Goat spermatozoa survival rate and freezability in different extenders with/without melatonin supplementation

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Abstract

The aim of this study was to compare preservation ability of five extenders-sodium citrate egg yolk (SCY), tris-citrate-glucose-glycerol-yolk(TGGY),Tris-citrate-fructose-glycerol-yolk (TFGY), Cornell University(CU-16) and 14 extenders-by means of sperm motility (SM) %, alive sperm (AS) %, sperm abnormalities (SA) % and acid phosphatase levels (AcP) of goat extended semen stored at 5°C for seven days. Also, the effects of melatonin (at the doses of 0.0, 10.0, 15.0 and 20.0 g/ 100 x 10^6 sperm) on SM %, AS %, SA %, AcP levels and post-thawing motility of goat extended semen stored at 5°C for seven days were studied.

The findings of the current investigation showed that CU-16 and 14 extenders ascertained high significant difference ($P<0.0001$), in the previous parameters than Tris extenders for goat semen preservation. Melatonin particularly the high concentrations (15.0 and 20.0 g) improved SM% ($P<0.0001$), reduced dead sperm % ($P<0.0001$) and AcP levels ($P<0.0001$) as well as post-thawing motility. In conclusion, the use of CU-16 and 14 extenders was better than Tris extenders for goat semen preservation. Moreover the inclusion of melatonin (particularly 15.0 and 20.0 g) improved the extended goat semen quality and its freezability.

Introduction

It necessary to extend the semen in special extenders to achieve good preservation of goat semen. These generally, should have proper pH and buffering capacity, suitable osmolarity and should protect the spermatozoa from cryogenic injury (Salmon and Maxwell, 1995). It was found that tris-buffer extenders were effective for dilution and storage of goat, sheep and rabbit (Chehadeh et al., 2001, Evans and Maxwell, 1987 and Roca et al., 2000).

Unfortunately, there is no available literature regarding the use of glycine containing extenders to keep the viability of goat semen. On the other hand, the replacement of citrate buffer with glycine was reported to improve the survival

of ram spermatozoa (Ahmed, 1955 and Schindler and Amir, 1961). Moreover, the use of glycine and glucose containing extenders maintain high fertility over a period of several days in bull (Shannon, 1964).

The inclusion of antioxidant such as catalase, superoxide dismutase, glutathione peroxidase besides thiouria improved the viability of goat spermatozoa during 6 days of storage at 5°C (Stojanov et al., 1994, Pomares et al., 1995 and Waheed et al., 2003).

Melatonin (N-acetyle-5-Methoxytryptamine) is a hormone produced mainly by the pineal gland besides other tissues like retina (Dubocovich et al. (1989). Mammdouh et al. (1996) recorded that the addition of melatonin to liquid bull semen improved its storage and preservation for 6 days.

So the aim of the present study is to investigate the preservation of goat semen in TGGY, TFGY and 14 extenders, to study the influence of melatonin on sperm motility, sperm alive %, sperm abnormalitities % and acid phosphatase activity. And sperm freezability.

Materials and Methods

This investigation was carried out at Experimental Research Farm that follows the National Research Center directory.

A- Experimental Animals.

Six male Zaraibi goats aged 19 month approximately and weighed 30-40 kg were used in the present investigation. Each buck was fed one Kg balanced concentrate and berseem hay ad libidium.

B- Experimental Materials.

Melatonin (N-acetyl-S-methoxytrytamine) was imported form Twinlab Specialty Corporation, Ronkon Koma, New York, USA.

C- Semen collection and evaluation.

Semen was collected twice weekly by means of an artificial vagina using an anestrous doe as teaser. Within 2-3 minutes and after collections, the ejaculates were transferred to the laboratory of the farm and kept in water bath at 37°C.

D- Semen extenders.

Five types of extenders were used for preserving the goat semen:

1- SCY extender was prepared according to Pellicer-Rubio et al., 1997) for goat semen.
2- TGGY extendernwas prepared according to Roca et al. (2000) + 10% egg yolk and 6.7% glycerol.
3- TFGY extender was formulated according to Foote (1970) using 10% egg yolk.
4- CU-16 extender wa sprepared according to Shannon (1964)
5- Extender 14 was prepared according to Shannon (1964) using 10% egg yolk.

