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EVALUATION OF SEMEN QUALITY, FREEZABILITY AND FERTILITY OF HOLSTEIN BULLS UNDER EGYPTIAN NEWLY-RECLAIMED LAND CONDITIONS

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SUMMARY

The objective of this study was to evaluate semen quality, freezability and field fertility of five Holstein bulls used for AI under Egyptian conditions. Spermiogram and freezability of raw semen collected twice weekly at a frequency of two ejaculates in succession were assessed throughout a full-year. Tris-yolk, skimmilk-yolk and citrate-yolk extenders were used for dilution either in one- or two-steps to achieve a final concentration of 30 x 10^6 motile sperm/ml. Diluted-equilibrated semen was packaged in 0.5 ml French straws and frozen on nitrogen vapor (at -100°C for 15 minutes) before plunging into liquid nitrogen for storage. Samples of each semen batch were thawed at 40°C for 30 seconds and incubated at 37°C for assessment of motility and sperm acrosomal, head and tail defects after 0 and 3 hours of incubation post-thaw. Field inseminations were conducted in small-scale (2-5 cows), medium-scale (20-50 cows) and large-scale (200-500 cows) breeder-farms using insemination doses proven to have minimal values of post-thaw progressive motility and sperm normalcy of 60 and 70 %, respectively. Spermiogram of raw semen was significantly ($P< 0.01$) affected by the prevailing temperature. Ejaculate volume and sperm concentration, motility and normality decreased in semen collected in hot summer months, however, the extent of dead cells increased. Semen collected in hot months revealed a poorer freezability ($P< 0.01$) compared to that collected in cooler months. Dilution in citrate-yolk extender significantly ($P< 0.01$) improved sperm freezability compared with dilution in Tris-yolk or skimmilk-yolk. Glycerolation after a sufficient holding period at 5°C with egg-yolk lipoproteins significantly improved sperm freezability. Semen collected in hot months revealed the least post-thawing sperm longevity ($P< 0.01$) compared to that collected in cooler months. Dilution in citrate-yolk extender significantly ($P< 0.01$) improved post-thawing sperm livability compared with dilution in Tris-yolk or skimmilk-yolk. Post-thawing sperm longevity significantly ($P< 0.01$) improved in semen glycerolated at 5°C.

A total 6063 field first-inseminations revealed an overall conception rate (non-return rate % within 60 days) of about 60%, however, a significant (P< 0.01) difference in conceptions had recorded between the three types of breeder-farms. The least conception (39%) recorded in the small-scale breeder-farms, while the best conceptions (71 %) recorded in the large-scale ones. Hormonal control of estrus before AI significantly (P< 0.01) improved fertility by about 32 % compared to insemination of cows observed standing natural heat.

Keywords: Holstein bulls, semen, spermiogram, extenders, glycerolation, freezability, post-thaw longevity, fertility.

INTRODUCTION

Artificial insemination (AI) technology is the first generation reproductive biotechnology that made a profound contribution in genetic improvement of livestock. In dairy cattle, the combined use of genetic evaluation, AI and frozen semen and more recently several other reproductive technologies, has resulted in a high genetic gain in Holstein Friesian breeds. The art of AI has now become a practical technology in commercial dairy cattle programs in both developed and developing countries, covering a worldwide total of about 50 million first inseminations (Chupin and Thibier, 1995). The desired results of AI-programs would not have been possible without successful freezing of semen. Before using of a sire for AI-programs, its potential fertility must be assessed, including clinical and laboratory evaluations. Male fertility is complex and depends upon a heterogeneous population of spermatozoa interacting at various levels of the female genital tract, the vestments of the oocyte and the oocyte itself (Rodriguez-Martinez, 2003). There are a number of causes for male infertility which include genetic, environmental, anatomic, hormonal, infectious and nutritional causes. In AI-programs, the male ejaculates are divided into multiple doses for AI and can contribute hundreds of thousands of pregnancies through AI (Tardif et al., 1999; Foote, 2003). Therefore, it would be economically beneficial to know the functional quality of its semen before use in field inseminations. In vitro semen evaluation is of a high diagnostic value to assess testicular and epididymal function and/or of the genital tract, allowing the elimination of infertility or even sub-fertility cases. Moreover, it facilitates the determination of the degree of normality of the semen before being processed into semen doses for AI.

Cryopreservation causes irreversible damage to the plasma membrane leading to cell death in a major number of cells (Holt, 2000) or, in those surviving spermatozoa, to changes similar to those seen during sperm capacitation (Watson, 2000a). The deleterious effects of cryopreservation process appear to be caused by the remotion of the seminal plasma, changes in temperature that cause modifications in the membrane structure or increments in intracellular calcium (Green and Watson, 2000). Osmotic stress and toxicity during the exposure of spermatozoa to cryoprotectants, in addition to the processes of dehydration and hydration occurring during the phase changes (formation and dissolution of ice) of the water that surrounds the spermatozoa, play an ef-

fect too. It has become evident that preservation greatly affects many sperm attributes and consequently, viability is reduced, transport in the female reproductive tract is inhibited, the timing of fertilization is altered and embryo development is affected following insemination of preserved, compared to fresh spermatozoa (Gillan et al., 2004).

The aim of this study was to evaluate the potential fertility of Holstein bulls under Egyptian conditions. Spermiogram of the raw semen, sperm freezability and post-thaw survival, in addition to the non-return rate (%) within 60 days of field first-inseminations were assessed.

MATERIALS AND METHODS

This study was conducted during the period from 2001 to 2004. Five Holstein bulls were regularly used for semen collection at AI-center of the International Agriculture Center for Training and Development in the newly-reclaimed lands [IACTD] (El-Amriyia, 33 km of Alexandria/Cairo desert road). Collection of semen was tried to be carried out twice weekly at a frequency of two ejaculates in succession from each bull. Only ejaculates with characteristics equal to or greater than 80% morphologically normal sperm, 70% estimated progressive motility and a concentration of 700 x 10^6 sperm/ml were processed for AI. Progressive motility (PPM) was estimated by diluting 5µl of raw semen in 1 ml of a warm extender buffer and one drop of the mixture was spread on a warm slide. Several microscopic fields (x 40) were evaluated for sperm progressive motility using a phase contrast microscope equipped with a heating stage adjusted at 40°C. Ejaculate concentration was assessed by using a photometer (533B MOD1 Densimeter, Anim. Reprod. Sys. 14395 Ramona Avenue, Chino, California, USA). Percentage of dead sperm in raw semen was assessed using slide smears stained with eosin-aniline stain (Saacke, 1970). Percentage of deteriorated and intact acrosomes (PIA) and sperm head and tail abnormalities in raw semen were estimated in wet semen mounts by fixing 5µl of raw semen in 200µl of 0.2 % glutaraldehyde solution and using oil immersion objective (Johnson et al., 1976). Four types of morphological state of acrosomes were distinguished (normal, swollen, detached and lost acrosomes) according to Watson and Martin (1972).

Freezability:

During 2001, evaluation of semen freezability for each bull was precisely conducted using the Tris-yolk, skimmilk-yolk and citrate-yolk extenders. Assessment of the effect of seasons of the year, extender-types and glycerolation-times on freezability of bull spermatozoa was conducted to achieve an efficient protocol for production of insemination doses of high post-thawing viability. Having met the minimum criteria of neat semen, the two ejaculates from each bull were pooled and split for partial dilution in three extenders, i.e. Tris-yolk, skimmilk-yolk and citrate-yolk, and thereafter, the partially diluted semen was transferred to the Laboratory of Artificial Insemination and Embryo Transfer (LAIET) of Desert Re-

search Center at Maryout Research Station (close to IACTD) for completion of processing semen. Extenders used in dilution were freshly prepared, the night before collection, according to the formulae listed in Table (1).

