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EFFECT OF SEMEN PRESERVATION PERIOD ON ITS QUALITY AND FERTILITY IN GIMMIZAH AND SINAI LOCAL STRAINS

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ABSTRACT

This study aimed at investigating the effect preservation time (0, 24, 48 and 72 hours) on physical characteristics, activity of transaminases (AST and ALT) in seminal plasma as well as fertility of semen collected from two strains (Gimmizah and Sinai). Eighty males (38 wk of age) were used in this study (40 males of each strain), they divided into four experimental groups (10 males/group) and hundred females from each strain at the same previous age were divided into four groups (25 females per group). Semen was collected from each group of males day by day at the same time of insemination and diluted with semen extender (1:1) at 37°C in water bath in the poultry house. Semen of the 1st, 2nd and 3rd groups was preserved at 5°C for 24, 48 and 72 hours, respectively. While, semen of the 4th group was directly used for insemination. Results indicated that semen of Gimmizah cocks had significantly higher percentages of sperm motility and live spermatozoa. However, Sinai strain was significantly better in early embryonic mortality, late dead embryos and unhatched eggs with live or dead embryos. Lengthening storage time significantly ($P<0.05$) decreased fertility and semen characteristics and increased all traits of embryonic mortality, activity of AST and ALT. The interaction between strain and cooling period had significant effects on the rate of late dead embryos, unhatched eggs with piped dead embryos and all semen characteristics.

INTRODUCTION

Artificial insemination with preserved spermatozoa has long been recognized as important to poultry production. Semen diluents are used for improving the reproductive efficiency of males and for reducing the cost of artificial insemination. Dilution of semen allows more extensive use of individual ejaculate of semen which can be cover larger number of females. Diluents include different components as osmotic regulators, energy sources, buffers and chelating agents to provide a protection against the harmful effects of cooling or freezing.

Seminal plasma as diluter had a negative effect on fertilizing ability of stored semen (Blesbois, 1990 and Lake and Ravie, 1987), and clear toxic effects of seminal plasma on the spermatozoa was observed when the semen was stored (Blesbois, 1986 and Sexton, 1988). Storage up to 24 hours at 5°C affects fertilizing capacity of fowl semen to a shorter extent than cryopreservation (Bellagamba *et al.*, 1993). Storage period was found to affect livability, abnormality and viability of Dandarawi and Fayuomi spermatozoa (Dowidar *et al.*, 2004), however, high fertility after insemination with fowl semen stored for 24 hours at 4°C was reported by Lake and Ravie (1979).

Salisbury *et al.* (1976) reported that rapid decrease in temperature of semen from 37 to 5°C causes cold shock and damage to the sperm cell which is caused by changes in gas solubility, metabolism, fluid viscosity and chemistry of semen. Cold shock increased the release of aspartate transaminase (AST) in seminal plasma, which was attributed to an increased permeability of cell membrane (Bruke and Pickett, 1971). Leakage of AST into the extra-cellular medium may clarify the process of sperm cell damage which occurs due to the preservation (Graham *et al.*, 1970 and Garbers *et al.*, 1971). Activity of AST in seminal plasma was found to be associated with semen characteristics and might play a role in the protection of semen during preservation (Hadarrage *et al.*, 1976).

The drop in fertilizing ability among eggs laid at a later date after insemination is a good indicator of the adverse effect on the spermatozoa by storage (Van Voorest and Leenstra, 1995). Genetic influences appear to affect response and tolerance of spermatozoa to thermal treatments resulting in differences in subsequent fertility (Bellagamba *et al.*, 1993).

The current study aimed at investigating the effect of storage period up to 72 h at 5^{o}C on quality and fertility of semen collected from Gimmizah and Sinai local strains of chickens.

MATERIALS AND METHODS

This study was carried out at Gimmizah Research Station, Animal Production Institute, Agricultural Research Center, during the period from 10/2006 to 4/2007

Birds:

Total of 80 males (38 wk of age) from two local strains (Gimmizah and Sinai, 40 males in each strain) were divided into four groups (10 males in each group). While, hundred females from each strain at the same previous age were divided into four groups (25 females in each group) as for male groups.

