Effect of GnRH Immunization on Testicular Function in Colts

U. Tshewang[1], K. F. Dowsett[2], L. Knott[2] and A. Jackson[3]
Research Extension Irrigation Division, Ministry of Agriculture, P.O. Thimphu, Bhutan

ABSTRACT : Ten Australian Stock Horses colts (five yearling and five 3-year old colts) of which 2 yearlings and 2 three year old colts served as control animals while 3 yearlings and 3 three year old colts received two GnRH immunizations within 4 weeks interval were used in this study. By the 5th to 6th week after immunization, the GnRH antibody titres in the plasma rose above 1:1000 and attained peak levels of 1:6500 by the 8th week and gradually declined to about 1:3000 by the 10th week in both the age groups. The testosterone and androstenedione concentrations of the control colts in both age groups were significantly greater ($p<0.05$) than that of the vaccinated groups. During the immunosuppression period, the vaccinated colts behaved like geldings. Semen could not be collected from 2 of the 3 three-year old vaccinated colts. The testicular dimensions, testicular weight, parenchymal weight, seminiferous tubule volumes, interstitial space volumes, Leydig cell volume, seminiferous tubule % of the control colts were significantly greater than those of the vaccinated colts in both the age groups. The 3-year old control colts had a significantly ($p<0.05$) greater % of Leydig cells than the control and vaccinated 1-year old colts. There was arrest of spermatogenesis with complete absence of sperm in the testes of the vaccinated colts while there was various stages of spermatogenesis in those of the control colts. Morphometric analysis demonstrated that the 3-year old colts had significantly ($p<0.05$) greater DSP/gm of testis and DSP/testis than those of the 1-year old control colts. This study elucidated that the GnRH immunization could suppress the testicular function of the 3-year old and yearling colts. ***(Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 3 : 348-353)***

Key Words : Colts, GnRH, Immunization, Immunosuppression, Spermatogenesis, Morphometric Analysis

INTRODUCTION

Microscopic studies on testicular involution caused by active immunization against GnRH have revealed reduction in the size of seminiferous tubules and arrest of spermatogenesis in rats (Fraser et al., 1974), marmoset monkeys (Hedges and Hearn, 1977), rabbits (Arimura et al., 1973) and dogs (Schanbacher, 1984). These studies reported that primary spermatocytes were still present in the testes of immunized rats, marmoset monkeys and dogs while the arrest of spermatogenesis was complete and no primary spermatogenesis were found in the testes of immunized rabbits. These studies also reported reduction in the volume density of seminiferous tubules and interstitial tissue and in the size and number of Leydig cells.

The aim of this experiment was to determine the relationship between sexual behaviour, semen characteristics, testicular morphology, testicular histology, and daily sperm production of yearling and 3-yr old colts while they were suppressed by a GnRH vaccine.

MATERIALS AND METHODS

Animals and experimental design

Ten Australian Stock Horses colts (five yearlings and five 3-yr olds) were randomly allocated into two groups of: (1) Unvaccinated Controls - 4 colts; (2) Vaccinated - 6 colts, with equal numbers of colts from each age group in each group, that received 400 mg of an injectable water soluble GnRH vaccine intramuscularly followed by a booster injection of the same dose 4 weeks later. Once GnRH antibody titres reached effective levels and marked testicular atrophy associated with low testosterone concentrations (0.17 ± 0.07 ng/ml and 0.21 ± 0.11 ng/ml in the 3-yr old and yearling colts respectively) and androstenedione concentrations (0.14 ± 0.05 ng/ml and 0.16 ± 0.07 ng/ml in the yearling and 3-yr old colts respectively) was apparent in the vaccinated colts surgical castration was performed for histological examination, morphometric analysis and evaluation of DSP some 10 weeks after the primary vaccination.

One of the vaccinated yearling colts died from accidental causes during the experimental period.

