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Impact of Glucose Solutions in Tris-Based Extender on Sperm Variables in Turkey Semen Preserved at Cool Temperature for 96 Hours

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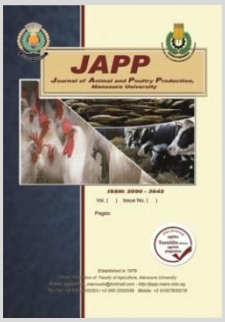

ABSTRACT

This work aimed to study the effects of extenders containing sugar (300- and 370-mM glucose) on the storability and fertility of turkey semen. Semen was collected twice a week from 12 Bronze turkey toms of 40 wk old by abdominal massage for 4 wk. The collected semen of toms was pooled, mixed and extended at a rate of 1:2 with saline (E1), tris-eggyolk-300 mM glucose (E2), and tris-eggyolk-370 mM glucose (E3). After dilution, semen was stored for 96 hours at cool temperature (4-5ºC). Semen was evaluated for motility, normality, and kinetic parameters of spermatozoa, then sperm fertility was determined for semen stored for appropriate storage time for each extender. Results show that overall mean of all sperm motility variables (total motility, total progressive, rapid and slow progressive, and non-progressive motility, and immotile spermatozoa), sperm-morphological parameters (head and neck abnormalities, mono and dual deformations, and sperm deformity index, SDI), and sperm-dynamic parameters (VCL, VSL, VAP linearity, straightness, and wobble) were improved by E2 and E3 as compared to E1. The fertility rate was higher (89.5%) for eggs produced from hens inseminated with E3-semen stored for 72 h ($P<0.05$) as compared to E1-semen stored for 2 h (77.5%), but E2-semen (85.83%) showed insignificant differences with both E1 and E3. Semen of toms could be stored with maintaining sperm function and fertility in tris-glucose-based extender for 72 h.

***Keywords*:** Turkey semen, glucose-extenders, sperm motility, sperm normality, sperm kinetics, fertility.

INTRODUCTION

For improving the genetic programs and preserving the genetic biodiversity, cryopreservation of the semen has long been used in the production of animals and poultry. Advantages of the AI opportunities in the poultry industry has not been able to achieve due to high costs and preparing procedures and the low fertility levels achievable of preserved semen (Blesbois, 2007).

Semen is stored at cool temperature (4-5ºC) to decrease sperm metabolism and to keep sperm function for a long time. In poultry, maintaining the fertilizability of spermatozoa for up to two days required the provision of oxygen, a diluent containing sugar (fructose or glucose) as a source of production of ATP, a buffer to maintain pH, and a temperature of 4-7ºC (Wishart, 1982). Improvement of storage protocol would allow maintenance of semen for longer periods and consequently hen sperm fertility (Iaffaldano & Meluzzi, 2003).

In poultry, several diluents have been achieved for semen preservation (Sexton, 1988; Morrell *et al*., 2005); most of these extenders are saline buffers required for the immediate sperm survival by providing an osmotic pressure of 330–400 mOsm and pH value of 7.0–7.4 in a simulator with the seminal plasma (Thurston, 1995). In addition, extenders of poultry semen must contain carbohydrates in terms of glucose or fructose and other components like citrate, glutamate, and acetate to provide energy (Christensen, 1995).

The effect of the variability in biological material on fertilizability of preserved semen, a multiplicity of preservation procedures affects the consistency of the fertilization results. Differences in diluent types, supplements, and environmental conditions make it difficult to get variable efficiency estimations of different procedures (Lake and Ravie, 1982). Spermatozoa must be pre-cooled to 2–8 °C to preserve the sperm fertilizability of *in vitro*-stored semen (Akçay *et al*., 1997; Donoghue and Wishart, 2000) and extended in appropriate extenders (Thurston *et al*., 1994; Akçay *et al*., 1997).

Turkey sperm cells differ from fowl spermatozoa in many aspects including sperm metabolism and maintenance at a cool temperature. The glucose and acetate metabolism are higher in mammalian than avian sperm cells. The supplemented substrates to semen extender of turkey may be insufficient or not appropriate for the energy requirements of spermatozoa preserved at a cool temperature (Akçay *et al*., 2006). Sperm viability and fertilizability of turkey is loosed by storing undiluted or diluted semen at physiological temperatures (Lake and Ravie, 1982). Satisfied survival and fertility of turkey spermatozoa were reported stored by storing the diluted/liquid semen at a cool temperature for 6 hours pre-AI (Iaffaldano and Meluzzi, 2003).

Storage of liquid turkey semen beyond 6 hours reduced the viability of spermatozoa for commercial use (Thurston, 1995). Also, other authors found a problem for preserving semen quality after liquid storage of turkey semen up to 24 hours (Bakst and Cecil, 1992; Thurston *et al*., 1994),

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and the problem is increased with older animals (Douard *et al*., 2003). However, turkey semen was stored for 24 h with minimum fertility and hatchability values (Wishart, 1982). Optimum conditions of turkey semen preservation for 48 hours have not been fully determined. The improvement of long-term liquid storage procedures of turkey semen is important since the commercial production of this bird relies almost entirely on AI. Many authors have been described different methods for turkey semen to store at cool temperature and cryopreservation.

Preservation of turkey semen with liquid storage is of practical interest in male turkey management. Lost fertilizability and motility were accompanied by marked losses in the phospholipids of the plasma membrane (Blesbois *et al*., 1999; Douard *et al*., 2000), lipid peroxidation (Long & Kramer, 2003; Douard *et al*., 2005; Bréque *et al*., 2003) and membrane damage of sperm cells during cold preservation (Donoghue *et al*., 1996). Also, during the liquid storage, disturbances to the acrosome occur due to increased sperm acrosin-like activity (Kotłowska *et al*., 2007), and the ability of spermatozoa to undergo the acrosome reaction was affected (Lemoine *et al*., 2011).

Akçay *et al*., 2006 studied the impact of glucose at a level of 0.3 or sucrose at a level of 0.6 M and ringer solution as simple carbohydrate-based solutions on semen quality and fertilizability of turkey semen stored for 48 hours at a cool temperature. In this context, they reported the minimal reduction in fertility and hatchability of semen stored in a glucose-based extender for 24 or 48 hours.

The search for an optimal composition of extenders for semen storage for longer times is required for turkey semen. Therefore, the current study aimed to evaluate the effect of simple carbohydrate-based solutions in Tris-based extender (300- or 370-mM glucose) versus saline as an extender on freezability, fertility, and hatchability of turkey semen stored at cool temperature (4-5ºC) for 96 hours.

MATERIALS AND METHODS

Study location and ethics:

This study was conducted in Animal Production Station, Turkey branch, Mehalet Mousa, belonging to Animal Production Research Institute (APRI), Egypt during the period from December 2020 to March 2021. The Institute's ethical rules for animal research were followed and the study plan was approved by the Institute's Research Committee on 18 December 2017 (code no. 020203429).

Birds:

Total of 12 Bronze toms at 36-40 weeks of age and 10-12 kg body weight were used in this study as semen donors. Toms were kept in a temperature-regulated (20 Max. and 10 Min.) building provided with an artificial light regimen (8 h dark:16 h light) and *ad libitum* feeding and watering systems.

