with built-in valve and handle.
- (b) and (c) - Two liners, each measuring 20 inches in length, 7 inches wide at anterior end and 3.5 inches wide at rear end.
- (d) - Cone - 8 inches long, 3 inches wide at anterior end and 1 inch wide at rear end.
- (e) - Filter assembly — Includes collection bottle, filter and filter ring.
- (f) - Insulating jacket — Plastic mug shaped jacket to protect the collection bottle from environmental temperature and sunlight.

Figure 4: Assembled artificial vagina - NISHIKAWA MODEL.



Broad end of the AV-cone was attached to narrow rear end of aluminium case and held in position by a single rubber band. The narrow end of the AV-cone was attached with filter assembly. Filter was placed inside the collection bottle and its free top end was fixed in the mouth of collection bottle with a plastic ring. Insulating jacket covering the filter assembly was fixed on the rear end of the aluminium case. Three and half to four litre of warm water (55 to 60 °C — to equilibrate at 45 °C) was poured in the artificial vagina. The AV-liner was lubricated with K-Y Jelly™ (Johnson and Johnson, USA) just prior to semen collection.

### 3.3.3: SEMEN COLLECTION PROCEDURE

During collection attempts the horse and donkey stallions were allowed their normal courtship activities near the receptive horse and donkey mares, respectively. The artificial vagina was held in left hand on the rear side of the stallion in line but approximately two meters away from its hind legs during courtship. Once the stallion mounted, its penis was deflected into the artificial vagina with the right hand and during service penis was held lightly from its base and pulsation of the urethra during ejaculation were counted. After ejaculation, as the stallion dismounted the penis was 'stripped' in to the artificial vagina to prevent loss of 'tail end' ejaculate (Figures 5 to 17).

### 3.3.4: SEMEN EVALUATION

#### 3.3.4.1: Volume and pH

Immediately after collection, the gel filter was removed from the collection bottle and turned upside down in to a 500 ml graduated glass cylinder to measure the gel volume. Gel free semen from the collection bottle was poured in to a pre-warmed measuring cylinder (500 ml) held at 45° angle and volume of the ejaculate was recorded and semen was placed at 38 °C. Semen pH was also noted with a close range BDH paper.

#### 3.3.4.2: Mass Activity and Individual Motility

Soon after semen collection, a drop of the gel free ejaculate was placed on a pre-warmed glass slide. First drop was examined under low power without cover slip for mass activity. Semen samples were scored from 0 to 3 for mass activity (Dowsett 1982). Second drop of semen was covered with cover-slip and examined under high power to record percentage of progressively motile spermatozoa. Both mass activity and individual motility were scored as described by Dowsett (1982).

Figure 5: Mare in heat.

Figure 6: Horse stallion just before approaching towards mare.



Figure 7: Partial erection of penis in horse stallion.

Figure 8: Complete erection of penis in horse stallion.

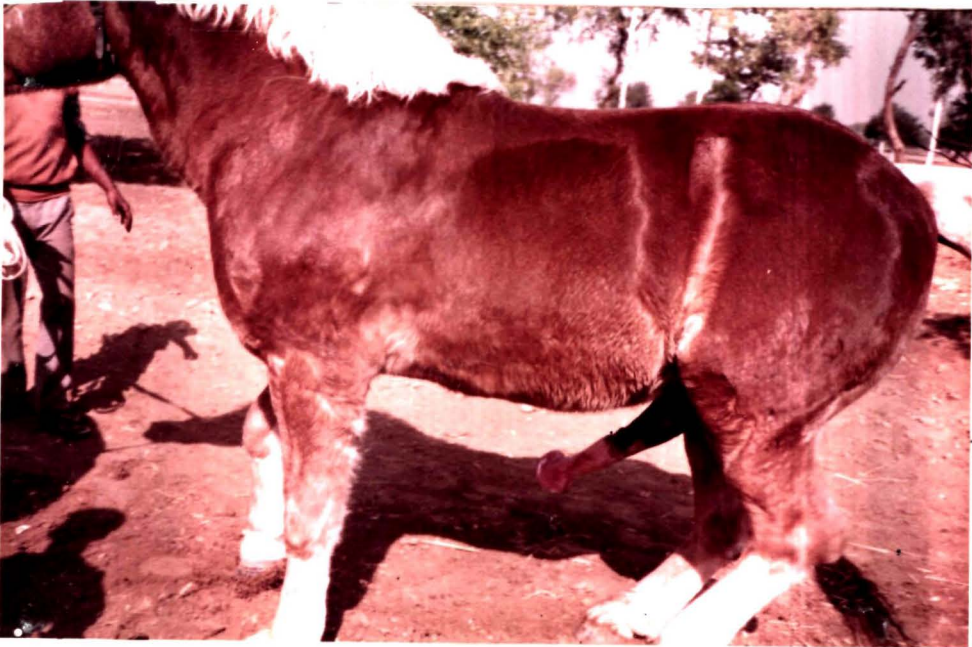


Figure 9: Horse stallion mounted on mare and penis deflected into AV.

Figure 10: Horse stallion performing rhythmic pelvic thrust.

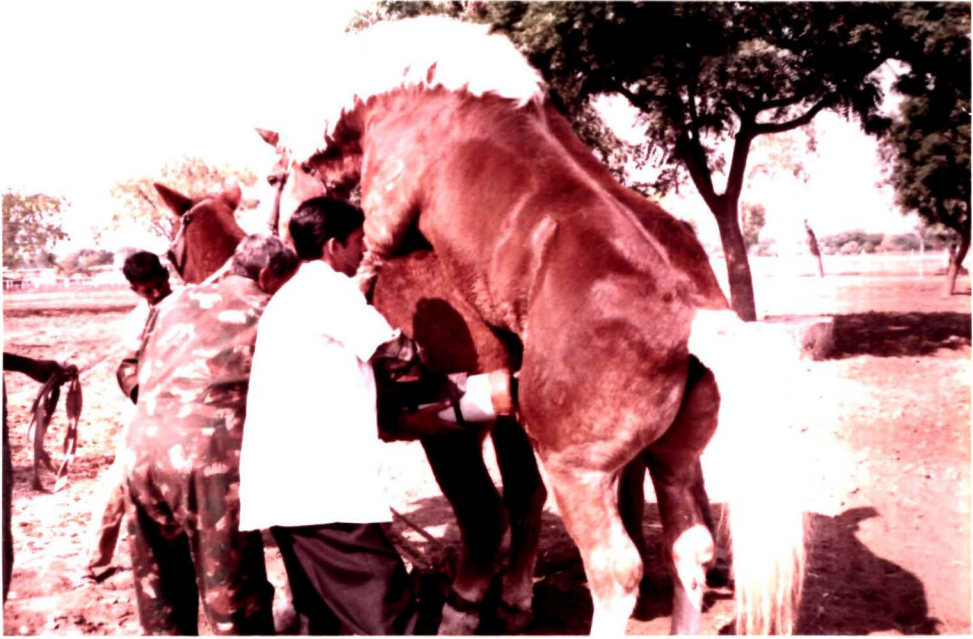


Figure 11: Horse stallion ejaculating semen.

Figure 12: Mare in oestrus.



Figure 13: *Partial erection of penis in donkey stallion.*

Figure 14: *Complete erection of penis in donkey stallion.*



Figure 15: Donkey stallion mounted on mare and, penis deflected into AV.

Figure 16: Donkey stallion performing rhythmic pelvic thrust.



Figure 17: Donkey stallion ejaculating semen.



### *3.3.4.3: Sperm Concentration*

Gel free semen was diluted with 10% Hancocks buffered formal saline (Hancocks & Trevan 1957) in 1:20 ratio and diluted semen was used for determination of sperm concentration using Neubauer Haemocytometer Chamber (Pickett & Black 1973).

### *3.3.4.4: Live and Dead Sperm Count (percentage)*

One drop of raw semen was placed on a pre-warmed glass slide and 9 drops of eosin and nigrosine stain were added and mixed with platinum loop and their uniform smears were prepared from this mixture on glass slides. Smears were dried and examined under high magnification and oil immersion for counting live and dead spermatozoa. Dead spermatozoa were stained red or pink by eosin, while live sperms remained unstained. Percentage of live and dead spermatozoa was calculated from 200 spermatozoa counted in the smear in zig-zag manner.

### *3.3.4.5: Seminal Biochemical Determination*

Gel-free ejaculates after evaluation were centrifuged at 15,000 rpm for 15 minutes to obtain seminal plasma and were transferred in to 5 ml screw capped plastic vials to store at -20 °C for biochemical analysis.

Frozen seminal plasma samples were thawed at room temperature for biochemical assay of citric acid (Mann 1956) using spectrophotometer (Spectronic-20™). Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase by using Autopak kits (Miles India Ltd.) and Blood Auto-analyse (Polimak).

## 3.4: BLOOD COLLECTION SCHEDULE

Jugular venous blood was collected in sterilised and heparinized glass vials from experimental horse and donkey stallions.

Blood samples were collected from horse and donkey stallions of Group-H-I, Group-H-II, Group-H-III, Group-H-IV, Group-D-I, Group-D-II, Group-D-III and Group-D-IV, in heparinized and sterilised glass tubes at 144, 72 and at 0 hour before, and 2, 4, 72 and 120 hours after hormonal treatment or saline injection.

### *3.4.1: PROCESSING OF BLOOD PLASMA SAMPLES*

Heparinized blood samples were centrifuged at 5,000 rpm for 20 minutes and blood plasma was transferred in to 5 ml screw capped plastic vials

containing a drop of merthiolate (1:10,000) and were frozen at  $-20^{\circ}\text{C}$  pending testosterone assay.

### 3.5: TESTOSTERONE ASSAY

Testosterone was quantified in unextracted blood plasma samples by solid phase  $\text{I}^{125}$  radio immuno assay technique. For this assay 'Radio Immuno Assay Kit' supplied by Diagnostic Products Corporation, Los Angeles, USA, was used. Material supplied in the radio immuno assay kit is given in Annexure - I.

#### 3.5.1: RADIO IMMUNO ASSAY PROCEDURE

Testosterone antibody coated tubes and testosterone  $\text{I}^{125}$  and testosterone calibrations (20, 100, 400, 800 and 1,600 ng/dl) were brought to room temperature before use and testosterone assay was done by the following procedure:

1 - Four plain tubes (uncoated, 12 X 75 mm) were labelled as non specific (NSB) and total count (TC). Testosterone antibody coated tubes were labelled in duplicate for maximum binding (A) and testosterone standards (3200, 6400 reconstituted in the concentrations of 10, 20, 50, 100, 200, 400, 800 and 1,600 coated pg/ml). Similarly, testosterone antibody tubes were numbered in duplicate for unknown plasma samples and assay was done using the following protocol:

Sets	Calibrators	Samples	$\text{I}^{125}$ Testosterone
Non Specific Binding	50 $\mu\text{l}$ (A)	—	1,000 $\mu\text{l}$
Total Count	—	—	1,000 $\mu\text{l}$
MB	50 $\mu\text{l}$ (A)	—	1,000 $\mu\text{l}$
Standard	50 $\mu\text{l}$ (10 - 6400 pg/ml)	—	1,000 $\mu\text{l}$
Unknown (Plasma)	—	50 $\mu\text{l}$	1,000 $\mu\text{l}$

2 - After an incubation of three hours at  $37^{\circ}\text{C}$ , all tubes (Except TC) were decanted thoroughly and were allowed to drain for 2 to 3 minutes. Tubes were then struck sharply on absorbent paper to shake off all residual droplets.

3 - Counts per minute (cpm) were recorded using RIA autogamma spectrophotometer (1271 LKB).

### 3.5.2: VALIDITY OF ASSAY

#### 3.5.2.1: Specificity

Testosterone antiserum was highly specific for testosterone. Per cent cross reactivity calculated on weight-per-weight basis at approximately 50 per cent binding is tabulated in Annexure - II.

#### 3.5.2.2: Sensitivity

The sensitivity of testosterone radio immuno assay in terms of minimum detectable concentration, was 4 pg/tube.

#### 3.5.2.3: Precision of Assay

The intra-assay coefficient of variation for testosterone is given in the following table.

Quality Control	Mean (pg/ml)	Coefficient of variation Intra-assay (%)
Low	5.0	6.2
High	10.0	6.4
Overall Mean		6.3

### 3.6: STATISTICAL ANALYSIS.

The data obtained was subjected to following statistical analysis using StatView 4.0 (Abacus Concepts Inc. 1992) on Macintosh computer.

- Arithmetic mean
  - Standard error
  - Analysis of variance test
  - Correlation coefficient
  - Pair 't' test.
-

# Results

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## 4.1: EFFECT OF GNRH ON LIBIDO (*REACTION TIME*) IN HORSE AND DONKEY STALLIONS.

Reaction time varied from  $182 \pm 14$  to  $203 \pm 16$  seconds (mean  $\pm$  standard error) and from  $198 \pm 12$  to  $203 \pm 11$  seconds in control group of horse and donkey stallions respectively (Table 1 and Figure 18). Variations in reaction time before or after saline administration were not statistically significant ( $P > 0.05$ ). In GnRH treatment groups. Pre-treatment (that is, -144 and -72 hour intervals) reaction time values were significantly ( $P \leq 0.05$ ) higher than that of saline groups (saline treatment) at same observation intervals. GnRH treatment significantly decreased the reaction time in both, horse and donkey stallions (Table 1 and Figure 18).

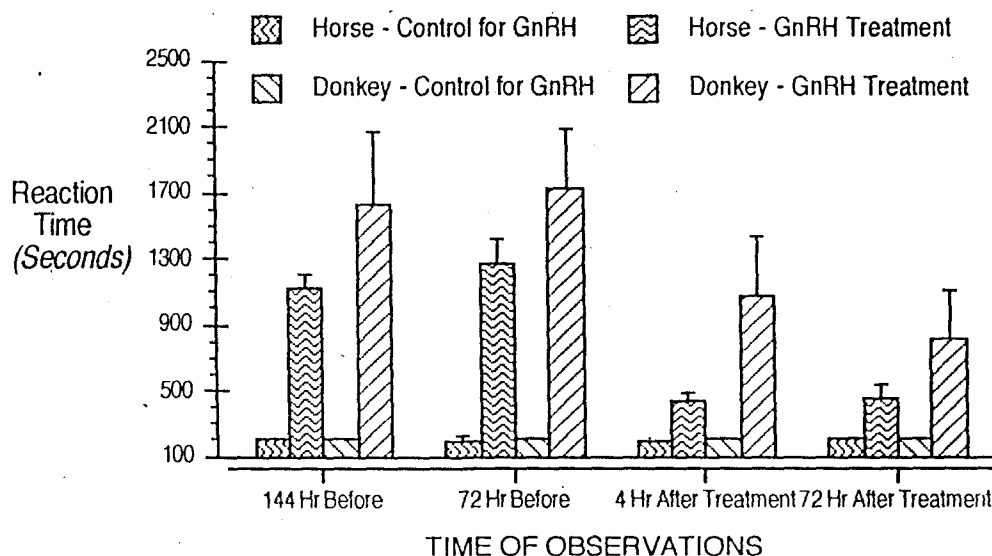
## 4.2: EFFECT OF LH ON LIBIDO (*REACTION TIME*).IN HORSE AND DONKEY STALLIONS.

