SEMEN CHARACTERSTISCS OF BUCKS IN CROSSBREEDING PROJECT INVOLVING SAUDI GABALI WITH V-LINE RABBITS IN SAUDI ARABIA

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Semen characteristics of bucks were evaluated for five genetic groups produced from a crossbreeding project involving Spanish breed called V-line (V) and Saudi Gabali (G) rabbits. For the first two generations of this project, volume of ejaculate, sperms abnormalities, living sperms, dead sperms, sperms concentration, motility of sperms and libido of bucks of these genetic groups were evaluated for 195 ejaculate given by 74 bucks. Seasonal variations in semen ejaculated by bucks and effect of litter size in which the buck was weaned were also investigated.

Sperms concentration (being 525, 543 and 488 sperm in 0, 1st and 2nd generation, respectively) and motility of sperms (being 47.4, 68.1 and 79.9 % in 0, 1st and 2nd generation, respectively) increased or improved from the parental generation to the two subsequent generations. Significant improvements in semen parameters of crossbred bucks than in purebred ones were recorded in terms of sperms concentration (P<0.01) and percentages of motile sperms (P<0.001), abnormal sperms (P < 0.001) and dead sperms (P < 0.05). Most semen parameters of V-line bucks were better than in G rabbits. Lower volume of ejaculate (0.56 ml) in addition with lower motility rate of sperms (50.6%) was noticed in the Gabali rabbits than in the other genetic groups. Autumn and winter recorded the best semen parameters in terms of sperm concentration, motility of sperms, abnormal sperms and libido relative to spring and summer. The estimates during autumn, winter, spring and summer were 537, 564, 405 and 437 for sperms concentration (sperm multiplied by 106 per ml), 72.0, 68.5, 61.6 and 58.4% for motility of sperms, 7.6, 5.3, 9.6 and 10.2% for abnormal sperms and 4.03, 3.38, 3.51 and 3.04 for libido. Moderate litter size of 6 or 7 born by the doe gave the lowest abnormality rate of sperms in the semen of bucks (about 7.8%) and the largest volume of ejaculate (about 0.68 ml) associated with the highest sperm concentration (about 522 sperm multiplied by 106 per ml), sperm motility (about 78%), living sperms (about 91.5%) and libido (about 4%).

Keywords: Gabali Saudi rabbits, crossbreeding, semen parameters, genetic groups, seasonal variation.

Undoubtedly bucks are the basis of the reproductive success in the rabbit farms, but they have not received the attention they should have, mainly if we consider that one single buck is affecting the fertility and prolificacy of about one hundred does especially when artificial insemination (AI) is performed as a routine in the rabbit farms (Alvarino et al, 1996 a& b; Alvarino, 2000). Many factors are influencing the production and quality of semen such as the buck (Castellini, 1996), the genetic origin of bucks (paternal lines have worse seminal qualities than maternal lines; Egea et al., 1992), the season (poor quality of semen in summer; Amin et al., 1987), the photoperiod (16L: 8D; Theau-Clement et al., 1995) and the frequency of ejaculate (Bencheijh, 1993). The literature concerning evaluation of semen parameters of V-line rabbits and their crosses raised in hot climate countries are scarce. Publications concerning also semen characteristics of Gabali rabbits and their crossbreds with standard breeds (e.g. Spanish V-line) in hot climate countries particularly in Saudi Arabia are not available. The objective of the present study was to investigate some physical and microscopic characteristics of the semen of bucks in crossbreeding project involving Spanish V-line rabbits and Saudi Gabali rabbits.

MATERIALS AND METHODS

A crossbreeding project was started in October 2000 in the experimental rabbitry, Collage of Agriculture and Veterinary Medicine, El-Qassim region, King Saud University, Saudi Arabia using rabbits of one desert Saudi breed (Gabali, G) and one exotic breed (Spanish V-line, V). The breeding plan used was permitted to produce V, G, ½V½G, ½G½V, ¾V¼G and ¾G¼V bucks within two generations. The project is still running and we have got the 3rd generation. Distribution of bucks in different genetic groups across the generations is presented in Table 1.

Bucks were housed separately in individual wired-cages arranged in flatdeck batteries in semi-closed rabbitry. During the experimental period, temperature ranged from 24 to 32 °C and photoperiod was 16L: 8D. Rabbits were fed on a commercial grower pelleted diet contained 18.5% crude protein (CP), 8.0% crude fiber (CF), 3.0% ether extract (EE) and 6.5% ash. Feed and water were available ad libitum.