Semen collection:

Semen was taken from 12 toms and collected twice/week for 3 weeks by dorso-abdominal massage. Abnormal ejaculates were discarded and about 72 ejaculates (12 Toms x 3 wk x 2 times) were taken throughout the study. On the day of semen collection, ejaculates from six toms were taken, pooled to avoid the individual differences in semen quality among toms. Only ejaculates showing high mass motility (≥75%) were used for evaluation. The semen and diluents were kept at 4-5 °C, the pooled semen was diluted by 3 types of diluents on split samples at a rate of 1: 2 to a semen concentration of about 1 x 10^9/ml at least.

Semen extenders:

Three types of extenders were used for semen dilution including saline (0.9% NaCl, E1), Tris-with 300 mM glucose (E2) and Tris with 370 mM glucose (E3). The composition of the extenders used in this study is shown in Table 1. Diluted samples were mixed thoroughly with different extender types and placed in 10 ml Erlenmeyer flasks. All flasks were covered with paraffin and stored in a refrigerator (4-5ºC) for, 0, 2, 24, 48, 72, and 96 hours.

Table 1. Composition of different types of extenders.

Component	E_1	E_2	E_3
Tris(hydroxymethyl-methyl-2-aminoethanesulfonic acid), g	-	0.363	0.363
DMSO (%)	-	5	5
Egg yolk	-	15	15
D-glucose (g)	-	5.96	7.35
Streptomycin	-	0.5	0.5
Lincomycin	-	0.01	0.01
NaCl (%)	0.9	-	-
Osmolarity (mOsm/L)	308	325	370
pH value	5.5	6.9	7.2
Distilled water up to (ml)	-	100	100

Semen evaluation by computer-assisted sperm analysis (CASA):

Video recordings of CASA 0, 4, 24, 48, 72, and 96h post-dilution and liquid storage at a cool temperature, were made using a light microscope and white video camera (SPERMOLAB®, China) and then analyzed using a computer program (SPERMOLAB, Cairo, Egypt) for turkey spermatozoa. A drop of semen (5 µL) extended with each extender was loaded into a pre-warmed slide (dis-posable Leja). A buffer (50 mM Tris buffer pH 7.4, 120 mM NaCl, 10 mM glucose, 2 mM CaCl2) was supplemented with 0.5% bovine serum albumin to prevent adherence of spermatozoa to the glass slides. Before the analysis, the sample was allowed to settle on the mini-thermal heating stage (38 °C). For each specimen, about 200 spermatozoa from 2-3 drops of each sample were evaluated. CASA data included the following parameters: percentages of total motility ™, progressive motility (PM), rapid (RPM) and slow (SPM) progressive motility, non-progressive motility (NPM), and immotility (IM) of spermatozoa.

Sperm velocity including curve linear velocity (VCL), straight linear velocity (VSL), and average path velocity (VAP) of sperm cells as well as sperm linearity (LIN), sperm straightness (STR), and wobble (WOB) were determined. Morphological sperm normality and abnormality classes as abnormalities in head, neck, and tail or mono, dual, and tri deformation was included in CASA data. Also, Sperm Deformity Index (SDI) was included in CASA data.

SDI=No. of defects in head, mid-piece and tail/total No. of spermatozoa counted

Fertility study:

The fertilizing ability of semen extended with different extenders and stored for an acceptable time was tested in this study. Hens were inseminated with E1 semen stored for 2 h, and glucose-based extenders (E2 and E3) after storage of 72 h. Total of 60 turkey hens (ageing 36-38 weeks and weighing 5-6 kg) were divided into similar three groups (20 hens in each group) and intravaginal inseminated for the

1st time with semen extended with different extenders. The inseminating dose per hen was of 0.25 ml in volume and a concentration of 250 x 10^6 sperm per insemination dose.

In particular, for 2 weeks, two intravaginal artificial inseminations were performed at the same conditions, one on 15 January and another on 23 January, using stored semen, respectively. At the moment of insemination, the stored semen at 5ºC was used. Egg collection began after the second insemination and went on for two weeks, for each group. Total of 120 intact and normal eggs were collected for each group, then all collected eggs were stored at 3-5ºC and 75% relative humidity before incubation.

The eggs were incubated at 37.8ºC and relative humidity of around 60% (Reform® Zeddem, Holland) hatchery. Eggs were candled after 7 days of incubation, unfertilized eggs and eggs with dead embryos were discarded. The fertility rate was calculated:

Fertility rate = (No. fertile eggs/total No. of incubated eggs) x100.

Statistical analysis:

To compare the effect of different extenders *in vivo*, a general linear model (GLM) procedure was used to determine the fixed effect of the extender type, storage time and their interaction for different sperm CASA parameters. These parameters were measured across the treatments of semen (3 extenders x 6 storage time) and were compared by analysis of variance (ANOVA) followed by Duncan's comparison test. The significance differences were set at P<0.05 and every statistical test was performed using the software package SPSS (SPSS 15.0 for Windows, 2006; SPSS, Chicago, IL, USA). The effect of the type of extender on *in vivo* fertility and hatchability rates was tested by Chi-square test.

RESULTS AND DISCUSSION

Sperm-motility variables:

Results in Table 2 show that, as affected by extender type, all sperm motility variables were improved by E2 and E3 as compared to E1. The improvement was observed for the overall mean of sperm motility parameters by increasing (P<0.05) percentage of total motility, total progressive, rapid and slow progressive, and non-progressive motility, and decreasing immotile sperm percentage (P<0.05). Although E3 show a higher (P<0.05) overall mean of total progressive and total motility percentages, and lower (P<0.05) sperm immotility percentage than E2, the percentages of rapid and slow progressive motilities were higher (P<0.05) in E2 than in E3.

As affected by storage time, the overall mean of total, rapid and slow progressive, and total motility percentages showed marked reduction (P<0.05), while sperm immotility percentage increased (P<0.05) by advancing storage time. However, the percentage of non-progressive motility showed an inconsistent trend of change at all storage times (Table 3).

Significant (P<0.05) interaction between extender type and storage time was detected on total progressive, rapid and slow progressive, total motility, and immotility percentages, and insignificant (P≥0.05) on non-progressive motility. Results illustrated in Fig. 1 showed marked effects on all sperm motility variables by advancing storage time. All sperm motility parameters were maintained by both E2 and E3 up to 96 h and by E1 only up to 72 h. At all storage times, E3 showed the highest protective effects on all sperm motility parameters, except on rapid progressive motility, whereas both E2 and E3 showed an impact on maintaining rapid progressive motility by increasing storage time more than 2 h. The positive impact of E3 on total progressive motility was in terms of maintaining the reduction in slow progressive motility and a slight increase in immotility as compared to E2 and E1, respectively.

Sperm-morphology variables:

Results in Table 4 show a significant (P<0.05) effect of extender type on all sperm-morphological parameters and sperm deformity index (SDI). The overall mean of normal sperm percentage was higher (P<0.05), while percentages of head and neck abnormalities, and mono and dual deformations, as well as SDI, were lower (P<0.05) by E2 and E3 than by E1. However, the percentage of tail abnormality and tri deformation decreased (P<0.05) only by E3.

The effect of storage time on percentages of all sperm-morphological parameters and SDI was significant. Normality percentage gradually decreased (P<0.05), while head, neck, and tail abnormalities, mono, dual, and tri deformation, and SDI sharply increased (P<0.05) by advancing storage time (Table 5).

Table 2. Effect of extender type on turkey sperm-motility parameters.

