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El Gaafary M.N.

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The effects of gonadotropin releasing hormone on reproductive performance of low fertile male rabbits

M.N. EL-GAAFARY
ANIMAL WEALTH DEPARTMENT, INSTITUTE OF EFFICIENT PRODUCTIVITY, ZAGAZIG UNIVERSITY, ZAGAZIG, EGYPT

SUMMARY - Eighteen NZW bucks were used in this experiment. Six of them were fertile and the other twelve bucks were shown to have low libido, bad semen quality and poor fertility records. These twelve bucks were divided randomly into two groups. The bucks in the first group (n = 6) were injected intramuscularly with 20 μg GnRH every three days for a total of six times. The other 6 bucks (second group) were injected with saline. Semen was collected by using artificial vagina 6 days after every treatment and evaluated. Testosterone concentration and mating activity were also determined at last injection. In the fertility trail, 51 NZW does were injected intramuscularly with 20 μg GnRH and immediately inseminated with fresh semen collected from each of the three experimental bucks. For each insemination, a sperm dose of $30x10^6$ was used. The main results could be summarized as follows: Treatment of low fertile bucks with 20 μg GnRH increased ($P < 0.01$) sperm motility, sperm concentration/ml and total sperm output/ejaculate and decreased ($P < 0.01$) the percentages of dead spermatozoa, abnormal spermatozoa and acrosome abnormalities as compared to the saline-treated bucks, whereas, ejaculate volume showed insignificant differences. The differences between the fertile bucks and the GnRH-treated bucks in all semen characteristics were not significant. Plasma testosterone concentration and mating activity were elevated ($P < 0.01$) following injection of low fertile bucks with GnRH as compared with saline-injected bucks, however, the differences between the GnRH-injected and the fertile bucks were not significant. Moreover, injection of low fertile bucks with 20 μg GnRH improved ($P < 0.01$) the kindling rate by about 46.8% as compared with the fertility of the saline-injected bucks. The difference between the GnRH-treated and the fertile bucks in the kindling rate was not significant. These data suggest that injection of low fertile bucks with GnRH improved their reproductive performance.

Key words : gonodotropin, semen, mating activity, testosterone, kindling rate.

Introduction

Improving the reproductive performance of female rabbits has received much attention far ago. However, very scanty efforts were done on the male side. It has been reported that male rabbits showed seasonal fluctuations in semen characteristics and breeding pattern under Egyptian environmental conditions (Ahmed *et al.*, 1991). Moreover, presence of some bucks with low libido and bad semen quality are a common phenomenon at the end of summer and at the beginning of autumn (Hsu *et al.*, 1987, Ahmed *et al.*, 1991 and Marai *et al.*, 1992). The reason for this besides environment, nutrition and diseases, is chiefly the disturbance or

imbalance of the sex hormone after a period of high temperature (Yan *et al.*, 1985; Lin and Ramirez, 1991 and McNitt, 1992).

The aim of this work was to study the effect of gonadotropin releasing hormone (GnRH) injection on reproductive performance of low fertile bucks during summer under Egyptian conditions.

Material and methods

Eighteen New Zealand White (NZW) bucks (aged, 12-18 months) chosen from a Rabbit Farm located in the East of the Nile Delta, Sharkia Province, Zagazig, Egypt, were used in this investigation during summer (June to August), 1993. Maximum and minimum values of ambient temperature (^{o}C) and relative humidity (%) in the rabbitry during the period of the study are shown in Table 1. Management and feeding were kept constant as far as possible to maintain good body condition through the experimental work. Six bucks of them were of proven fertility and the other twelve bucks were shown to have low libido, bad semen characteristics and poor fertility results. The twelve bucks with bad reproductivity were then divided randomly into two groups. The animals in the first group (n=6) were chosen for a gonadotropin releasing hormone (Gonadoreline, Fertagyl, Intervet Lab.) treatment. Every buck was injected intramuscularly once with 20 μg GnRH (this level was previously used by Rebollar *et al.*, 1992, with rabbits) every three days for a total of six times. The second group was injected with physiological saline solution (0.9% NaCl). Six days after treatment, semen was collected (*n*=6) with an artificial vagina. The ejaculate volume (ml), sperm motility (%), dead spermatozoa (%), abnormal spermatozoa (%), sperm concentration per ml (x10^6) and total sperm output per ejaculate (x10^6) were examined microscopically according to Smyth and Gordon (1967) and El-Gaafary (1987). The percentages of spermatozoa with abnormal acrosomes were determined by using a Giemsa stain procedure as described by Watson (1975) and El-Gaafary (1987). At the last injection (*6th* injection), a single blood sample (2 ml) was taken from each buck (1 h post-injection) from the ear vein into heparinized tubes. The samples were then centrifuged at 2000 rpm for 15 minutes and the plasma were removed and frozen at - 20 ^{o}C until assayed for testosterone concentration with a double antibody radioimmunoassay (Diagnostic Products Corporation Kits). Mating activity of each buck was determined with sexually receptive doe showing lordosis. The number of completed matings in a 10 minutes test period (mating activity) was averaged for each of the three experimental