Semen collection and analysis:

Bucks were housed in individual cages and the semen was collected with artificial vaginas (AV). A mature female was used for stimulating the males. Three or four ejaculates per buck were collected with one-week interval. Once the ejaculate had been collected, the volume (ml) was measured using a graduated tube. The ejaculates with abnormal colours (if there were blood or

urine, etc.) were not evaluated. If the ejaculates had gel, they were measured and removed. The samples were placed in an incubator (37 °C) to prevent cold shock. Each ejaculate was evaluated manually and examined microscopy. Semen was diluted with 2.9% sodium citrate dihydrate solution (37°C) and individual spermatozoa motility (%) was recorded on a subjective scale of 0 to 100% after viewing several microscopic fields. Percentages of live and abnormal sperms were recorded as described by Chemineau et al. (1991). One smear from each ejaculate were stained with easin-nigrosin stain and a total of 200 spermatozoa were examined randomly. Sperm cell concentration (multiplied by 106/ml) was quantified by direct cell count using the improved Neubauer haemocytometer (Boussit, 1989). Libido was recorded on a subjective scale of 0-5 where 0 score was given to those males who do not show any interest of mating and score of 5 to the very active ones. Records of five genetic groups of V, G, ½V½G, ½G½V and ¾V¼G were analyzed. The six genetic group of \(^1/4V^3/4G\) was excluded from the analysis due to its small number.

Traits investigated and model of analysis:

Semen parameters were represented by volume of the ejaculate in ml (VOL), pH of semen (pH), concentration or count of sperms (CS), motility of sperms % (MS%), percent of abnormal sperms (AS%) and percent of living (LS%) and dead (DS%) sperms and libido of bucks (LIB). Semen characteristics were statistically analyzed using the following mixed model (SAS, 1996):

 $Y_{ijklmnp} = \mu + G_i + S_{ij} + A_k + B_l + C_m + D_n + e_{ijklmnp}$ Where $Y_{ijklmnp}$ is the observation on the ijklmnpth semen trait; μ is the overall mean; G_i is the fixed effect of i^{th} genetic group; S_{ij} is the random effect of j^{th} buck within i^{th} genetic group; A_k is the fixed effect of the k^{th} generation; $B_l = is$ the fixed effect of the l^{th} season of kindling; C_m is the fixed effect of m^{th} litter size in which the buck was weaned; D_n is the fixed effect of m^{th} ejaculate order, and $e_{ijklmnp}$ is the random deviation particular to the p^{th} ejaculate of buck, NID $(0, \sigma^2 e)$. Genetic group by season interaction was non-significant and therefore was deleted from the model; other interactions were not possible.

RESULTS AND DISCUSSION

Generation effect:

Least square means for different semen characteristics in the parental generation and the other two subsequent generations of the study are presented in Table 2. No significant differences in the three generations were observed for VOL, AS%, LS%, DS% and LIB. The estimates were 0.50, 0.62 and 0.69 ml for volume of ejaculate, 3.5, 9.7 and 11.2 % for sperms abnormalities, 91.3,

91.2 and 90.3 % for living sperms, 8.7, 8.7 and 9.7 % for dead sperms, and 3.58, 3.47 and 4.14 for libido of bucks in parental, first and second generation, respectively. On the other hand, pH of semen (being 5.67, 7.23 and 7.07 in 0, 1st and 2nd generation, respectively), sperms concentration (being 525, 543 and 488 sperm in 0, 1st and 2nd generation, respectively) and motility of sperms (being 47.4, 68.1 and 79.9 % in 0, 1st and 2nd generation, respectively) increased from the parental generation to the two subsequent generations.

In general, most semen parameters in the 2nd generation were better than in the 1st generation (Table 2). This increment can improve the pregnancy rate especially if the does were bred to males that had been selected for semen quality. These results are encouraging factor in the project and showed that considerable gains could be attained for different semen parameters in the subsequent generations. Such improvement could be due to the fact that bucks of the new generations adapted much better to the local environment than bucks of their parental generation.