Sperm motility (%)	E1 (0.9% NaCl)	E2 (Tris-300 mM glucose)	E3 (Tris-370 mM glucose)
Total progressive	31.01±6.79^{c}	50.54±4.15^{b}	59.45±2.01^{a}
Non-progressive	10.45±1.79^{b}	15.01±1.64^{a}	16.85±1.39^{a}
Total motility	41.46±7.81^{c}	65.55±4.08^{b}	76.31±2.28^{a}
Rapid progressive	16.49±4.33^{c}	32.23±4.47^{a}	25.56±1.88^{b}
Slow progressive	14.51±2.92^{c}	18.31±1.85^{b}	33.88±1.01^{a}
Immotility	58.53±7.81^{a}	34.44±4.08^{b}	23.69±2.28^{c}

$^{a, b \text{ and } c}$: Means denoted with the same superscripts in the same row are significantly different at P<0.05.

Table 3. Effect of storage time on turkey sperm-motility parameters.

Sperm motility (%)	Storage time (hour)					
	0	2	24	48	72	96
Total progressive	76.82±1.43^{a}	59.81±1.21^{b}	46.48±3.83^{c}	38.75±6.45^{d}	34.53±7.75^{e}	25.61±7.44^{f}
Non-progressive	9.33±0.95^{c}	20.99±1.95^{a}	17.91±2.28^{ab}	13.79±1.73^{bc}	13.57±1.97^{bc}	9.03±2.85^{c}
Total motility	86.15±0.73^{a}	80.81±1.77^{a}	64.39±4.60^{b}	52.54±7.66^{c}	48.11±9.03^{c}	34.63±10.00^{d}
Rapid progressive	52.17±4.86^{a}	31.50±2.68^{b}	21.67±3.28^{c}	17.33±2.60^{cd}	14.60±3.31^{de}	11.30±2.93^{e}
Slow progressive	24.64±3.87^{ab}	28.31±2.12^{a}	24.81±2.65^{ab}	21.41±4.18^{b}	19.931±4.9^{b}	14.30±5.01^{c}
Immotility	13.84±0.73^{d}	19.19±1.77^{d}	35.60±4.60^{c}	47.45±7.66^{b}	51.89±9.03^{b}	65.36±10.00^{a}

$^{a,\ldots\ldots f}$: Means denoted with the same superscripts in the same row are significantly different at P<0.05.

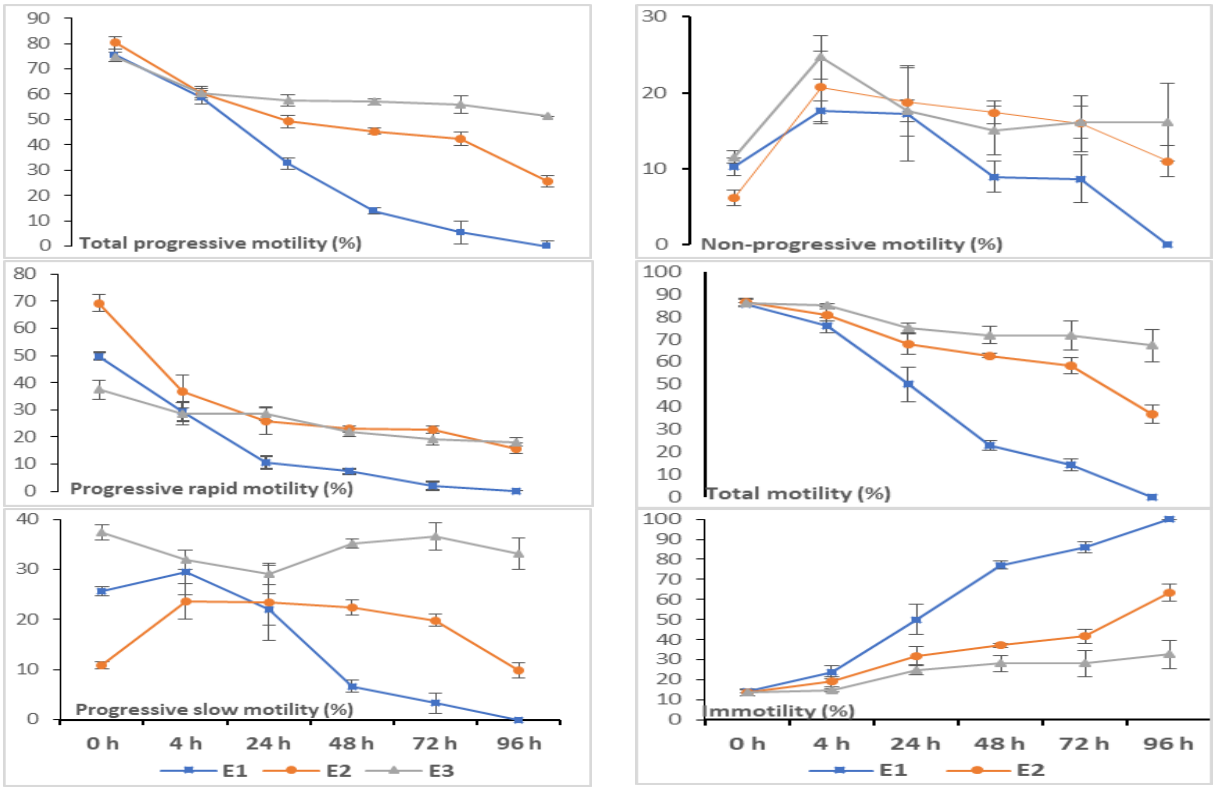

Fig. 1. Change in turkey sperm-motility parameters at different storage times.

Table 4. Effect of extender type on turkey sperm-morphology parameters.

Sperm morphology (%)	E1 (0.9% NaCl)	E2 (Tris-300 mM glucose)	E3 (Tris-370 mM glucose)
Normal forms	29.04±4.76^{c}	40.26±4.17^{b}	47.98±3.63^{a}
Abnormal head	42.41±4.43^{a}	34.39±4.67^{b}	27.58±3.03^{c}
Abnormal neck	31.87±3.02^{a}	24.17±3.04^{b}	19.28±1.82^{c}
Abnormal tail	34.66±4.40^{a}	36.22±5.19^{a}	23.32±2.93^{b}
Mono deformation	26.33±2.11^{a}	19.45±1.47^{b}	16.03±1.82^{b}
Dual deformation	21.30±1.67^{a}	14.31±1.05^{b}	13.25±1.11^{b}
Tri deformation	23.31±3.09^{ab}	25.96±4.17^{a}	22.73±2.28^{b}
Sperm deformity index (SDI)	1.08±0.11^{a}	0.94±0.12^{b}	0.70±0.07^{c}

$^{a, b \text{ and } c}$: Means denoted with the same superscripts in the same row are significantly different at P<0.05.

Table 5. Effect of storage time on turkey sperm-morphology parameters.