Dilution of semen was carried out to achieve a final concentration of 30 x 10^6 motile sperm/ml either in one-step (glycerolation at 37°C) or in two-steps (glycerolation at 5°C). For one-step dilution, the required volume of each extender was estimated, according to the obtained values of ejaculate volume, percent progressive motility and sperm concentration in raw semen, and slowly added to semen at 37°C with gentle mixing. Diluted semen was then transferred into the cooling cabinet (adjusted at 5°C) in a beaker containing tap water for equilibration to about 6 hours. For two-steps dilution, the estimated volume of part (A) of each extender was added with gentle mixing to semen at 37°C. Semen diluted with part (A) was then transferred into the cooling cabinet in a beaker containing tap water. After 3-hours of equilibration, estimated volume of the cooled part (B) of each extender (containing glycerol) was added in a dripping manner with gentle mixing.

After completion of the six hours equilibration period at 5°C, diluted semen (whether in one- or two-steps) was packaged in 0.5 ml French straws and frozen on nitrogen vapor at -100°C for 15 minutes, using a programmable LN2 vapor freezer, before plunging into liquid nitrogen for storage. Few days after freezing, three straws from each semen batch were thawed at 40°C for 30 seconds, pooled and incubated at 37°C in a programmable circulator water bath. Progressive motility and morphological status of acrosomes and sperm head and tail were evaluated in frozen-thawed semen immediately and after 3 hours of incubation post-thaw.

Table 1: The composition of the extenders used in dilution of bull semen.

Constituents		Tris-yolk	Skimmilk-yolk	Citrate-yolk
Tris-buffer solution*	% (v/v)	72	------	------
Skimmed milk	% (v/v)	-----	72	------
Sodium citrate (2.9 gm/100ml)	% (v/v)	-----	------	72
Egg yolk	% (v/v)	20	20	20
Glycerol	% (v/v)	8	8	8
Antibiotics:				
Penicillin	(I.U./ml)	500	500	500
Polymixin B	(I.U./ml)	500	500	500
Streptomycin	(µg/ml)	1000	1000	1000

* Tris-buffer: 3.0 gm Tris, 1.3 gm fructose and 1.7 gm citric acid monohydrate/100 ml double distilled water.

Fertility:

Field inseminations were conducted, during 2002-2004, with insemination doses proven to have a post-thaw progressive motility greater than or equal to 60% and morphologically normal acrosomes and sperm of at least 70%. Artificial breeding was carried out in three types of breeder-farms: small-scale breeders (owned 2-5 cows), medium-scale breeders (20-50 cows) and large scale breeders (200-500 cows). Cows were artificially inseminated either after detecting signs of natural estrus or after synchronization of estrus using prostaglandin ($PGF_{2\alpha}$) (two injections of $PGF_{2\alpha}$ 11-days apart and heat detection and AI during days 2 to 5 after each injection). Cows were observed for signs of estrus at least three times daily (at early morning, afternoon and late evening) and were considered to be in estrus if they stood to allow mounting by a herdmate. Inseminations were carried out after 10-12 hrs of the onset of heat. Fertility estimates were based on the percentage of the non-returns to service, within a 60 days period following the field first-inseminations.

The percentage values of the obtained results were subjected to arcsine square root % transformation (angle values) before analysis using Statgraph Computer Program (version 5).

RESULTS AND DISCUSSION

Seasonal Spermiogram

The mean values of seasonal semen characteristics of Holstein bulls are presented in Table (2). Libido of bulls, reflected as successful mounting and ejaculation/days scheduled for semen collection, was markedly affected by the prevailing temperature within each season, i.e. the number of semen ejaculates that have been collected in hot months was markedly less than that collected in cooler ones. Foote (1978) reported that long-term heat stress will reduce sex drive (mounting activity) of bovine bulls. Similarly, a negative impact of high temperature on libido of buffalo bulls was earlier reported by Misra and Sengupta (1965).

Results revealed a highly significant seasonal variation ($P< 0.01$) in spermiogram of Holstein bulls under prevailing environmental conditions. The mean ejaculate volume of semen collected in summer (2.8 ml) was lower than that collected in autumn, winter and spring (5.8, 6.3 and 5.1 ml, respectively). Summer ejaculates of bulls had poorer sperm PPM (43.1 %) and lower concentration (453.5 x 10^6 sperm/ml) than winter ejaculates (85.3 % and 1029.2 x 10^6 sperm/ml, respectively). Similar seasonal fluctuations in semen quality were reported for bovine (Godfrey et al., 1990; Vogler et al., 1993), buffalo (Heuer et al., 1987) and stallions (Janett et al., 2003).

Substantially, the northwestern region of Egypt is semi-arid and is characterized by the prevalence of the hot-wetted climate throughout the summer months (Fig. 1). In spring, the region is characterized by blowing of consecutive waves of seasonal winds which are hot and carrying sand and dust (known as khamsin). Elevation in ambient temperature (up to 45°C at mid-day) and relative humidity always prolonged to several consecutive days causing nocuous thermal stress on animals

raised in that region, particularly the high producing breeds. Heat stress during the dry period of cows reduces milk yield and prolongs days open (Moore et al., 1992) and markedly reduces the estrual behavior (Stevenson, 2001). During the active AI-breeding period, heat stress reduces uterine blood flow (Roman-Ponce et al., 1978), oocyte quality, embryo development, luteal function and endometrial function (Wolfenson et al., 2000) and overall reproductive performance (Armstrong, 1994). Recently, Derensis and Scaramuzzi (2003) reported a decrease in fertility of dairy cows during hot months of the year and attributed this situation to different factors, the most important of which is the consequence of increased temperature and humidity that result in a reduction in appetite and dry matter intake. Reduction in appetite and dry matter intake might reflect difficulties in thermoregulation mechanism in the animal body. Under the northwestern region prevailing summer conditions of Egypt, the Holstein sires of our AI-center obviously descended their testes in the scrotum which became reddish in color reflecting the thermally stressed status of the sires. Reduction in semen quality of the sires during the hot months, particularly in summer, might be due to alteration in testicular and epididymal function under heat stress. Spermiogram is considered a histological snapshot of what happened at the time of spermatogenesis in the testis and during maturation, transit and storage within the epididymis. Therefore, modifications of testicular function through either disturbance of heat regulation or stress-related endocrine disturbances would result in a change in the spermiogram. Extreme climatic heat-stress can interfere with temperature regulation within the scrotum resulting in germ-cell destruction, a temporary decrease in sperm production and fertility (Wellemann et al., 1985) and suppression of

Table 2: Average seasonal values (means ± s.e.) of semen characteristics of Holstein bulls under Egyptian newly-reclaimed land conditions.