GnRH immunogen

A water-based adjuvant vaccine containing 400 mg of the GnRH-ovalbumin conjugate was dissolved in sterile saline to give a final dose volume of 2 ml (the vaccine was supplied by Polypeptide Technology Ltd, N.S.W., Australia). The response to immunization was monitored by determining the plasma antibody titres, testosterone and androstenedione concentrations every week.

Physical measurements

Scrotal width and length of both the testes of experimental animals were measured at weekly intervals. Height was measured at intervals of 5 weeks. During each measurement, the testicles were examined for their tone and texture.

Hormone assays

The plasma testosterone and androstenedione concentrations were determined from the blood samples collected weekly before vaccination (16 weeks) to

[1] Corresponding Author: U. Tshewang.
[2] University of Queensland, FAMP, St. Lucia 4072, Brisbane
[3] University of Queensland, Department of Anatomy, St. Lucia 4072, Brisbane
Received July 15, 1998; Accepted October 24, 1998

ascertain the levels for 1-yr old and 3-yr old colts. After vaccination, the concentrations of these hormones were determined until the conclusion of the experiment. A modification of the radioimmunoassay procedure used by Cox et al. (1973) was followed to quantify plasma testosterone and androstenedione concentrations. For testosterone analysis, the inter-assay variation at 0.75 ng/ml was 14.8% and at 1.23 ng/ml was 7.8%. The intra-assay variability was 6.5% while the sensitivity of the assay was 0.08 ng/ml. The intra-assay variation for the androstenedione assay was 6.4% while the inter-assay variation was 17% at 0.54 ng/ml and 13% at 0.98 ng/ml and the sensitivity 0.08 ng/ml.

GnRH antibody titres

Antibody titres were assessed weekly after immunization until the conclusion of the experiment. GnRH antibody titres were determined using a modification of the method used by (Hoskinson et al, 1988) for the detection of antisomatostatin antibodies. Iodinated GnRH (3-[^{125}I] iodotytosyl) was prepared from I-radioisotope obtained from Amersham Australia Ltd., N.S.W. The specific activity was 74 TBq/mM. This tracer was reconstituted in 100 μl distilled water and then diluted in sodium phosphate buffer (0.05 M, pH 7.25) to a concentration of 10,000 cpm/100 mi and stored at 4℃ before use.

Semen collection

Two semen samples were collected from the 3-yr old colts before they were vaccinated using the artificial vagina for stallions developed by Dowsett and Pattie (1980). After vaccination, one semen collection attempt was made with the 3-yr old colts. The sexual behaviour of the immunosuppressed colts was also studied during this semen collection attempt.

Evaluation of semen

The gel fraction of the ejaculate, if present, was separated from the semen before examination and the gel-free fraction was immediately assessed for volume (ml), density score (0-3), mass activity (0-3); and percentage motile spermatozoa. The percentages of live normal, live abnormal, dead normal and dead abnormal spermatozoa were estimated by staining the semen with nigrosine-eosin stain (Dowsett and Pattie, 1980). A few drops of semen were mixed with 8 drops of the stain in a vial and incubated at 39℃ for 3 minutes before making a smear on microscope slides.

The concentration of spermatozoa per ml of semen was assessed by use of a haemocytometer. Semen (0.1 ml) was mixed with buffered formal saline (4.9 ml) and a small drop of this mixture was placed on the haemocytometer (Neubauer's Chamber) and allowed to settle under a cover slip. Spermatozoal concentration was determined by counting on the red cell grid and multiplying the result by 2.5×10^6 to give the concentration per ml of semen. The total number of spermatozoa per ejaculate was calculated by multiplication of the spermatozoal concentration with the volume (ml) of the gel-free semen.