Sperm morphology (%)	Storage time (hours)					
	0	2	24	48	72	96
Normal forms	64.36±1.06^{a}	55.83±2.45^{b}	42.09±4.62^{c}	29.39±4.60^{d}	23.31±2.55^{e}	19.58±3.09^{f}
Abnormal head	14.01±1.17^{f}	20.40±1.76^{e}	26.12±3.21^{d}	40.44±4.30^{c}	50.38±3.15^{b}	57.41±3.48^{a}
Abnormal neck	10.82±1.55^{e}	15.95±1.31^{d}	21.21±2.87^{c}	31.08±3.10^{b}	34.31±3.73^{ab}	37.27±2.28^{a}
Abnormal tail	8.44±1.24^{e}	15.55±1.15^{d}	27.79±2.69^{c}	33.00±3.43^{b}	49.63±4.02^{a}	53.99±3.98^{a}
Mono deformation	14.56±2.09^{c}	21.12±2.70^{ab}	23.93±3.01^{ab}	21.20±3.81^{ab}	24.62±2.73^{a}	18.18±2.11^{bc}
Dual deformation	10.36±0.95^{b}	13.03±1.21^{b}	17.71±1.67^{a}	19.22±1.61^{a}	16.96±1.86^{a}	20.41±3.31^{a}
Tri deformation	10.71±2.27^{e}	10.01±1.16^{e}	16.26±1.61^{d}	30.17±2.08^{c}	35.09±1.69^{b}	41.81±2.85^{a}
SDI	0.33±0.03^{f}	0.52±0.03^{e}	0.75±0.06^{d}	1.04±0.09^{c}	1.34±0.09^{b}	1.48±0.08^{a}

$^{a,\ldots,f}$: Means denoted with the same superscripts in the same row are significantly different at P<0.05.
SDI: Sperm deformity index.

The effect of interaction between extender type and storage time was significant on all sperm-morphological parameters. Results shown in Fig. 2 cleared a similar trend of change of all types of extenders throughout the storage period. Sperm normality showed a marked reduction, while other morphological abnormalities in head, neck, and tail, dual and tri deformation, and SDI increased by storage time progress. However, mono deformation showed an increase within 2 h of storage, nearly stable up to 72 h of storage, then decreased to the minimum values at 96 h of storage. It is of interest to note that the marked reduction in mono deformation was associated with a clear increase in dual and tri deformation.

Sperm dynamic variables:

Results in Table 6 show significant (P<0.05) effect of extender type on all sperm-dynamic parameters, in terms of a remarkable increase in overall mean of curvilinear (VCL), straight-line (VSL), and average path (VAP) sperm velocity as well as percentages of linearity (LIN), straightness (STR) and wobble (WOB) by E2 and E3 in comparing with E1. Sperm velocity parameters were better in E3 than in E2.

All sperm-dynamic parameters were affected significantly by storage time. The overall mean of sperm velocities including VCL, VSL, and VAP showed a continuous reduction by advancing storage time, being the lowest after 96 h of storage. These results were reflected in non-significant changes in LIN, STR, and WOB after 72 h, as a result of the lacking sperm motility in E1 and lower sperm velocities after 96 h of storage, Generally, the dramatic changes in all sperm-dynamic parameters occurred between 72 and 96 h of storage (Table 7).

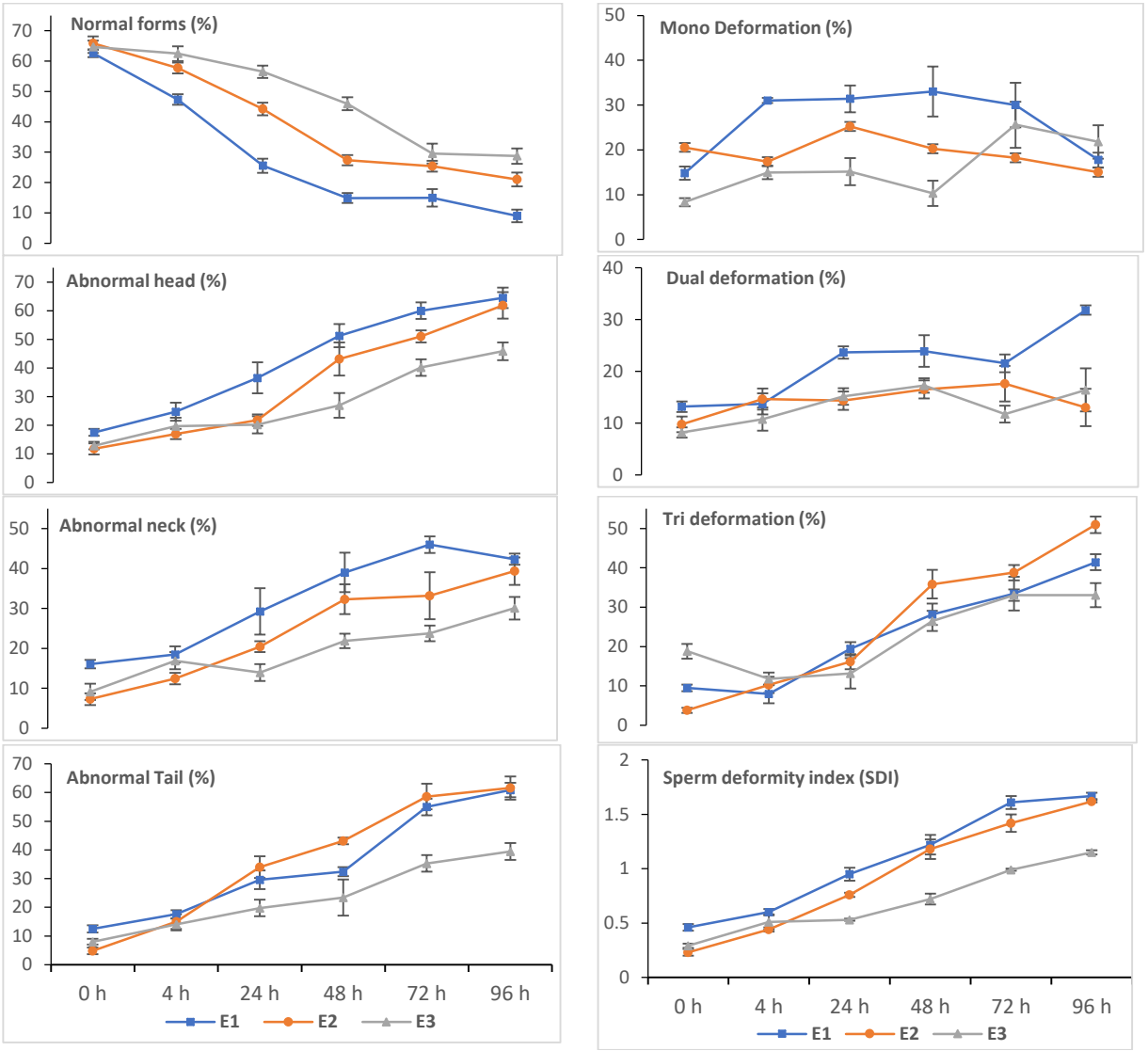

Fig. 2. Change in turkey sperm-morphology parameters at different storage times.

The effect of interaction between extender type and storage time was significant on all sperm-dynamic parameters. Results illustrated in Fig. 3 showed all sperm-dynamics had the same trend of change for all types of extenders up to 72 h. At these times E3 showed the highest positive impact on maintaining sperm dynamics, followed by E2, while E1 showed the poorest results on sperm storage. It is of interest to observe that sperm dynamics were not observed in E1 only after 96 h of storage, being stable at this time.

Table 6. Effect of extender type on turkey sperm-dynamic parameters.