E- semen processing and experimental design:

Only ejaculates of >70% initial motility and 2000 x 10^6 sperm cells/ ml were used in the following experiments:

Experiment I:

This experiment was carried out to test the motility of chilled-stored semen in SCY; TGGY, TFGY and 14 extenders. Semen samples were divided and diluted (1:4 according to Evans and Maxwell, 1987) at 30°C with semen extenders. Immediately after dilution, the extended semen was incubated at 5°C to be examined daily for seven days for SM %, AS % and SA % using eosin-aniline stain according to Shaffer and Almquist (1948).

Experiment 2:

This experiment was designed to find out the impact of melatonin on the previous parameters viability of chilled semen in TGGY, TFGY and 14 extenders. Semen samples were split and processed as previously mentioned in experiment I, then melatonin was added to the extended semen as 0.0 g (control sample), 10, 15 and 20 g/100x10^6 sperm (Mamdouh et al., 1996). After daily examination, the samples were centrifuged at 3000 rpm for 20

minutes to get supernatant for immediate determination of acid phosphatase (AcP) according to Moss (1984).

Experiment 3:

This experiment was designed to investigate influence of melatonin on freezability of goat semen in 0.25 ml straws. Semen samples were cooled and frozen as prescribed by El-Battawy et al. (2003). After few weeks, frozen goat semen was thawed in a water bath at 40°C for 30 seconds . The straws were wiped and deplugged. The thawed semen was emptied in pre-warmed tubes and incubated in water bath at 30°C for assessment of sperm motility.

F- Statistical analysis

Data were transformed from percentage to absolute figures using arcsin tables. The ANOVA test was used at a confidence not less than limit 95% using SAS program (1988). LSD test was used to evaluate the significant difference between means at $P<0.05$.

Results

Data were shown in tables (1-14). Table (1) declared the significant ($P<0.0001$) effect of various extenders, time factor and their interaction on SM %. SM % (Table 2), AS % (Table 3), SA % (Table 4) and non prostatic moiety of AcP activity (Table 5) were significantly ($P<0.0001$) influenced by the addition of melatonin to TGGY extender. The high concentration of melatonin had increased the motility, the live sperm significantly ($P<0.0001$) in comparison with the other concentrations, all over the storage duration. On the other hand, it decreased significantly ($P<0.0001$) the sperm abnormalities and acid phosphatase activity. The same results were obtained using the same melatonin's concentrations with the TFGY ($P<0.0001$) (Tables 6, 7, 8 and 9). Concerning the effect of melatonin on SM % and AS %, using glycine containing extender (14 extender), the overall means trend elaborated the improvement of motility and alive sperm significantly ($P<0.0001$) with the high concentrations (15 and 20 g) compared to the other concentrations (0.0 and

10.0 g). This was shown in tables (10 and 11), while a significant (P<0.0001) decrease was shown in case of SA % and AcP activity (Tables 12 and 13). No significant differences were found concerning the interaction between time of storage and the concentration of melatonin (Tables 10 – 13).

Regarding the effect of melatonin on the post-thawed sperm motility, only there was significant (P<0.0001) differences between the different concentrations of melatonin, while no significant differences were found in case of extenders or the interaction between the melatonin concentrations and the extenders factor (Table 14).

Table 1. The effect of various extenders on the sperm motility % stored at 5°C for 7 days.