In LH controls, reaction time ranged from  $198 \pm 19$  to  $228 \pm 42$  seconds in horse stallions, whereas it varied from  $196 \pm 8$  to  $203 \pm 11$  seconds in donkey stallions (Table 2 and Figure 19). Variations in reaction time were non significant between pre and post saline treatment periods in horse and donkey stallions. Pre-treatment (-144 and -72 hours) reaction time was significantly higher in the LH group of stallions

Table 1: Effect of GnRH and saline (*control*) treatment on reaction time (*seconds*) in horse and donkey stallions. (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Reaction Time (Seconds)			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH ( <i>n</i> =4)	202.5 $\pm$ 15.95	196.3 $\pm$ 21.06	182.3 $\pm$ 13.49	199.3 $\pm$ 13.22
<i>P</i> Value (Two tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treated with GnRH ( <i>n</i> =4)	1114.5 $\pm$ 79.54	1263.0 $\pm$ 149.33	429.0 $\pm$ 48.48	451.5 $\pm$ 86.96
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = 0.0045	<i>P</i> = 0.0222
Compared with -72 hour values			<i>P</i> = 0.0056	<i>P</i> = 0.0037
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = <0.0001	<i>P</i> = <0.0001	<i>P</i> = 0.0004	<i>P</i> = 0.0117
Donkey Control for GnRH ( <i>n</i> =4)	200.3 $\pm$ 13.50	202.5 $\pm$ 11.21	198.0 $\pm$ 12.07	202.0 $\pm$ 10.5
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treated with GnRH ( <i>n</i> =4)	1626.0 $\pm$ 439.4	1732.0 $\pm$ 356.2	1070.3 $\pm$ 362.9	802.5 $\pm$ 294.8
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = 0.0166	<i>P</i> = 0.0298
Compared with -72 hour values			<i>P</i> = 0.0064	<i>P</i> = 0.0388
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0048	<i>P</i> = 0.0016	<i>P</i> = 0.0086	<i>P</i> = 0.0277

Figure 18: Effect of GnRH and saline (*control*) treatment on reaction time (*seconds*) in horse and donkey stallions. (Mean  $\pm$  SE).

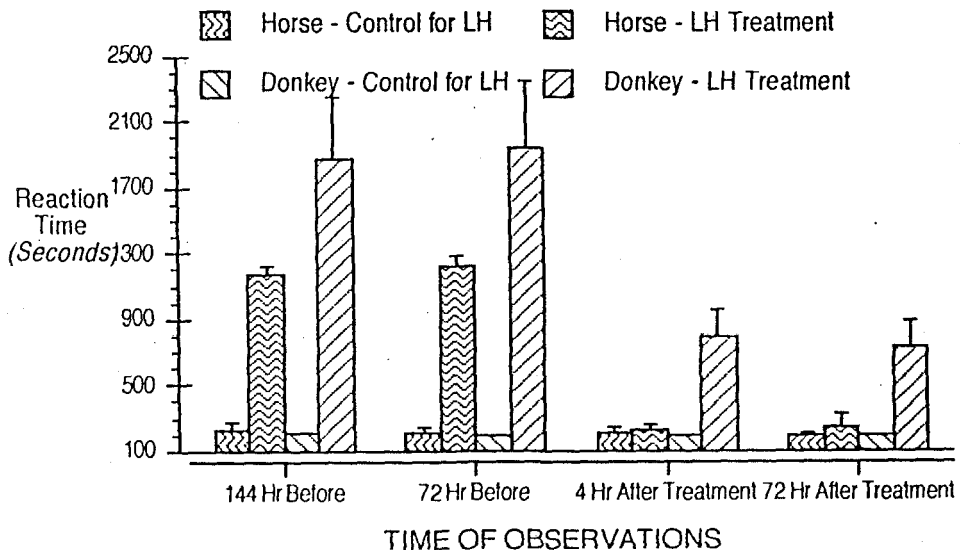


compared to saline treated (-144 and -72 hours) stallions, respectively. LH administration significantly decreased post-treatment reaction time compared to pre-treatment periods in horse and donkey stallions (Table 2 and Figure 19).

Table 2: Effect of LH and saline (*control*) treatment on reaction time (*seconds*) in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Reaction Time ( <i>Seconds</i> )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH ( <i>n</i> =4)	227.9 $\pm$ 41.5	210.8 $\pm$ 36.3	215.8 $\pm$ 30.7	198.0 $\pm$ 18.6
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment with LH ( <i>n</i> =4)	1179.0 $\pm$ 45.46	1222.0 $\pm$ 72.4	219.0 $\pm$ 42.32	241.8 $\pm$ 79.8
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = < 0.0001	<i>P</i> = 0.0011
Compared with -72 hour values			<i>P</i> = 0.0030	<i>P</i> = 0.0037
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = 0.9505	<i>P</i> = 0.5764
Donkey Control for LH ( <i>n</i> =4)	202.5 $\pm$ 11.3	201.3 $\pm$ 9.0	196.3 $\pm$ 8.3	200.5 $\pm$ 10.0
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treatment with LH ( <i>n</i> =4)	1869.0 $\pm$ 386.1	1935.0 $\pm$ 417.9	805.5 $\pm$ 151.0	729.5 $\pm$ 166.8
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = 0.0214	<i>P</i> = 0.0364
Compared with -72 hour values			<i>P</i> = 0.0258	<i>P</i> = 0.0260
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0016	<i>P</i> = 0.0008	<i>P</i> = 0.0490	<i>P</i> = 0.0476

Figure 19: Effect of LH and saline (*control*) treatment on reaction time (*seconds*) in horse and donkey stallions (Mean  $\pm$  SE).



#### 4.3: EFFECT OF GNRH ON PERIPHERAL TESTOSTERONE PROFILES IN HORSE AND DONKEY STALLIONS.

In GnRH controls, plasma testosterone levels ranged from 850.8  $\pm$  81.4 to 994.3  $\pm$  84.6  $\mu$ g/ml in horse stallions while it varied from 640.0  $\pm$  92.8 to 812.5  $\pm$  67.3

pg/ml in donkey stallions (Table 3 and Figure 20). Variations in plasma testosterone profiles were non-significant between pre and post saline treatment periods in stallions. Pre-treatment (-144 and -72 hours) testosterone values were significantly lower in GnRH group of stallions compared to pre-saline treatment periods (-144 and -72 hours), respectively in horse and donkey stallions. GnRH administration in "slow breeder" stallions resulted in significant rise in plasma testosterone concentration at 2 hr post-treatment and remained elevated till 72 hr post-treatment before declining significantly at 120 hr post-treatment (Table 3 and Figure 20).

Table 3: Effect of GnRH and saline (control) treatment on plasma testosterone profiles (pg./ml) in horse and donkey stallions (Mean  $\pm$  SE).

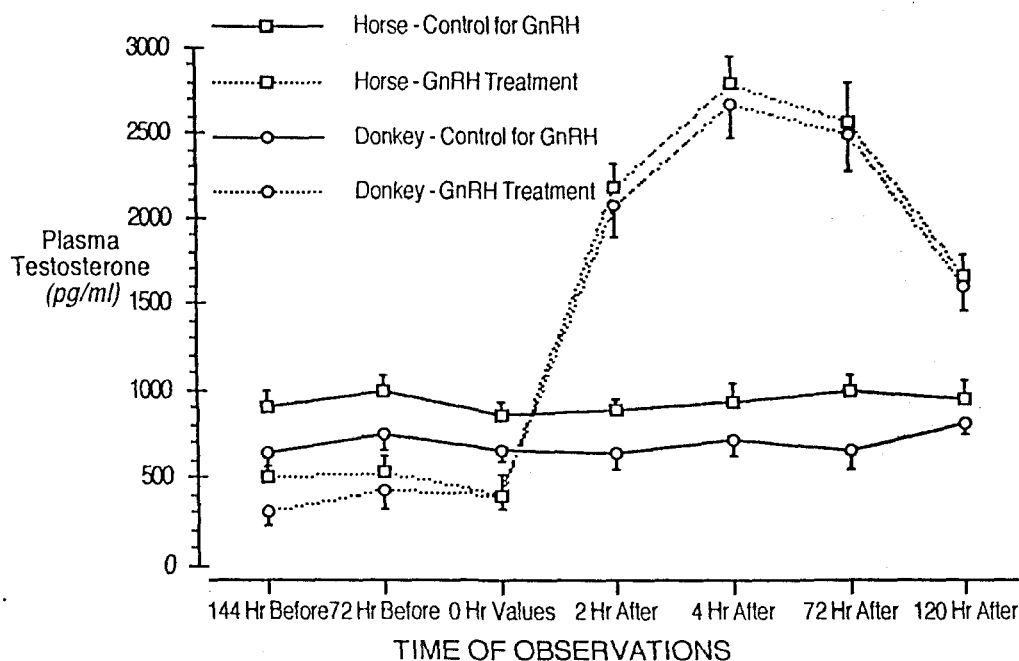
GROUPS AND STATISTICS	Plasma Testosterone (pg/ml)						
	144 Hours Before	72 Hours Before	At Zero Hour	2 Hours After	4 Hours After	72 Hours After	20 Hours After
Horse Control for GnRH (n=4)	904 $\pm$ 83	987 $\pm$ 105	851 $\pm$ 81	885 $\pm$ 67	931 $\pm$ 105	994 $\pm$ 85	942 $\pm$ 107
<i>P</i> Value (Two tail Pair t-test) Compared with -144 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with Zero hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment, GnRH (n=4)	495 $\pm$ 68	526 $\pm$ 102	400 $\pm$ 114	2177 $\pm$ 140	2785 $\pm$ 160	2564 $\pm$ 229	1648 $\pm$ 128
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hr values				<i>P</i> = 0.0033	<i>P</i> = 0.0006	<i>P</i> = 0.0017	<i>P</i> = 0.0045
Compared with -72 hr values				<i>P</i> = 0.0090	<i>P</i> = 0.0025	<i>P</i> = 0.0056	<i>P</i> = 0.0114
Compared with Zero hr values				<i>P</i> = 0.0003	<i>P</i> = < 0.0001	<i>P</i> = 0.0011	<i>P</i> = < 0.0001
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0031	<i>P</i> = 0.0268	<i>P</i> = < 0.0039	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001
Donkey Control for GnRH (n=4)	644 $\pm$ 82	743 $\pm$ 85	656 $\pm$ 63	640 $\pm$ 93	715 $\pm$ 92	655 $\pm$ 103	813 $\pm$ 67
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with Zero hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treatment, GnRH (n=4)	298 $\pm$ 73	424 $\pm$ 114	394 $\pm$ 81	2065 $\pm$ 173	2670 $\pm$ 198	2490 $\pm$ 216	1586 $\pm$ 127
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hr values				<i>P</i> = 0.0004	<i>P</i> = 0.0014	<i>P</i> = 0.0008	<i>P</i> = 0.0017
Compared with -72 hr values				<i>P</i> = 0.0004	<i>P</i> = 0.0009	<i>P</i> = 0.0011	<i>P</i> = 0.0008
Compared with Zero hr values				<i>P</i> = 0.0004	<i>P</i> = 0.0011	<i>P</i> = 0.0014	<i>P</i> = 0.0009
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0082	<i>P</i> = 0.0433	<i>P</i> = 0.0253	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001

#### 4.4: EFFECT OF LH ON PERIPHERAL TESTOSTERONE PROFILES IN HORSE AND DONKEY STALLIONS.

In controls, peripheral testosterone levels varied from 749.0  $\pm$  76.9 to 971.8  $\pm$  106.8 pg/ml and from 633.5  $\pm$  87.1 to 834.0  $\pm$  113.9 pg/ml in horse and donkey stallions, respectively (Table 4 and Figure 21). Variations in plasma testosterone

profiles were non-significant between pre and post-saline treatment periods in stallions. Pre-treatment (-144 and -72 hours) testosterone concentration was significantly lower in LH groups of stallions compared to pre-saline treatment periods (-144 and -72 hours), respectively in horse and donkey stallions. LH administration caused significant increase in peripheral testosterone levels at 2 hr post-treatment and followed an increasing trend up to 72 hr post-treatment and then showed a significant drop at 120 hr post-treatment in stallions (Table 4 and Figure 21).

Figure 20: Effect of GnRH and saline (*control*) treatment on plasma testosterone profiles (*pg./ml*) in horse and donkey stallions (Mean  $\pm$  SE).



#### 4.5: EFFECT OF GNRH ON SEMINAL CHARACTERISTICS IN HORSE AND DONKEY STALLIONS.

##### 4.5.1: GEL VOLUME.

In controls, gel volume varied from  $12.83 \pm 0.74$  to  $13.80 \pm 0.45$  ml in horse stallions while it ranged from  $10.20 \pm 0.77$  to  $10.60 \pm 0.46$  ml in donkey stallions (Table 5 and Figure 22). Gel volume did not vary between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) gel volume was significantly lower in GnRH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. GnRH administration significantly increased post-treatment gel volume compared to pre-treatment periods in stallions (Table 5 and Figure 22).

Table 4: Effect of LH and saline (*control*) treatment on plasma testosterone profiles (*pg./ml*) in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Plasma Testosterone ( <i>pg/ml</i> )						
	144 Hours Before	72 Hours Before	At Zero Hour	2 Hours After	4 Hours After	72 Hours After	20 Hours After
Horse Control for LH ( <i>n</i> =4)	860 $\pm$ 103	972 $\pm$ 107	749 $\pm$ 77	777 $\pm$ 97	922 $\pm$ 76	894 $\pm$ 103	914 $\pm$ 97
<i>P</i> Value (Two tail Pair t-test) Compared with -144 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with Zero hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment with LH ( <i>n</i> =4)	547 $\pm$ 50	362 $\pm$ 66	488 $\pm$ 81	3673 $\pm$ 179	4139 $\pm$ 166	3953 $\pm$ 208	2842 $\pm$ 133
<i>P</i> Value (Two tail Pair t-test) Compared with -144 hr values				<i>P</i> = 0.0002	<i>P</i> = < 0.0001	<i>P</i> = 0.0004	<i>P</i> = 0.0010
Compared with -72 hr values				<i>P</i> = 0.0001	<i>P</i> = 0.0004	<i>P</i> = 0.0004	<i>P</i> = 0.0011
Compared with Zero hr values				<i>P</i> = 0.0011	<i>P</i> = < 0.0001	<i>P</i> = 0.0008	<i>P</i> = 0.0003
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0186	<i>P</i> = 0.0008	<i>P</i> = 0.503	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = 0.0003
Donkey Control for LH ( <i>n</i> =4)	647 $\pm$ 74	722 $\pm$ 100	687 $\pm$ 76	634 $\pm$ 87	834 $\pm$ 114	682 $\pm$ 110	756 $\pm$ 82
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with Zero hr values				<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treated with LH ( <i>n</i> =4)	298 $\pm$ 80	403 $\pm$ 98	372 $\pm$ 69	2994 $\pm$ 174	3523 $\pm$ 263	3324 $\pm$ 150	2012 $\pm$ 146
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hr values				<i>P</i> = 0.0008	<i>P</i> = 0.0015	<i>P</i> = 0.0003	<i>P</i> = 0.0002
Compared with -72 hr values				<i>P</i> = < 0.0001	<i>P</i> = 0.0007	<i>P</i> = 0.0003	<i>P</i> = 0.003
Compared with Zero hr values				<i>P</i> = 0.0014	<i>P</i> = 0.0013	<i>P</i> = 0.0008	<i>P</i> = 0.002
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0078	<i>P</i> = 0.0435	<i>P</i> = 0.0096	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001	<i>P</i> = < 0.0001

Figure 21: Effect of LH and saline (*control*) treatment on plasma testosterone profiles (*pg./ml*) in horse and donkey stallions (Mean  $\pm$  SE).