Item	E1 (0.9% NaCl)	E2 (Tris-300 mM glucose)	E3 (Tris-370 mM glucose)
Sperm velocity (µm/s):			
Curvilinear velocity (VCL)	56.53±8.77^{c}	72.87±6.97^{b}	82.61±5.49^{a}
Straight line velocity (VSL)	30.01±4.72^{c}	37.32±3.42^{b}	41.87±2.47^{a}
Average path velocity (VAP)	49.46±8.19^{c}	59.55±6.73^{b}	67.59±5.40^{a}
Dynamic index (%):			
Linearity (LIN)	44.41±4.95^{b}	51.91±1.42^{a}	51.51±1.36^{a}
Straightness (STR)	52.52±6.16^{b}	66.75±3.27^{a}	64.85±2.77^{a}
Wobble (WOB)	71.28±7.88^{b}	79.28±2.42^{a}	80.44±1.68^{a}

$^{a, b and c}$: Means denoted with the same superscripts in the same row are significantly different at P<0.05.

Table 7. Effect of storage time on turkey sperm-dynamic parameters.

Item	Storage time (hours)					
	0	2	24	48	72	96
Sperm velocity (µm/s):						
VCL	114.71±2.46^{a}	94.32±3.33^{b}	76.56±4.68^{c}	60.51±4.22^{d}	48.88±5.48^{e}	29.04±7.49^{f}
VSL	57.08±2.37^{a}	48.72±2.59^{b}	38.96±2.38^{c}	31.26±2.14^{d}	26.44±2.54^{d}	15.94±4.26^{e}
VAP	99.55±3.31^{a}	84.42±2.80^{b}	62.33±4.39^{c}	49.53±3.19^{d}	37.43±4.30^{e}	19.93±5.33^{f}
Dynamic index (%):						
LIN	49.84±2.01^{a}	51.42±1.45^{a}	51.08±1.75^{a}	51.93±1.87^{a}	55.09±2.01^{a}	36.30±9.26^{b}
STR	57.31±1.41^{b}	57.53±2.07^{b}	63.24±2.83^{ab}	63.44±2.92^{ab}	72.54±4.19^{a}	54.20±14.21^{b}
WOB	86.79±2.23^{ab}	89.62±1.04^{a}	81.17±1.67^{bc}	82.21±1.37^{bc}	76.90±2.57^{c}	45.30±11.53^{d}

$^{a,\ldots,f}$: Means denoted with the same superscripts in the same row are significantly different at P<0.05.

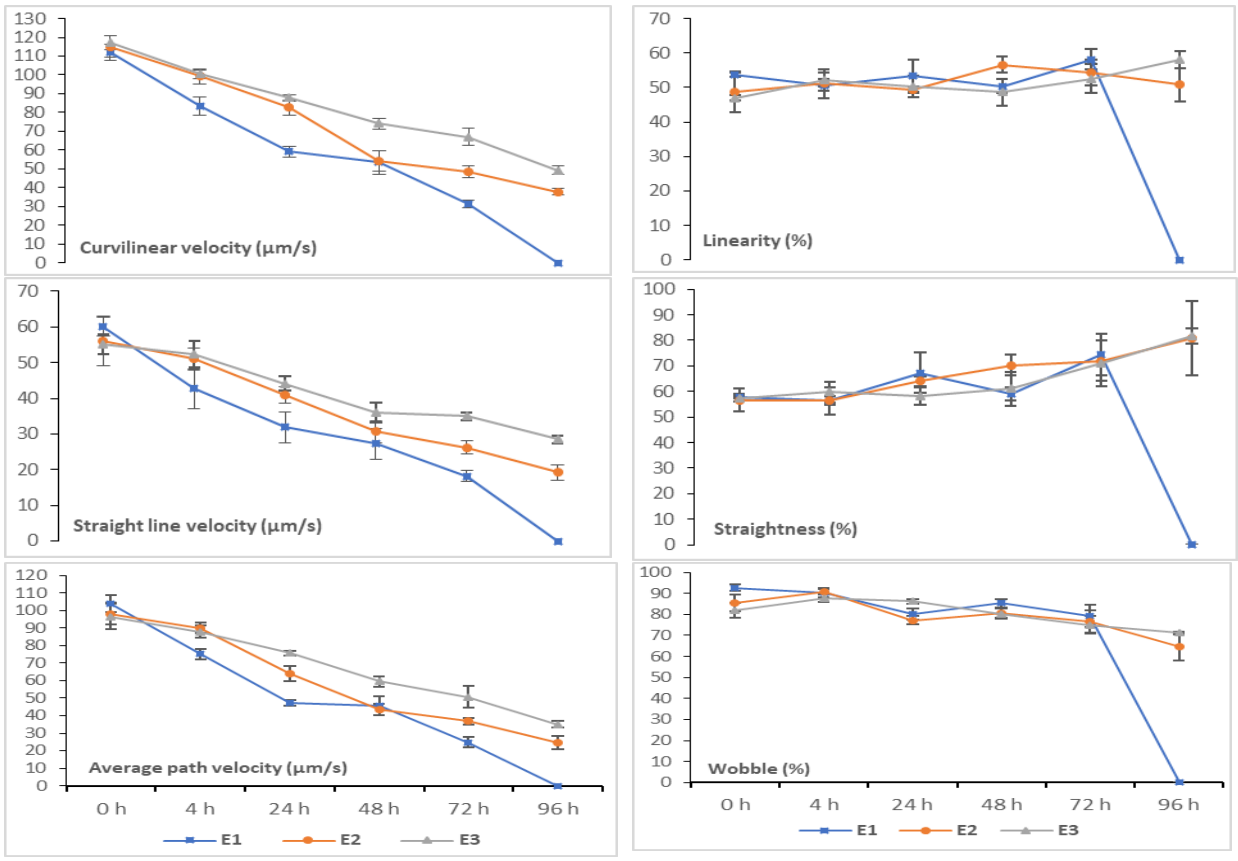

Fig. 3. Change in turkey sperm-dynamic parameters at different storage times.

Sperm fertility:

Fertility of eggs produced from hens inseminated by semen with different extenders revealed a significant ($P<0.05$) increase of E3 (87.5%) as compared to E1 (77.5%), but E2 (85.83%) showed insignificant differences with both E1 and E3 (Fig. 4).

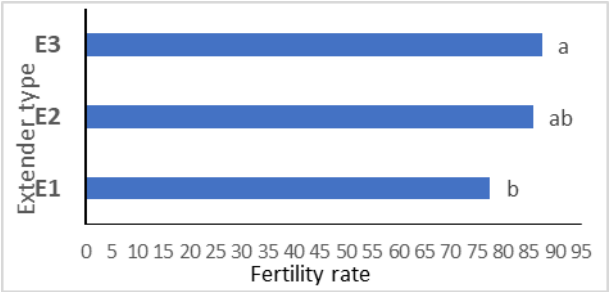

Fig. 4. The fertility of turkey semen extended with different types of extenders.