All birds were housed in individual cages. Daily lighting was 16 hours. Birds were fed a commercial layer diet (16% crude protein and 2850 Kcal ME/kg). Females were fed *ad-libitum*, while males were restricted to feed intake of 120 g/day. Water was available all day for female and male groups.

Collection, dilution and evaluation of semen:

Before the main collection period, semen was individually collected from males in each group and microscopically examined to determine sperm motility for 5 days. Throughout the main collection period, semen was collected from all males day by day for four days.

Semen was diluted with egg yolk-citrate extender at a rate of 1:1 at 37°C in water bath within the poultry house. The diluted semen was gradually cooled from 37 up to 5^{o}C, then the diluted semen of the 1st, 2nd and 3rd groups were stored at 5^{o}C for 72, 48 and 24 hours, respectively. While, semen of the 4th group was evaluated and used directly for insemination without storage period. Five samples of each experimental group of semen were stored at -20°C for determination of transaminases activity (AST and ALT) in seminal

plasma. Activity of AST and ALT transaminases were determined calorimetrically by using commercial kits (Bio-Merieux, Franc

Semen at each storage time for each experimental group was evaluated for the percentages of live, dead and abnormal spermatozoa in semen smears stained by eosin and nigrosin (Van der Schaaf, 1952).

Insemination, egg incubation and fertility test:

Intravaginally insemination (about 4 cm depth) was carried out using a rabbit insemination pistol by about 0.5 ml from diluted semen/hen. All inseminations were carried out on the same afternoon at the same times of semen storage period for each experimental group.

Eggs of each female group were collected and individually weighed after insemination for seven days. The collected intact eggs were incubated at 37.6°C and 55% relative humidity for 18 days, and then eggs were weighed individualy and examined by candling to determine fertility rate. The infertile eggs and eggs with dead embryos were calculated for each group. Thereafter, eggs were transferred in the hatchery at 37.2°C and 65% relative humidity.

Unhatched eggs were examined and classified as eggs with piped live embryos, eggs with piped dead embryos and eggs with late dead embryos and expressed as a percentage of the total incubated eggs.

Statistical analysis:

Data collected were subjected to analysis of variance as a factorial design (2 strains x 4 experimental groups) using the statistical analysis of SPSS (1997) version 10. The significant differences among treatment means were tested using multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Sperm characteristics:

Data listed in Table (1) show that strain had significant ($P<0.05$) effect only on sperm motility percentage, being higher in Gimmizah than in Sinai semen by about 11.5%. However, percentages of dead and abnormal spermatozoa were nearly similar in semen of both strains. The present results agreed with those of Afifi and Dowidar (2000), who found better semen quality in Dandarawi than Fayoumi strain and this difference could be attributed to the genetic make-up of each strain.

The effect of storage time on sperm characteristics was significant ($P<0.05$). Percentages of sperm motility significantly ($P<0.05$) reduced, while percentages of dead and abnormal spermatozoa significantly ($P<0.05$) increased by increasing storage time from 0 up to 72 hours (Table 1).

It is of interest to note that the observed reduction in sperm motility percentage was significantly ($P<0.05$) higher between 0 and 24 or 24 and 48 h than that occurred between 48 and 72 h. However, the recorded rate of increase in dead and abnormal sperm percentages was significant ($P<0.05$) between 0 and 24 or 48 and 72 h and insignificant between 24 and 48 h. Generally, the lowest sperm motility percentage and the highest dead and abnormal sperm percentages were significantly ($P<0.05$) obtained at 72 h of storage period (Table 1).

Table (1): Sperm characteristics as affected by strain, storage period and their interaction.