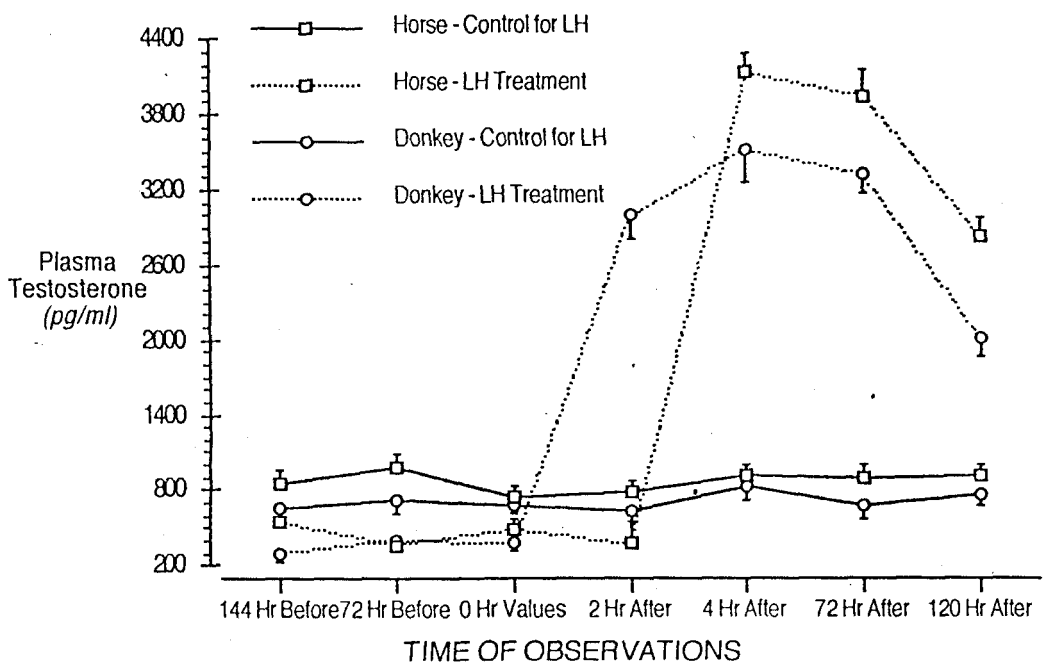
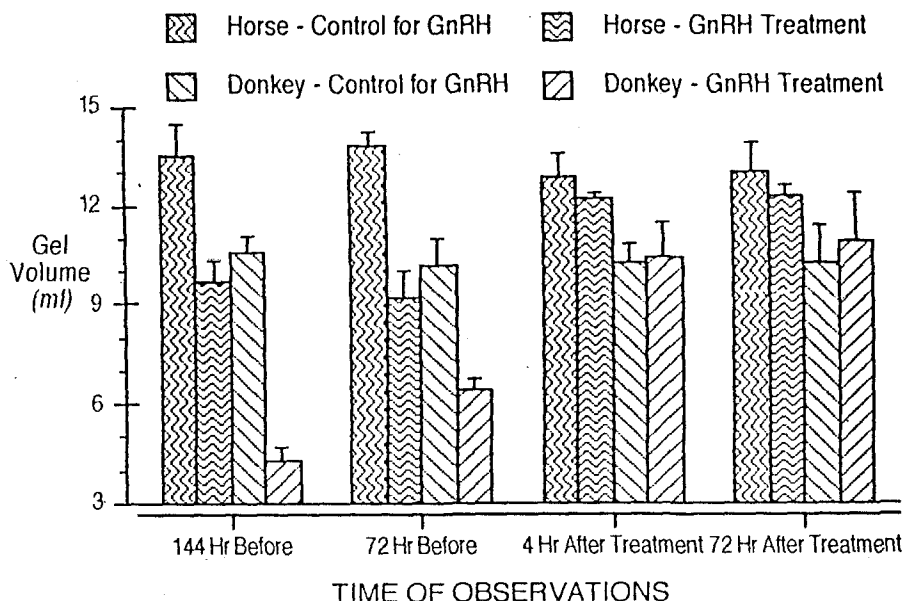


Table 5: Effect of GnRH and saline (*control*) treatment on gel alone, gel free and total semen volume in horse and donkey stallions (Mean ± SE).

GROUPS AND STATISTICS	144 Hours Before			72 Hours Before			4 Hours After			72 hours After		
	GEL VOL	GEL FREE	TOTAL VOL	GEL VOL	GEL FREE	TOTAL VOL	GEL VOL	GEL FREE	TOTAL VOL	GEL VOL	GEL FREE	TOTAL VOL
Horse: Control for GnRH (n=4)	13.5 ± 0.93	70.5 ± 2.09	84.0 ± 3.20	13.8 ± 0.45	75.2 ± 2.32	89.0 ± 2.73	12.8 ± 0.74	70.17 ± 3.16	83.0 ± 3.87	13.1 ± 0.83	73.2 ± 2.00	86.3 ± 3.19
P Value (Two tail Pair t-test) Compared with -144 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Compared with -72 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Horse: GnRH Treatment (n=4)	9.7 ± 0.56	54.0 ± 3.21	63.7 ± 3.77	9.2 ± 0.79	51.5 ± 4.42	60.8 ± 5.21	12.2 ± 0.18	66.3 ± 3.5	78.5 ± 3.59	12.3 ± 0.31	62.4 ± 2.98	74.2 ± 1.69
P Value (Two Tail Pair t-test) Compared with -144 hour values							0.032	0.029	0.021	0.044	0.028	0.034
Compared with -72 hour values							0.040	0.037	0.019	0.046	0.021	0.025
P Value (ANOVA test) Compared with Control at same time interval.	0.009	0.016	0.013	0.001	0.001	0.001	0.519	0.044	0.063	0.426	0.018	0.089
Donkey: Control for GnRH (n=4)	10.6 ± 0.46	54.2 ± 1.92	64.8 ± 2.39	10.2 ± 0.76	56.1 ± 2.73	66.3 ± 3.42	10.3 ± 0.64	52.3 ± 3.19	62.5 ± 3.50	10.3 ± 1.12	53.7 ± 4.48	64.0 ± 5.58
P Value (Two Tail Pair t-test) Compared with -144 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Compared with -72 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Donkey: GnRH Treatment (n=4)	4.3 ± 0.41	34.7 ± 3.30	39.0 ± 3.71	6.4 ± 0.30	36.9 ± 5.69	43.3 ± 5.79	10.4 ± 1.11	46.1 ± 4.51	56.2 ± 3.17	10.9 ± 1.43	51.3 ± 5.61	62.1 ± 5.43
P Value (Two Tail Pair t-test) Compared with -144 hour values							0.026	0.008	0.030	0.043	0.008	0.007
Compared with -72 hour values							0.049	0.012	0.034	0.038	0.011	0.010
P Value (ANOVA test) Compared with Control at same time interval.	<0.0001	0.001	0.001	0.006	0.009	0.006	0.147	0.181	0.158	0.619	0.137	0.086

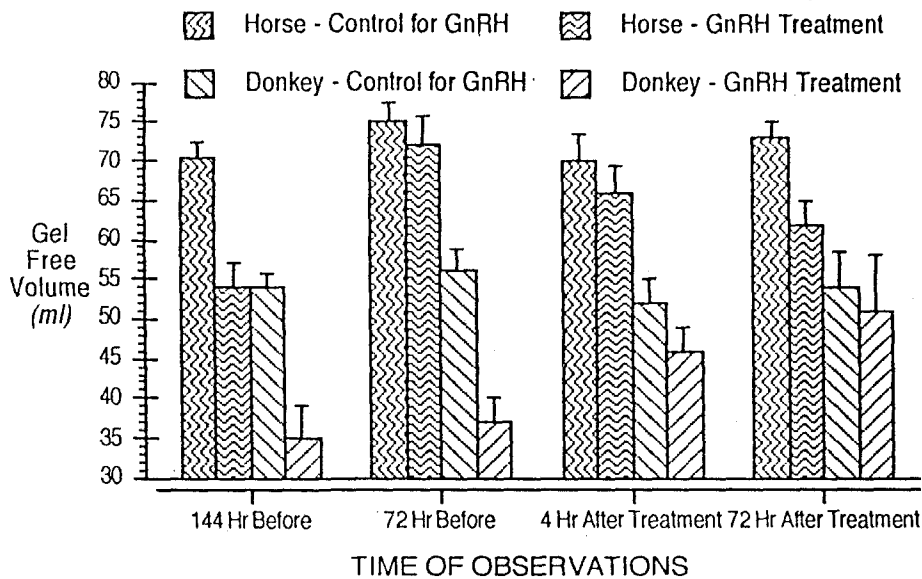
Figure 22: Effect of GnRH and saline (*control*) treatment on gel volume in horse and donkey stallions (Mean ± SE).



#### 4.5.2: GEL FREE VOLUME.

Gel free volume varied from  $70.17 \pm 3.16$  to  $75.20 \pm 2.32$  ml and  $52.25 \pm 3.19$  to  $56.10 \pm 2.73$  ml in saline treated horse and donkey stallions, respectively (Table 5 and Figure 23). Variations in gel free volume were not significantly different between pre and post-saline treatment periods in stallions. Pre-treatment (-144 and -72 hours) gel free volume was significantly lower in GnRH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. Gel free volume was significantly increased in stallions after GnRH administration compared to pre-treatment values (Table 5 and Figure 23).

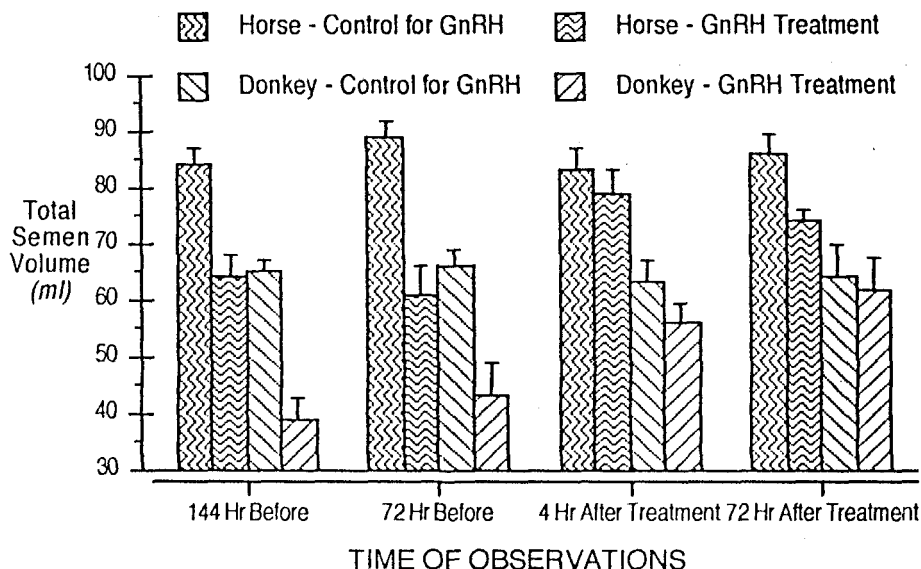
Figure 23: Effect of GnRH and saline (*control*) treatment on gel free volume in horse and donkey stallions (Mean  $\pm$  SE).



#### 4.5.3: TOTAL SEMEN VOLUME.

In controls, total semen volume varied from  $83.0 \pm 3.87$  to  $89.0 \pm 2.73$  ml and from  $62.50 \pm 3.79$  to  $66.3 \pm 3.43$  ml in saline treated horse and donkey stallions, respectively (Table 5 and Figure 24). Total semen volume did not vary between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) total semen volume was significantly lower in GnRH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. GnRH administration significantly increased post-treatment total semen volume compared to pre-treatment periods in stallions (Table 5 and Figure 24).

Figure 24: Effect of GnRH and saline (*control*) treatment on total semen volume in horse and donkey stallions (Mean  $\pm$  SE).



#### 4.5.4: PH.

In saline treated controls, pH ranged from 7.3 to 7.5 and 7.3 to 7.4 in horse and donkey stallions, respectively. The pH of ejaculates did not vary significantly between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) in GnRH groups of horse and donkey stallions was similar to saline treated (-144 and -72 hours) stallions, respectively. GnRH administration did not affect pH of ejaculates in stallions.

#### 4.5.6: MASS ACTIVITY.

Mass activity ranged from 2.75 to 3.0 score in saline treated horse and donkey stallions (Table 6 and Figure 25). Mass activity did not vary significantly between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) mass activity in GnRH group of stallions was similar to saline treated (-144 and -72 hours) stallions, respectively. GnRH administration did not influence mass activity in stallions (Table 6 and Figure 25).

#### 4.5.7: INDIVIDUAL SPERM MOTILITY:

In controls, individual sperm motility ranged from  $70.0 \pm 12.90$  to  $80.0 \pm 11.55$  per cent and from  $70.0 \pm 12.90$  to  $85.0 \pm 9.57$  per cent in horse and donkey stallions, respectively (Table 7 and Figure 26). Variations in individual sperm motility were non-significant between pre and post-saline treatment periods in stallions. Pretreatment (-144 and -72 hours) individual sperm motility was significantly lower in

GnRH group of horse and donkey stallions than in saline treated (-144 and -72 hours) stallions, respectively. GnRH administration significantly increased post-treatment individual sperm motility in stallions (Table 7 and Figure 26).

Table 6: Effect of GnRH and saline (control) treatment on mass activity (score) in gel free ejaculate of horse and donkey stallions (Mean ± SE).

GROUPS AND STATISTICS	Mass Activity (Scoring: 0 to 3)			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH (n=4)	2.75 ± 0.25	3.0 ± 0.0	2.75 ± 0.25	2.75 ± 0.25
P Value (Two tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
Horse treatment, GnRH (n=4)	2.50 ± 0.29	2.50 ± 0.29	2.50 ± 0.29	3.0 ± 0.0
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
P Value (ANOVA test) Compared with Control at same time interval.	P = > 0.05	P = > 0.05	P = > 0.05	P = > 0.05
Donkey Control GnRH (n=4)	2.75 ± 0.25	3.00 ± 0.00	3.00 ± 0.00	2.75 ± 0.25
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
Donkey treatment, GnRH (n=4)	2.75 ± 0.25	2.50 ± 0.29	2.75 ± 0.25	3.00 ± 0.00
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
P Value (ANOVA test) Compared with Control at same time interval.	P = > 0.05	P = > 0.05	P = > 0.05	P = > 0.05

Figure 25: Effect of GnRH and saline (control) treatment on mass activity (score) in gel free ejaculate of horse and donkey stallions (Mean ± SE).

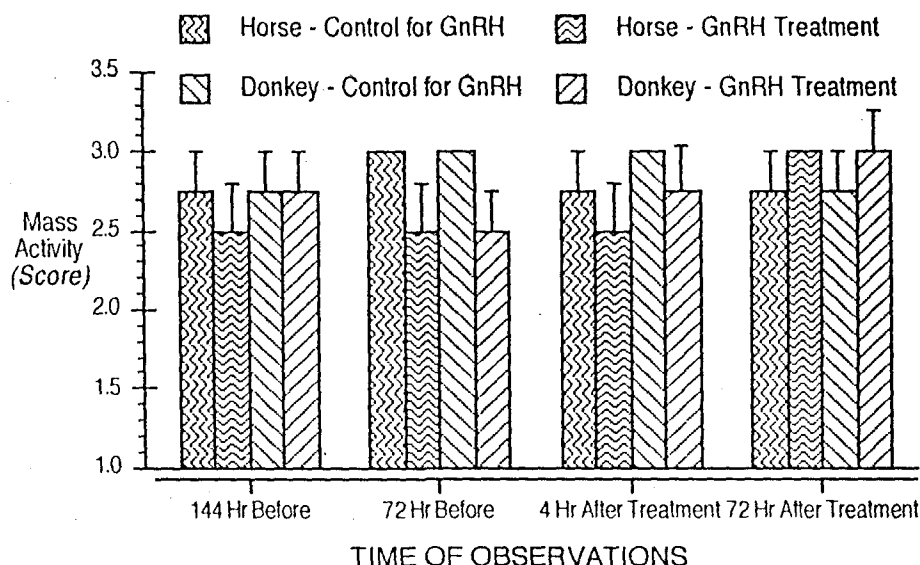
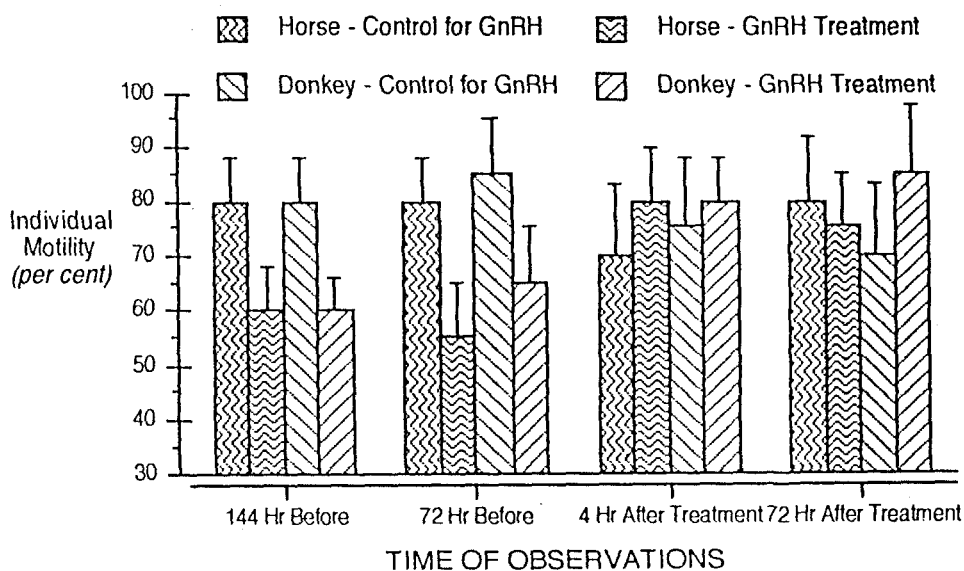


Table 7: Effect of GnRH and saline (control) treatment on individual sperm motility (per cent) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).