Evaluation of testis

By the tenth week after primary vaccination the colts were surgically castrated in order to examine testicular histology and calculate daily spermatozoa production (DSP). Both the right end the left testes were weighed immediately after removal using electronic scales. The *tunica albuginea* was carefully dissected from the testicular parenchyma. The parenchymal weight was then determined by subtracting the weight of the (*tunica albuginea*) from the total testicular weight. The parenchyma of the right testis was cut into about 0.50 cm slices and stored in plastic bottles. They were frozen at -20℃ until Daily Sperm Production (DSP) analysis was performed.

Testicular histology

The testicular tissue was fixed by immersion in Bouin's solution for 24 hrs, and placed in 70% alcohol for 48 hrs. Each specimen was blocked with wax and a number of 5 μM sections, were cut and placed on glass slides. Haematoxylin and eosin stains were used to stain the tissues and then examined under a light microscope (Zeiss Axiophot, Germany) at the magnification of $\times10$, $\times20$ and $\times40$. Testicular histology was examined by light microscopy. Photomicrographs of the representative areas of testis were taken with a Zeiss photo microscopeⅢ on Kodak EPY film.

Evaluation of daily sperm production (DSP)

The frozen pieces of testicular parenchyma were thawed and blotted dry prior to use. Samples as small as 2-3 gm were weighed and then added to 100 ml of an isotonic solution containing 150 mM sodium chloride, 3.8 mM sodium azide and 0.05% Triton X-100 (Johnson & Neaves, 1981) and homogenized in a blender for 6 min. The homogenate was then incubated st 4℃ until evaluated within 24 hr, occasionally being mixed and stirred during the interim period. Drops of the homogenate solution were examined in a haemo-cytometer under a phase contrast microscope and two haemocytometer counts were carried out for each sample. The number of elongated spermatids (the maturation-phase found in stages 5 to 8 of spermatogenesis which are resistant to homogenization) were counted (Amann et al., 1979).

The total number of spermatids per gm of testis was calculated from the average number of elongated spermatid nuclei counted on the haemocytometer, the final volume of suspension and the weight of the testicular parenchyma homogenized. Since the cycle of seminiferous epithelium is 12.2 days in the stallion and the stages 5 to 8 comprise 49.2% of one cycle the life span (time divisor) for enumerated spermatids was estimated to be 6 days. The DSP per gm of testis was therefore estimated by dividing the number of spermatids by 6 days and the DSP per testis was obtained by

Table 1. Mean (±S.D.) plasma testosterone concentrations of the yearling and 3-yr old colts immunized with a GnRH vaccine during the different experimental periods and surgically castrated during the immunosuppression period

Testosterone conc. ng/ml	Control		Treated	
	1-yr (n=2) Mean (±S.D.)	3-yr (n=2) Mean (±S.D.)	1-yr (n=2) Mean (±S.D.)	3-yr (n=2) Mean (±S.D.)
Before vaccination	0.40^{a} (±0.10)	0.70^{b} (±0.11)	0.43^{a} (±0.09)	0.99^{c} (±0.35)
After primary vaccination	0.44^{a} (±0.14)	0.69^{b} (±0.13)	0.31^{c} (±0.07)	0.38^{ac} (±0.17)
After secondary vaccination	0.67^{a} (±0.27)	0.83^{a} (±0.33)	0.21^{a} (±0.11)	0.17^{b} (±0.07)
After surgical castration	0.18 (±0.04)	0.16 (±0.05)	0.18 (±0.03)	0.19 (±0.04)

a,b,c Means with different superscripts in the same rows are significantly different ($p<0.05$).
n=number of colts.