Table 1. Distribution of ejaculates of bucks in different genetic groups

Sire genotype	Dam genotype	Buck genotype ⁺	Parental generation	First generation	Second generation	Total
V-Line (V)	V-Line	V-Line	10	37	5	52
	(V)	(V)				
Gabali (G)	Gabali (G)	Gabali (G)	7	21	6	34
V	G	½V½G		47	-	47
G	v	1/2G1/2V		9	24	33
G	1/2V1/2G	3/4G1/4V		-	29	29
Total						195

V = V-line rabbits: G = Gabali Saudi rabbits, * Sire-breed of buck listed first.

Table 2: Least square means for semen characteristics in the two generations of the study

Traits	Parental generation [†]	First generation	Second generation	Sig ⁺⁺	
	Mean + SE	Mean + SE	Mean + SE		
- Control Hamilton	N = 17	N= 64	N= 114		
Volume of ejaculate (VOL)	0.50±0.07	0.62±0.06	0.69±0.12	*	
pH of semen (pH)	5.67±0.69	7.23±0.37	7.07±0.43	*	
Concentration of sperms (CS)	525±84	543±45	388±52	**	
Motility of sperms % (MS%)	47.4±5.6	68.1±4.9	79.9±9.1	***	
Abnormal sperms % (AS%)	3.5±2.47	9.7±1.3	11.2±1.5	**	
Living sperms % (LS%)	91.3±2.4	91.2±1.3	90.3±1.5	NS	
Dead sperms % (DS%)	8.7±2.4	8.7±1.3	9.7±1.5	NS	
Libido (LIB)	3.58±0.31	3.47±0.16	4.14±0.19	**	

^{*}Parental generation included only V-line and Gabali bucks.

 $^{++}NS = Non-significant; * = P < 0.05; ** = P < 0.01.$

Table 3: Least square means of purebred and crossbred groups and their standard errors (± SE) for semen characteristics

Traits		Sig+				
	VV	GG	½V½ G	⅓G½V	%V%G	
	N = 59	N = 27	N = 47	N = 33	N = 29	Total N = 195
VOL	0.62 ± 0.07	0.56±0.09	0.59 ± 0.08	0.66±0.08	0.60±0.09	NS
PH	7.12±0.42	5.47±0.53	6.89±0.48	6.86±0.49	6.95±0.54	
CS	385±50	513±63	409±57	553±59	568±66	**
MS %	56.7±5.4	50.6±6.85	62.7±6.22	79.3±6.41	76.3±7.10	***
AS %	10.7±1.48	8.79±1.87	6.85±1.70	6.05±1.74	8.55±1.93	***
LS %	90.8±1.5	89.1±1.8	91.9±1.7	93.8±1.7	89.1±1.9	*
DS %	9.2±1.47	10.9±1.86	8.1±1.69	6.2±1.74	10.9±1.92	
LIB	3.79 ± 0.18	3.33±0.23	4.12±0.21	3.35±0.22	3.57±0.24	***

*NS = Non-significant; * = P < 0.05; ** = P < 0.01. *** = < 0.001

Genetic groups:

In most cases, V-line bucks showed better semen characteristics compared to the Gabali bucks (Table 3). These results were expected and reflected the superiority of V-line rabbits in maternal behavior, milk production, and post-weaning growth of bucks (Estany et al, 1989). Therefore, V-line bucks could reproduce efficiently under hot climatic conditions of Saudi Arabia.

Least-square means for semen characteristics in the five genetic groups of the study (Table 3) verify that some of the semen parameters were improved in crossbreds compared with purebreds of V-line and Gabali. Crossbred bucks gave high concentrated semen (Figure 1) and this was an improvement in semen parameters especially if one likes to use the semen in artificial insemination technique. Alvarino et al (1996 a & b) stated that the number of does that can be artificially inseminated would increase as the ejaculate concentration increased.

Crossbreeding affects the growth rate of the whole body and this can leads to the early maturation of the hypothalamus and the pituitary, which directly affects the growth of the testes and finally the performance of the males. Crossbreds bucks were associated with positive effects on sperm motility compared with bucks of purebreds (Table 3). High percentages of 93.8% for live sperms in ½G ½V group (Table 3) affect positively the conception rate through the rapid transport of the sperm from the site of insemination into the site of fertilization. The low volume of semen in the bucks of the five genetic groups (Table 3) might be due to that semen was collected from those bucks at an early age (4-6 months). This notation was observed also in some studies (Dubiel et al, 1985; Regriguez et al, 1996; Luzi et al, 1996; Minelli et al, 1999). However, the volumes of ejaculates or sperm concentrations in semen are influenced by the age of the bucks. Luzi et al (1996) and Minelli et al

(1999) reported a significant effect of age of buck on sperm concentration, libido, ejaculate volume, sperm motility and pH. Arroita et al (2000) stated that young bucks at the beginning of their mating life showed high percentages of cytoplasmic droplets.