An optimal composition of extenders for chilled-semen storage for longer times is required for turkey semen. Poultry sperm storage, or the ability of sperm to survive and remain functional for the short term, is known to be marginal and often requires invasive techniques such as extender types to produce live offspring (Iaffaldano *et al.*, 2005). In the commercial field, improving the procedures of semen preservation is important because turkey production relies almost entirely on artificial insemination. A loss of fertilizability of turkey semen occurs by storing the semen for a long time more than 6 hours. Thus, improving the storage regimens may allow longer preservation and high fertility of turkey semen (Iaffaldano and Meluzzi, 2003). The studies for obtaining an optimal extender composition for semen preservation is still in progress, therefore the current study aimed to test a simple carbohydrate-based solution containing 300- or 370-mM glucose, as a source of energy, in Tris-based extender, in comparing with saline for turkey-semen preservation for long-term at a cool temperature. The obtained results indicated higher sperm motility parameters of both glucose-based extenders (E2 and E3) as compared to saline (E1), in addition to maintaining sperm characteristics for 72 h for E2 and E3 versus 2 h for saline (E1). The presence of glucose levels ensuring energy in E2 and E3 may explain the better quality of turkey semen in comparison with saline (E1) free from these components. The exogenous glucose added to tris-extenders (E2 and E3), which is not found in E1, is sufficient and appropriate for the energy required for spermatozoa during *in vitro* cool preservation. The extenders used in this study for storage of turkey semen are considered as buffers for maintaining spermatozoa by providing the osmotic pressure of 330-400 mOsm/L and pH value of 7.0-7.5, being similar to those of the seminal plasma as well as they save energy substrates as glucose (Iaffaldano *et al.*, 2005). The motility of turkey spermatozoa might be affected due to hypertonic media (Akçay *et al.*, 2006).

Also, the source of energy in the semen extender affects the osmolarity level and pH value of each extender. Following our results, Akçay *et al.* (2006) found that sperm motility of turkey semen in glucose-based extender for 24-48 h at 4ºC was better than in semen stored in sucrose-based extender or ringer + glucose solution. Also, Iaffaldano *et al.* (2005) reported that semen quality parameters in semen extender at a level of 366 mOsm/L and 7.5 pH value were better than the other extenders at a level of 380 and 390 mOsm/l and 6.95 and 7.1 pH value during cooling

preservation. In our study, semen stored by E1 maintained sperm motility parameters within 2 h in comparison with E2 and E3. Inclusion of egg yolk in tris-extenders gave a superiority for these extenders to show higher values at 5°C at 0 time than saline. In this respect, Miškeje *et al.* (2013) found that egg yolk extender showed a higher efficiency compared to the saline solution at storage time from 0 to 60 minutes. Our results indicated a marked decrease of saline (E1) at 5°C within a storage time of 2 hours, whereas a reduction was observed in sperm motility parameters after 2 h of storage. The dilution with financially undemanding egg yolk diluents (E2 and E3) in comparison with E1was reported as a positive impact on motility, viability and membrane integrity in semen cooled stored for 48 h, as functional sperm parameters (Iaffaldano *et al*., 2005). Similar results were reported by Miškeje *et al*. (2013) up to 180 minutes.

In avian spermatozoa, glucose metabolism is lower than in mammalian spermatozoa. Also, the activity of the glycolytic enzyme was reported to be lower 2–10 folds in turkey than in fowl spermatozoa (Wishart, 1982). In older studies, turkey-semen maintained motility when was stored in glucose solution for 24-48 hours at a cool temperature in comparison with unstored control semen (Akçay *et al*., 2006). Also, higher sperm motility percentages were obtained when approximately half of the extender was replaced by iso-osmolar glucose solution with fresh semen (Graham *et al*., 1982). During cooling preservation, turkey spermatozoa can tolerate hypertonic solutions (Huyghebaert *et al*., 1983) as reported in fowl semen (Van Wambeke, 1977). In glucose-based extenders used in our study, increasing glucose molarity from 300 (E2) to 370 mM (E3) improved ($P<0.05$) total progressive, total motility, slow progressive motility, and immotility at all storage times, being with acceptable values up 72 of storage, but rapid progressive motility was nearly similar for E2 and E3 at all storage times. These differences may be due to the observed variable in osmolarity level (325 vs.370) or pH value (6.9 vs.7.2) of E2 and E3, respectively. In a study of Akçay *et al*. (2006) found that sperm motility was lower in semen stored with 0.6 M sucrose solution (600 mOsm/L and pH value 5) in comparing with 0.3 M glucose solution (300 mOsm/L and pH value 5.5) after 24 -48 h.

In our study, advancing the storage time caused deleterious effects on sperm motility parameters in semen extended with all types of extenders. The pH of the extender and agitation, which is used to aerate the semen, may have created bad conditions for the spermatozoa during semen preservation (Akçay *et al*., 2006). In this way, a constant agitation was observed in turkey semen during storage in an extender with a pH 7.5 than pH 6.5 extender for 18 h at 5ºC with or without intermittent agitation (Giesen and Sexton, 1983; Lake *et al*., 1984). In agreement with the present results, Gündoğan *et al*. (2003) found that seawater did not affect primary motility. Sperm motility in this diluent was 0. However, glucose-phosphate extender had the highest impact on sperm motility (80.5 ± 0.88%) in comparison with blood serum (69.5 ± 1.70%) after semen incubation for 24 h and creamy yolk extender (78.0 ± 1.17%). Glucose-phosphate and egg yolk-citrate extenders maintained ≥50% of spermatozoa to be motile for 4 days of cultivation. Also, Parkhurst *et al*. (2000) showed a curvilinear decrease in turkey spermatozoa stored for 48 hours. About 36, 18, and 30% of initial activity were losses within the first 6 hours, 6-24, and after 48 hours of storage, respectively.

According to sperm morphological parameters by CASA, E3 showed the highest normality forms in terms of increasing the percentages of normal sperm and decreasing the percentages of head, neck, and tail abnormality, mono, dual, and tri deformation, and SDI. These results are in parallel with sperm motility parameters. Respecting the sperm abnormality, SDI value increases the clinical value of semen analysis and should also be used to differentiate between fertile and infertile males in addition to other semen parameters. Values of SDI were significantly lesser in the fertile than in infertile men (Zaheer and Khan, 2009). It is suggested that SDI should also be used to differentiate between fertile and infertile males in addition to other semen parameters as it can be useful in identifying potential infertile males (Ahmad and Tariq, 2011). They reported that SDI was significantly lesser in the proven fertile men (1.58±0.19) than in infertile men (1.8 ±0.57).

Sperm motility should be considered an important determinant for fecundity (Froman *et al*., 1997) because sperm kinetics reflect the energy status and the functional integrity of spermatozoa (Quintero-Moreno *et al*., 2004). In our study, the present results cleared a positive relationship between sperm motility parameters and sperm velocity parameters (VCL, VSL, and VAP) for each extender type. At all storage times, the highest value of VCL was observed in E3, followed by E2, while E1 showed the lowest values. However, Iaffaldano *et al*. (2005) found the highest VCL at 0 time of turkey semen diluted with saline stored at 5ºC. Significant differences were found later at the time from 60 to120 minutes in egg yolk diluent and saline solution.