Storage time (days)	Control (sod. Citrate egg yolk)	TGGY	TFGY	CU-16	14	Overall mean
1	53.76 ± 1.23 (65.05)	67.36 ± 1.66 (85.20)	66.41 ± 1.93 (84.00)	67.58 ± 2.73 (85.45)	65.55 ± 2.49 (82.85)	64.13^{A} (80.95)
2	45.00 ± 2.36 (50.00)	57.59 ± 0.80 (71.25)	60.12 ± 1.92 (75.20)	60.92 ± 1.60 (76.46)	60.06 ± 1.36 (75.08)	56.74^{B} (69.90)
3	36.93 ± 3.85 (36.10)	53.02 ± 1.44 (63.80)	56.83 ± 1.28 (70.10)	56.06 ± 1.50 (68.80)	56.83 ± 1.28 (70.10)	51.93^{C} (62.00)
4	33.00 ± 2.59 (29.70)	47.89 ± 1.67 (55.00)	52.29 ± 1.92 (62.60)	53.02 ± 1.44 (63.85)	53.83 ± 2.48 (65.20)	48.01^{D} (55.25)
5	27.52 ± 2.15 (21.35)	40.67 ± 1.44 (42.45)	43.56 ± 1.44 (47.50)	49.34 ± 1.88 (57.55)	50.83 ± 2.41 (60.10)	42.39^{E} (45.45)
6	19.89 ± 1.03 (11.60)	31.39 ± 3.05 (27.10)	36.22 ± 1.74 (34.90)	47.16 ± 1.38 (53.76)	45.74 ± 2.99 (51.30)	36.08^{F} (34.70)
7	14.76 ± 1.30 (6.50)	21.56 ± 1.96 (13.50)	25.25 ± 3.13 (18.20)	43.56 ± 1.44 (47.50)	39.17 ± 2.41 (39.90)	28.86^{G} (23.30)
Overall mean	32.98^{D} (29.60)	45.64^{C} (51.10)	48.67^{B} (56.40)	53.95^{A} (65.40)	53.14^{A} (64.00)	

Mean ± SE.; (%); LSD for days 2.556 (P<0.05); LSD for treatments 2.159 (P<0.05)
LSD for interaction 5.703 (P<0.05)

Table 2. The effect of melatonin on the sperm motility % stored at 5°C for 7 days using TGGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	65.55 ± 2.49 (82.90)	68.44 ± 1.96 (86.50)	73.23 ± 2.39 (91.65)	73.23 ± 2.39 (91.65)	70.12^{A} (88.40)
2	57.57 ± 0.81 (71.25)	60.86 ± 0.86 (76.30)	64.38 ± 0.94 (81.30)	68.30 ± 1.09 (86.30)	62.78^{B} (79.10)
3	53.02 ± 1.44 (63.80)	58.40 ± 0.93 (72.55)	60.86 ± 0.86 (76.30)	64.38 ± 0.94 (81.30)	56.16^{C} (69.00)
4	47.89 ± 1.67 (55.00)	55.26 ± 0.88 (67.50)	58.40 ± 0.93 (72.50)	60.86 ± 0.86 (76.30)	55.60^{D} (68.10)
5	42.12 ± 1.67 (45.00)	52.25 ± 0.85 (62.50)	55.26 ± 0.88 (67.50)	57.59 ± 0.80 (71.30)	51.80^{E} (61.80)
6	34.56 ± 3.02 (32.20)	48.60 ± 1.38 (56.25)	51.53 ± 1.40 (61.30)	53.76 ± 1.23 (65.05)	47.11^{F} (53.70)
7	23.84 ± 1.91 (16.34)	42.84 ± 1.38 (46.25)	46.44 ± 0.83 (52.50)	50.05 ± 0.73 (58.75)	40.79^{G} (42.70)
Overall mean	46.36^{D} (52.40)	55.24^{C} (67.50)	58.58^{B} (72.80)	61.17^{A} (76.75)	

Mean ± SE.; (%); LSD for days 2.073 (P<0.05); LSD for treatments 1.567 (P<0.05); LSD for interaction 4.149 (P<0.05).

Table 3. The effect of melatonin on the alive sperm % stored at 5°C for 7 days using TGGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	84.50 ± 3.38	88.50 ± 2.72	93.75 ± 2.84	93.75 ± 2.84	90.13^{A}
2	73.25 ± 1.25	78.25 ± 1.25	83.00 ± 1.00	87.75 ± 1.75	80.56^{B}
3	66.50 ± 2.18	74.50 ± 1.44	78.25 ± 1.25	83.00 ± 1.00	75.56^{C}
4	58.50 ± 2.60	70.00 ± 1.15	74.50 ± 1.44	75.75 ± 1.25	69.69^{D}
5	48.50 ± 3.18	65.50 ± 1.44	70.00 ± 1.15	73.25 ± 1.25	64.31^{E}
6	35.00 ± 5.05	59.50 ± 2.18	64.25 ± 2.39	67.75 ± 1.84	56.63^{F}
7	26.50 ± 2.18	49.75 ± 2.66	56.00 ± 1.15	61.75 ± 1.25	48.50^{G}
Overall mean	56.11^{D}	69.43^{C}	74.25^{B}	77.57^{A}	