Table 2. Mean (±S.D.) plsma androstenedione concentrations of the yearling and 3-yr old colts immunized with GnRH vaccine and surgically castrated during the immunosuppression period

Androstenedione conc. ng/ml	1-yr old colts		3-yr old colts	
	Control Mean (±S.D.)	Treated Mean (±S.D.)	Control Mean (±S.D.)	Treated Mean (±S.D.)
Before vaccination	0.38^{a} (±0.08)	0.33^{a} (±0.11)	0.54^{b} (±0.18)	0.82^{c} (±0.40)
After primary vaccination	0.31^{a} (±0.06)	0.21^{b} (±0.06)	0.53^{c} (±0.07)	0.31^{a} (±0.16)
After secondary vaccination	0.46^{a} (±0.19)	0.14^{c} (±0.05)	0.58^{b} (±0.24)	0.16^{c} (±0.07)
After surgical castration	0.18 (±0.04)	0.18 (±0.05)	0.16 (±0.06)	0.19 (±0.04)

a,b,c Means with different superscripts in the same rows are significantly different ($p<0.05$).
n=number of colts.

multiplication of the weight of the testicular parenchyma by the DSP per gm of testis.

Morphometric analysis

This analysis was used to determine the quantitative histological aspects of the testis. The volume fractions of the Leydig cells, inter-tubular areas and the seminiferous tubules were estimated by the point counting method using a Reichert projection microscope which had a 63 point grid placed over it. The sections were viewed under high power (×100) objective and the points falling over seminiferous tubules, inter-tubular areas and Leydig cells were counted. Formulas used by (Johnson and Neaves, 1981) for the calculation of percentages and volumes of seminiferous tubules, inter-tubular space and Leydig Cells were followed.

Statistical analysis

The statistical software package SAS version 6.03 (1988) was used for performing the analysis of all the experimental data. The effects of treatment, age, time and their interactions on body weight, testosterone and androstenedione concentrations, testis weight parenchymal weight seminiferous tubule volume, interstitial tubule volume, Leydig cell volume, % seminiferous tubule, % interstitial tubule, % Leydig cell, dsp/gm and dsp/testis of the left testis of the colts were determined by the repeated measures analysis of variance using generalized interactive modelling. When there were significant differences their means were compared by using least significant differences.

RESULTS

GnRH antibody titre

The control colts in both age groups did not produce any GnRH antibody titres while the vaccinated colts produced effective antibody titres 1-2 weeks after the booster vaccination. The pattern of GnRH antibody titre development is illustrated along with the scrotal width and plasma testosterone concentrations in figure (1) and figure (2) respectively. There were no significant differnces ($P>0.05$) in the mean antibody titres between the vaccinated yearling and 3-yr old colts.

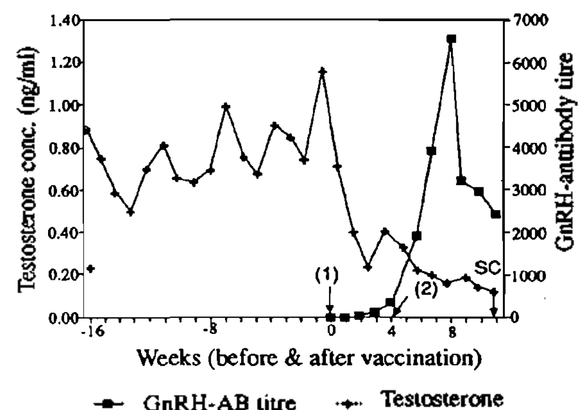

Figure 1.

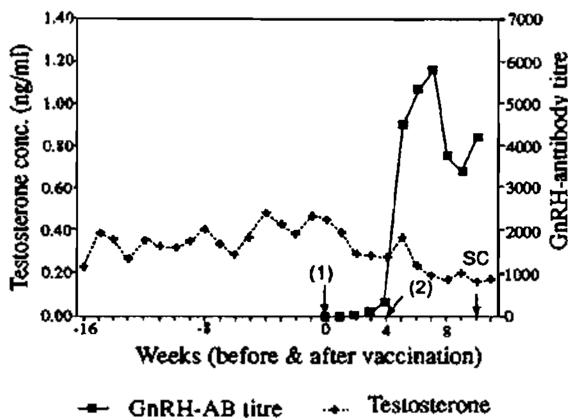

Figure 2.