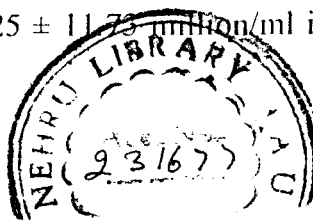
GROUPS AND STATISTICS	Individual Motility (per cent)			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH ( $n=4$ )	80.0 $\pm$ 8.16	80.0 $\pm$ 8.16	70.0 $\pm$ 12.90	80.0 $\pm$ 11.55
<i>P</i> Value (Two tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment, GnRH ( $n=4$ )	60.0 $\pm$ 8.16	55.0 $\pm$ 9.57	80.0 $\pm$ 9.57	75.0 $\pm$ 9.57
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0500$	$P = 0.0311$
Compared with -72 hour values			$P = 0.0148$	$P = > 0.05$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0489$	$P = 0.0431$	$P = 0.07423$	$P = 0.7350$
Donkey Control for GnRH ( $n=4$ )	80.0 $\pm$ 8.16	85.0 $\pm$ 9.57	75.0 $\pm$ 12.58	70.0 $\pm$ 12.9
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment, GnRH ( $n=4$ )	60.0 $\pm$ 5.77	65.0 $\pm$ 9.57	80.0 $\pm$ 8.16	85.0 $\pm$ 12.90
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0053$	$P = 0.0082$
Compared with -72 hour values			$P = 0.0198$	$P = 0.0252$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0453$	$P = 0.0427$	$P = 0.6981$	$P = 0.0000$

Figure 26: Effect of GnRH and saline (control) treatment on individual sperm motility (per cent) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).



#### 4.5.8: SPERM CONCENTRATION.

Sperm concentration varied from 182.25  $\pm$  13.49 to 199.25  $\pm$  11.73 millions/ml and from 179.75  $\pm$  9.73 to 219.25  $\pm$  11.73 million/ml in saline treated



horse and donkey stallions, respectively (Table 8 and Figure 27). Sperm concentration did not vary between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) sperm concentration was significantly lower in GnRH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. GnRH treatment in slow breeder horse and donkey stallions significantly increased post-treatment sperm concentration compared to pre-treatment periods in stallions (Table 8 and Figure 27).

Table 8: Effect of GnRH and saline (control) treatment on sperm concentration ( $10^6/\text{ml}$ ) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Sperm Concentration ( $10^6/\text{ml}$ )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH ( $n=4$ )	185.75 $\pm$ 5.80	191.50 $\pm$ 11.18	182.25 $\pm$ 13.49	199.25 $\pm$ 13.22
<i>P</i> Value (Two tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment, GnRH ( $n=4$ )	121.25 $\pm$ 11.74	110.65 $\pm$ 9.49	176.00 $\pm$ 6.02	183.25 $\pm$ 9.08
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0459$	$P = 0.0393$
Compared with -72 hour values			$P = 0.0438$	$P = 0.0371$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0409$	$P = 0.0311$	$P = 0.3517$	$P = 0.0879$
Donkey Control for GnRH ( $n=4$ )	179.75 $\pm$ 9.88	190.25 $\pm$ 8.89	206.00 $\pm$ 16.70	219.75 $\pm$ 11.73
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment, GnRH ( $n=4$ )	103.00 $\pm$ 15.01	121.25 $\pm$ 13.02	164.30 $\pm$ 12.65	182.00 $\pm$ 19.25
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0349$	$P = 0.0145$
Compared with -72 hour values			$P = 0.0365$	$P = 0.0254$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0009$	$P = 0.0076$	$P = 0.0035$	$P = 0.0004$

#### 4.5.9: LIVE SPERM COUNT.

In controls, live sperm count varied from  $80.50 \pm 2.72$  to  $86.50 \pm 2.72$  per cent and from  $77.50 \pm 3.23$  to  $81.25 \pm 1.93$  per cent in horse and donkey stallions, respectively (Table 9 and Figure 28). Variations in live sperm count were non significant between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) live sperm count was significantly lower in GnRH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. GnRH administration significantly increased post-treatment live sperm count compared to pre-treatment periods in horse and donkey stallions (Table 9 and Figure 28).

Figure 27: Effect of GnRH and saline (control) treatment on sperm concentration ( $10^6/ml$ ) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).

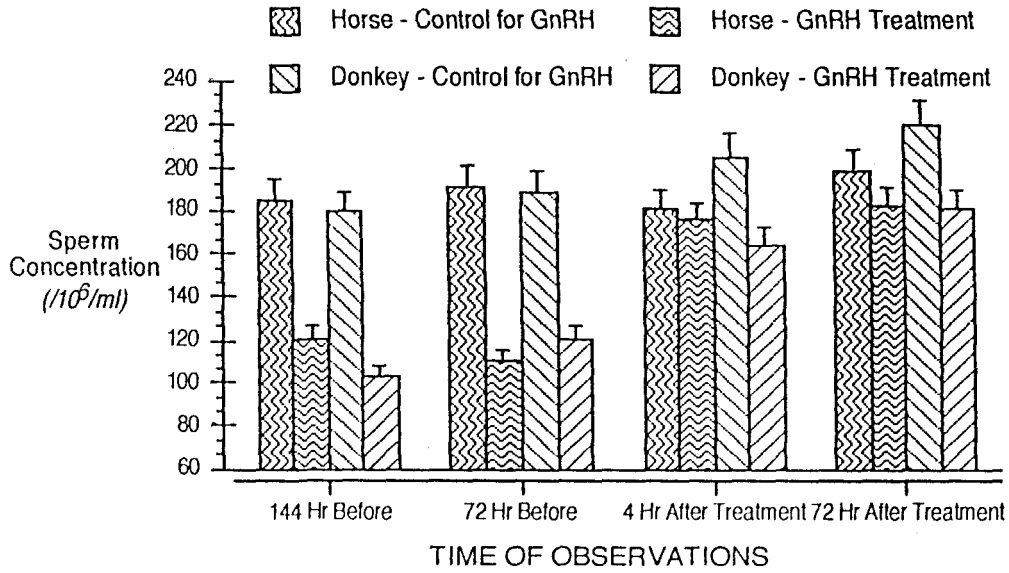
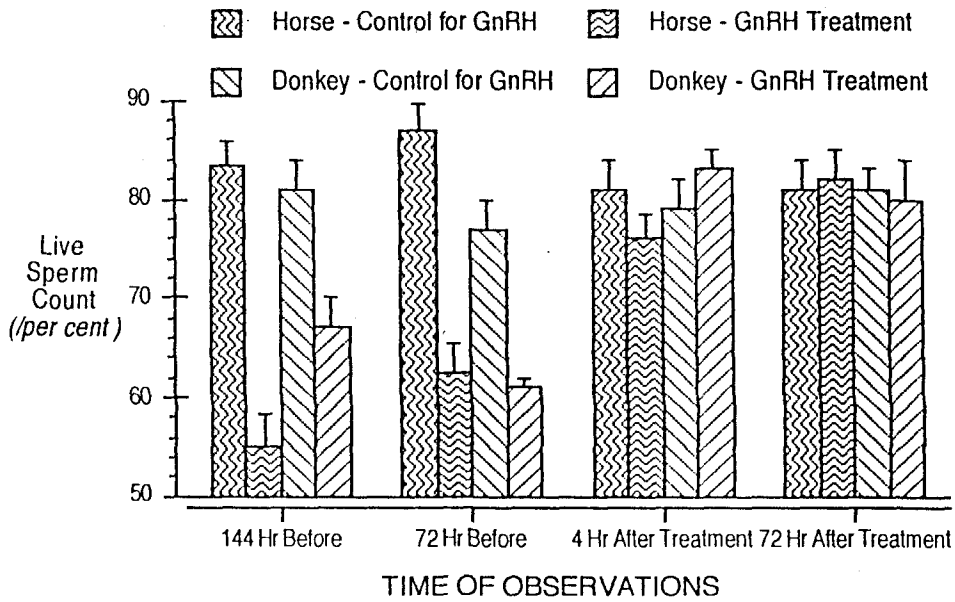


Table 9: Effect of GnRH and saline (control) treatment on live sperm count (per cent) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Live Sperm Count (per cent)			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH (n=4)	83.25 $\pm$ 2.53	86.50 $\pm$ 2.72	81.00 $\pm$ 3.02	80.50 $\pm$ 2.72
P Value (Two tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
Horse treatment, GnRH (n=4)	54.75 $\pm$ 3.50	62.50 $\pm$ 3.30	76.25 $\pm$ 2.69	81.50 $\pm$ 3.12
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = 0.0067	P = 0.0091
Compared with -72 hour values			P = 0.0456	P = 0.0153
P Value (ANOVA test) Compared with Control at same time interval.	P = 0.0433	P = 0.0405	P = 0.1943	P = 0.8127
Donkey Control for GnRH (n=4)	80.75 $\pm$ 3.20	76.50 $\pm$ 3.23	79.25 $\pm$ 3.22	81.25 $\pm$ 1.93
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
Donkey treatment, GnRH (n=4)	67.00 $\pm$ 3.16	61.50 $\pm$ 1.03	83.25 $\pm$ 1.88	80.50 $\pm$ 3.70
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = 0.0067	P = 0.0153
Compared with -72 hour values			P = 0.0021	P = 0.0079
P Value (ANOVA test) Compared with Control at same time interval.	P = 0.0471	P = 0.0372	P = 0.0000	P = 0.0000

Figure 28: Effect of GnRH and saline (*control*) treatment on live sperm count (*per cent*) in ejaculate (*gel free*) of horse and donkey stallions (Mean  $\pm$  SE).



#### 4.6: EFFECT OF LH ON SEMINAL CHARACTERISTICS IN HORSE AND DONKEY STALLIONS.

##### 4.6.1: GEL VOLUME.

In saline treated controls, gel volume ranged from  $14.10 \pm 0.54$  to  $14.80 \pm 0.59$  ml in horse stallions while it varied from  $10.02 \pm 0.75$  to  $11.0 \pm 0.99$  ml in donkey stallions (Table 10 and Figure 29). Variations in gel volume were not significant between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) gel volume was significantly lower in LH group of stallions compared to saline treated (-144 and -72 hours) stallions respectively. LH infusion significantly increased post-treatment gel volume compared to pre-treatment values in stallions (Table 10 and Figure 29).

##### 4.6.2: GEL FREE VOLUME.

Gel free volume varied from  $73.7 \pm 3.20$  to  $75.9 \pm 2.65$  ml and from  $52.2 \pm 3.52$  to  $55.3 \pm 4.85$  ml in saline treated horse and donkey stallions, respectively (Table 10 and Figure 30). Differences in gel free volume of ejaculate were non-significant between pre and post-saline treatment periods in horse and donkey stallions (Table 3 and Fig 4). Pre-treatment (-144 and -72 hours) gel free volume was significantly lower in LH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. Gel free volume was significantly increased in stallions after LH treatment compared to pre-treatment values (Table 10 and Figure 30).

Table 10: Effect of LH and saline (*control*) treatment on gel alone, gel free and total semen volume in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	144 Hours Before			72 Hours Before			4 Hours After			72 Hours After		
	GEL VOL	GEL FREE	TOTAL VOL	GEL VOL	GEL FREE	TOTAL VOL	GEL VOL	GEL FREE	TOTAL VOL	GEL VOL	GEL FREE	TOTAL VOL
Horse: Control for LH (n=4)	148 $\pm$ 1.22	75.9 $\pm$ 2.56	90.8 $\pm$ 3.19	14.8 $\pm$ 0.59	74.5 $\pm$ 3.00	89.3 $\pm$ 3.56	14.3 $\pm$ 0.72	73.7 $\pm$ 3.40	88.0 $\pm$ 4.10	14.1 $\pm$ 0.54	73.7 $\pm$ 3.20	87.8 $\pm$ 3.75
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Compared with -72 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Horse: LH Treatment with LH (n=4)	11.1 $\pm$ 1.22	54.8 $\pm$ 6.85	65.9 $\pm$ 8.04	10.5 $\pm$ 0.95	52.5 $\pm$ 0.95	63.0 $\pm$ 4.79	14.7 $\pm$ 0.20	62.1 $\pm$ 7.20	76.8 $\pm$ 5.90	13.9 $\pm$ 0.12	67.1 $\pm$ 4.72	81.5 $\pm$ 4.62
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hour values							0.038	0.038	0.048	0.049	0.036	0.027
Compared with -72 hour values							0.031	0.043	0.048	0.046	0.038	0.024
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<0.001	0.0010	0.0040	0.0012	0.0008	0.0008	0.0018	0.0083	0.0060	0.3086	0.0148	0.0107
Donkey: Control for LH (n=4)	10.0 $\pm$ 0.75	52.2 $\pm$ 3.52	62.3 $\pm$ 4.26	10.3 $\pm$ 1.26	55.3 $\pm$ 4.86	65.5 $\pm$ 6.11	11.0 $\pm$ 0.99	53.0 $\pm$ 4.88	64.0 $\pm$ 5.84	10.9 $\pm$ 0.86	53.4 $\pm$ 3.77	64.3 $\pm$ 4.17
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Compared with -72 hour values							>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Donkey: LH Treatment (n=4)	5.4 $\pm$ 0.57	37.9 $\pm$ 3.67	43.3 $\pm$ 3.94	7.0 $\pm$ 0.54	34.8 $\pm$ 3.43	41.8 $\pm$ 3.85	10.0 $\pm$ 0.54	48.5 $\pm$ 3.33	58.5 $\pm$ 3.50	11.9 $\pm$ 2.63	45.9 $\pm$ 6.88	56.1 $\pm$ 5.10
<i>P</i> Value (Two Tail Pair t-test) Compared with -144 hour values							0.042	0.027	0.033	0.005	0.033	0.039
Compared with -72 hour values							0.049	0.017	0.030	0.008	0.034	0.029
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<0.0001	0.007	0.014	0.008	0.009	0.128	0.049	0.225	0.522	0.049	0.137	0.131