Mean ± SE.; LSD for days 3.058 (P<0.05); LSD for treatments 2.312 (P<0.05); LSD for interaction 6.120 (P<0.05).

Table 4. The effect of melatonin on the sperm abnormalities % stored at 5°C for 7 days using TGGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	10.50 ± 0.65	8.75 ± 0.48	7.50 ± 0.29	6.75 ± 0.48	8.38^{G}
2	12.75 ± 0.25	10.75 ± 0.25	9.50 ± 0.29	7.75 ± 0.25	10.19^{F}
3	12.75 ± 0.25	11.50 ± 0.29	11.50 ± 0.50	9.75 ± 0.25	11.38^{E}
4	14.00 ± 0.58	12.50 ± 0.29	11.75 ± 0.49	10.75 ± 0.25	12.25^{D}
5	15.00 ± 0.29	13.50 ± 0.29	12.50 ± 0.29	11.75 ± 0.25	13.31^{C}
6	18.25 ± 1.11	14.00 ± 0.41	13.50 ± 0.29	13.00 ± 0.41	14.69^{B}
7	22.50 ± 0.96	15.25 ± 0.25	14.50 ± 0.29	13.75 ± 0.25	16.50^{A}
Overall mean	15.18^{A}	12.32^{B}	11.54^{C}	10.50^{D}	

Mean ± SE.; LSD for days 0.622 (P<0.05); LSD for treatments 0.470 (P<0.05); LSD for interaction 1.245 (P<0.05).

Table 5. The effect of melatonin on the acid phosphatase activity stored at 5°C for 7 days using TGGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	22.50 ± 1.55	16.00 ± 0.71	17.25 ± 0.48	16.50 ± 0.65	18.06^{E}
2	25.50 ± 2.40	25.50 ± 0.65	20.75 ± 0.85	18.50 ± 0.65	22.56^{D}
3	31.25 ± 1.31	30.75 ± 1.55	25.25 ± 1.55	23.25 ± 1.38	27.63^{C}
4	33.75 ± 1.75	32.75 ± 0.85	31.00 ± 1.08	26.25 ± 0.85	30.94^{B}
5	43.50 ± 1.04	40.75 ± 0.85	36.25 ± 1.11	34.00 ± 1.68	38.63^{A}
6	43.25 ± 0.85	39.00 ± 1.29	39.25 ± 0.75	34.25 ± 1.38	38.94^{A}
7	44.25 ± 0.85	39.75 ± 1.31	39.75 ± 1.49	35.00 ± 1.47	39.69^{A}
Overall mean	34.86^{A}	32.07^{B}	29.92^{C}	26.82^{D}	

Mean ± SE.; LSD for days 1.734 (P<0.05); LSD for treatments 1.311 (P<0.05).

Table 6. The effect of melatonin on the sperm motility % stored at 5°C for 7 days using TFGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	69.39 ± 1.26 (87.60)	74.32 ± 1.59 (92.70)	74.32 ± 1.59 (92.70)	74.32 ± 1.59 (92.70)	71.11^{A} (89.50)
2	60.12 ± 1.92 (75.20)	63.61 ± 2.08 (80.30)	67.50 ± 2.34 (85.35)	69.39 ± 1.26 (87.95)	65.15^{B} (82.30)
3	56.83 ± 1.28 (70.10)	60.86 ± 0.86 (76.30)	63.52 ± 1.47 (80.10)	67.36 ± 1.66 (85.20)	78.15^{C} (78.15)
4	52.29 ± 1.92 (63.10)	58.40 ± 0.93 (72.55)	60.86 ± 0.86 (76.30)	64.27 ± 0.98 (81.15)	58.95^{D} (73.40)
5	43.56 ± 1.44 (47.50)	52.99 ± 0.74 (63.80)	56.03 ± 0.77 (68.80)	59.20 ± 0.80 (73.50)	52.94^{E} (63.70)
6	36.22 ± 1.74 (34.90)	49.32 ± 0.84 (57.50)	52.25 ± 0.85 (62.50)	55.26 ± 0.88 (67.50)	48.26^{F} (55.70)
7	25.25 ± 3.13 (18.20)	42.84 ± 1.38 (46.20)	47.15 ± 0.72 (53.80)	50.77 ± 0.00 (60.00)	41.50^{G} (43.50)
Overall mean	48.67^{D} (56.40)	56.77^{C} (70.00)	60.23^{B} (75.35)	62.94^{A} (79.30)	