Item	Sperm characteristics (%)			
	Motility	Live and normal	Dead	Abnormal
Effect of strain:				
Gimmizah (G)	65.71[a]	64.39	20.50	15.11
Sinai (S)	58.93[b]	63.75	21.04	15.21
±SEM	1.01	0.66	0.37	0.33
Effect of storage time (h):				
0	83.57[a]	71.29[a]	16.50[c]	12.21[c]
24	67.50[b]	64.36[b]	21.00[b]	14.64[b]
48	52.86[c]	63.79[c]	20.86[b]	15.36[b]
72	45.36[d]	56.86[d]	24.71[a]	18.43[a]
±SEM	1.41	0.93	0.52	0.46
Effect of interaction between strain and storage time:				
G x 0	81.43	66.57	19.00	14.43
G x 24	74.29	70.00	18.00	12.00
G x 48	57.86	63.43	20.86	15.71
G x 72	49.29	57.57	24.14	18.29
S x 0	85.71	76.00	14.00	10.00
S x 24	60.71	58.71	24.00	17.29
S x 48	47.86	64.14	20.86	15.00
S x 72	41.43	56.14	25.29	18.57
±SEM	1.99*	0.75*	0.74*	0.65*

a, b….d: Means within the same column with different superscripts are significantly different (P<0.05). * Significant interaction at P<0.05.

These results agreed with those of reported by Dowidar *et al.* (2004), who observed that lengthening the storage period from 6 up to 12 h at 5 ^{o}C decreased percentages of live sperm and advanced motility percentages and increased total abnormalities in Dandarawi and Fayuomi semen. The observed deterioration of semen characteristics with increasing storage period may be attributed to increasing the metabolic by-products of spermatozoa (lactic acid), which resulted in decreasing pH value of extracellular medium of spermatozoa leading to reduced sperm motility and sperm death.

In addition, the increase of total abnormalities with increasing storage period could be attributed to peroxidative damage (Al-Daraji, 2001).

Although sperm quality was higher in Gimmizah than Sinai, the significant (P<0.05) effect of interaction between strain and storage period on motility, dead and abnormality percentages was reflected in the highest sperm motility and the lowest dead and abnormality percentages were obtained for Sinai strain immediately after dilution at 0 h of storage period. However, an opposite situation was noticed for the same strain at 72 h of storage period (Table 1). These results may suggest higher preserve ability of Gimmizah than Sinai spermatozoa at 5^{o}C for 72 h.

Activity of transaminases (AST and ALT):

Data presented in Table (3) show that strain had no significant effect on activity of AST and ALT, although there was a tendency of higher values in Gimmizah than Sinai semen.

Storage time had significant effects on activity of transaminases (AST and ALT), showing marked increase by increasing storage time.

Activity of AST significantly (P<0.05) increased by advancing storage time from 0 up to 72 h. This increase is coincident with the higher percentage of dead sperms as the storage period increased. In this respect, it was reported that the leakage of these enzymes (AST and ALT) is related to dead spermatozoa (EL-Wardany, *et al.*, 1995); inadequate storage (Bilgili, *et al.*, 1985) and had handling of semen samples (Kundu and Panda, 1991). However, activity of ALT showed insignificantly slight increase between 0 and 24 h and significantly higher increase between 24 and 48 or 48 and 72 h. Generally, the highest values of all parameters studied were recorded at 72 h of storage period (Table 2). The present results are in agreement with those obtained by Dhami *et al.* (1987), who found that activity of AST and ALT in seminal plasma of buffalo bulls significantly increased in frozen-thawed semen.

Table (2): Activity of transaminases (AST and ALT) and concentration of prostatic acid in seminal plasma as affected by strain, storage period and their interaction.

Item	Activity in seminal plasma (IU/l)	
	AST	ALT
Effect of strain:		
Gimmizah (G)	131.88	66.25
Sinai (S)	127.94	64.94
±SEM	2.95	1.43
Effect of storage time (h):		
0	100.88^{d}	51.25^{c}
24	122.50^{c}	56.63^{c}
48	141.25^{b}	69.38^{b}
72	155.00^{a}	85.13^{a}
±SEM	4.17	2.02
Effect of interaction between strain and storage time:		
G x 0	106.25	51.25
G x 24	122.50	57.25
G x 48	142.50	70.00
G x 72	156.25	86.50
S x 0	95.50	51.25
S x 24	122.50	56.00
S x 48	140.00	68.75
S x 72	153.75	83.75
±SEM	5.90	2.86

a, b….d: Means within the same column with different superscripts are significantly different (P<0.05). * Significant interaction at P<0.05.