Semen Characteristics		Spring (Mar.-May)	Summer (June-Aug.)	Autumn (Sep.-Nov.)	Winter (Dec.-Feb.)	F-test
No. Ejaculates		175	85	150	210	
Ejac. Volume (ml)		5.1 ± 0.09^{b}	2.8 ± 0.08^{c}	5.8 ± 0.06^{ab}	6.3 ± 0.04^{a}	**
Ejac. Concentration ($x10^6$/ml)		854.3 ± 1.41^{b}	453.5 ± 1.89^{c}	926.7 ± 0.98^{ab}	1029.2 ± 0.74^{a}	**
Progressive Motility (PPM) (%)		73.5 ± 0.71^{b}	43.1 ± 1.34^{c}	79.6 ± 0.62^{ab}	85.3 ± 0.35^{a}	**
	Normal	87.4 ± 0.73^{b}	72.2 ± 0.97^{c}	92.0 ± 0.61^{a}	93.6 ± 0.45^{a}	*
	Swollen	6.2 ± 0.17^{b}	10.1 ± 0.35^{a}	3.1 ± 0.09^{c}	2.6 ± 0.06^{c}	**
Acrosomal State: (%)	Detached	4.3 ± 0.41^{b}	8.4 ± 0.62^{a}	2.7 ± 0.23^{c}	2.3 ± 0.14^{c}	**
	Lost	2.1 ± 0.31^{b}	9.3 ± 0.64^{a}	2.2 ± 0.13^{b}	1.5 ± 0.11^{b}	**
Sperm	Head	2.7 ± 0.56^{b}	9.5 ± 0.82^{a}	3.1 ± 0.34^{b}	2.3 ± 0.20^{b}	**
Abnormalities: (%)	Tail	6.1 ± 0.78^{b}	18.2 ± 0.95^{a}	6.4 ± 0.52^{b}	5.1 ± 0.34^{b}	**
Dead sperm (%)		8.9 ± 0.76^{b}	13.5 ± 0.95^{a}	9.5 ± 0.61^{b}	8.3 ± 0.45^{b}	*

a, b values in the same row, in each block, with different superscripts are significantly different at least at $p< 0.05$;
* $p< 0.05$; ** $p< 0.01$.

spermatogenesis (Flowers et al., 1997). Sperm morphology evaluation is a major component of spermiogram and indicates testicular and epididymal pathologies and it can also provide some clues regarding the ability of spermatozoa to sustain freezing-thawing damage (Gravance et al., 1998; Madan, 2002). Therefore, the assessment of integrity and functionality of different sperm parameters is considered a prerequisite for fertilization and a priority for semen evaluation.

A clear indication of the adverse effect of summer heat on the bulls was a high extent of acrosomal anomalies in summer ejaculates (up to 28%) as compared with semen ejaculates that have been collected in autumn and winter months which exhibited a permissible extent of the acrosomal defects that ranged from 6 to 8 %. Deterioration of acrosomal integrity in summer ejaculates was also associated with elevation in sperm head and tail abnormalities (9.5 and 18.2 %, respectively) and more frequent dead cells in ejaculates (up to 13.5 %). Similar observations of deleterious heat effect on acrosomal status were earlier reported for bovine (Vogler et al., 1993), buffaloes (Heuer et al., 1987) and stallions (Janett et al., 2003).

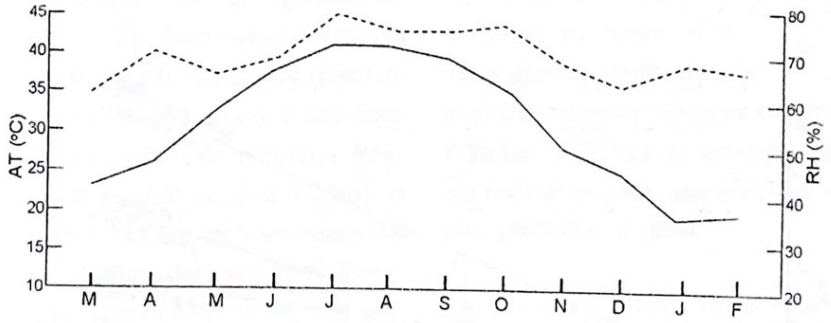

Fig. (1): Monthly averages of ambient temperature (AT: ——) and relative humidity (RH: ----------) prevailed at med-day along the experimental period.

In the present study, regression analysis (Fig. 2) revealed that sperm acrosomes and tails in bull ejaculates are more sensitive to heat-stress than the sperm heads. Saacke (1970) reported that acrosomal defects may reflect errors in nuclear morphology during formation of sperm in the testis. Insulation of bull testis for only 48 hours led to increase sperm with cytoplasmic droplets, number of decapitated sperm heads followed these and then nuclear vacuolation defects, pyriform heads and acrosomal defects (Saacke et al., 1994). Furthermore, Saacke et al. (1994) showed that insemination of semen from scrotally insulated bulls with a high proportion of nuclear vacuoles on otherwise normal appearing sperm yielded increased proportions of degenerate embryos.

The obtained results of seasonal spermiogram of the Holstein bulls under Egyptian newly reclaimed areas conditions have led to use semen for AI only during the favorable months of the year and to discard semen ejaculates during hot months. In that way, AI fertility might increase, but simultaneously cost of semen production would be higher because of the lower yearly production per bull. Utilization of cooling systems may have beneficial effect on the bulls under hot conditions and improve the quality of delivered semen in hot months. Adequate ventilation and fine water sprays with fans can be used to cool bulls to avoid deleterious effect of heat stress and therewith improve their semen traits. However, even when sires at bull studs were maintained in air-conditioned facilities or well ventilated barns during summer, the quality of semen often was compromised to some degree (Stevenson, 2001).

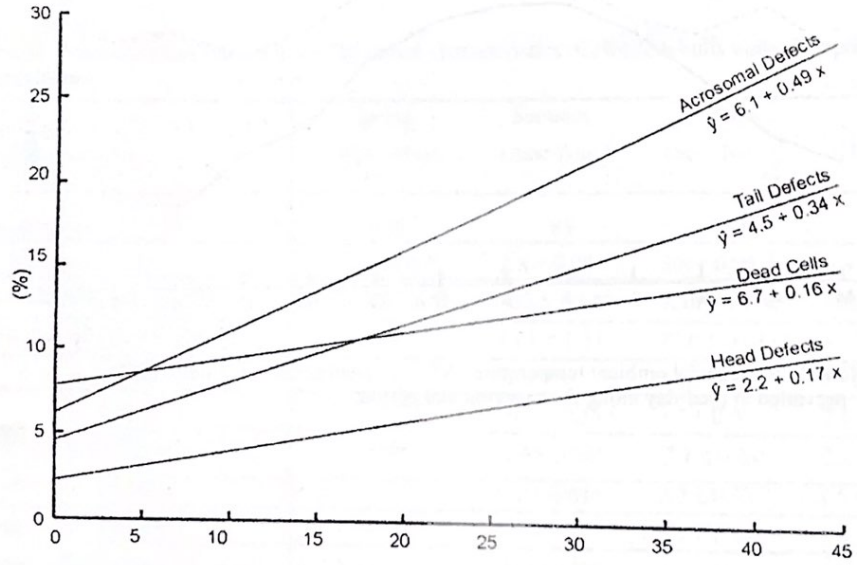

Fig. (2): Fitted lines of regression of percent acrosomal, sperm head and tail defects and dead cells in bull semen ejaculates on ambient temperature.

Sperm Freezability

Mean values of post-thawing sperm progressive motility, acrosomal state and sperm head and tail abnormalities are presented in Table (3). Results reflected significant seasonal differences ($P< 0.01$) in sperm freezability of Holstein bulls under the locally-prevailing conditions. Frozen-thawed semen that has been processed in winter months revealed the highest post-thaw motility (52.1 %) and the least extent of acrosomal, head and tail anomalies (38.2, 12.6 and 20.9 %, respectively) as compared with that frozen-thawed in other seasons. The poorest post-thaw sperm quality was recorded in semen that has been frozen-thawed in hot summer months, where post-thaw motility and sperm acrosomal, head and tail defects averaged 31.9, 52.4, 20.4 and 29.0 %, respectively. Regarding the different types of acrosomal defects (Fig. 3), a non-significant seasonal variation has been recorded in the frozen-thawed semen for sperm having swollen and detached acrosomes (ranges: 13-16 and 14-19 %, respectively). However, a significant seasonal variation ($P< 0.01$) in the extent of sperm that lost their acrosomes had been recorded. Semen that has been frozen-thawed in winter months revealed the least post-thaw extent of decapitated sperm (8 %) as compared with that frozen-thawed in hot summer months (18 %). Deleterious effect of heat stress on bull semen freezability was earlier reported by Sullivan (1970) who found a marked decline in spermatozoal motility and elevations in total and primary abnormalities post-thaw in bull semen frozen-thawed in summer months. Meanwhile, Saacke et al. (1994) found significant differences between semen samples for cryopreservation collected from bulls prior to and after heat stress.