Mean ± SE.; (%); LSD for days 2.064 (P<0.05); LSD for treatments 1.560 (P<0.05); LSD for interaction 4.131 (P<0.05).

Table 7. The effect of melatonin on the alive sperm % stored at 5°C for 7 days using TFGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	85.75 ± 2.59	89.50 ± 2.02	95.50 ± 1.44	95.50 ± 1.44	91.56^{A}
2	77.00 ± 2.89	81.50 ± 2.60	87.50 ± 3.18	89.50 ± 2.02	83.88^{B}
3	72.25 ± 1.84	78.25 ± 1.25	81.75 ± 1.84	86.75 ± 2.29	79.75^{C}
4	65.25 ± 3.04	74.50 ± 1.44	78.25 ± 1.25	81.75 ± 1.84	74.94^{D}
5	51.25 ± 2.75	66.75 ± 1.25	71.00 ± 1.00	75.75 ± 1.25	66.19^{E}
6	37.50 ± 3.18	60.50 ± 1.44	65.50 ± 1.44	70.00 ± 1.15	58.38^{F}
7	27.00 ± 2.48	49.75 ± 2.66	57.00 ± 1.00	63.00 ± 0.00	49.19^{G}
Overall mean	59.43^{D}	71.54^{C}	76.64^{B}	80.32^{A}	

Mean ± SE.; LSD for days 2.857 (P<0.05); LSD for treatments 2.160 (P<0.05); LSD for interaction 5.718 (P<0.05).

Table 8. The effect of melatonin on the sperm abnormalities % stored at 5°C for 7 days using TFGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	10.25 ± 0.48	9.00 ± 0.58	7.50 ± 0.29	6.50 ± 0.29	16.38^{G}
2	12.00 ± 0.58	10.00 ± 0.58	9.00 ± 0.58	7.50 ± 0.29	9.63^{F}
3	12.00 ± 0.41	10.75 ± 0.25	10.00 ± 0.41	9.00 ± 0.41	10.44^{E}
4	13.00 ± 0.41	11.75 ± 0.25	10.75 ± 0.25	10.25 ± 0.63	11.44^{D}
5	15.50 ± 0.29	13.25 ± 0.25	12.25 ± 0.25	11.25 ± 0.25	13.06^{C}
6	17.25 ± 0.75	14.00 ± 0.00	13.50 ± 0.29	12.50 ± 0.29	14.31^{B}
7	22.00 ± 1.29	15.25 ± 0.25	14.50 ± 0.29	13.75 ± 0.25	16.38^{A}
Overall mean	14.57^{A}	12.00^{B}	11.07^{C}	10.11^{D}	

Mean ± SE.; LSD for days 0.648 (P<0.05); LSD for treatments 0.490 (P<0.05); LSD for interaction 1.296 (P<0.05).