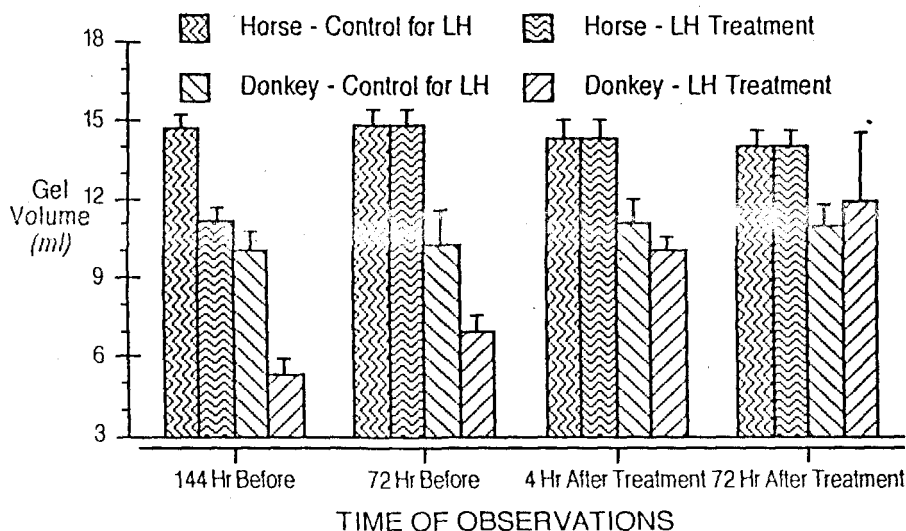
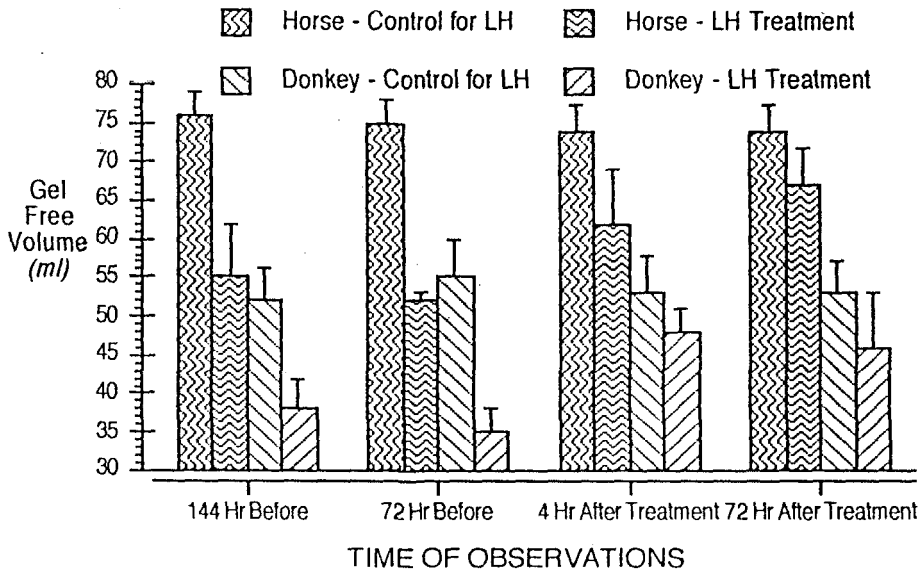
Figure 29: Effect of LH and saline (*control*) treatment on gel volume (ml) in horse and donkey stallions (Mean  $\pm$  SE).

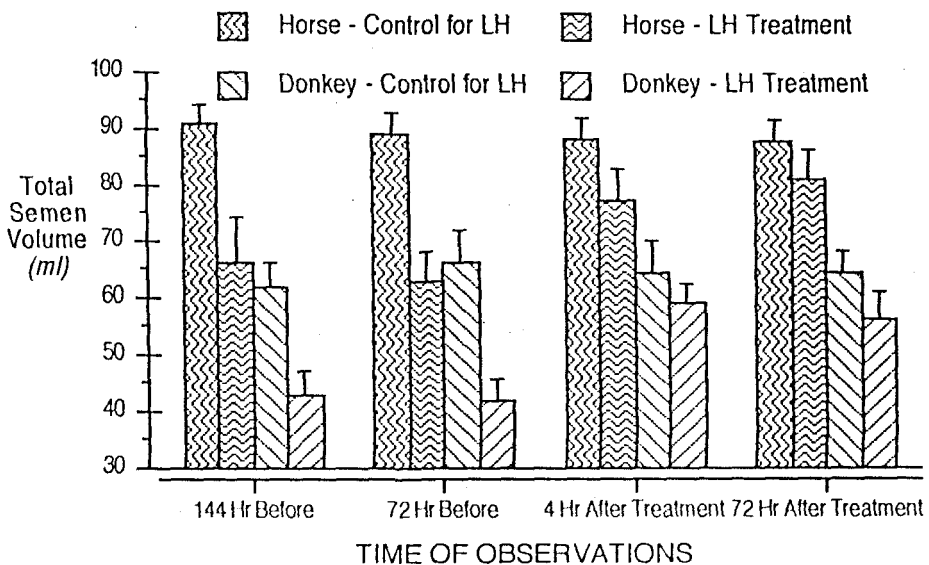
Figure 30: Effect of LH and saline (*control*) treatment on gel free volume (*ml*) in horse and donkey stallions (Mean  $\pm$  SE).



4.6.3: TOTAL SEMEN VOLUME.

In saline treated controls, total semen volume ranged from  $88.0 \pm 4.10$  to  $90.8 \pm 3.19$  ml and from  $62.3 \pm 4.26$  to  $65.50 \pm 6.11$  ml in horse and donkey stallions, respectively (Table 10 and Figure 31). Variations in total semen volume were non-significant between pre and post-saline treatment periods in horse and donkey stallions. Pre-treatment (-144 and -72 hours) total semen volume was significantly lower in LH group of stallions compared to saline treated (-144 and -72

Figure 31: Effect of LH and saline (*control*) treatment on total semen volume (*ml*) in horse and donkey stallions (Mean  $\pm$  SE).



hours) stallions, respectively (Table 3 and Figure 5). LH injection significantly increased post-treatment total semen volume in stallions (Table 10 and Figure 31).

#### 4.6.4: PH

The mean pH ranged from 7.3 to 7.5 and 7.3 to 7.4 in saline treated horse and donkey stallions, respectively. Variations in pH were not significant between pre and post-saline treatment periods in stallions. Pre-treatment (-144 and -72 hours) in LH groups of horse and donkey stallions was similar to saline treated (-144 and -72 hours) control stallions, respectively. The pH of ejaculates was not affected by LH treatment in stallions.

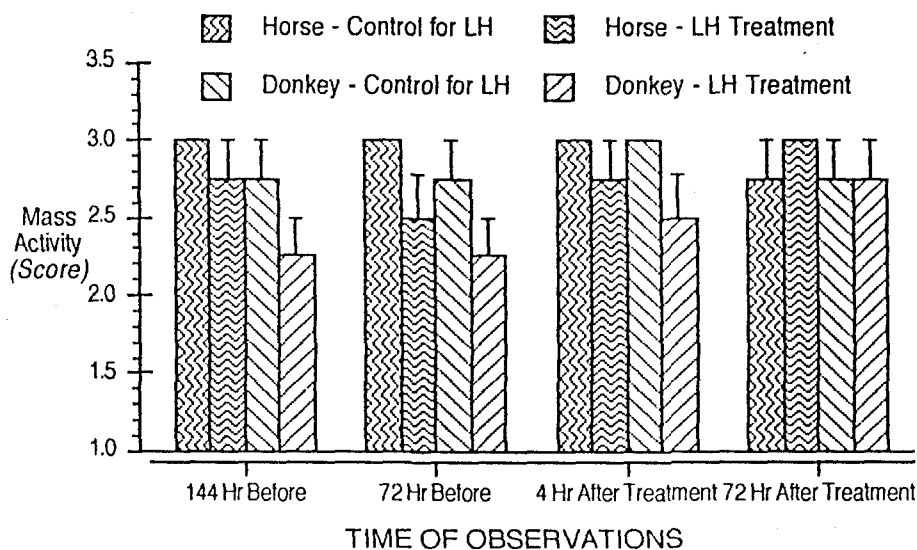
#### 4.6.5: MASS ACTIVITY.

In controls, mass activity of ejaculates varied from  $2.75 \pm 0.25$  to  $3.00 \pm 0.00$  scores in horse and donkey stallions (Table 11 and Figure 32). Variations in mass activity of ejaculates were also non-significant between pre and post-saline treatment periods in stallions. Pre-treatment (-144 and -72 hours) mass activity in LH group of stallions was similar to saline treated (-144 and -72 hours) stallions, respectively. LH treatment in slow breeder stallions did not influence mass activity.

Table 11: Effect of LH and saline (*control*) treatment on mass activity (*score*) in gel free ejaculate of horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Mass Activity (Scoring: 0 to 3)			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH ( $n = 4$ )	$3.00 \pm 0.00$	$3.00 \pm 0.00$	$3.00 \pm 0.00$	$2.75 \pm 0.25$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment, LH ( $n = 4$ )	$2.75 \pm 0.25$	$2.50 \pm 0.29$	$2.75 \pm 0.25$	$3.00 \pm 0.00$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = > 0.05$	$P = > 0.05$	$P = > 0.05$	$P = > 0.05$
Donkey Control for LH ( $n = 4$ )	$2.75 \pm 0.25$	$2.75 \pm 0.25$	$3.00 \pm 0.00$	$2.75 \pm 0.25$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment, LH ( $n = 4$ )	$2.25 \pm 0.25$	$2.25 \pm 0.25$	$2.50 \pm 0.29$	$2.75 \pm 0.25$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = > 0.05$	$P = > 0.05$	$P = > 0.05$	$P = > 0.05$

Figure 32: Effect of LH and saline (*control*) treatment on mass activity (*score*) in gel free ejaculate of horse and donkey stallions (Mean  $\pm$  SE).



#### 4.6.6: INDIVIDUAL SPERM MOTILITY:

Individual sperm motility of ejaculates ranged from  $86.0 \pm 5.0$  to  $90.0 \pm 10.0$  per cent and from  $80.0 \pm 8.16$  to  $80.0 \pm 9.57$  per cent in saline treated horse and donkey stallions, respectively (Table 12 and Figure 33). Non-significant differences in individual sperm motility were recorded between pre and post-saline treatment periods in stallions. Pretreatment (-144 and -72 hours) individual sperm motility in LH group of horse and donkey stallions was significantly lower than in saline treated (-144 and -72 hours) stallions, respectively. LH treatment significantly increased post-treatment individual sperm motility compared to pre-treatment periods in stallions (Table 12 and Figure 33).

#### 4.6.7: SPERM CONCENTRATION.

In controls, sperm concentration was significantly ( $P < 0.05$ ) higher in horse ( $187.25 \pm 19.33$  to  $205.25 \pm 28.78$   $10^6/\text{ml}$ ) than in donkey ( $184.75 \pm 18.88$  to  $210.50 \pm 11.06$   $10^6/\text{ml}$ ) stallions (Table 13 and Figure 34). Non-significant variations were found in sperm concentration between pre and post-saline injection periods in horse and donkey stallions. Pre-treatment (-144 and -72 hours) sperm concentration was significantly lower in LH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. LH administration in slow breeder horse and donkey stallions significantly increased post-treatment sperm concentration compared to pre-treatment periods in horse and donkey stallions (Table 13 and Figure 34).

Table 12: Effect of LH and saline (*control*) treatment on individual sperm motility (*per cent*) in ejaculate (*gel free*) of horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Individual Motility ( <i>per cent</i> )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH ( <i>n</i> = 4)	85.0 $\pm$ 5.0	85.0 $\pm$ 9.57	90.0 $\pm$ 5.77	90.0 $\pm$ 10.0
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment with LH ( <i>n</i> = 4)	55.0 $\pm$ 9.57	60.0 $\pm$ 5.77	85.0 $\pm$ 9.57	75.0 $\pm$ 9.57
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = 0.0027	<i>P</i> = 0.0448
Compared with -72 hour values			<i>P</i> = 0.0176	<i>P</i> = 0.0382
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0199	<i>P</i> = 0.0315	<i>P</i> = 0.7243	<i>P</i> = 0.3192
Donkey Control for LH ( <i>n</i> = 4)	90.0 $\pm$ 9.57	80.0 $\pm$ 8.16	80.0 $\pm$ 8.16	80.0 $\pm$ 8.16
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treatment with LH ( <i>n</i> = 4)	70.0 $\pm$ 12.58	65.0 $\pm$ 9.57	85.0 $\pm$ 5.0	80.0 $\pm$ 5.77
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = 0.0491	<i>P</i> = 0.0493
Compared with -72 hour values			<i>P</i> = 0.0471	<i>P</i> = 0.0449
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0500	<i>P</i> = 0.0496	<i>P</i> = 0.6981	<i>P</i> = 0.5098

Figure 33: Effect of LH and saline (*control*) treatment on individual sperm motility (*per cent*) in ejaculate (*gel free*) of horse and donkey stallions (Mean  $\pm$  SE).

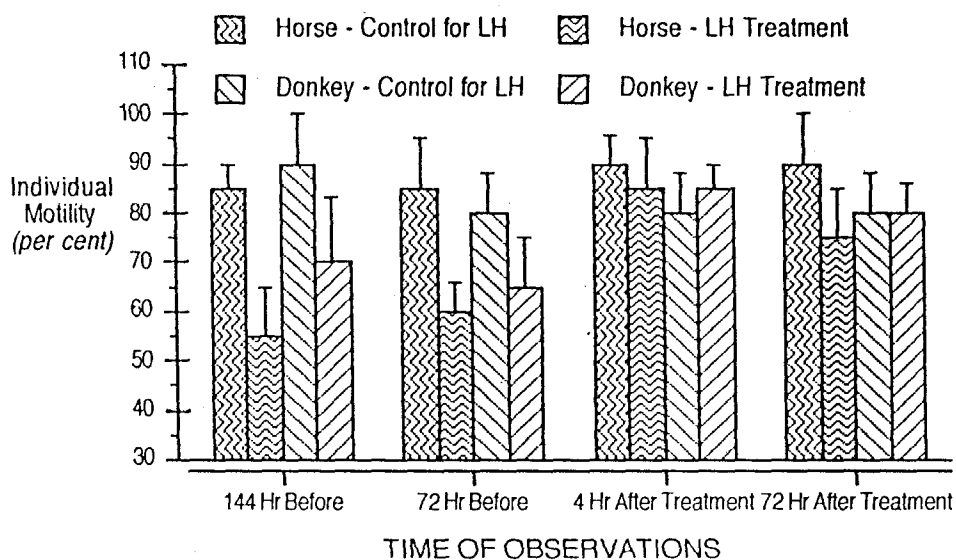
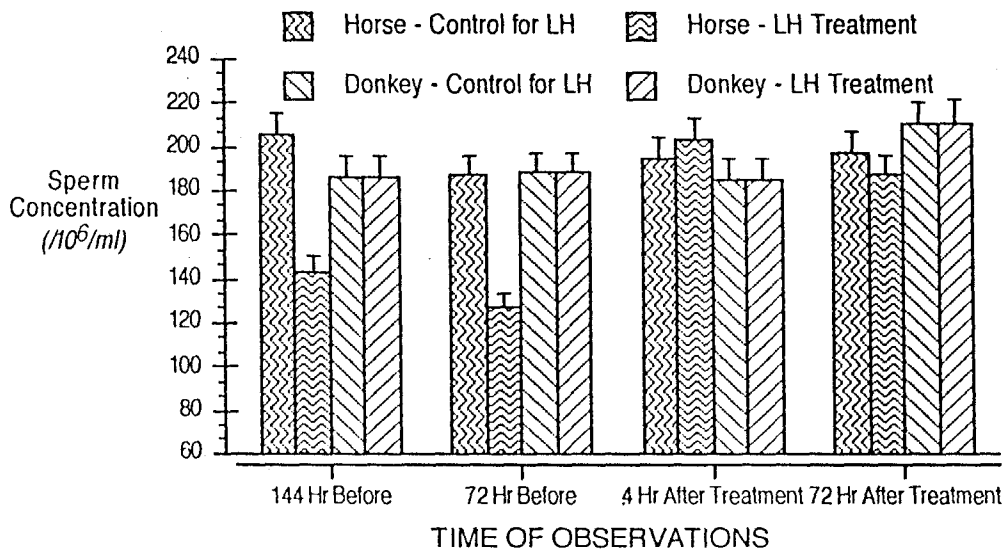


Table 13: Effect of LH and saline (*control*) treatment on sperm concentration ( $10^6/ml$ ) in ejaculate (*gel free*) of horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Sperm Concentration ( $10^6/ml$ )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH ( $n=4$ )	205.25 $\pm$ 28.78	187.25 $\pm$ 19.33	195.00 $\pm$ 28.62	197.25 $\pm$ 19.64
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment with LH ( $n=4$ )	142.75 $\pm$ 13.73	126.50 $\pm$ 12.19	203.45 $\pm$ 9.46	186.61 $\pm$ 11.33
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0407$	$P = 0.0481$
Compared with -72 hour values			$P = 0.0382$	$P = 0.0442$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0305$	$P = 0.0432$	$P = 0.3642$	$P = 0.1625$
Donkey Control for LH ( $n=4$ )	186.00 $\pm$ 14.47	187.75 $\pm$ 12.33	184.75 $\pm$ 18.88	210.50 $\pm$ 11.06
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment with LH ( $n=4$ )	109.50 $\pm$ 9.07	100.75 $\pm$ 10.55	186.20 $\pm$ 16.05	169.25 $\pm$ 9.03
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0211$	$P = 0.0415$
Compared with -72 hour values			$P = 0.0206$	$P = 0.0427$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0009$	$P = < 0.0001$	$P = 0.0076$	$P = 0.0003$

Figure 34: Effect of LH and saline (*control*) treatment on sperm concentration ( $10^6/ml$ ) in ejaculate (*gel free*) of horse and donkey stallions (Mean  $\pm$  SE).