The obtained results indicated early release of AST than ALT from spermatozoa to extra-cellular medium during storage period. Also, the gradual release of AST, ALT and prostatic acid was associated with gradual reduction in semen quality with progress of storage period. Dhami *et al.*

(1987) mentioned that the release of AST pre- and post-freeze had a significant positive correlation with initial motility and post thaw- motility. Also, Hadarrage *et al.* (1976) found that activity of AST in seminal plasma of bulls was associated with semen characteristics.

The Interaction of storage time with strain on activity of transaminases (AST and ALT) was not significant, reflecting the highest values in Gimmizah semen at 72 h of storage period, while the lowest values wee obtained in Sinai strain in diluted semen (0 storage time). These results indicated also higher release of AST from Sinai than Gimmizah spermatozoa, while the release of ALT and prostatic acid was nearly similar for both strains throughout different storage times (Table 2).

Based on the obtained results of sperm characteristics throughout different storage times, results of enzyme activity indicated higher preserve ability of Gimmizah than Sinai spermatozoa at 5^{o}C for 72 h.

Fertility rate:

Data presented in Table (3) show that strain had no significant effect on fertility rate, being similar in both strains (80.39 and 80.87% for Gimizah and Sinai, respectively). These results agreed with those reported by Nofal (1997), who found insignificant differences in both fertility and hatchability percentage between Gimmizah and Mamourah pullets.

Table (3): Fertility and embryonic mortality as affected by strain, storage time and their interaction.

Item	Fertility rate (%)	Embryonic mortality (%)			
		Early dead	Late dead	Live pipped	Dead pipped
Effect of strain:					
Gimmizah (G)	80.39	5.49^{a}	4.84^{a}	3.07^{a}	2.35^{a}
Sinai (S)	80.87	4.61^{b}	3.90^{b}	2.80^{b}	2.26^{b}
±SEM	0.79	0.12	0.04	0.03	0.02
Effect of storage time (h):					
0	91.04^{a}	4.16^{c}	4.21^{bc}	2.81^{b}	2.19^{b}
24	85.66^{b}	4.82^{b}	4.32^{b}	2.85^{b}	2.31^{a}
48	80.86^{c}	5.07^{b}	4.41^{ab}	2.99^{a}	2.33^{a}
72	64.94^{d}	6.14^{a}	4.54^{a}	3.07^{a}	2.39^{a}
±SEM	1.1	0.18	0.06	0.04	0.03
Effect of interaction between strain and storage time:					
G x 0	90.47	4.36	4.64	2.93	2.31
G x 24	85.59	5.40	4.64	3.01	2.37
G x 48	80.68	5.59	4.86	3.11	2.34
G x 72	64.80	6.63	5.20	3.21	2.37
S x 0	91.60	3.97	3.77	2.70	2.07
S x 24	85.74	4.24	4.00	2.69	2.24
S x 48	81.04	5.56	3.97	2.87	2.32
S x 72	65.09	5.66	3.87	2.93	2.41
±SEM	1.58	0.25	0.08*	0.06	0.04*

a, b….d: Means within the same column with different superscripts are significantly different (P<0.05). * Significant interaction at P<0.05.

As affected by storage time of semen, fertility rate significantly (P<0.05) decreased with by increasing storage time. Eggs produced from hens inseminated directly with diluted fresh semen had significantly (P<0.05) the highest fertility rate (91.04%) when compared with those inseminated with semen stored for 24, 48 or 72 h before insemination (85.6, 80.86 and 64.94%, respectively). These findings are in agreement with those of Van Voorst and Leenstra (1995), who reported that semen stored for 24 hours at 5^{o}C reduced fertilizing ability of spermatozoa in the 1st week after insemination, especially when no dialysis was applied. A rapid decrease in semen temperature from 37 to 5^{o}C causes cold shock and damage to sperm cell which is caused by changes in gas solubility, metabolism, fluid viscosity and chemistry of semen (Salisbury *et al.*, 1976).