Processing of semen such as freezing and thawing is detrimental to sperm functionality and usually result in death of a large number of spermatozoa. It has been recently reported that between 30 and 40% of the living spermatozoa do not survive cryopreservation and those among the survivors that maintain post-thaw motility are modified in a way that prevents their ability to interact with the oocyte during fertilization (Rodriguez-Martinez, 2002). Since there is a need of a certain population of viable, motile and non-capacitated spermatozoa with intact acrosomes in the frozen-thawed AI-dose to obtain fertility, a scrutiny of these parameters is necessary. Evaluation of freezing protocols should include the assessment of different sperm parameters post-thaw in order to monitor the number of surviving spermatozoa and to provide insights upon the fertilizing capacity of the cryopreserved semen (Januskauskas and Zilinskas, 2002) and for identifying and monitoring bulls of very high and very low potential fertility (Phillips et al., 2004).

Obviously, the obtained results reflected a negative correlation between freezability of semen and the prevailing climatic conditions, i.e. as the temperature and humidity increased the freezing ability of the collected semen was markedly reduced, and vice versa. These results were in agreement with those reported by Sullivan (1970) and Saacke et al. (1994) in frozen-thawed bull semen. Spermatozoa undergoing freezing are challenged not for their ability to endure storage at very low temperatures, but rather by the lethality of an in-

Table (3): Average values (mean±s.e.) of seasonal post-thawing physical charcteristics in frozen-thawed semen of Holstein bulls diluted in one or two steps in Tris-yolk, Skimmilk-yolk and citrate-yolk under Egyptian newly-reclaimed land conditions.

Season	Semen Characteristics		Tris-yolk Extender		Skimmilk-yolk Extender		Citrate-yolk Extender		F-test
			One-step	Two-steps	One-step	Two-steps	One-step	Two-steps	
Spring	Progressive motility (%)		36.9±0.89^{c}	47.4±0.63^{b}	30.4±0.92^{d}	45.1±1.05^{b}	31.4±0.87^{b}	56.9±1.12^{a}	**
	Acrosomal state (%)	Normal	56.1±1.21^{b}	60.8±0.98^{a}	54.9±1.05^{b}	59.4±0.91^{ab}	60.0±0.84^{ab}	64.2±0.64^{a}	*
		Swollen	15.1±1.31^{a}	11.7±1.51^{b}	16.4±0.87^{a}	12.5±1.23^{b}	15.4±1.07^{a}	11.3±1.43^{b}	*
		Detached	13.7±1.05^{b}	14.1±1.12^{b}	12.4±0.98^{b}	15.1±1.13^{ab}	12.3±0.98^{b}	16.4±0.79^{a}	**
		Lost	15.1±0.93^{ab}	13.4±1.52^{bc}	16.3±0.76^{a}	13.0±1.55^{c}	12.3±1.42^{cd}	8.1±1.74^{a}	
	Sperm Abnormalities (%)	Head	17.3±0.89^{a}	14.2±1.21^{bc}	18.4±0.75^{a}	13.4±0.98^{cd}	16.2±1.05^{ab}	11.7±1.12^{d}	*
		Tail	25.5±1.54^{ab}	21.1±0.88^{c}	27.7±1.13^{a}	22.2±1.43^{bc}	23.1±1.54^{bc}	18.3±0.98^{d}	**
Summer	Progressive motility (%)		25.9±0.95^{c}	39.5±0.74^{a}	25.8±1.13^{c}	30.4±0.79^{b}	30.8±0.94^{b}	39.1±1.05^{a}	**
	Acrosomal state (%)	Normal	42.0±1.31^{d}	47.0±1.05^{bcd}	43.0±0.98^{cd}	48.2±1.12^{bcd}	49.2±1.42^{abc}	56.4±1.63^{a}	*
		Swollen	20.1±0.54^{a}	13.1±0.87^{c}	18.4±0.62^{ab}	13.7±0.98^{c}	16.1±0.35^{b}	13.7±1.04^{c}	**
		Detached	15.7±0.97^{c}	16.8±0.69^{bc}	22.1±1.12^{a}	20.2±1.05^{a}	19.3±1.12^{ab}	17.5±1.43^{bc}	*
		Lost	22.2±0.89^{a}	23.1±1.01^{a}	16.5±0.78^{bc}	17.9±0.67^{b}	15.4±1.02^{c}	12.4±1.31^{d}	**
	Sperm Abnormalities (%)	Head	22.7±1.31^{a}	17.8±0.88^{c}	22.4±1.12^{ab}	19.6±0.97^{bc}	21.5±1.05^{ab}	18.5±0.84^{c}	*
		Tail	35.5±1.45^{a}	28.2±0.97^{b}	31.2±1.12^{ab}	31.4±1.31^{ab}	27.4±1.05^{b}	20.4±0.86^{c}	**
Autumn	Progressive motility (%)		31.3±1.67^{c}	43.1±1.54^{c}	37.4±1.52^{d}	50.1±1.15^{b}	45.1±1.05^{bc}	68.3±0.84^{a}	**
	Acrosomal state (%)	Normal	54.6±1.72^{cd}	61.5±0.98^{ac}	52.2±1.51^{d}	59.8±1.67^{acd}	62.9±0.87^{a}	67.0±0.63^{a}	*
		Swollen	17.7±0.79^{a}	13.3±1.05^{b}	16.3±0.89^{a}	16.7±0.93^{a}	16.2±0.84^{a}	13.7±1.05^{b}	*
		Detached	14.3±0.74^{b}	18.1±0.82^{a}	19.1±0.69^{a}	15.3±1.05^{b}	9.6±0.54^{d}	12.1±0.89^{c}	**
		Lost	13.4±0.54^{a}	7.1±0.63^{d}	12.4±0.87^{ab}	8.2±0.71^{c}	11.3±0.92^{b}	7.2±0.57^{cd}	*
	Sperm Abnormalities (%)	Head	16.3±0.67^{a}	11.5±0.56^{b}	18.1±0.87^{a}	12.4±0.45^{b}	12.3±0.63^{b}	9.3±0.81^{c}	*
		Tail	25.4±0.87^{a}	22.3±0.67^{ab}	25.4±0.95^{a}	20.1±0.65^{b}	23.5±0.58^{a}	17.2±0.54^{c}	*
Winter	Progressive motility (%)		41.1±1.05^{c}	53.8±1.21^{b}	41.0±1.31^{c}	52.7±1.01^{b}	50.5±0.98^{b}	73.2±1.42^{a}	**
	Acrosomal state (%)	Normal	56.1±1.13^{c}	59.1±1.05^{bc}	59.9±1.42^{abc}	62.2±1.05^{abc}	64.8±0.97^{ab}	68.8±0.68^{a}	*
		Swollen	11.7±0.63^{c}	16.1±0.54^{a}	15.2±0.71^{ab}	15.4±0.43^{ab}	13.4±0.67^{bc}	15.1±0.81^{ab}	*
		Detached	20.0±0.67^{a}	16.5±0.45^{b}	16.2±0.52^{b}	15.1±0.41^{bc}	13.7±0.63^{c}	10.7±0.37^{d}	**
		Lost	12.2±0.81^{a}	8.3±0.63^{bc}	8.7±0.72^{b}	7.3±0.61^{c}	8.1±0.59^{bc}	5.4±0.45^{d}	**
	Sperm Abnormalities (%)	Head	15.3±0.73^{a}	10.1±0.57^{c}	17.4±0.92^{a}	11.7±0.75^{b}	13.2±0.67^{b}	8.1±0.43^{d}	**
		Tail	24.2±0.89^{a}	20.4±0.68^{b}	23.2±0.54^{ab}	21.3±0.78^{ab}	20.1±0.59^{b}	16.4±0.46^{c}	**

a,b values in the same row, in each block, with different superscripts are significantly different (P<0.05); * P<0.05; **P<0.01.