#### 4.6.8: LIVE SPERM COUNT.

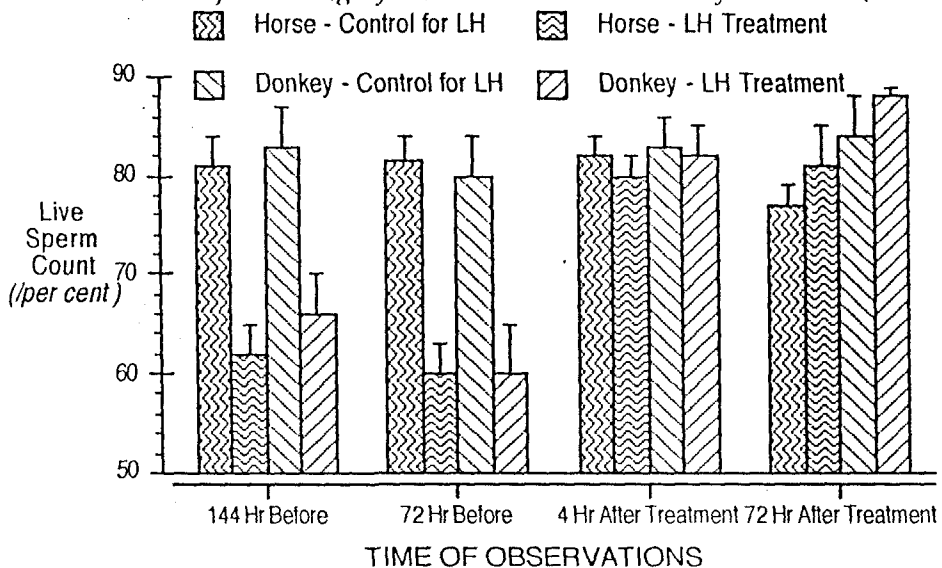
In controls, live sperm count varied from  $77.50 \pm 2.10$  to  $82.25 \pm 2.01$  per cent and from  $79.75 \pm 13.63$  to  $83.75 \pm 4.25$  per cent in horse and donkey stallions, respectively (Table 14 and Figure 35). Live sperm count did not vary

significantly between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) live sperm count of ejaculates in LH treated stallions was significantly lower than saline treated stallions (-144 and -72 hours), respectively. LH treatment significantly increased post-treatment live sperm count in horse and donkey stallions (Table 14 and Figure 35).

Table 14: Effect of LH and saline (control) treatment on live sperm count (per cent) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Live Sperm Count (per cent)			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH (n=4)	81.25 $\pm$ 2.63	81.50 $\pm$ 2.53	82.25 $\pm$ 2.01	77.50 $\pm$ 2.10
P Value (Two Tail Pair t-test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment with LH (n=4)	62.25 $\pm$ 2.95	60.25 $\pm$ 3.03	79.25 $\pm$ 1.85	81.02 $\pm$ 3.53
P Value (Two Tail Pair t-test) Compared with -144 hour values			$P = 0.0337$	$P = 0.0072$
Compared with -72 hour values			$P = 0.0341$	$P = 0.0206$
P Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0319$	$P = 0.0419$	$P = 0.0745$	$P = 0.1413$
Donkey Control for LH (n=4)	83.00 $\pm$ 4.02	79.75 $\pm$ 3.63	82.50 $\pm$ 2.98	83.75 $\pm$ 4.25
P Value (Two Tail Pair t-test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment with LH (n=4)	65.50 $\pm$ 3.48	60.00 $\pm$ 4.88	82.00 $\pm$ 3.24	87.50 $\pm$ 0.64
P Value (Two Tail Pair t-test) Compared with -144 hour values			$P = 0.0073$	$P = 0.0041$
Compared with -72 hour values			$P = 0.0151$	$P = 0.0037$
P Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0266$	$P = 0.0311$	$P = 0.9046$	$P = 0.3937$

Figure 35: Effect of LH and saline (control) treatment on live sperm count (per cent) in ejaculate (gel free) of horse and donkey stallions (Mean  $\pm$  SE).



## 4.7: EFFECT OF GnRH ON SEMINAL BIOCHEMICAL CONSTITUENTS IN HORSE AND DONKEY STALLIONS.

### 4.7.1: CITRIC ACID.

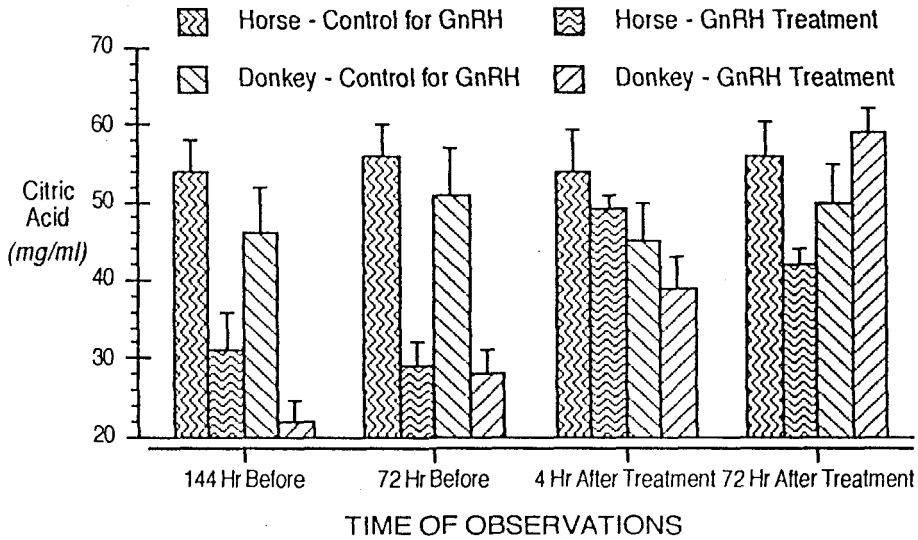
In GnRH controls, citric acid content of seminal plasma ranged from  $54.10 \pm 5.30$  to  $56.35 \pm 4.14$  mg/dl in horse stallions while it varied from  $45.45 \pm 5.44$  to  $51.20 \pm 6.10$  mg/dl in donkey stallions (Table 15 and Figure 36). Variations in citric acid content were non-significant between pre and post-saline treatment periods in horse and donkey stallions.

Table 15: Effect of GnRH and saline (*control*) treatment on citric acid content of seminal plasma ( $mg.dl^{-1}$ ) in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Citric Acid ( $mg.dl^{-1}$ )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH ( $n=4$ )	$54.20 \pm 3.56$	$56.35 \pm 4.14$	$54.10 \pm 5.30$	$55.65 \pm 4.44$
<i>P</i> Value (Two tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment, GnRH ( $n=4$ )	$30.60 \pm 4.79$	$28.50 \pm 3.35$	$48.75 \pm 2.15$	$42.01 \pm 1.83$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0304$	$P = 0.0473$
Compared with -72 hour values			$P = 0.0315$	$P = 0.0412$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0023$	$P = 0.0016$	$P = 0.0342$	$P = 0.0088$
Donkey Control, GnRH ( $n=4$ )	$45.72 \pm 5.97$	$51.20 \pm 6.10$	$45.45 \pm 5.44$	$50.00 \pm 5.25$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment, GnRH ( $n=4$ )	$21.85 \pm 2.83$	$28.25 \pm 3.22$	$38.65 \pm 3.72$	$58.73 \pm 2.73$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0096$	$P = 0.0058$
Compared with -72 hour values			$P = 0.0291$	$P = 0.0496$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0070$	$P = 0.0131$	$P = 0.4555$	$P = 0.0484$

Pre-treatment (-144 and -72 hours) citric acid content of seminal plasma was significantly lower in GnRH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. GnRH treatment significantly increased post-treatment citric acid content compared to pre-treatment periods in horse and donkey stallions (Table 15 and Figure 36).

Figure 36: Effect of GnRH and saline (*control*) treatment on citric acid content of seminal plasma ( $mg.dl^{-1}$ ) in horse and donkey stallions (Mean  $\pm$  SE).



#### 4.7.2: GLUTAMIC OXALOACETIC TRANSAMINASE

Glutamic oxaloacetic transaminase (GOT) activity of seminal plasma varied from  $175.02 \pm 18.67$  to  $194.47 \pm 39.46$  IU/ml and from  $283.42 \pm 57.30$  to  $299.0 \pm 57.35$  IU/ml in saline treated horse and donkey stallions, respectively (Table 16 and Figure 37). Variations in GOT activity were non-significant between pre and post-saline treatment periods in horse and donkey stallions. Pre-treatment (-144 and -72 hours) GOT activity of seminal plasma was significantly higher in GnRH group of stallions compared to saline treated periods (-144 and -72 hours) in stallions, respectively. Significant drop in GOT activity was recorded at 72 hr after GnRH administration in horse and donkey stallions (Table 16 and Figure 37).

#### 4.7.3: GLUTAMIC PYRUVIC TRANSAMINASE.

In saline treated controls, glutamic pyruvic transaminase (GPT) activity of seminal plasma varied from  $28.57 \pm 2.30$  to  $32.80 \pm 4.20$  IU/ml and from  $21.22 \pm 5.82$  to  $25.0 \pm 2.53$  IU/ml in horse and donkey stallions, respectively (Table 17 and Figure 38). Variations in GPT activity were non-significant between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) GPT activity of seminal plasma was significantly higher in GnRH group of stallions compared to saline treatment periods (-144 and -72 hours) in control stallions. Significant decline in GPT activity was noticed at 72 hr after GnRH treatment in horse and donkey stallions (Table 17 and Figure 38).

Table 16: Effect of GnRH and saline (*control*) treatment on glutamic oxaloacetic transaminase activity (*IU/ml*) of seminal plasma in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Glutamic Oxaloacetic Transaminase ( <i>IU.ml<sup>-1</sup></i> )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH ( <i>n=4</i> )	194.47 $\pm$ 39.46	176.67 $\pm$ 16.33	190.67 $\pm$ 37.15	175.02 $\pm$ 18.67
<i>P</i> Value (Two tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment, GnRH ( <i>n=4</i> )	461.50 $\pm$ 45.89	443.25 $\pm$ 54.16	316.40 $\pm$ 69.74	230.22 $\pm$ 40.25
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0496
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0441
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0119	<i>P</i> = 0.0032	<i>P</i> = 0.0023	<i>P</i> = 0.0040
Donkey Control, GnRH ( <i>n=4</i> )	288.00 $\pm$ 49.11	297.05 $\pm$ 52.05	283.42 $\pm$ 57.30	299.00 $\pm$ 57.35
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treatment, GnRH ( <i>n=4</i> )	429.12 $\pm$ 32.32	421.45 $\pm$ 53.38	296.10 $\pm$ 51.69	221.13 $\pm$ 49.26
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0247
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0253
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0348	<i>P</i> = 0.0372	<i>P</i> = 0.0954	<i>P</i> = 0.1962

Figure 37: Effect of GnRH and saline (*control*) treatment on glutamic oxaloacetic transaminase activity (*IU/ml*) of seminal plasma in horse and donkey stallions (Mean  $\pm$  SE).

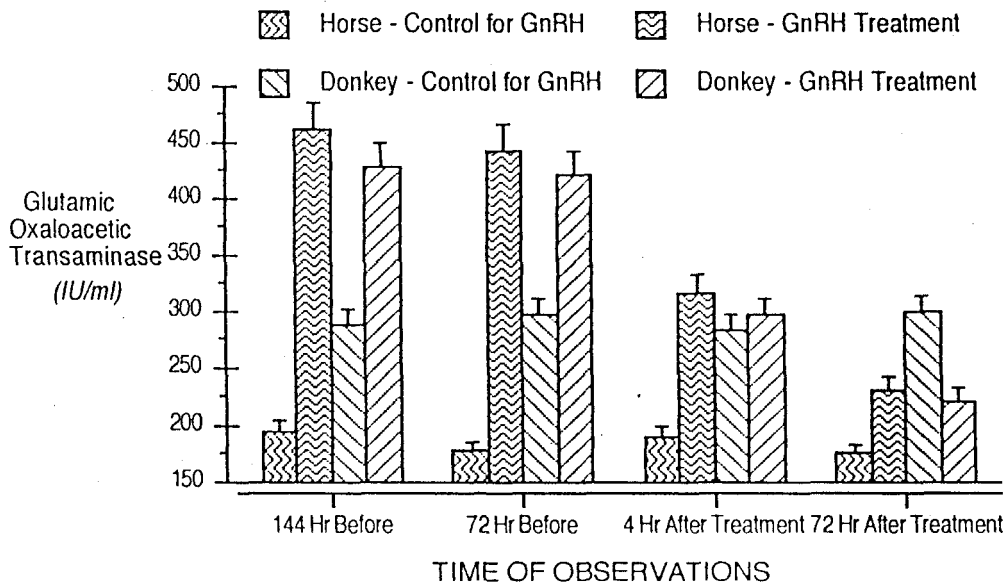
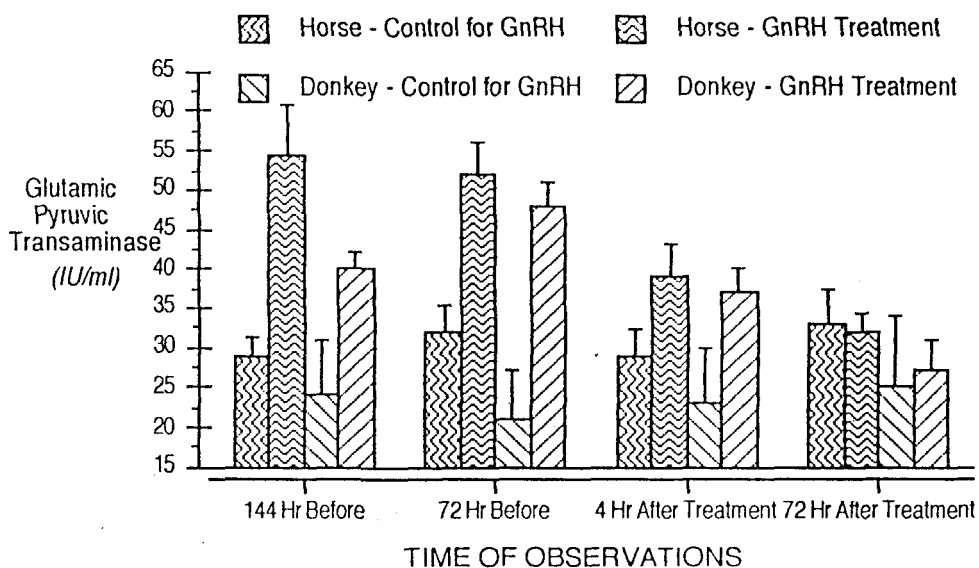


Table 17: Effect of GnRH and saline (*control*) treatment on glutamic pyruvic transaminase activity (*IU/ml*) of seminal plasma in four horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Glutamic Pyruvic Transaminase ( <i>IU.ml<sup>-1</sup></i> )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for GnRH ( <i>n</i> =4)	28.57 $\pm$ 2.30	32.00 $\pm$ 3.28	28.70 $\pm$ 3.43	32.80 $\pm$ 4.20
<i>P</i> Value (Two tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment, GnRH ( <i>n</i> =4)	54.40 $\pm$ 6.33	51.87 $\pm$ 3.97	39.15 $\pm$ 4.14	31.57 $\pm$ 2.44
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0280
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0271
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0025	<i>P</i> = 0.0087	<i>P</i> = 0.0105	<i>P</i> = 0.4662
Donkey Control, GnRH ( <i>n</i> =4)	23.75 $\pm$ 6.99	21.22 $\pm$ 5.82	22.75 $\pm$ 7.25	25.00 $\pm$ 2.53
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treatment, GnRH ( <i>n</i> =4)	40.05 $\pm$ 4.68	48.22 $\pm$ 6.06	37.05 $\pm$ 9.72	26.82 $\pm$ 9.16
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0242
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0276
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0046	<i>P</i> = 0.0032	<i>P</i> = 0.6697	<i>P</i> = 0.6429

Figure 38: Effect of GnRH and saline (*control*) treatment on glutamic pyruvic transaminase activity (*IU/ml*) of seminal plasma in four horse and donkey stallions (Mean  $\pm$  SE).