Crossbred bucks showed significant increase in sperm concentration (P<0.01) and motility of sperms (P<0.001) along with less sperms abnormalities (P<0.001) and dead sperms (P<0.05) than bucks of purebreds (Table 4 and Figure 1). Lower volume of ejaculate (0.56 ml) in addition with lower motility rate of sperms (50.6%) were noticed in Gabali rabbit than in the other genetic groups (Table 3) and this may be due the fact that they had not fully mature at the time they were collected. The recorded decrease in volume of ejaculate and motility of sperms in Gabali bucks were compensated with lower pH of semen. The reason behind this decrease in volume and motility could be due to the incomplete maturity of the pituitary. This can affect the secretion of lutinizing hormone (LH) which affects the secretion of testosterone from the interstitial tissue of the testes. The low amount of testosterone will not have its full stimulation to the accessory sex gland and the epididymis, which influence the volume and the maturity of the ejaculate.

The high variations in spermogram parameters could be attributed to breeds variations (Dubiel et al., 1985; El-Ezz et al., 1985). Alvarino (2000) reported wide variations in the figures of semen parameters in some breed groups to be: (1) the volume of semen varies from 0.3 to 0.6 ml depending on secretion of accessory sex glands (gel fractions), (2) sperm concentration ranges from 50 to 500 x 10⁶ / ml, and (3) the pH ranges between 6.8-8.4 and this is a good index to estimate semen quality. With Black Tan, New Zealand White, New Zealand Red and German Pied Giant, Dubiel et al. (1985) reported significant differences in semen parameters of the four breeds; being 0.68, 0.97, 0.83 and 1.51 ml for volume of ejaculate, 54, 66, 49 and 71% for motility of sperms, 97.6, 309.6, 221.7 and 502.5 for sperm concentration, respectively.

Table 4: Purebreds average v. crossbreds average for semen

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Traits	Purebreds average	Crossbreds average	Improvement % *	
VOL	0.59	0.62	5.1	
CS	449	510	13.6	
MS %	53.6	72.8	35.8	
AS %	9.7	7.1	-26.8	
LS %	89.9	91.6	1.9	,
	9.1	8.4	-16.8	
LIB	3.56	3.85	8.1	
DS % LIB				

^{*}Improvement % = [(crossbreds average / purebreds average) x 100] - 100

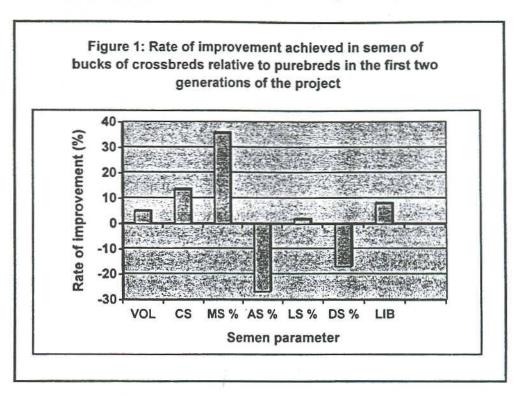


Table 5: Least square means for semen characteristics in the four seasons of the year

Traits	Autumn	Winter	Spring	Summer	Sig. ⁺	
	N=36	N=39	N=58	N=62	Total N=195	
VOL	0.63 ± 0.08	0.63 ± 0.08	0.62 ± 0.07	0.56 ± 0.07	NS	
PH	6.99±0.08	6.45 ± 0.48	6.58±0.43	6.61±0.44	NS	
CS	537±62	564±58	405±52	437±53	***	
MS %	72.0±6.7	68.5±6.2	61.6±5.6	58.4±5.8	*	
AS %	7.6±1.8	5.3±1.7	9.6±1.5	10.2±1.6	*	
LS %	92.4 1.8±	89.8±1.7	90.6±1.5	90.9±1.6	NS	
DS %	7.6 ± 1.8	10.2 ± 1.7	9.3±1.5	9.1 ± 1.6	NS	
LIB	4.03 ± 0.22	3.38 ± 0.21	3.51±0.19	3.02 ± 0.19	**	

*NS = Non-significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

The percentages of abnormal sperms in the five genetic groups (Table 3) were lower than those estimates reported in the other studies (Dubiel et al, 1985; El-Ezz et al, 1985; Castellini, 1996; Alvarino et al, 1996 a & b; Alvarino, 2000; Arroita et al., 2000). However, cytoplasmic droplets in different genetic groups were the most common cause of sperm abnormalities. This was expected and not strange thing since bucks of the present study were examined at young age at 4 months.