Table 9. The effect of melatonin on the acid phosphatase activity stored at 5°C for 7 days using TFGY extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	23.50 ± 1.85	16.00 ± 1.08	13.25 ± 0.85	15.50 ± 1.55	17.06^{E}
2	26.50 ± 1.76	23.75 ± 1.75	19.00 ± 0.91	16.00 ± 1.08	21.31^{D}
3	32.50 ± 1.32	28.00 ± 0.91	22.25 ± 1.11	22.25 ± 1.49	26.25^{C}
4	35.75 ± 1.89	33.50 ± 1.76	28.00 ± 0.91	25.25 ± 0.85	30.63^{B}
5	43.75 ± 1.25	40.75 ± 1.25	34.50 ± 1.32	33.75 ± 2.50	37.56^{A}
6	43.50 ± 2.02	37.25 ± 2.17	35.75 ± 1.65	33.75 ± 1.49	38.19^{A}
7	43.75 ± 1.93	42.00 ± 1.29	38.25 ± 2.21	34.00 ± 1.58	39.50^{A}
Overall mean	35.60^{A}	31.61^{B}	27.29^{C}	25.79^{C}	

Mean ± SE.; LSD for days 2.188 (P<0.05); LSD for treatments 1.654 (P<0.05).

Table 10. The effect of melatonin on the sperm motility % stored at 5°C for 7 days using 14 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	65.55 ± 2.49 (82.90)	68.44 ± 1.96 (86.50)	73.23 ± 2.39 (91.70)	73.23 ± 2.39 (91.70)	70.12^{A} (88.45)
2	60.06 ± 1.36 (75.10)	64.61 ± 2.45 (81.60)	68.74 ± 2.92 (86.90)	70.77 ± 2.34 (89.20)	66.04^{B} (83.50)
3	56.83 ± 1.28 (70.10)	60.06 ± 1.36 (75.10)	63.52 ± 1.47 (80.10)	67.36 ± 1.66 (85.20)	61.94^{C} (77.90)
4	53.83 ± 2.68 (65.20)	57.69 ± 2.05 (71.40)	61.00 ± 2.20 (76.50)	64.61 ± 2.45 (81.60)	59.28^{C} (73.90)
5	50.83 ± 2.41 (60.10)	54.56 ± 1.94 (66.40)	54.69 ± 2.05 (66.60)	61.00 ± 2.20 (76.50)	56.02^{D} (68.80)
6	45.74 ± 2.99 (51.30)	53.08 ± 2.31 (63.90)	56.09 ± 1.93 (68.90)	57.59 ± 2.11 (71.30)	53.12^{D} (64.00)
7	39.17 ± 2.41 (39.90)	50.06 ± 1.40 (58.80)	53.02 ± 1.44 (63.80)	55.86 ± 1.61 (68.50)	49.53^{E} (57.90)
Overall mean	53.14^{D} (64.00)	58.36^{C} (72.50)	61.80^{B} (77.80)	64.35^{A} (81.25)	

Mean ± SE.; (%); LSD for days 2.988 (P<0.05); LSD for treatments 2.559 (P<0.05).

Table 11. The effect of melatonin on the alive sperm % stored at 5°C for 7 days using 14 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	84.50 ± 3.38	88.50 ± 2.72	93.75 ± 2.84	93.75 ± 2.84	90.13^{A}
2	77.00 ± 2.04	83.50 ± 3.38	88.00 ± 3.46	90.75 ± 2.93	84.81^{B}
3	72.25 ± 1.84	77.00 ± 2.04	81.75 ± 1.84	86.75 ± 2.29	79.44^{C}
4	67.76 ± 3.88	73.50 ± 2.99	78.00 ± 2.92	83.50 ± 3.38	75.69^{CD}
5	63.00 ± 3.67	71.50 ± 3.62	73.50 ± 2.99	78.00 ± 2.92	71.50^{D}
6	54.45 ± 5.12	66.50 ± 3.50	71.00 ± 2.92	73.50 ± 2.99	66.44^{E}
7	43.00 ± 4.49	61.75 ± 2.39	66.50 ± 2.13	68.75 ± 4.03	60.00^{F}
Overall mean	66.04^{C}	74.61^{B}	78.93^{A}	82.14^{A}	

Mean ± SE.; LSD for days 4.430 (P<0.05); LSD for treatments 3.349 (P<0.05).