#### 4.8: EFFECT OF LH ON SEMINAL BIOCHEMICAL CONSTITUENTS IN HORSE AND DONKEY STALLIONS.

##### 4.8.1: CITRIC ACID.

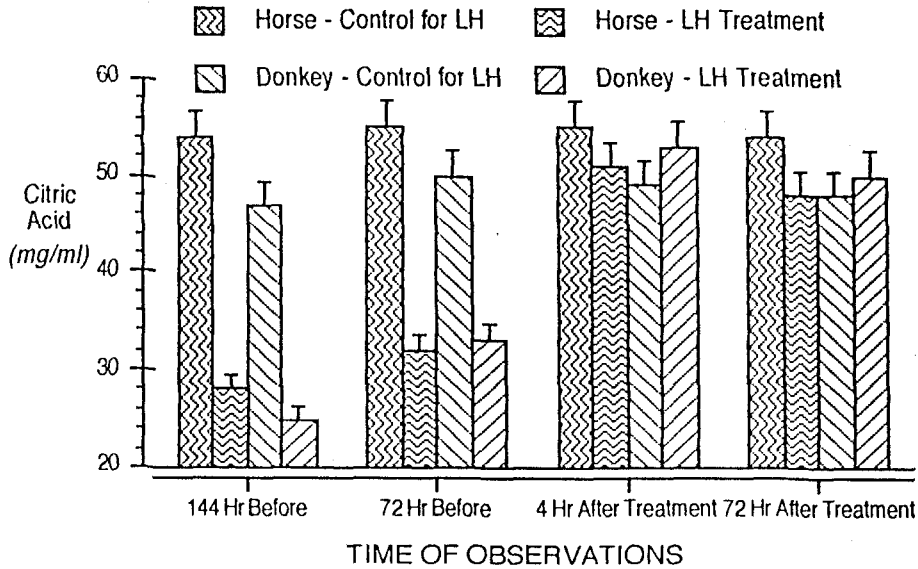
Citric acid content of seminal plasma varied from  $53.95 \pm 3.03$  to  $55.07 \pm 7.0$  mg/dl and from  $46.60 \pm 7.61$  to  $49.80 \pm 6.35$  mg/dl in saline treated horse and donkey stallions, respectively (Table 18 and Figure 39). Variations in citric acid content were non-significant between pre and post-saline treatment periods in horse and donkey stallions.

Table 18: Effect of LH and saline (*control*) treatment on citric acid content of seminal plasma ( $mg.dl^{-1}$ ) in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Citric Acid ( $mg.dl^{-1}$ )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH ( $n=4$ )	$54.42 \pm 3.64$	$54.85 \pm 5.70$	$55.07 \pm 7.00$	$53.95 \pm 3.03$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Horse treatment with LH ( $n=4$ )	$28.10 \pm 5.17$	$31.62 \pm 5.18$	$51.25 \pm 6.08$	$47.65 \pm 3.76$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0473$	$P = 0.0381$
Compared with -72 hour values			$P = 0.0412$	$P = 0.0302$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0017$	$P = 0.0055$	$P = 0.2111$	$P = 0.0022$
Donkey Control for LH ( $n=4$ )	$46.60 \pm 7.61$	$49.80 \pm 6.35$	$48.75 \pm 8.12$	$48.10 \pm 7.84$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = > 0.05$	$P = > 0.05$
Compared with -72 hour values			$P = > 0.05$	$P = > 0.05$
Donkey treatment, LH ( $n=4$ )	$25.37 \pm 2.51$	$33.30 \pm 2.46$	$53.27 \pm 5.78$	$49.70 \pm 2.60$
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			$P = 0.0083$	$P = 0.0096$
Compared with -72 hour values			$P = 0.0110$	$P = 0.0481$
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	$P = 0.0136$	$P = 0.0331$	$P = 0.3354$	$P = 0.0870$

Pre-treatment (-144 and -72 hours) citric acid content of seminal plasma was significantly lower in LH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. LH treatment significantly increased post-treatment citric acid content compared to pre-treatment periods in horse and donkey stallions (Table 18 and Figure 39).

Figure 39: Effect of LH and saline (*control*) treatment on citric acid content of seminal plasma ( $mg.dl^{-1}$ ) in horse and donkey stallions (Mean  $\pm$  SE).



#### 4.8.2: GLUTAMIC OXALOACETIC TRANSAMINASE

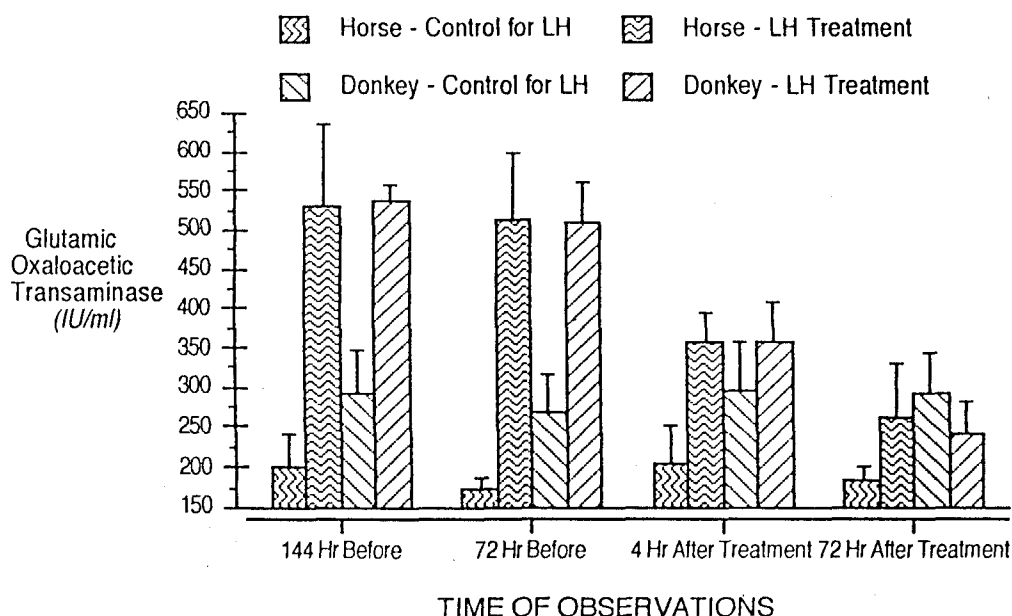
In saline treated controls, glutamic oxaloacetic transaminase (GOT) activity of seminal plasma varied from  $172.0 \pm 13.87$  to  $204.32 \pm 45.90$  IU/ml and from  $266.95 \pm 49.44$  to  $297.0 \pm 59.41$  IU/ml in horse and donkey stallions,

Table 19: Effect of LH and saline (*control*) treatment on glutamic oxaloacetic transaminase activity (IU/ml) of seminal plasma in horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Glutamic Oxaloacetic Transaminase (IU.ml <sup>-1</sup> )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH (n=4)	199.15 $\pm$ 42.74	172.00 $\pm$ 13.87	204.32 $\pm$ 45.90	183.50 $\pm$ 15.66
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
Horse treatment with LH (n=4)	531.50 $\pm$ 103.72	514.87 $\pm$ 84.50	357.25 $\pm$ 38.13	261.40 $\pm$ 69.74
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = 0.0460
Compared with -72 hour values			P = > 0.05	P = 0.0394
P Value (ANOVA test) Compared with Control at same time interval.	P = 0.0242	P = 0.0005	P = 0.0717	P = 0.0039
Donkey Control for LH (n=4)	292.25 $\pm$ 56.10	266.95 $\pm$ 49.44	297.00 $\pm$ 59.46	291.05 $\pm$ 52.19
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = > 0.05
Compared with -72 hour values			P = > 0.05	P = > 0.05
Donkey treatment, LH (n=4)	538.47 $\pm$ 20.78	509.77 $\pm$ 53.39	356.80 $\pm$ 53.42	243.41 $\pm$ 39.71
P Value (Two Tail Pair t-test) Compared with -144 hour values			P = > 0.05	P = 0.0201
Compared with -72 hour values			P = > 0.05	P = 0.0196
P Value (ANOVA test) Compared with Control at same time interval.	P = 0.0298	P = 0.0463	P = 0.1142	P = 0.2896

respectively (Table 19 and Figure 40). Variations in GOT activity were not significant between pre and post-saline treatment periods in horse and donkey stallions. Pre-treatment (-144 and -72 hours) GOT activity of seminal plasma was significantly higher in LH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. LH treatment significantly reduced GOT activity of seminal plasma in horse and donkey stallions (Table 19 and Figure 40).

Figure 40: Effect of LH and saline (*control*) treatment on glutamic oxaloacetic transaminase activity (IU/ml) of seminal plasma in horse and donkey stallions (Mean  $\pm$  SE).



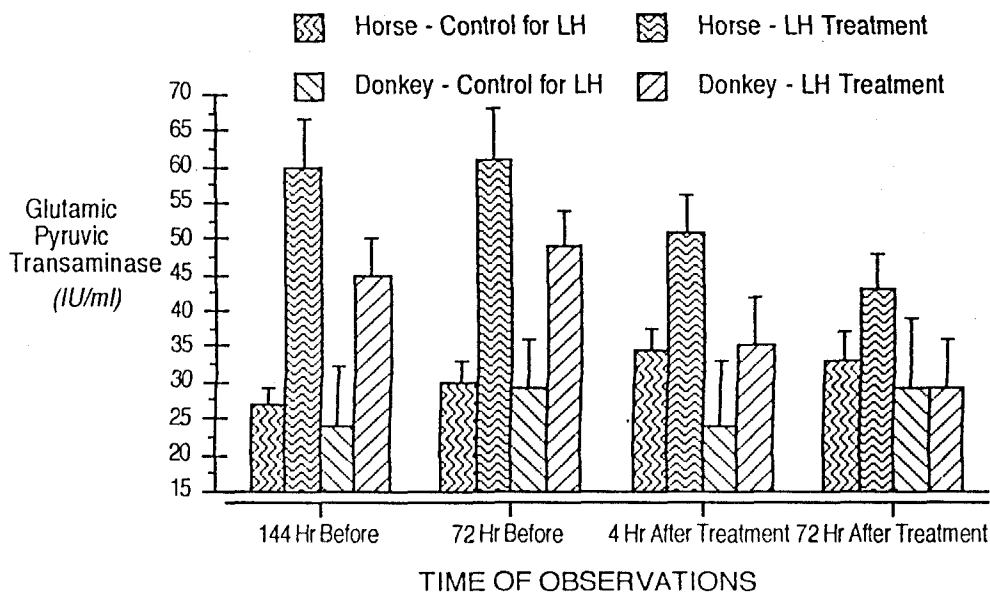
#### 4.8.3: GLUTAMIC PYRUVIC TRANSAMINASE.

In controls, glutamic pyruvic transaminase activity of seminal plasma varied from  $27.0 \pm 1.99$  to  $34.27 \pm 2.93$  IU/ml and from  $24.57 \pm 7.73$  to  $29.32 \pm 9.72$  IU/ml in horse and donkey stallions, respectively (Table 20 and Figure 41). Variations in glutamic pyruvic transaminase activity were non-significant between pre and post-saline treatment periods in control stallions. Pre-treatment (-144 and -72 hours) glutamic pyruvic transaminase activity of seminal plasma was significantly higher in LH group of stallions compared to saline treated (-144 and -72 hours) stallions, respectively. Significant drop in glutamic pyruvic transaminase activity of seminal plasma occurred at 72 hr after LH administration in horse and donkey stallions (Table 20 and Figure 41).

Table 20: Effect of LH and saline (*control*) treatment on glutamic pyruvic transaminase activity (*IU/ml*) of seminal plasma in four horse and donkey stallions (Mean  $\pm$  SE).

GROUPS AND STATISTICS	Glutamic Pyruvic Transaminase ( <i>IU.ml<sup>-1</sup></i> )			
	144 Hours Before	72 Hours Before	4 Hours After	72 Hours After
Horse Control for LH ( <i>n</i> =4)	27.00 $\pm$ 1.99	29.75 $\pm$ 2.92	34.27 $\pm$ 2.93	32.65 $\pm$ 4.05
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Horse treatment with LH ( <i>n</i> =4)	59.65 $\pm$ 6.53	61.00 $\pm$ 6.75	50.65 $\pm$ 5.37	42.65 $\pm$ 5.37
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0173
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0180
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0004	<i>P</i> = 0.00050001	<i>P</i> = 0.1717	<i>P</i> = 0.0111
Donkey Control for LH ( <i>n</i> =4)	24.57 $\pm$ 7.73	28.60 $\pm$ 7.00	24.82 $\pm$ 9.16	29.32 $\pm$ 9.72
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = > 0.05
Donkey treatment, LH ( <i>n</i> =4)	45.27 $\pm$ 4.97	49.15 $\pm$ 4.54	35.13 $\pm$ 7.26	29.41 $\pm$ 6.71
<i>P</i> Value (Two Tail Pair <i>t</i> -test) Compared with -144 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0306
Compared with -72 hour values			<i>P</i> = > 0.05	<i>P</i> = 0.0346
<i>P</i> Value (ANOVA test) Compared with Control at same time interval.	<i>P</i> = 0.0129	<i>P</i> = 0.0146	<i>P</i> = 0.8678	<i>P</i> = 0.1994

Figure 41: Effect of LH and saline (*control*) treatment on glutamic pyruvic transaminase activity (*IU/ml*) of seminal plasma in four horse and donkey stallions (Mean  $\pm$  SE).



# Discussion

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## 5.1: EFFECT OF GNRH AND LH ON LIBIDO (REACTION TIME) IN HORSE AND DONKEY STALLIONS.

GnRH injection (100 µg, i.v.) in slow breeder horse and donkey stallions improved libido as evidenced by significant reduction in post-treatment reaction time. Similarly, GnRH treatment (80 µg i.v.) restored libido in two horse stallions with deficient libido (Bader *et al* 1988). Prolonged GnRH infusion (500 mg, i.v. for 7 days) also reduced reaction time in five stallions (Stonina and Pozor 1989). Low libido stallions have low LH profiles due to deficient GnRH secretion (Hyland and John 1990). Significant reduction in post-treatment reaction time in GnRH treated stallions could be due to LH mediated testosterone rise (Kilroy and Dobson 1986).