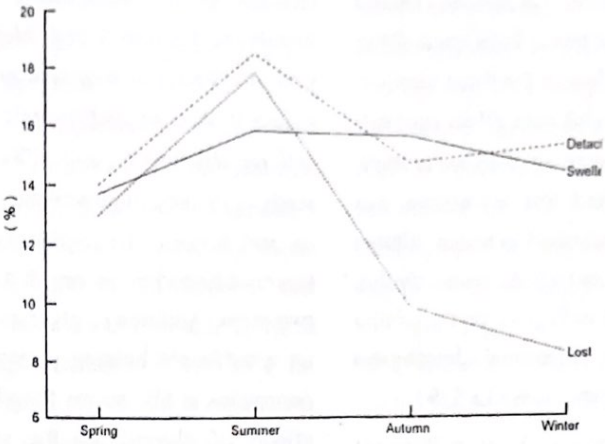

Fig. 3: Post-thawing sperm acrosomal defects in frozen-thawed Holstein bull semen in different seasons under Egyptian newly-reclaimed areas conditions.

termediate zone of temperature which they must traverse both during cooling/freezing and thawing. Loss of motility of spermatozoa during cooling is particularly pronounced when the temperature of the semen after collection is quickly lowered to below 15°C, and further to 5°C and 0°C, the so-called "cold shock". Sensitivity of spermatozoa to cold shock is suggested to be caused by special composition in phospholipids and cholesterol of their plasma membrane (Watson and Pummer, 1985). Changes in temperature impose changes in the composition and structures of various sperm plasma membrane domains (Krogenaes et al., 1994) and thereby modifying their function. In the present study, alteration in testicular and epididymal functions due to exposure of bulls to thermal stress may have led to production of sperm of more sensitivity to cold shock than that collected in cooler months. However, considerable differences be ween breeds and between males in the freezability f semen have been detected, and as a consequence, frozen semen of some genetically interesting breeds or males not be suitable as a gene bank resource or can be used only with a poor efficiency (Hiemstra et al., 2005).

Freezability of bull semen varied significantly ($P< 0.01$) amongst the three used extenders. Dilution of semen in the citrate-yolk extender revealed the best post-thaw progressive motility (49.4 %) and the least extent of acrosomal, head and tail defects (38.4, 13.9 and 20.8 %, respectively). Superiority of the citrate-based extender on Tris-yolk and milk-yolk extenders in improving sperm freezability has earlier been reported in bovine semen (Schenk et al., 1987) and recently reported in sheep semen (El-Hassanein, 2004). Dilution in Tris-yolk and Skimmilk-yolk extenders revealed relatively comparable post-thawing average values for motility and acrosomal, head and tail abnormalities (39.5, 45.3, 16.2 and 25.3%, respec-

tively). Similar observations of comparable freezability of semen diluted in Tris- and milk-based extenders have been reported for bovine (Dhami et al., 1995), canine (Rota et al., 2001) and sheep (El-Hassanein, 2004). Although the three used extenders revealed a non-significant effect on post-thaw extent of sperm having swollen acrosomes, the extent of detached and lost acrosomes was significantly ($P< 0.05$) decreased in semen diluted in the citrate-yolk (13.9 and 10 %, respectively). Dilution in Tris-yolk and milk-yolk had a similar effect on the post-thaw acrosomal detachment (about 16.6 %) and acrosomal loss (13.5 %).

Dilution of bull semen in two-steps significantly ($P< 0.01$) improved sperm freezability as compared with dilution in one-step. Glycerolation of bull semen at 5°C, in comparison with that carried out at 37°C, increased the post-thaw motility by about 40 % (50.0 vs. 35.7 %) and decreased the post-thaw extent of acrosomal anomalies by about 11 % (40.5 vs. 45.4 %), head defects by about 25 % (13.2 vs. 17.6 %) and tail defects by about 17 % (21.6 vs. 26.0 %). Similar improvement in post-thaw spermatozoal viability and acrosomal integrity has been recently detected in frozen-thawed ram semen that has been glycerolated at 5°C (El-Hassanein, 2004). Although glycerolation-time revealed a non-significant effect on the extent of swollen and detached acrosomes in frozen-thawed semen, the post-thaw sperm that lost their acrosomes were significantly ($P< 0.01$) reduced by about 20 % in frozen-thawed semen that has been glycerolated at 5°C as compared with that glycerolated at 37°C (11.0 vs. 13.7 %, respectively). One of the major causes of reduced motility and acrosomal integrity after glycerolation is the negative effect of glycerol that may cause changes in the permeability of the sperm cell membrane (Maxwell and Watson, 1996). Addition of glycerol to semen in stepwise manner can reduce its toxicity and osmotic stress to the sperm cell membrane (Watson, 2000b). In the present study, a marked improvement in post-thaw viability and acrosomal integrity has been detected in frozen-thawed bull semen that has been diluted in two-steps. Addition of glycerol to spermatozoa after a sufficient holding period with egg-yolk lipoproteins at 5°C seems to reduce the deleterious effects of glycerol on the spermatozoal membranes.

A thorough examination of the obtained results has obviously shown that the best post-thaw semen quality (freezability) has been achieved in semen diluted in two-steps in the citrate-yolk extender in winter months. The post-thaw progressive motility has improved to about 73 % and the extent of acrosomal, head and tail anomalies in frozen-thawed semen has been diminished to about 31, 8 and 16 %, respectively. Substantially, sperm quality in frozen-thawed semen that has been diluted in citrate-yolk in two-steps in months of favorable temperature, i.e. autumn, winter and spring months had ordinarily exceeded the criteria limitations of post-thaw sperm quality control for use in AI-programs. In cattle AI-industry, in which bulls are selected for freezability of their semen, the post-thaw semen quality is quite good, featured 50-70% motile spermatozoa and pregnancy or calving rates after insemination is relatively the same as that of fresh semen (Hiemstra

et al., 2005).

Sperm Post-thaw Survival

Reduction in fertilizing capacity of frozen thawed semen may be due to a loss of viable spermatozoa during the freezing-thawing process and apparently to more subtle changes in the surviving sperm population (Watson, 2000 a). Spermatozoa that survive cryopreservation should be able to resist the stress of incubation between 37 and 39°C for several hours, a situation that might prevail in the female genital tract (Rodriguez-Martinez, 2002). Reduced post-thaw longevity and fertilizing ability of spermatozoa was suggested resulting from their early capacitation status (Watson, 1995). Generally, normal and motile frozen-thawed sperm need about 6 to 10 hours to reach the lower portion of the oviduct during which the process of capacitation is completed (Hawk, 1987) and the subsequent 12 to 16 hours represent the period of maximal fertility of the sperm, followed by rapidly declining motility and fertility. It has been earlier reported that if the frozen-thawed semen from most bulls is handled properly, it is estimated to have a viable life span of about 48 hours in the female reproductive tract (McLaren, 1974). For this reason, post-thaw sperm longevity and their resistance to the thermal stress of incubation should be assessed. After a certain period of incubation, aliquots are removed to evaluate motility, morphology, status of plasma membrane, acrosomal status, ATP contents, --- etc.

In the present study, the mean values of sperm progressive motility, acrosomal state and sperm head and tail abnormalities in the frozen-thawed semen after 3-hours of incubation at 37°C are presented in Table (4). The obtained results indicated that the post-thaw longevity of the incubated frozen-thawed spermatozoa was significantly ($P< 0.01$) affected by season. Semen that has been frozen-thawed in winter months revealed the best motility (38.9 %) and the least extent of deterioration in sperm acrosomes (48.6 %), heads (17.1 %) and tails (25.8 %) after 3-hours incubation at 37°C post-thaw. The poorest post-thaw sperm livability has been recorded in semen that has been frozen-thawed in hot summer months. Progressive motility and acrosomal, head and tail abnormalities that have been recorded after incubation were 20.8, 63.1, 25.0 and 36.1 %, respectively. Considering types of acrosomal defects, results revealed non-significant seasonal differences in the extent of different types of acrosomal anomalies in frozen-thawed semen after 3-hours incubation post-thaw.