Seasonal variation:

Across the four seasons of the year, it can be observed that the differences in ejaculate volume, pH, LS% and DS% were not significant (Table 5). On the other hand, sperm concentration, motility, abnormal sperms and the sexual drive of the bucks were not the same through the seasons of the year. Sperm concentration and percentages of motility of sperms and libido during autumn and winter were much better than in spring and summer (Table 5). In addition, autumn and winter recorded the lowest rate of abnormal sperms (7.6 and 5.3%, respectively) compared with the rates recorded by spring and summer (9.6 and 10.2%, respectively). This indicates that some semen characteristics of the bucks in El-Qassim region might be much affected by ambient high temperature than by day length and photoperiod. Variabilities in spermogram values of V-line rabbits may be due to that V-line bucks had changed their environmental conditions from cold climate in Spain to hot ambient temperature in Saudi Arabia. Under hot climatic areas, strong seasonal breeding patterns are not observed in the bucks, while the highest ejaculate volume and sperms concentrations were found during autumn and winter and the lowest at summer and the end of the spring (Amin et al., 1987). Changes in the pH of semen and morphological alterations of the sperm could be increased during the summer (Amin et al., 1987). Temperatures higher than 27 °C can affect fertility due to increasing semen pH values and morphological alterations (abnormalities), as well as a decrease in sperm motility and libido (Brockhausen et al., 1979; Bagliacci et al., 1987). In our study, the semen pH of V-line (7.21) was higher than in Gabali rabbits (5.03). Amin et al. (1987) and Ibrahim (1994) in Egypt observed also a decrease in sperm concentration for V-line bucks raised in hot ambient temperature. This could be attributed to the decrease in sertoli cells activity (El-Masry et al., 1994). Recently, Moce et al. (2000) stated that high ambient temperature affected the total sperm production.

The semen parameters may be influenced by the changes in photoperiod (Boyd, 1985 & 1986). The increase of day length affects the pineal gland to decrease its secretion of melatonin hormone, which affect the hypothalamus negatively. Therefore, GnRH release is influenced by the photoperiod (Lin and Ramirez, 1988). The effect of photoperiod start with the retina, which receives the

change in day length and ends with the pineal gland which, increases its release of melatonin hormone. Melatonin is a hormone that produced during the hours of darkness and play an important role in the control of the annual breeding season in some mammals. This hormone affects the reproductive procedure either positively or negatively and this is species dependent. In rabbits, photoperiod (acting via melatonin) limits fertility by controlling release of

gonadotropin releasing hormone (GnRH) from the hypothalamus and consequently decreases lutinizing hormone and follicle stimulating hormone (FSH). Therefore, gonads are inactive during the non-breed season. However, spermatogenesis in rabbits shows seasonal variations related chiefly to photoperiod and also temperature (Alvarineo, 2000). Several studies (e.g. Boyd and Myhill, 1987) showed that peak of fecundity occurring during spring in which testes exhibiting active spermatogenesis. The results of our study did not show this trend. Changes in the scrotal testis length provide a good indicator of the reproductive status of the buck (Boyd, 1985). This parameter may be influenced by changes in photoperiod (Boyd, 1985 & 1986). Gonadotrophine releasing hormone (GnRH) secretion is influenced by the photoperiod with a higher level of GnRH release in the evening than in the afternoon hours (Lin and Ramirez, 1988). Seasonal variations in GnRH release occurred even in bucks maintained in a fixed photoperiod of 12L: 12D (Lin and Ramirez, 1991). There were seasonal changes in the secretion of GnRH pulses with the lowest values occurring just before the winter solstice. The frequency of GnRH pulses increased after the winter solstice, and GnRH release increased during and within one month after the summer solstice (Lin and Ramirez, 1991).