Table 1. Maximum and minimum values of ambient temperature (°C) and relative humidity (%) in the rabbitry, during the experimental period.

Summer months	Ambient temperature (°C)		Relative humidity (%)	
	Maximum	Minimum	Maximum	Minimum
June	30.28±0.54	22.97±0.36	83.00±0.41	30.03±1.35
July	33.81±0.24	25.49±0.15	86.00±0.98	45.00±1.22
August	31.22±0.20	24.52±0.14	89.10±0.70	42.23±1.10

bucks. In the fertility trial, fifty-one lactating does of NZW rabbits were used. Each doe was injected intramuscularly with 20 μg GnRH (Gonadoreline, Fertagyl, Intervet Lab.). Inseminations were carried out immediately following GnRH injection with pooled fresh semen collected from each of the three experimental bucks (i.e. GnRH-treated, saline-treated and untreated fertile bucks). For each insemination, a sperm dose of $30x10^6$ was used. The insemination procedure was same as described by Adams (1981). Number of does kindled was recorded at kindling.

For statistical analysis, data were examined by the analysis of variance according to Snedecor and Cochran (1982). Duncan's New Multiple Range test was used for the multiple comparisons. Kindling rate was analyzed using a log linear model for the analysis of Contingency Tables according to Everitt (1977).

Results and discussion

Semen Quality

Injection of the infertile bucks with 20 μg GnRH significantly increased ($P < 0.01$) sperm motility (%), sperm concentration/ml ($x10^6$) and total sperm output/ejaculate ($x10^6$) and significantly decreased ($P < 0.01$) the percentages of dead and abnormal spermatozoa and spermatozoa with abnormal acrosomes as compared to the saline-injected bucks, whereas, ejaculate volume (ml) showed insignificant differences (Table 2). The apparent improvement in semen characteristics may suggest that GnRH has a beneficial effect on increasing the steroidogenic activity of the interstitial cells which may account for the improvement in semen quality in the treated animals. The influence of GnRH on the reproductive performance of the bucks may be mediated through the activity of the

Table 1. Semen characteristics of low fertile bucks treated with gonadotropin releasing hormone as compared with saline-injected or fertile bucks.

Experimental bucks	Treatment	Semen Characteristics						
		Ejaculate+ Volume (ml)	Sperm motility (%)	Dead spermatozoa (%)	Abnormal spermatozoa (%)	Sperm concentration ($x10^6$/ml)	Total sperm output ($x10^6$/ejaculate)	Abnormal acrosomes (%)
Fertile	Untreated	0.36±0.08	72.8±3.37[a]	19.1±2.11[a]	15.4±2.42[a]	160.9±10.12[a]	57.9± 5.37[a]	14.5±2.13[a]
Low fertile	a- GnRH	0.39±0.07	68.3±6.93[a]	22.0±4.78[a]	14.9±2.73[a]	141.4±18.38[a]	55.1±15.98[a]	17.7±2.74[a]
	b- Saline	0.30±0.06	23.1±3.23[b]	46.6±3.60[b]	31.6±2.28[b]	93.3± 9.88[b]	27.9± 4.45[b]	29.0±2.08[b]
Significance		N.S.	**	**	**	**	**	**

Values are expressed as means ± standard error (6 replications x 6 bucks).
Values in the same column bearing different superscripts differ significantly ($P < 0.01$).
** $P < 0.01$
N.S. = not significant.
+ Any jelly present in the ejaculate was discarded.