Of concern, is the obtained significant effect ($P< 0.01$) of diluents on sperm post-thaw longevity after 3-hours of incubation. The best motility and the least extent of defects in acrosomes and sperm heads and tails have been recorded after incubation in frozen-thawed semen that has been diluted in citrate-yolk (36.5, 48.3, 16.8 and 24.2 %, respectively). Dilution in Tris-yolk and Skimmilk-yolk pre-freezing revealed a comparable effect (28.6, 56.5, 21.5 and 32.8 %, respectively). Recently, a significant ($P< 0.01$) decrease in post-thaw motility and percentage of live sperm with intact acrosomes and sperm with detached acrosomes with the successive time of incubation was found in frozen-thawed bull semen that has been

Table (4): Average values (mean±s.e.) of seasonal physical characteristics after 3 hrs of post-thawing incubation of friozen-thawed semen of Holstein bulls diluted in one or two steps in Tris-yolk, Skimmilk-yolk and Citrate-yolk under Egyption newly-reclaimed land conditions.

Season	Semen Characteristics		Tris-yolk Extender		Skimmilk-yolk Extender		Citrate-yolk Extender		F-test
			One-step	Two-steps	One-step	Two-steps	One-step	Two-steps	
Spring	Progressive motility (%)		28.4±1.11^{c}	34.3±0.98^{b}	19.2±0.74^{d}	33.4±0.96^{b}	21.3±0.76^{d}	42.5±1.35^{a}	**
	Acrosomal state (%)	Normal	41.2±1.31^{d}	54.1±1.52^{ab}	45.0±0.97^{cd}	50.4±0.86^{b}	47.4±1.14^{bc}	58.4±1.05^{a}	*
		Swollen	22.1±0.65^{a}	15.4±0.63^{c}	23.2±0.83^{a}	17.7±0.57^{bc}	18.2±0.63^{b}	12.7±0.45^{d}	**
		Detached	17.4±0.52^{ab}	16.1±0.47^{b}	19.4±0.64^{a}	18.2±0.53^{ab}	18.1±0.46^{ab}	17.4±0.39^{ab}	*
		Lost	19.3±0.56^{a}	14.4±0.43^{bc}	12.4±0.35^{cd}	13.7±0.61^{c}	16.3±0.72^{b}	11.5±0.39^{d}	*
	Sperm Abnormalities (%)	Head	24.2±0.87^{a}	19.1±0.63^{b}	24.2±1.05^{a}	18.1±0.57^{b}	19.2±0.65^{b}	15.5±0.57^{c}	*
		Tail	35.3±1.05^{a}	26.7±0.89^{b}	36..7±1.12^{a}	29.3±0.78^{b}	28.3±0.83^{b}	22.4±0.52^{c}	*
Summer	Progressive motility (%)		16.3±0.54^{c}	26.9±0.42^{a}	16.2±0.35^{c}	22.1±0.73^{b}	16.7±0.64^{c}	26.7±0.56^{a}	**
	Acrosomal state (%)	Normal	31.2±0.63^{c}	39.4±0.87^{ab}	30.3±0.56^{c}	37.4±0.81^{b}	38.2±0.94^{b}	45.1±1.01^{a}	*
		Swollen	23.8±0.76^{a}	19.4±0.58^{b}	20.2±0.59^{b}	18.1±0.43^{b}	18.3±0.51^{b}	18.1±0.44^{b}	*
		Detached	24.3±0.51^{a}	24.4±0.63^{a}	22.4±0.47^{a}	24.2±0.52^{a}	24.1±0.62^{a}	20.4±0.41^{b}	**
		Lost	20.7±0.43^{b}	16.8±0.36^{c}	27.1±0.72^{a}	20.3±0.51^{b}	19.4±0.42^{b}	16.4±0.37^{c}	*
	Sperm Abnormalities (%)	Head	26.4±0.78^{ab}	24.1±0.82^{bc}	29.1±0.97^{a}	26.1±0.65^{ab}	23.2±0.73^{bc}	21.2±0.54^{c}	**
		Tail	43.9±1.25^{a}	36.4±1.05^{b}	43.2±0.98^{a}	38.5±0.87^{ab}	31.3±0.79^{c}	23.4±0.73^{d}	**
Autumn	Progressive motility (%)		22.2±0.65^{d}	35.4±0.72^{b}	26.4±0.54^{c}	38.4±0.86^{b}	38.5±1.05^{b}	51.2±1.21^{a}	**
	Acrosomal state (%)	Normal	44.6±0.89^{b}	45.4±0.91^{b}	38.1±0.76^{c}	47.4±0.68^{b}	51.0±0.99^{ab}	57.1±1.12^{a}	**
		Swollen	19.2±0.52^{a}	16.5±0.61^{b}	18.4±0.58^{ab}	18.7±0.49^{ab}	20.1±0.73^{a}	18.1±0.64^{ab}	*
		Detached	17.7±0.43^{c}	20.4±0.51^{b}	25.3±0.78^{a}	18.5±0.61^{bc}	12.4±0.47^{d}	16.1±0.54^{c}	**
		Lost	18.5±0.51^{a}	17.7±0.43^{a}	18.2±0.61^{a}	15.4±0.57^{b}	16.5±0.45^{ab}	10.7±0.36^{c}	**
	Sperm Abnormalities (%)	Head	21.4±0.51^{a}	16.2±0.45^{b}	22.4±0.82^{a}	17.0±0.65^{b}	15.7±0.43^{b}	12.7±0.32^{c}	*
		Tail	32.1±0.72^{a}	26.7±0.56^{b}	33.4±0.87^{a}	29.2±0.54^{ab}	26.4±0.47^{b}	19.1±0.51^{c}	**
Winter	Progressive motility (%)		27.1±0.67^{c}	42.4±0.81^{b}	29.4±0.58^{c}	39.9±0.91^{b}	38.4±0.87^{b}	56.4±1.12^{a}	**
	Acrosomal state (%)	Normal	43.4±0.81^{b}	53.7±0.85^{a}	40.7±0.67^{b}	54.4±0.91^{a}	56.2±1.05^{a}	60.3±1.21^{a}	**
		Swollen	16.3±0.53^{ab}	17.1±0.41^{ab}	18.2±0.61^{a}	16.1±0.43^{ab}	15.2±0.52^{b}	18.4±0.45^{a}	*
		Detached	22.7±0.61^{a}	19.1±0.54^{b}	17.0±0.43^{bc}	17.4±0.41^{bc}	15.3±0.38^{c}	13.2±0.31^{d}	**
		Lost	17.6±0.57^{b}	10.1±0.42^{d}	24.1±0.74^{a}	12.1±0.43^{c}	13.3±0.35^{c}	8.1±0.31^{e}	**
	Sperm Abnormalities (%)	Head	19.9±0.45^{b}	15.1±0.42^{c}	23.4±0.59^{a}	17.3±0.61^{c}	16.4±0.42^{c}	10.2±0.34^{d}	**
		Tail	31.7±0.71^{a}	25.3±0.62^{b}	29.5±0.73^{a}	25.5±0.54^{b}	24.3±0.67^{b}	18.4±0.48^{c}	**

a,b values in the same row, in each block, with different superscripts are significantly different (P<0.05); * P<0.05; **P<0.01.

diluted in Tris-yolk-fructose extender (Shitta, 2001).