Litter size in which the buck was born:

Males which born by does that gave a litter size of 6 or 7 (moderate litter size) had the best semen motility and concentration (Table 6). These are the two important characteristics of semen that directly affect the conception rate of mated females. Normally, the hypothalamus and pituitary of a female that gives large litter size are more active and synthesize and secrete more reproductive hormones. The hypothalamus secretes gonadotrophine-releasing hormone (GnRH), which enhances the anterior pituitary to secrete two important hormones: follicle-stimulating hormone (FSH) and lutinizing hormone (LH). In the female, FSH and LH stimulate the growth, maturation and ovulation of new follicles of the primary gonads, the ovary. Female that genetically has this high level of GnRH, FSH and LH will pass this trait to their males and females offspring's. In the male, the increase of FSH enhances the production of semen while LH hormone affects the sexual drive, libido. LH stimulates the interstitial, leydig cells, to increase their secretion of testosterone hormone that positively affects libido. Males which came from does gave large litter size might have great genetic features but they did not get sufficient milk from their dams. Therefore, they did not grow and mature as fast as those who came from does gave small litter size. On the other hand, those males who came from females do not have high litter size, allow them to give excellent ejaculate and strong sexual drive. For these reasons just explained above, we

Table 6: Least square means for semen characteristics in different litter-size classes in which the buck was born

Trait	Litter-size class								Sig.	
	2 10	3	4	5	6	7		8	9	+
	N =6	N=2	N=21	N=48	N=36	N=19	N =29	N =26	N =8	
VOL	0.57	.49	.60	.64	.71	.66	.55	.51	.72	NS
	±.12	$\pm .20$	±.08	±.06	±.07	±.09	±.08	±.07	±.12	
PH	6.40	7.07	6.73	6.50	6.76	7.28	6.30	6.71	6.18	NS
	±.72	±1.18	±.48	±.36	±.42	±.51	±.48	±.44	±.71	
CS	469	462	591	452	518	527	389	522	415	
	±87	±42	±58	±43	±51	±61	±58	±53	±85	
MS %	85.7	51.6	56.2	64.9	75.7	79.0	63.6	58.7	50.8	
	±9.5	±5.3	±6.2	±4.7	±5.5	±6.6	±6.3	±5.8	±9.2	
AS %	12.9	11.9	9.7	8.8	6.8	6.0	8.5	10.4	8.5	
	±2.6	±4.2	± 1.7	±1.2	±1.5	±1.8	±1.7	±1.6	±2.5	
LS %	86.4	83.5	90.7	90.3	91.8	91.1	91.5	88.2	95.0	
	±2.6	±4.1	± 1.7	±1:3	±1.5	±1.7	±1.7	±1.6	±2.4	
DS %	13.6	16.5	9.3	9.7	8.2	8.9	8.5	11.8	5.0	
	±2.6	±4.1	±1.7	±1.3	±1.5	±1.7	±1.7	±1.6	±2.4	
LIB	3.85	2.82	3.89	3.57	3.90	4.14	3.52	3.74	4.18	
	±.32	±.52	±.21	±.16	±.19	±.22	±.21	±.19	±.31	

*NS = Non-significant; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

can understand the reasons behind the high reproductive performance of those males that were born by females gave moderate litter size.

CONCLUSIONS

- (1) Preliminary results of the project could be an encouraging factor to produce crossbred bucks involving V-line genes that could be used as a suitable genetic resource for production of rabbits under hot climate conditions. Since performances of bucks for most semen parameters in the second generation were better than in the first one, these results could also be considered as an encouraging factor in the project and showed that considerable gains could be attained for different semen parameters in the subsequent generations.
- (2) Since semen parameters in Spanish V-line are significantly better than in Saudi Gabali rabbits, one may use V-line bucks as sires. For commercial rabbits industry in Saudi Arabia, V-line bucks could be used in terminal crossbreeding system especially in such areas of hot climate.
- (3) Crossbred bucks resulted from mating Saudi Gabali sires with V-line dams is recommended (i.e. ½G½V or ¾V¼G) and producers and processors could potentially benefit economically through commercial production by this synthetic cross.

(4) Crossing V-line with Saudi Gabali rabbits was associated with an increase in ejaculate volume (P<0.05), sperm concentration (P<0.05), percentages of motile and living sperms, and libido of bucks (P<0.001) along with a reduction in percentages of abnormal and dead sperms.

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