As such, we obtained better results of fertility for semen diluted with E3, followed by E2 undergoing storage at 4-5ºC for 72 h than that extended in E1 for 2 h and stored at 4-5ºC. These were reflected by improving motility and kinetic parameters of spermatozoa at the same storage conditions. Improving stored semen quality by decreasing the percentage of immotile sperm cells or sperm with impaired motility are retained in the vagina, while there are only spermatozoa with progressive motility to the site of sperm storage in the avian female reproductive tract (Allen and Grigg, 1957). About the storage time of the semen, the fertility rates from stored turkey semen are consistently different from chicken semen fertility according to the elongation of storage time (Long *et al*., 2014). In previous studies, turkey semen usually cannot be stored longer than 6 hours without loss of fertilizing ability, even if oxygenic and stored in the diluent at a reduced temperature (Iaffaldano *et al*., 2005). In accordance with the obtained results, Sexton and Giesen (1983) found that fertilizing capacity and motility in storage was suppressed more if diluted with extender at pH 5.5 than at pH 6.5 or 6.0. This may explain a high fertility rate of turkey semen diluted with E3 (pH of 7.2) and E2 (pH of 6.9) than E1 (pH of 5.5). Similar results were obtained by Iaffaldano *et al*. (2005), who studied the effect of diluent, pH, size, volume of insemination dose and frequency of insemination on motility and fertilization capacity of turkey spermatozoa stored at an interval of 6 hours at 15°C. In comparison with the results of sperm fertility in our study, sperm fertilizability of turkey semen stored at a cool temperature for 24-48 h was 79.1% in

glucose-extender, 0% in sucrose-extender, and 30.9% ringer + glucose (Akçay *et al*., 2006). In our study, higher fertility was associated with glucose-extenders than with saline. The glucose extender gives better fertility results than a sucrose extender (Akçay *et al*., 2006). Sperm fertility was not affected by using extenders with an osmolarity of 280 mOsm/L (Sexton and Giesen 1982). In the current study, increasing sperm fertility for semen stored in E2 and E3, being with higher osmolality level than in E1 during cooled storage. Therefore, it remains difficult to pronounce an optimal tonicity per se since other physical and chemical characteristics of the extender varied in these different studies (Akçay *et al*., 2006). The fertility rate was not affected by the preservation of turkey semen at 5ºC for 18 h, but fertility was lowered in semen preserved at 15, 25, or 36ºC for 18 h (Giesen and Sexton, 1983).

It was confirmed that the count of spermatozoa ≥50 million and < 200 million will assure consistently high fertility rates (Donoghue *et al*., 1995). In our study, the concentration of spermatozoa used for artificial insemination was $250x10^6$/ejaculate for semen diluted at a rate of 1:3. Sexton (1987) recorded a lower fertility rate of stored turkey semen extended at a rate of 1:2 than 1:1 or 2:1. In this respect, the high rate of dilution (1:4) showed negative effects on sperm fertility due to increasing sperm metabolism activity (Sexton, 1976). Finally, Akçay *et al*. (2006) showed a positive impact of using simple glucose-based extenders on sperm fertility of cooled-stored turkey semen (24-48 h, at 4ºC).

CONCLUSION

Tris-glucose extenders used for stored turkey semen are buffers appropriate for the immediate survival of spermatozoa because they provide osmotic pressure (325-370 mOsm/L) and pH (6.9-7.2) similar to those of seminal plasma but they also provide energy substrates as carbohydrates (glucose) or other components such as egg yolk. Based on the foregoing results, turkey semen has an efficacy when was diluted with tris-glucose extender (with 300 and 370 Mm) and cooled stored for 48 or 72 h at 4-5ºC with a minimal loss in sperm characteristics and fertility rate. Further researches are needed to study the effect of glucose at various molecular levels in chilled semen on sperm function and fertility.

REFERENCES

Ahmad, M. O. and Tariq, M. (2011). A study of sperm deformity index at Islamabad. Pakistan Journal of Physiology, 7(1): 1–3.

Akçay, E.; Varışlı, Ö. and Tekin, N. (2006). Fertilizing ability of turkey semen diluted with simple sugar-based extenders after cooled storage. XII European Poultry Conference, 11–13.

Akçay, E. (1997). Hindi spermasinin değişik sulandiricilarda 4° c'de saklanmasi. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 44(02.03).

Allen, T. E. and Grigg, G. W. (1957). Sperm transport in the fowl. Australian Journal of Agricultural Research, 8(6): 788–789.

Bakst, M. R. and Cecil, H. C. (1992). Research Note: Effects of Agitation and Temperature Changes on Turkey Sperm Viability After Twenty-Four Hour Storage. Poultry Science, 71(2): 395–397.

Blesbois, E. (2007). Current status in avian semen cryopreservation. World's Poultry Science Journal, 63(2): 213–222.

Blesbois, E.; Grasseau, I. and Hermier, D. (1999). Changes in lipid content of fowl spermatozoa after liquid storage at 2 to 5 C. Theriogenology, 52(2): 325–334.

Bréque, C.; Surai, P. and Brillard, J. (2003). Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. Molecular Reproduction and Development: Incorporating Gamete Research, 66(3): 314–323.

Christensen, V. L. (1995). Diluents, dilution, and storage of poultry semen for six hours. In: Proceedings of the First International Symposium on Artificial Insemination of Poultry (M. Bakst, G. Wishart, eds), Illinois, Savoy, 90-106.

Donoghue, A. M.; Garner, D. L.; Donoghue, D. A. N. J. and Johnson, L. A. (1995). Viability assessment of turkey sperm using fluorescent staining and flow cytometry. Poultry Science, 74(7): 1191–1200.

Donoghue, A. M.; Garner, D. L.; Donoghue, D. J. and Johnson, L. A. (1996). Assessment of the membrane integrity of fresh and stored turkey spermatozoa using a combination of hypo-osmotic stress fluorescent staining and flow cytometry. Theriogenology, 46(1): 153–163.

Donoghue, A. M. and Wishart, G. J. (2000). Storage of poultry semen. Animal Reproduction Science, 62(1–3): 213–232.

Douard, Veronique; Hermier, D. and Blesbois, E. (2000). Changes in turkey semen lipids during liquid in vitro storage. Biology of Reproduction, 63(5): 1450–1456.

Douard, Véronique; Hermier, D.; Labbe, C.; Magistrini, M. and Blesbois, E. (2005). Role of seminal plasma in damage to turkey spermatozoa during in vitro storage. Theriogenology, 63(1): 126–137.

Douard, Veronique; Hermier, D.; Magistrini, M. and Blesbois, E. (2003). Reproductive period affects lipid composition and quality of fresh and stored spermatozoa in turkeys. Theriogenology, 59(3–4): 753–764.

Froman, D. P.; Feltmann, A. J. and McLean, D. J. (1997). Increased fecundity resulting from semen donor selection based upon in vitro sperm motility. Poultry Science, 76(1): 73–77.

Giesen, A. F. and Sexton, T. J. (1983). Beltsville poultry semen extender. 9. Effect of storage temperature on turkey semen held eighteen hours. Poultry Science, 62(7): 1305–1311.

Graham, E. F.; Nelson, D. S. and Schmehl, M. K. L. (1982). Development of Extender and Techniques for Frozen Turkey Semen: 1. Development. Poultry Science, 61(3): 550–557.

Gündoğan, M.; Tekerli, M.; Uçar, M. and Türkmenoğlu, I. (2003). Effect of diluents on motility of ram sperm during storage at 5 C. Archives of Andrology, 49(1): 69–75.

Huyghebaert, G.; Wambeke, F. and Groote, G. (1983). The effect of diluent, storage temperature and number of spermatozoa on fertility and hatchability results obtained with turkey semen stored for 6 hours. Archiv Fuer Gefluegelkunde (Germany, FR), 47: 103-109.

Iaffaldano, N.; Rosato, M. P.; Manchisi, A.; Centoducati, G. and Meluzzi, A. (2005). Comparison of different extenders on the quality characteristics of turkey semen during storage. Italian Journal of Animal Science, 4(sup2), 513–515.