Figure 1 and 2 illustrate mean GnRH antibody titres superimposed with the mean plasma testosterone concentration (ng/ml) of the 3-yr old and yearling colts vaccinated with GnRH vaccine, respectively

Plasma testerone concentrations

Plasma testosterone concentrations of the yearling and 3-yr old colts decreased following the week after primary vaccination and declined significantly ($p<0.05$) to gelding levels of about 0.20 ng/ml after the booster vaccination. They continued to increase much faster in the yearling control colts than the 3-yr old control colts, figure 1 and 2. The overall mean plasma testosterone concentrations of the control and vaccinated colts of both age groups before vaccination, after primary vaccination and after booster vaccination are presented in table 1. The 3-yr old controls had significantly ($p<0.05$) greater mean plasma concentrations of testosterone than the yearling control colts, table 1. The plasma testosterone concentrations of the 3 yr old and yearling colts fell rapidly but decreased to gelding levels, by 5th to 6th week, only after the GnRH antibody titres rose to effective levels (>1:1000), figure 1. There were no significant differences ($p>0.05$) in plasma testosterone concentrations between age groups after primary and booster vaccinations. There were no significant differences ($p>0.05$) in mean plsma testosterone concentrations of plasma samples taken 24 hr after surgical castration between control and the treated yearling and 3-yr old control colts, nor were there significant differences between the control colts.

Plasma androstenedione concentrations

The plasma androstenedione concentrations of the treated 1-yr and 3-yr old colts differed significantly ($p<0.05$) from their respective controls after primary vaccination with a significant ($p<0.05$) age interaction between the control colts. The plasma androstenedione concentrations remained below 0.20 ng/ml by 4th week after the primary vaccination. There were no significant ($p>0.05$) differences in plasma androstenedione concentrations of plasma samples collected after 24 hr between control and the treated colts nor was there any age interaction.

Semen characteristics

The semen characteristics of 3-yr old colts collected during immunosuppression period are presented in table 3. Semen could not be collected from two of the GnRH immunized 3-yr old colts while semen was successfully collected from the controls and one of the GnRH immunized 3-yr old colts.

Table 3. Semen characteristics of the 3-yr old colts collected (27/10/93) after immunization with a water soluble GnRH vaccine

Semen characteristics	Control		GnRH treated		
	Romeo	Serenity	Potion	Mascot	Shout
Volume (ml)	40	5	-	3	-
Density (0-3)	3	3	-	3	-
Mass activity (0-3)	3	2	-	2	-
Motility %	85	65	-	80	-
Live normal %	85	50	-	22	-
Live abnormal %	8	20	-	68	-
Dead normal %	4	25	-	4	-
Dead abnormal %	3	5	-	10	-
Sperm conc./ml ($\times 10^6$)	237.5	197.5	-	195	-
Total sperm/ ejaculate ($\times 10^6$)	9,500	987.5	-	585.0	-

Testicular dimension and weight

The scrotal widths and the testicular lengths of the treated colts were significantly ($p<0.05$) smaller than those of the control colts in both age groups after the primary and booster vaccinations. They decreased significantly ($p<0.05$) after the booster vaccination. The testes of the control colts continued to grow normally with time. The testicular and parenchymal weights of the control colts were significantly ($p<0.05$) greater than those of the vaccinated colts table 5. The testicular and parenchymal weights of the 3-yr old control colts were non-significantly greater than those of the 1-yr old control colts.

Testicular histology

There were numerous Leydig cells in the testes of the 3-yr old control colts while those of the immunized colts appeared atrophied (Plate 1). The testes of yearling control colts also had Leydig cells. The seminiferous tubules of the treated colts were lined predominantly with Sertoli cells towards the basal lumina while the interstitial space was expanded with infiltration of fibroblast cells in the testes of both the yearling and 3-yr old colts (Plate 2). The GnRH immunized colts of both the age groups had 'disrupted' spermatogenesis. Occasional spermatocytes were present in the tubules with a total lack of spermatids in the treated colts while the control colts had different stages of spermatogenesis. Occasional Leydig cells that were present appeared atrophied in the vaccinated 1-yr old colts.