The interaction between strain and storage time was insignificant on fertility rate (Table 3). Nearly similar trend of change in fertility of hens in both strains inseminated with semen stored at different storage times, being the highest for Sinai strain inseminated with diluted semen and the lowest in Gimmizah hens inseminated with semen stored for 72 h (Table 3).

Embryonic mortality:

Results shown in Table (3) reveal that strain had significant (P<0.05) effect on all embryonic morality parameters, being higher in Gimmizah than Sinai strain. Genetic variation between strains plays a role in early and late embryonic mortality. In accordance with the previous findings, Galal (1990) observed that naked neck birds had higher dead embryos compared to normal plumage birds at all stages of embryonic mortality.

Also, storage time affected all embryonic mortality parameters, being the highest for eggs produced from hens inseminated with semen stored at for 72 h, while eggs of hens inseminated with diluted fresh semen had the lowest embryonic motility parameters.

It is worthy noting that the observed increase in morality rate with increasing storage time was more pronounced on early and late embryonic mortality than on mortality of live piped and dead pipped embryos. Late dead and pipped live embryos were not affected by storage of semen for 24 hours, but significantly (P<0.05) increased by increasing storage period to 48 and 72 h (Table 3). The present results disagreed with those obtained by Williamson *et al.* (1981), who reported that embryonic mortality was unaffected by cooling and freezing procedures.

The interaction between strain and storage time was significant (P<0.05) on late and dead piped embryonic mortality, indicating higher increase in dead piped mortality in Sinai than Gimmizah and an opposite trend for late embryonic mortality with increasing storage time. While the insignificant interaction on early and live piped embryonic mortality reflected similar trends of change in both strains with storage time (Table 3).

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تـأثير مـدة حفـظ السـائل المنـوي علـى خصائصـه والخصـوبة فـي سـلالتي الجميـزة وسيناء

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أجري هذا البحث لدراسة تأثير السلالة وفترة التبريد على الخصوبة والنفوق الجنيني وخواص السائل المنوي ومستوى إنزيمي AST,ALT في السائل المنوي.

استخدم في هذه الدراسة ٨٠ ديك(عمر ٣٨ أسبوع) من سلالتي الجميزة وسيناء (٤٠ ديك من كل سلالة) قسمت كل منها إلى ٤ مجموعات (١٠ ديوك في كل مجموعة) , كما استخدم ١٠٠ دجاجة من كل سلالة قسمت كل منها إلى ٤ مجموعات (٢٥ دجاجة في كل مجموعة). تم جمع السائل المنوي على مدار أربعة أيام (مجموعة/يوم) بحيث يتم التلقيح في وقت واحد كما تم تخفيف السائل المنوي بالمخفف بنسبة ١:١ على درجة حرارة ٣٧م في حمام مائي في مسكن الطيور. حيث خصصت المجموعات الأولى والثانية والثالثة للتلقيح بعد التبريد على درجة ٥م لمدة ٢٤, ٤٨, ٧٢ ساعة على الترتيب بينما خصصت المجموعة الأولى للتلقيح المباشر. وجاءت النتائج كما يلي:-

- كانت سلالة سيناء هي الأفضل معنويا في معدل النفوق الجنيني في الفترتين الأولى والأخيرة من التفريخ وأيضا معدل الأجنة الناقرة الميتة أو الحية بالبيض الغير فاقس ، بينما تفوقت ديوك سلالة الجميزة في معدل الحيوانات المنوية الحية وأيضا معدل حركتها.
- كان لإطالة فترة التبريد تأثيرا معنويا وتدريجيا في نقص معدلات الخصوبة وخواص السائل المنوي بينما (AST, ALT). زادت معدلات النفوق الجنيني وإنزيمي
- كان للتداخل بين السلالة وفترة التبريد تأثيرات معنوية على معدل النفوق الجنيني في الفترة الأخيرة من التفريخ ومعدل البيض الغير فاقس الذي يحوي أجنة ناقرة ميتة وأيضا على جميع خواص السائل المنوي.