In the present study, extender-type revealed a non-significant effect on the extent of swollen acrosomes in frozen-thawed semen after 3-hours incubation post-thaw. However, significant variations ($P< 0.05$) in the extent of detached and lost acrosomes have been recorded within the different diluents after 3-hours of incubation. Dilution in citrate-yolk revealed the least extent of acrosomal detachment (17.1 %) and loss (16.0 %) after incubation, nevertheless, Tris-yolk and Skimmilk-yolk revealed a comparable effect (20.3 and 19.0 %, respectively). Sperm post-thaw longevity was also significantly affected ($P< 0.01$) by glycerolation-time. Dilution of bull semen in two-steps, i.e. glycerolation at 5°C, improved post-thaw motility (37.5 %) and decreased the extent of deterioration in acrosomes (49.8 %), heads (17.7 %) and tails (26.7 %) after 3-hours incubation post-thaw as compared with dilution in one-step before freezing of bull semen.

Reduction in motility and viability of frozen-thawed semen during storage might be due to formation of lipid peroxide radicals (Sonmer and Demirci, 2004). The morphological structures of spermatozoa which are especially sensitive to the process of peroxidation are the acrosome and plasmalemma (Jones and Mann, 1977). The lipid peroxidation destroys the structure of the lipid matrix of the membranes of the spermatozoa and is associated with a loss of motility and membrane integrity (Aitken et al., 1989; Sharma and Agrawal, 1996). Deep freezing increases sensitivity to lipid peroxidation and frozen-thawed semen is more susceptible to lipid peroxidation than fresh semen (Beconi et al., 1993; Bell et al., 1993). In the present study, frozen-thawed sperm that have been processed in hot months might be more sensitive to membrane lipid peroxidation than sperm processed in cooler ones as judged by the marked decrease in post-thaw motility and acrosomal integrity in semen processed in summer that in that processed in cooler months. Dilution in citrate-yolk may be more efficient in reducing sperm sensitivity to peroxidative damage during post-thaw incubation than dilution in the other two extenders. Furthermore, glycerolation of semen after a sufficient holding period with egg-yolk lipoproteins at 5°C proved to prevent excessive peroxide formation and, thereby, improved motility and acrosomal integrity of sperm incubated for 3 hours post-thaw.

A thorough screening of the obtained results can obviously dictate that dilution of Holstein bull semen in citrate-yolk extender in two-steps during winter months had markedly improved the post-thaw sperm longevity as compared with the other processing protocols. The progressive motility and the extent of deteriorations in sperm acrosomes, heads and tails that have been recorded after 3-hours incubation post-thaw were 56.4, 39.7, 10.2 and 18.4 %, respectively. In addition, the extents of swollen, detached and lost acrosomes that have been detected in frozen-thawed and incubated semen were 18.4, 13.2 and 8.1 %, respectively.

Field Fertility

The results of total 6063 field first-inseminations and conception rates of frozen-thawed Holstein bull semen in three different types of animal breeder-farms in the newly reclaimed lands of Egypt are presented in Table (5).

Fertility results revealed an overall conception rate of about 60 %. However, results indicated highly significant differences ($P< 0.01$) in conception rates between the three types of animal breeder-farms. Higher conception rates of about 71% were achieved in the large-scale breeding farms (200-500 cows); while conceptions recorded in small-scale ones (2-5 cows) were only about 39%. Variation in percent conceptions within breeding farms is mainly attributed to the level of management system that applied in the breeding farm. It is well known that AI-programs should be integrated with other programs and services that influence their efficiency and efficiency of animal production. On the contrary to the small-holding villages, large-scale breeding farms are distinguished by persistent abundance of feed stuffs and available veterinarian and skilled-inseminators which are the critical factors limiting success of AI-programs and fertility in cattle breeding farms.

On the other hand, hormonal control of ovarian activity before insemination, i.e. collective synchronization of heat by injection of $PGF_{2\alpha}$, had significantly improved the conception rates by about 32% as compared with insemination after detection of natural heat. Out of 3786 first-inseminations of synchronized cows 2517 have been conceived (about 67 %). However, only about 50 % of 2277 cows observed standing natural heat have conceived after first-insemination. It has been recently reported that reproductive performance of synchronized cows, as measured by the pregnancy rate during AI-breeding period or

Table (5): Fertility results of frozen-thawed bull spermatozoa preserved with citrate-yolk extender under Egyptian newly-reclaimed land conditions.

Fertility	Small-scale Breeder Farms		Medium-scale Breeder Farms		Large-scale Breeder Farms		Total
	N. E.	S. E.	N. E.	S. E.	N. E.	S. E.	
No. Inseminated Cows	840	462	501	1290	936	2034	6063
No. Pregnant Cows*	306	204	243	792	597	1521	3663
Index of Pregnancy (%)	36.4^{d}	43.2^{cd}	48.5^{bc}	61.4^{b}	63.8^{b}	74.8^{a}	60.4
Overall Index of Pregnancy (%)	39.2^{c}		57.8^{b}		71.3^{a}		

* Conceived cows after first-insemination (60-day non-return rate).
N. E.: Natural estrus; S. E.: Synchronized estrus.
$^{a, b}$values in the same row, in each block, with different superscripts are significantly different ($P< 0.05$).

the interval from start of the breeding season to conception, was higher than that of control cows (Xu and Burton, 2000). Administration of $PGF_{2\alpha}$ or its analogues during luteal phase of the sexual cycle is usually followed by ovulation between days 3 and 5 after treatment (Larson et al., 1996; Martinez et al., 2000). Therefore, a synchronization/AI program results in earlier conception in a higher population of females (Smith et al., 1979), concentrates the calving period, reduces the age variability in the calf crop and, thereby, increasing the average market weights (Sprott, 1999). In the present study, improvement in conception rates of synchronized cows may be due to proper observation and detection of heat signs at a limited time-period after first and second hormonal injections (during days 2 to 5 after injection).

Obviously, conception rates of the field first-services varied significantly between the three types of breeder farms, where cows were artificially bred after hormonal synchronization or after standing natural heat (Table 5). Percent conceptions in cows that first-inseminated after standing natural heat or after hormonal treatment in large-scale farms were 64 and 75%, respectively. However, corresponding conceptions in small-scale farms were only 36 and 43%. Practically, optimum fertility can only be achieved by using frozen-thawed semen in AI when inseminating heifers and cows of acceptable age and weight at proper time after the onset of heat and preceding ovulation. It has been reported that the level of cow fertility depends upon a number of factors including the fertility of the service sire, correct thawing and handling of semen, AI-breeding technique and timing of insemination (Stevenson et al., 1983). Adequate nutrition increases pregnancy rates to synchrony (Odde, 1990) and using high-quality semen (Sprott et al., 2000) with proper semen handling from thaw to insemination (Barth, 1993) improves pregnancy rates in synchronized heifers and postpartum cows. The key to proper timing of insemination and maximizing fertilization rates is to inseminate cows at a time to allow ovulation to occur when adequate numbers of motile sperm are present in the oviduct (Stevenson, 2001), and cows submitted for insemination should be inseminated about 12 hours after first detection in estrus (Timberger, 1948).

In general, the obtained results have obviously revealed a marked effect of the prevailing environmental conditions in the newly-reclaimed lands of Egypt on semen quality of Holstein bulls. Processing of semen into AI-doses should be conducted only during the favorable months of the year to improve AI-fertility. For processing Holstein semen, it can be recommended to dilute semen in a citrate-based extender in two-steps before freezing to achieve the optimal post-thaw sperm progressive motility and acrosomal integrity. The obtained AI-doses have proven to attain up to 70% field conception rates especially in large scale farms where fundamentals of cattle husbandry are persistently obtainable.