adenohypophysis, stimulating the amounts of LH and FSH released. The LH stimulants the interstitial cells (or Leydig cells) to secrete androgen (testosterone), while FSH stimulates the Sertoli cells to secrete androgen binding protein (ABP). The androgen and the ABP bind together to stimulates the development of the germinal cells. The androgen can also stimulate the libido of the bucks, maintains the balance of hormones in the body and prolongs the life of the spermatozoa and promotes their motility (Hsu *et al.*, 1987). It was also demonstrated that a gonadotropin releasing hormone therapy was effective for improving the reproductive performance of either men with oligospermia (Schwarzstein *et al.*, 1975) and rams during the nonbreeding season (Schanbacher, 1978). The results of the present investigation also revealed that the differences between the GnRH-treated bucks and the fertile bucks in all semen characteristics were not significant (verified by Duncan's New Multiple Range test). Similar trend was previously reported by Schanbacher (1978) with rams.

Testosterone and Mating Activity

Plasma testosterone of low fertile bucks was elevated in response to chronic injections of GnRH as compared to injections of saline (Table 3). A typical 2-fold increase in testosterone concentration (5.70 vs 2.72 ng/ml, $P < 0.01$) was observed after a 20 μg of GnRH treatment. However, the difference between the GnRH-treated and the fertile bucks was not significant. The numbers of completed matings in a 10 minutes test period were considerably higher ($P < 0.01$) in the GnRH-injected bucks over the saline-injected bucks (3.17 vs 1.30), however, the difference between the GnRH-treated bucks and the fertile buck was not statistically significant. Injection of rams with 50 μg GnRH during the nonbreeding season increased serum testosterone concentration and improved the mating activity (Schanbacher, 1978). These findings suggest that treatment of bucks with GnRH may have influential on spermatogenesis via FSH release as via LH induced testosterone production.

Fertility Trial

The kindling rate was significantly higher ($P < 0.01$) following insemination of the does with semen collected from bucks treated with GnRH than from those inseminated with semen collected from saline-injected bucks (Table 4). Treatment of the infertile bucks with GnRH improved fertility as assessed by kindling rate by about 46.8% when compared with the fertility of the saline-injected bucks. However, there was no significant difference in the kindling rate between the

Table 3. Effects of gonadotropin releasing hormone on plasma testosterone concentrations and mating activity of low fertile bucks.

Experimental bucks	Treatment	Serum testosterone (ng/ml)	Completed matings in 10 min. test period
Fertile	Untreated	5.60 ± 0.35^{a}	3.50 ± 0.56^{a}
Low fertile	a- GnRH	5.70 ± 0.36^{a}	3.17 ± 0.31^{a}
	b- Saline	2.72 ± 0.22^{b}	1.30 ± 0.33^{b}
Significance		**	**

Values in the same column bearing different superscripts differ significantly ($P < 0.01$).
** $P < 0.01$

Table 4. Fertility results of low fertile bucks treated with GnRH as compared with the saline-treated and fertile bucks.

Experimental bucks	Treatment	No. of does inseminated	No. of does kindled	Kindling (%)
Fertile	Untreated	12	7	58.3^{a}
Low fertile	a- GnRH	18	11	61.1^{a}
	b- Saline	21	3	14.3^{b}

Values bearing different superscripts differ significantly ($P < 0.01$).

GnRH-treated bucks and the fertile bucks. These results emphasize the hypothesis that buck fertility could be improved by stimulation of testicular androgen secretion induced by chronic treatment with GnRH, since the physiological processes which involve the maintenance of the production of high quality semen is considered to be androgen dependent. The fertility results obtained in this investigation concerning the GnRH-treated bucks could be considered satisfactory as compared with those obtained by Sinkovics *et al.* (1983), Szendro *et al.* (1992) and El-Gaafary *et al.* (1993) using fresh semen collected from fertile bucks and inseminated into nursing does.

Data presented in this investigation indicated that injection of low fertile bucks with 20 μg GnRH every 3 days for a total of 6 times improved, in general, semen characteristics, testosterone concentration, mating activity and fertility as assessed by kindling rate. Therefore, GnRH could be used to overcome the temporary sterility which commonly occurred for some bucks during summer.

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