Plate 1

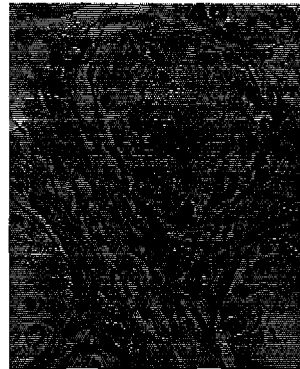

Plate 2

Plate 1 and 2 illustrate the light micrographs of the testes of the control and GnRH immunized 3-yr old colts. Arrows indicate the Leydig cells

Various stages of spermatogenesis were found in the histological sections of the testes of both the 1-yr and 3-yr old control colts, table 4. The 1-yr old control colts had a significantly ($p<0.05$) greater percentage of spermatogenesis in Stage Ⅰ without any in Stage Ⅷ compared with the 3-yr old control colts. The seminiferous tubules of the immunized colts had virtually no spermatocytes. Same of them that had a few spermatocytes were clumped towards the lumen of the tubules.

Morphometric analysis

The seminiferous tubule volumes, infer-tubular volumes and the Leydig cell volumes of the GnRH immunized colts were significantly ($p<0.05$) lower than those of the control colts in both age groups, table 5. The seminiferons tubule % of the control colts were significantly ($p<0.05$) greater than those of the the treated colts while the inter-tubular space % of the treated colts were significantly greater than those of the control colts. The 3 year old control colts had a significantly ($p<0.05$) greater % of Leydig cells than the control and vaccinated 1-yr old colts. However, the Leydig cell % in treated 3-yr old colts was not significantly ($p>0.05$) different from the control and vaccinated 1-yr old colts

Table 4. Percentage of seminiferous tubules in the stages of spermatogenesis (Ⅰ-Ⅷ) in the testes of GnRH immunized 1-yr and 3-yr old colts

Stages of	1-yr old colts		3-yr old colts	
spermatogenesis	Control	Treated	Control	Treated
Stage Ⅰ %	50.65^{a}	-	18.80^{b}	-
Stage Ⅱ %	30.50	-	28.80	-
Stage Ⅲ-Ⅶ %	10.90^{a}	-	39.33^{b}	-
Stage Ⅷ %	-	-	2.94	-
Disrupted %	28.28^{a}	100^{b}	10.15^{c}	100^{b}

^{a,b,c} Means with different superscripts in the same rows are significantly different ($p<0.05$).

Daily sperm production (DSP)

There was complete absence of sperm in the testes of the immunized colts. The 3-yr old colts had significantly ($p<0.05$) greater DSP/gm of testis than those of the 1-yr old control colts (table 5).

DISCUSSION

This study elucidated the microscopic changes in testicular tissue during immunosuppression of GnRH vaccinated 1 and 3-yr old colts. Plasma testosterone concentrations of the yearling and 3-yr old control colts were 0.67 ± 0.27 ng/ml and 0.83 ± 0.33 ng/ml respectively at the time of surgical castration while that of the vaccinated colts were below 0.21 ng/ml suggesting that the sexual activity of these colts was suppressed (Arighi and Bosu, 1988).

Testicular data such as testis weight, parenchymal weight tubular volume and Leydig cell volume of the vaccinated colts were significantly ($p<0.05$) reduced when compared to those of the controls. These findings suggest testicular degeneration due to the lack of sex

Table 5. Morphometric analysis and Daily Sperm Production (DSP) of the left testis of the yearling and 3-yr old immunized with a water soluble GnRH vaccine