Iaffaldano, Nicolaia. and Meluzzi, A. (2003). Effect of dialysis on quality characteristics of turkey semen during liquid storage. Theriogenology, 60(3): 421–427.

Kotłowska, M.; Dietrich, G.; Wojtczak, M.; Karol, H. and Ciereszko, A. (2007). Effects of liquid storage on amidase activity, DNA fragmentation and motility of turkey spermatozoa. Theriogenology, 67(2): 276–286.

Lake, P. E.; Cherms, F. L. and Wishart, G. J. (1984). Effect of aeration on the fertilising ability of turkey semen stored for 48 hours at 5 and 15 C: a study from the 33rd to the 47th week of age. Reproduction Nutrition Développement, 24(2): 147–153.

Lake, P. E. and Ravie, O. (1982). Effect on fertility of storing turkey semen for 24 hours at 10 C in fluids of different pH. British Poultry Science, 23(1): 41–47.

Lemoine, M.; Mignon-Grasteau, S.; Grasseau, I.; Magistrini, M. and Blesbois, E. (2011). Ability of chicken spermatozoa to undergo acrosome reaction after liquid storage or cryopreservation. Theriogenology, 75(1): 122–130.

Long, J. A. and Kramer, M. (2003). Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored turkey semen. Poultry Science, 82(11): 1802–1807.

Long, Julie A.; Purdy, P. H.; Zuidberg, K.; Hiemstra, S.-J.; Velleman, S. G. and Woelders, H. (2014). Cryopreservation of turkey semen: Effect of breeding line and freezing method on post-thaw sperm quality, fertilization, and hatching. Cryobiology, 68(3): 371–378.

Miškeje, M.; Slanina, T.; Kolenčiková, A.; Knižat, L.; Mirda, J.; Petrovičová, I. and Massanyi, P. (2013). The effect of different temperature and medium on turkey spermatozoa motility in vitro. Journal of Microbiology, Biotechnology and Food Sciences, *2 (Special issue 1)*, 1491–1501.

Morrell, J. M.; Persson, B.; Tjellström, H.; Laessker, A.; Nilsson, H.; Danilova, M. and Holmes, P. V. (2005). Effect of semen extender and density gradient centrifugation on the motility and fertility of turkey spermatozoa. Reproduction in Domestic Animals, 40(6): 522–525.

Parkhurst, A. M.; Korn, N. and Thurston, R. J. (2000). The effects of methylxanthines on the mobility of stored turkey sperm. Poultry Science, 79(12): 1803–1809.

Quintero-Moreno, A.; Rigau, T. and Rodrıguez-Gil, J. E. (2004). Regression analyses and motile sperm subpopulation structure study as improving tools in boar semen quality analysis. Theriogenology, 61(4): 673–690.

Sexton, T. J. (1988). Comparison of commercial diluents for holding turkey semen 24 hours at 5 C. Poultry Science, 67(1): 131–134.

Sexton, T. J. (1987). Effect of semen treatments and age of tom on fertility of unstored semen and semen held 18 hours. Poultry Science, 66(10): 1721–1726.

Sexton, T. J. (1976). Studies on the dilution of turkey semen. British Poultry Science, 17(2): 179–184.

Sexton, T. J. and Giesen, A. F. (1983). Beltsville poultry semen extender. 8. Factors affecting turkey semen held six hours at 15 C. Poultry Science, 62(6): 1063–1068.

Sexton, T. J. and Giesen, A. F. (1982). Beltsville poultry semen extender: 6. Holding turkey semen for six hours at 15 C. Poultry Science, 61(6): 1202–1208.

Thurston, R. J.; Rogoff, M. S.; Scott, T. R. and Korn, N. (1994). Perfluorochemical emulsions as turkey semen diluents: effects of varying aeration treatments and aqueous phase on fertilizing capacity of semen stored for twenty-four hours. Poultry Science, 73(5): 724–732.

Thurston, R. J. (1995). Storage of poultry semen above freezing for twenty-four to forty-eight hours. 107–122.

Van Wambeke, F. (1977). The effect of tonicity of storage media for fowl semen on the occurrence of neck-bending spermatozoa, fertility and hatchability. British Poultry Science, 18(2): 163–168.

Wishart, G. J. (1982). Maintenance of ATP concentrations in and of fertilizing ability of fowl and turkey spermatozoa in vitro. Reproduction, 66(2): 457–462.

Zaheer, M. and Khan, U. A. (2009). A Study of Teratozoospermic Index in Pakistani Men. Ann. Pak. Inst. Med. Sci, 5(3): 186–188.

تأثير محاليل الجلوكوز في مخفف التريس على خصائص الحيوانات المنوية في السائل المنوي للديوك الرومي المبرد لمدة 96 ساعة

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يهدف هذا العمل إلى دراسة تأثير مخفف الترس المحتوي على سكر الجلوكوز (300 أو370 مليمول) على قابلية تخزين وخصوبة السائل المنوي للديوك الرومي. تم جمع السائل المنوي مرتين في الأسبوع لمدة 4 أسابيع من عدد 12 من الديوك الرومي البرونزي بعمر 40 أسبوعًا عن طريق تدليك البطن. تم تجميع السائل المنوي للديوك بعد جمعه ثم تخفيفه بمعدل 1: 2 مع محلول ملحي (المخفف الأول) - مخفف الترس + 300 ملمول جلوكوز (المخفف الثاني) أو مخفف الترس + 370 ملمول جلوكوز (المخفف الثالث). بعد التخفيف، تم تخزين السائل المنوي لمدة 96 ساعة على درجة حرارة باردة (4-5 درجة مئوية). تم تقييم السائل المنوي من حيث الخصائص الحركية والمورفولوجية والديناميكية، ثم تم تحديد خصوبة الحيوانات المنوية للسائل المنوي المخزن لوقت التخزين المناسب لكل مخفف. أظهرت النتائج أن المتوسط العام لجميع خصائص حركة الحيوانات المنوية (الحركة الكلية، التقدمية السريعة، التقدمية السريعة والبطيئة، والغير تقدميه والغير متحرك)، الخصائص المورفولوجية للحيوانات المنوية (تشوهات الرأس والرقبة، التشوهات الأحادية والمزدوجة ومؤشر التشوه وكذلك الخصائص الديناميكية للحيوانات المنوية (السرعة الانحنائية والخطية ومتوسط السرعة، معدل الخطية، الاستقامة والتمايل تحسن معنوي بواسطة المخفف الثاني والثالث مقارنة بالمخفف الأول. كان معدل الخصوبة أعلى معنويا (89.5٪) للبيض المنتج من الدجاج الملقح بالسائل المنوي للمخفف الثالث المخزن لمدة 72 ساعة مقارنةً بالسائل المنوي للمخفف الأول المخزن لمدة ساعتين (77.5٪)، ولكن لم يختلف معدل الخصوبة للسائل المنوي للمخفف الثاني (85.83٪) معنويا عن المخفف الأول أو الثالث. ويمكن استنتاج أنه يمكن تخزين الحيوانات المنوية للديوك الرومي مع الحفاظ على وظيفة الحيوانات المنوية والخصوبة في مخفف تريس محتوى على الجلوكوز (300 أو 370 مليمول) لمدة 72 ساعة على الأكثر على درجة حرارة التبريد (4-5 درجه مئوية).