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تقييم جودة السائل المنوى وقابليته للتجميد وخصوبته للثيران الولشتاين تحت ظروف الاراضى حديثة الإستصلاح بمصر

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هدفت هذه الدراسة إلى تقييم خصائص السائل المنوى وقابليته للتجميد لعدد خمسة ثيران هولشتاين مستخدم للتلقيح الإصطناعى تحت الشروف المصرية ثم توصيف الحيوانات المنوية ونقييم قابليتها للتجميد بمحفظات التريس واللبن الغرز وسترات الصوديوم لتخفيف السائل المنوى إما فى مرحلة واحدة أو على مرحلتين للوصول إلى معدل تخفيف نهائى ٣٠ × ١٠٦ حيوان منوى متحرك / ملليلتر . ثم تعبئة السائل المنوى المخفف بعد فترة الإتزان الحرارى عند ٤°م فى القيبات الفرنسية سعة ٠.٥ ملليلتر ثم تم تجميد القصيبات المعبئة على بخار النيتروجين السائل (عند -١٠٠°م لمدة ١٥ دقيقة) قبل غمرها فى النيتروجين السائل للحفظ عند -١٩٦°م بعد التجميد بمدة أيام . تم إسالة عينات من كل معاملة لتخفيف وحفظ السائل المنوى عند ٤٠°م لمدة ٣٠ دقيقة وتحضين هذه العينات فى حمام مائى عند ٣٧°م لتقييم الحركة المتقدمة والنشوهات المختلفة بالحيوانات المنوية بعد الإسالة خلال الثلاثة أعوام التالية تم إجراء تلقيح إصطناعى حقلى (First Inseminations) لعدد ٦٠٦ بقرة فى ثلاث توعيات من المزارع :- المستوى الصغير (عدد ٥٠٢ بقرات) . والمستوى المتوسط (٢٠ - ٥٠ بقرة) . والمستوى الكبير (٢٠٠ - ٥٠٠ بقرة) وذلك بإستخدام القصيبات المجمدة التى ثبت إحتوائها على حيوانات منوية ذات حركة متقدمة لا تقل عن ٦٠٪ وتتميز بسلامتها من التشوهات بنسبة لا تقل عن ٧٠٪ بعد الإسالة .

تأثرت صفات السائل المنوى معنوياً بالتغيرات فى حرارة الجو السائد بالمنطقة . حيث إنخفض حجم القذفة وتركيزها ومعدلات الحركة المتقدمة وزادت نسبة التشوهات ونسبة الخلايا الميتة فى السائل المنوى الذى تم جمعه خلال أشهر الصيف الحارة كما أظهر السائل المنوى الذى تم جمعه صيفاً أقل قابلية للتجميد بالمقارنة بنظيره الذى تم جمعه خلال الأشهر الباردة أو معتدلة الحرارة وأظهرت النتائج أن رستخدام سترات الصوديوم فى تخفيف السائل المنوى قد ساعد على تحسين قابلية السائل المنوى للتجميد بالمقارنة بالتخفيف فى التريس أو اللبن الفرز كما أظهرت النتائج أن إضافة الجلسرين للسائل المنوى بعد فترة مناسبة من التحضين عند ٤°م للسائل المنوى المخفف جزئياً قد ساعد على تحسين قابلية الحيوانات المنوية للتجميد وأظهرت النتائج أيضاً أن السائل المنوى الذى تم جمعه صيفاً تميز بأقل طول عمر وحيوية للخلايا أثناء التحضين عند ٣٧°م بعد الإسالة وقد تبين أن التخفيف فى سترات الصوديوم قد ساعد على تحسين حيوية وطول عمر الحيوانات المنوية أثناء التحضين بعد الإسالة كما تبين أن إضافة الجلسرين إلى السائل المنوى المخفف جزئياً بعد فترة الإتزان الحرارى قد ساعد على تحسين الحيوية وطول العمر للحيوانات المثوبة أثناء التحضين بعد الإسالة .

وأظهرت النتائج الخاصة بخصوبة السائل المنوى المجمد نسبة إخصاب عامة تصل إلى ٦٠٪ بعد التلقيح الحقلى (First Inseminations) . ولكن كان هناك فروق معنوية بين نوعيات المزارع من حيث نتائج التلقيح ونسبة الإخصاب حيث تم الحصول على أقل نسبة إخصاب (٣٩٪) بعد التلقيح الحقلى بمزارع صغار المنتفعين (١-٥ بقرات) بينما تم الحصول على أعلى نسبة إخصاب (٧١٪) بمزارع كبار المربين (٢٠٠ - ٥٠٠ بقرة) كما أظهرت النتائج أن تنظيم موسم التناسل بالإستحداث الهرمونى للشباع والتلقيح الإصطناعى قد ساعد على زيادة نسبة الإخصاب بحوالى ٣٢٪ بالمقارنة بالتلقيح بعد إكتشاف الشباع الطبيعى للأبقار .

توصلت الدراسة إلى ضرورة توفير نظام تبريد مناسب للثيران الهولشتاين خلال الأشهر الحارة عالية الرطوبة لتحسين معدلات إنتاجيتها من جرعات السائل المنوى خلال هذه الفترة كما أوصت الدراسة بإستخدام مخفف سترات اصوديم لتخفيف سائل منوى الثيران الهولشتاين على أن يتم التخفيف على مرحلتين لتحسين قابلية الحيوانات المنوية للتجميد وللحفاظ على حيويتها وطول عمرها بعد الإسالة. كما أوصت الدراسة بضرورة توافر الخدمات البيطرية الملقح الإصطناعى الكفء بالإضافة إلى الرعاية الغذائية الكاملة لضمان الحصول على أعلى نسبة إخصاب بمزارع الأبقار بالأراضى الجديدة بمصر .

FREEZABILITY OF CAMEL CAMEL DUMMY" AND DI OR TRIS-BASED EXTEND

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SUMMARY

Eight adult male camels were lection, during rutting season 2002), by using the re Hassanein Camel Dummy Station of Desert Researc Tris and their combinatio ing five extenders for c 150 x 10^6 motile sperm two steps. Diluted sem Three freezing and t plied to evaluate sp post-thawing viabi (PPM) and acrosom ated post-dilution a thawing incubatio

* The manuscript w lished in summa

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FREEZABILITY OF CAMEL SEMEN COLLECTED BY "EL-HASSANEIN CAMEL DUMMY" AND DILUTED IN TWO STEPS IN SUCROSE AND/ OR TRIS-BASED EXTENDERS*.

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SUMMARY

Eight adult male camels were used for semen collection, during rutting season (Nov. 2001- March. 2002), by using the recently invented "El-Hassanein Camel Dummy " at Maryout Research Station of Desert Research Center. Sucrose and Tris and their combinations were used for preparing five extenders for camel semen. Dilution to 150 x 10^6 motile sperms/ml was carried out in two steps. Diluted semen filled in 0.5 ml straws. Three freezing and two thawing rates were applied to evaluate spermatozoa freezability and post-thawing viability. Progressive motility (PPM) and acrosomal integrity (PIA) were evaluated post-dilution and at 0, 2 and 4 hours of post-thawing incubation.

The new collection technique significantly improved the quantity and quality of the delivered semen. The obtained semen volume, ejaculate concentration, progressive motility and acrosomal integrity averaged 15.3 ± 1.15 ml, 810.3 ± 2.21 x 10^6 sperm/ml, 81.6 ± 1.01% and 89.7± 1.94%, respectively.

Dilution of camel spermatozoa in a Sucrose-Tris extender significantly reduced dilution effect on spermatozoa viability and improved their freezability and their post-thawing viability. Higher freezing rates significantly conserved post-thawing viability. Slow and rapid-thawing rates had a relatively comparable effect on post-thawing viability. However, the optimum sperm viability was achieved in semen frozen rapidly (at -140°C for 15 min.) and thawed slowly (at 40°C

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