LH administration (3000 IU, i.v.) in slow breeder horse and donkey stallions resulted in significant reduction in post-treatment reaction time. Bader *et al* (1988) also improved libido in poor libido stallions with a higher LH dose (10,000 IU, i.v.). Stallions with low libido have deficient pituitary LH secretion (Hyland and John 1990). Post-treatment reduction in reaction time in LH treated stallions could be ascribed to exogenous LH induced testosterone rise (Cox 1989).

## 5.2: EFFECT OF GnRH ON PLASMA TESTOSTERONE PROFILES IN HORSE AND DONKEY STALLIONS.

Recorded wide variations in testosterone values in GnRH control horse and donkey stallions supports earlier reports in horse (Johnson and Thompson 1983) and donkey stallions (Minoia *et al* 1983). GnRH induces LH secretion with subsequent rise in plasma testosterone (Blue *et al* 1991). GnRH administration in horse (Bader *et al* 1988), donkey (Minoia *et al* 1983) and bull (Kilroy and Dobson 1986) produced rise in peripheral testosterone concentration. Significant rise in plasma testosterone concentration occurred in GnRH treated horse and donkey stallions consequential to pituitary LH induced testicular androgen production (Blue *et al* 1991). First significant rise in testosterone at 2 hours post-treatment agrees with the findings in bulls (Schanbacher and Echterkamp 1978). The observed significant drop at 120 hr post-treatment in stallions remain un-compared due to non-availability of literature.

## 5.3: EFFECT OF LH ON PLASMA TESTOSTERONE PROFILES IN HORSE AND DONKEY STALLIONS.

Variations in plasma testosterone levels during pre and post-saline treatment periods agrees well with reported wide variations in testosterone levels in horse (Harris *et al* 1983) and donkey stallions (Minoia *et al* 1983). Testosterone values in LH control horse stallions were higher than reported values (500 pg/ml) in horse stallions (Johnson and Thompson 1983, Raeside 1979) but the pre-treatment testosterone profiles in low libido stallions agrees well with these workers.

Studies in bull (Sundby and Farahat 1978) ram (Garnier and Sacz 1980) and stallions (Cox and Redhead 1990) have shown that HCG administration increases circulatory testosterone level. Significant rise in plasma testosterone profiles following LH injection in stallions confirms earlier findings in bulls (Katongole *et al* 1971) and horse stallions (Cox 1989). Recorded peak testosterone profiles till 72 hr after HCG injection are consistent with the findings of Silberzahn and Zwan (1988) in horse stallions. The observed significant drop at 120 hr post-treatment in stallions compares well with the observations in horse stallions (Cox and Redhead 1990).

## 5.4: EFFECT OF GnRH AND LH ON SEMINAL CHARACTERISTICS IN HORSE AND DONKEY STALLIONS.

### 5.4.1: GEL VOLUME.

Mean gel volume in GnRH and LH control horse stallions (normal libido) at various intervals was higher than the reported low value (7.0 ml) by Pickett *et al* (1976). However, Komarek *et al* (1965) recorded higher gel volume (36.0 ml) in stallions.

Seminal vesicles are the major source of seminal gel in stallions (Humphry and Mann 1948). High peripheral testosterone concentration was accompanied with increased gel volume of ejaculates in stallions (Thompson *et al* 1977). Significant increase in seminal gel volume after GnRH and LH treatment in horse and donkey stallions could be ascribed to testosterone induced gel secretion from seminal vesicles (Byers *et al* 1983).

### 5.4.2: GEL FREE SEMEN.

Recorded gel free volume of ejaculates at various intervals in GnRH and LH control horse stallions agrees well with the reported gel free volume (68.0 ± 6.3 ml) in stallions (Schumacher and Riddell 1986). However, Pickett *et al* (1976) reported slightly low gel free semen volume (58.4 ml) in stallions.

Low pre-treatment gel free semen volume in "slow breeder" horse and donkey stallions could be due to low testosterone concentration (Berndtson *et al* 1974, Byers *et al* 1983) while significant post-treatment rise occurred due to rise in testosterone profiles after GnRH and LH administration.

### 5.4.3: TOTAL SEMEN VOLUME.

Mean total semen volume at various intervals in GnRH and LH control horse stallions were slightly higher than reported value (65 ml) in stallions (Pickett *et al* 1976). Total semen volume at various intervals in GnRH and LH control donkey stallions were comparable to reported semen volume (69 ml) by Mann *et al* (1963). Significant post-treatment increase in semen volume in GnRH and LH treated stallions was due to testosterone induced secretion of accessory sex glands (Thompson *et al* 1977, Byers *et al* 1983).

### 5.4.4: PH.

Mean pH of ejaculates in GnRH and LH control horse stallions at various intervals ranged from 7.3 to 7.5 which agrees well with the reported pH values (7.3 to 7.5) in normal horses (Pickett *et al* 1975). Recorded pH of the GnRH and LH

control donkey stallions at various intervals agrees with reported pH values (7.3 to 7.4) in donkey stallions (Singhvi 1989). The observed indistinct change in seminal pH between pre and post-treatment periods in GnRH and LH treated horse and donkey stallions could not be compared due to paucity of parallel literature.

#### 5.4.5: MASS ACTIVITY.

Recorded mass activity of ejaculate (Gel free) from GnRH and LH control horse and donkey stallions (normal libido) varied from 2 to 3 score, which agrees well with the reported mass activity in normal horse stallions (Hendriske 1966, Castro *et al* 1991). The observed indistinct change in mass activity of gel free ejaculates between pre and post-treatment periods in GnRH and LH treated horse and donkey stallions could not be compared due to lack of published reports.

#### 5.4.6: INDIVIDUAL SPERM MOTILITY.

Recorded individual sperm motility of ejaculates from GnRH and LH control horse stallions (normal libido) varied from 70 to 90 per cent, which is comparable with reported individual motility (75 to 80 per cent) in normal horse stallions (Bielanski 1956, Singhvi 1989). In GnRH and LH control donkey stallions (normal libido), the observed individual sperm motility is in agreement with the reported values (70 to 80%) by Singhvi (1989). Recorded significant increase in individual sperm motility in GnRH treated horse and donkey stallions contradicts the reported non-significant effect of pulsatile GnRH administration in horse stallions (Blue *et al* 1991).

Androgen administration increases motility of mature spermatozoa in cauda epididymis (Mann 1964). Further, castration adversely affects motility of mature epididymal spermatozoa which can be restored by androgen therapy (Mann 1964). Therefore, increased post-treatment motility in stallions occurred consequential to GnRH and LH induced testosterone rise.

#### 5.4.7: SPERM CONCENTRATION.

Mean sperm concentration of gel free semen at various intervals in GnRH and LH control horse stallions (normal libido) ranged from 182 to 191 millions/ml and from 187 to 205 millions/ml, respectively which is comparable with reported values (170 to 210 millions/ml) in normal horses (Pickett *et al* 1976, Singhvi 1989). However, Castro *et al* (1991) reported higher values (333 to 336 millions/ml) in normal stallions. In GnRH and LH control donkey stallions, mean sperm

concentration (gel free) at various intervals ranged from 179 to 219 million/ml and 184 to 210 million/ml, respectively which supports the reported observation in donkey stallions (188 to 207 million/ml) by Costa *et al* (1991).

Low pre-treatment sperm concentration of gel free ejaculates in "slow breeder" horse and donkey stallions could be due to low testosterone profiles adversely affecting sperm out put from cauda epididymis (Berndtson 1983, Byers *et al* 1983). Secretory and gametogenic activity of stallion reproductive tract are mediated by testosterone concentration (Berndtson 1974).

#### 5.4.8: LIVE SPERM COUNT.

In GnRH and LH control horse stallions, the mean live sperm count of ejaculates at various intervals ranged from 80 to 86 per cent and from 77 to 82 per cent, respectively. Similarly, 83 per cent live sperm count was reported in normal stallions (Singhvi 1989, Schumacher and Riddell 1986). Recorded mean live sperm count in GnRH and LH control donkey stallions at various intervals varied from 76 to 81 per cent and 76.9 to 83 per cent, respectively, which are in agreement with those reported by Singhvi (1989) and Gastal *et al* (1991). The observed significant increase in live sperm count in post-treatment ejaculates both in horse and donkey stallions remains un-compared due to non-availability of literature.

### 5.5: EFFECT OF GNRH AND LH ON SEMINAL BIOCHEMICAL CONSTITUENTS IN HORSE AND DONKEY STALLIONS.

#### 5.5.1: CITRIC ACID.

Citric acid content of seminal plasma at various intervals ranged from 54 to 56 mg/dl and from 53 to 55 mg/dl in GnRH and LH controls horse stallions, respectively, which agrees well with the reported citric acid content (53 mg/dl) by Matos *et al* (1977). However, Mann *et al* (1956) reported low citric acid values (8.8 mg/dl) in stallions. Citric acid values varied from 45 to 51 mg/dl in GnRH and LH control donkey stallions, which are much lower than the reported value (82 mg/dl) by Mann *et al* (1956).

Seminal vesicles are the major source of citric acid (Humphery and Mann 1948). Low pre-treatment citric acid content of seminal plasma in "slow breeder" horse and donkey stallions could be due to low testosterone concentration adversely affecting its synthesis from seminal vesicles (Skinner and Bowen 1968,

Byers *et al* 1983), whereas, post-treatment increase in citric acid content of seminal plasma could be attributed GnRH and LH induced testosterone rise in stallions.

#### 5.5.2: GLUTAMIC OXALOACETIC TRANSAMINASE

Mean glutamic oxaloacetic transaminase (GOT) activity of seminal plasma in GnRH and LH control horse stallions at different intervals ranged from 175 to 196 IU/ml and 172 to 204 IU/ml, respectively, which are lower than reported values (437 to 550 IU/ml) in normal stallions (Castro *et al* 1991). Elevated pre-treatment seminal GOT activity in "slow breeder" horse and donkey stallions was significantly reduced after GnRH and LH administration. However, parallel reports do not exist.

The post-treatment reduction in seminal GOT activity occurred due to greater sperm survival as evidenced by recorded increased live sperm count thereby reducing sperm derived GOT activity into seminal plasma (Roussel and Stallcup 1965).

#### 5.5.3: GLUTAMIC PYRUVIC TRANSAMINASE.

Recorded mean glutamic pyruvic transaminase (GPT) activity of seminal plasma at various intervals in GnRH and LH control horse and donkey stallions are fairly comparable with the reported values (33 to 38 IU/ml) in bulls (Pangawkar *et al* 1988).

Significant reduction in seminal GPT activity in GnRH and LH treated horse and donkey stallions was due to reduction in dead sperm count, reducing sperm derived GPT activity from dead spermatozoa in to seminal plasma as reported in bulls (Roussel and Stallcup 1965).

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# Summary & Conclusions

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Effect of GnRH (100 µg i.v.) and LH (3000 IU i.v.) was studied on libido, testosterone profiles and seminal characteristics in low libido horse (n = 4) and donkey stallions (n = 4). Four horse and four donkey stallions with normal libido and seminal characteristics were administered with normal saline (1 ml i.v.) and were used as control for both the treatments (GnRH, LH).

GnRH and LH injection significantly ( $P < 0.0025$ ) reduced post-treatment reaction time (4 and 72 hours post-treatment) in low libido horse and donkey stallions and significantly ( $P < 0.0025$ ) increased peripheral testosterone profiles at 2 and 4 hr post-treatment and testosterone remained significantly elevated till 72 hr post-treatment before a significant drop at 120 hr post-treatment.

Mean gel volume, gel free volume and total semen volume was significantly increased in post-treatment ejaculates (4 and 72 hours after GnRH and LH treatment) collected from slow breeder horse and donkey stallions. Both GnRH and H treatment significantly ( $P \leq 0.05$ ) increased individual sperm motility, sperm concentration and live sperm count in gel free semen of horse and donkey stallions. However, mass activity and pH of the gel free semen was not affected by either of the hormonal treatment in stallions.

Both GnRH and LH treatment significantly ( $P \leq 0.05$ ) increased citric acid content of seminal plasma in post-treatment ejaculates (4 and 72 hours) obtained from low libido stallions. GOT and GPT activity of seminal plasma was significantly reduced in post-treatment ejaculates (72 hours) of stallions.

Following conclusions were drawn from the present investigation:

- ☞ GnRH (100  $\mu$ g, i.v.) and LH (3000 IU, i.v.) administration can be used to restore libido in slow breeder horse and donkey stallions.
  - ☞ GnRH (100  $\mu$ g, i.v.) and LH (3000 IU, i.v.) treatment improves seminal characteristics (gel volume, gel free semen volume, total volume, individual motility and live sperm count) in "slow breeder" horse and donkey stallions.
  - ☞ GnRH (100  $\mu$ g, i.v.) and LH (3000 IU, i.v.) injection increases functional activity of seminal vesicles and also increases sperm survivability.
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# Annexure - I

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## MATERIAL SUPPLIED

### *TOTAL TESTOSTERONE AB-COATED TUBES (TTT-1)*

Five hundred polypropylene tubes coated with antibodies to testosterone were supplied by the manufacturer (stable at 2-8 °C until expiry date).

### *<sup>125</sup>I TOTAL TESTOSTERONE (TTT-2)*

Five vials of iodinated testosterone in liquid form (525 ml) were supplied by the manufacturer (stable at 2 - 8 °C until expiry date).

### *TOTAL TESTOSTERONE CALIBRATORS (TTT 3-8)*

Two sets of six vials of testosterone calibrators (0, 20, 100, 400, 800 and 1600 ng/dl) were supplied by manufacturer (stable at 2 - 8 °C until expiry date).

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# Annexure - II

## Specificity

The Coat-A-Count Total Testosterone antiserum is highly specific for testosterone. Percent crossreactivity was calculated on a weight-per-weight basis at approximately 50% binding.

	ng/dL Added	Apparent Conc. ng/dL	Percent Crossreactivity
Aldosterone	800,000	ND	ND
5 $\beta$ -Androstan-3 $\alpha$ ,17 $\beta$ -diol	200,000	740	0.4%
Androstenedione	100,000	490	0.5%
5 $\alpha$ -Androstan-3 $\beta$ ,17 $\beta$ -diol	100,000	38	0.04%
5-Androsten-3 $\beta$ ,17 $\beta$ -diol	25,000	57	0.2%
5 $\alpha$ -Androstan-3,17-dione	100,000	54	0.05%
Androsterone	10,000,000	430	0.004%
Corticosterone	500,000	9.0	0.002%
Cortisol	800,000	43	0.005%
Cortisone	800,000	150	0.02%
Danazol	20,000	17	0.09%
11-Deoxycortisol	100,000	ND	0
Dexamethasone	800,000	26	0.003%
DHEA	1,000,000	15	0.002%
DHEA-sulfate	100,000	6.0	0.006%

## ANNEXURE

5a-Dihydrotestosterone	5,000	164	3.3%
	2,500	77	3.1%
	1,250	35	2.8%
	625	21	3.4%
Estradiol	100,000	18	0.02%
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5(10)-Estren-17a-ethinyl 17B-01-3-one	5,000	11	0.2%
4-Estren-7a-methyl-17B-01-3-one	100,000	1,100	1.1%
4-Estren-17-o1-3-one	10,000	2,000	20%
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(19-Nortestosterone)	5,000	1,000	20%
	1,000	220	22%
Estrone	50,000	7.0	0.01%
Ethisterone	5,000	37	0.7%
Fluoxymesterone ("Halotestin")	500,000	67	0.01%
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19-Hydroxyandros-tenedione	100,000	2,000	2.0%
11-Ketotestosterone	1,300	210	16%
Methyltestosterone	3,000	52	1.7%
Norethindrone	5,000	5.0	0.1%
Norethyrodrel	5,000	13	0
Prednisone	80,000	ND	0
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Progesterone	100,000	ND	0
Spirolactone	100,000	ND	0
11B-Hydroxytestosterone	200,000	1,600	0.8%
	100,000	1,200	1.2%
Triamcinolone	5,000	11	0.2%

ND indicates nondetectable.