	Control				Treated			
	1-yr (n=2)		3-yr (n=2)		1-yr (n=2)		3-yr (n=2)	
	Mean	(±S.D.)	Mean	(±S.D.)	Mean	(±S.D.)	Mean	(±S.D.)
Testis weight (gm)	111.0^{a}	(±54.7)	181.3^{a}	(±17.9)	30.3^{b}	(±9.0)	66.0^{bc}	(±36.4)
Parenchymal weight (gm)	84.2^{abc}	(±49.8)	136.5^{a}	(±9.2)	17.6^{b}	(±4.7)	43.4^{c}	(±23.7)
DSP/gm $\times 10^{6}$	10.7^{a}	(±1.62)	78.2^{b}	(±51.5)	-		-	
DSP/testis $\times 10^{9}$	0.33^{a}	(±0.23)	1.04^{b}	(±0.63)	-		-	
Seminiferous tubule volume (ml)	93.5^{a}	(±4.7)	105.9^{a}	(±17.5)	22.2^{b}	(±0.4)	22.7^{b}	(±12.9)
Interstitial space volume (ml)	51.1^{a}	(±7.6)	43.0^{a}	(±4.5)	23.0^{b}	(±0.4)	20.7^{b}	(±10.8)
Leydig cell volume (ml)	10.6^{ab}	(±3.9)	16.0^{a}	(±1.8)	3.6^{b}	(±1.3)	6.1^{b}	(±3.8)
Seminiferous tubule %	67.5^{a}	(±0.4)	68.6^{a}	(±1.2)	49.2^{b}	(±0.1)	52.0^{c}	(±1.3)
Interstitial space %	32.6^{a}	(±1.3)	31.5^{a}	(±1.2)	50.85^{b}	(±0.4)	48.0^{c}	(±1.3)
Leydig cell %	7.0^{ac}	(±3.7)	16.6^{b}	(±1.4)	1.9^{a}	(±0.4)	4.7^{bc}	(±1.4)

^{a,b,c} Means with different superscripts in the same rows are significantly different ($p<0.05$).

steroids (as explained earlier) in the testes, thus leading to suppressed sexual behaviour. Histological sections of the atrophied testis of the vaccinated colts revealed that spermatogenesis was disrupted with tubular involution, infiltration of fibroblasts in the inter-tubular space and atrophy of the Leydig cells. Their tubules were mostly lined with Sertoli cells at the basal lumina. Similar histological findings were described by (Fraser, 1981; Schanbacher, 1984) in GnRH immunized rats, rabbits and dogs. Sertoli cells are responsible for structural support and germ cell development (Johnson and Nguyen, 1986). The lack of germ cells in the lumen of seminiferous tubules might have led the Sertoli cells to settle on the basement membrane indicating that this is one of the characteristic features during lack of spermatogenesis.

Evaluation of daily sperm production by the homogenate method also revealed a total absence of spermatids. The daily sperm productions of the 3-yr old control colts were similar to those reported by (Johnson and Neaves, 1981) while those of the 1-yr control colts were significantly lower than those of the 3-yr old colts. Lack of spermatogenesis can be further substantiated by the failure of two of the immunized 3-yr old colts to produce any semen although one of the colts produced same semen. The colt that produced semen had less testicular atrophy than the other two 3-yr old colts. Microscopic examination of this colt's seminiferous tubules showed total disruption of spermatogenesis which indicated that the effect of the vaccine was not complete at the time of semen collection.

While the control colts behaved normally, the GnRH immunized 3-yr old colts behaved like surgical castrates when they were presented to an oestrous mare during the immunosuppression period. The lack of libido and arrested spermatogenesis can be attributed to the greatly reduced testosterone secretion, testosterone being responsible for spermatogenesis (Sharpe, 1987).

It can be concluded that by 6 weeks after the booster vaccination i.e. 10 weeks after the primary vaccination, there was an arrest of spermatogenesis associated with testicular atrophy and the reduction in testosterone and androstenedione secretions. Evaluation of intra-testicular testosterone concentrations is recommended in order to examine the basal levels of this hormone during the immunosuppression period. Since Sertoli cells play an important role in spermatogenesis it would be appropriate to evaluate the total number of Sertoli cells during the immunosuppression period. Testicular biopsy at regular intervals after vaccination may help explain the different stages of the inhibition of spermatogenesis by GnRH immunization and should be considered in further research of this vaccine in colts.

ACKNOWLEDGEMENT

The author wish to thank the Polypeptide Technology, New South Wales, Australia for supplying the GnRH vaccine for the experiment. They alo wish to thank Mr Lockland Arthur and Bruce Wilson for their assistance in handling the experiment animals.

REFERENCES

Amann, R. P., D. L. Thompson, E. L. Squires and B. W. Pickett. 1979. Effects of age and frequency of ejaculation on Sperm production and extragonadal sperm reserves in stallions. J. Reprod. Fert. Suppl. 27:1-6.

Arighi M. and W. K. B. Bosu. 1988. Comparison of hormonal methods for diagnosis of cryptorchidism in horses. Equine Veterinary Science. 9(1):20-26.

Arimura, A., H. Sato, T. Kumasaka, R. B. Worobec, L. Debeljuk, J. Dunn and A. V. Schally. 1973. Production of anti-serum to LH-RH associated with gonadal atrophy in rabbits: Development of radioimmuaosssays for LHRH. Endocrinology. 93(5):092-1103.

Cox, J. E., P. H. Redhead and F. E. Dawson. 1986. Comparison of the measurement of plasma testosterone and plasma oestrogens for the diagnosis of cryptorchidism in the horse. Equine Vet. J. 18(3):179-182.

Cox, J. E., J. H. Williams, P. H. Rowe and J. A. Smith. 1973. Testosterone in normal, cryptorchid and castrated male horses. Equine Vet. J. 5(2):85-90.

Dowsett, K. F. 1983. A study of stallion semen characteristics and observations on their relationships with fertility. Doctor of Philosophy, University of Queensland.

Dowsett, K. F. and W. A. Pattie. 1980. Collection of semen from stallions at stud. Australian Vet. J. 56:373-377.

Fraser, H. M. 1976. Physiological effects of antibody to luteinising hormone releasing hormone. In Physiological effects of immunity against reproductive hormones., (Ed.) by Edwards R. G. and Johnson M. H., Cambridge University Press. pp.137-165.

Fraser, H. M., A. Gunn, S. L. Jeffcoate and D. T. Holland. 1974. Efftect of active immunization to LHRH on serum and pituitary gonadotropins, testis and accessory sex organs in male rats. J. Endocrinol. 63:399-406.

Fraser, H. M. 1980. Inhibition of reproductive function by antibodies to luteinizing hormone releasing hormone. In Immunological aspects of reproduction and fertility control., Ed. by Hearn J. P., MTP Press Limited, International Medical Publishers. pp. 143-171.

Hodges, J. K. and J. P. Hearn. 1977. Effect of immunization against LHRH on reproduction of the marmoset monkey *Callithrix jacchus*. Nature. 265:747-748.

Hoskinson, R. M., P. Djura, R. J. Welch, B. E. Harrison, G. H. Brown, J. B. Donnelly and M. R. Jones. 1988. Failure of antisomatostatin antibodies to stimulate the growth of crossbred lambs. Australian Journal of Experimental Agriculture. 28:161-165.

Johnson, L. and W. B. Neaves. 1981. Age related changes in the Leydig cell population, seminiferous tubules and sperm production in stallions. Biol. Reprod. 24:703-712.

Johnson, L. and H. B. Nguyen. 1986. Annual cycle of the Sertoli cell population in adult stallions. J. Reprod. Fert. 76:311-31620.

Schanbacker, B. D. 1984. Active immunization against LH-RH in the male. In Immunological aspects of reproduction in mammal. Edited by D. B. Creighton, Butterworths, London. pp. 345-362.

Sharpe, R. M. 1987. Testosterone and spermatogenesis. J. Endorinol. 113:1-2.