Table 12. The effect of melatonin on the sperm abnormalities % stored at 5°C for 7 days using 14 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	10.50 ± 0.65	8.75 ± 0.48	7.50 ± 0.29	6.75 ± 0.48	8.38^{G}
2	12.00 ± 0.41	10.00 ± 0.41	8.75 ± 0.48	7.50 ± 0.29	9.56^{F}
3	12.00 ± 0.41	11.00 ± 0.41	10.00 ± 0.41	9.00 ± 0.41	10.50^{E}
4	13.00 ± 0.41	11.75 ± 0.63	11.00 ± 0.41	10.00 ± 0.71	11.44^{D}
5	14.00 ± 0.41	12.75 ± 0.63	11.75 ± 0.25	10.75 ± 0.48	12.31^{C}
6	14.75 ± 0.63	13.50 ± 0.50	12.50 ± 0.50	11.75 ± 0.63	13.13^{B}
7	16.25 ± 1.25	14.25 ± 0.48	13.25 ± 0.48	12.50 ± 0.65	14.06^{A}
Overall mean	13.21^{A}	11.71^{B}	10.68^{C}	9.75^{D}	

Mean ± SE.; LSD for days 0.756 (P<0.05); LSD for treatments 0.571 (P<0.05).

Table 13. The effect of melatonin on the acid phosphatase activity stored at 5°C for 7 days using 14 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 g	15 g	20 g	
1	22.00 ± 1.29	18.25 ± 0.48	17.25 ± 0.48	16.25 ± 0.48	18.44^{F}
2	27.50 ± 0.65	24.75 ± 0.63	21.75 ± 0.48	19.50 ± 0.65	23.38^{E}
3	31.00 ± 0.71	30.00 ± 1.29	25.25 ± 1.11	24.50 ± 0.65	27.69^{D}
4	35.25 ± 0.85	33.50 ± 1.32	30.25 ± 0.48	27.50 ± 0.65	31.63^{C}
5	42.25 ± 1.11	40.50 ± 1.04	36.75 ± 0.85	34.25 ± 1.25	38.44^{B}
6	43.00 ± 0.71	40.00 ± 1.29	38.25 ± 0.75	35.50 ± 0.65	39.19^{AB}
7	44.50 ± 0.96	42.25 ± 1.11	39.25 ± 0.85	35.75 ± 1.11	40.44^{A}
Overall mean	35.07^{A}	32.75^{B}	29.82^{C}	27.61^{D}	

Mean ± SE.; LSD for days 1.263 (P<0.05); LSD for treatments 0.955 (P<0.05).

Table 14. The effect of melatonin on the sperm motility % of goat spermatozoa after freezing and thawing for various extenders.

Melatonin concentrations	TGGY	TFGY	CU-16	14	Overall mean
0.0 g (Control)	36.99 ± 1.44 (36.20)	34.68 ± 1.99 (32.40)	36.73 ± 4.22 (35.80)	39.09 ± 3.25 (31.40)	36.87^{A} (36.00)
10 g	34.74 ± 0.88 (32.45)	38.43 ± 2.51 (38.60)	39.15 ± 3.16 (39.90)	40.66 ± 1.88 (42.45)	38.24^{A} (38.30)
15 g	42.12 ± 1.18 (45.00)	43.56 ± 1.86 (47.50)	42.12 ± 1.18 (45.00)	44.28 ± 1.37 (48.75)	43.02^{B} (46.60)
20 g	45.72 ± 1.37 (51.30)	46.44 ± 0.83 (52.50)	46.44 ± 0.83 (52.50)	47.15 ± 0.72 (53.75)	46.44^{A} (52.50)

Mean ± SE.; (%); LSD for melatonin concentration 2.906 (P<0.05).

Discussion

This study compared the preservation ability of five extenders-SCY,TGGY, TFGY), CU-16 and 14 extenders-by means of SM %, AS %, SA % and AcP levels of goat extended semen stored at 5°C for seven days. It was noticed that the use of TGGY, TFGY, CU-16 and 14 extenders improved significantly the storage of extended semen. These results are compatible with Evans and Maxwell (1987), Roca et al. (2000) and Chehadeh et al. (2001) who reported that Tris-buffer extenders were the best diluents for sheep, rabbit and goat respectively. With respect to the preservation of goat semen in glycine containing extenders (CU-16 and 14), the present findings are in agreement with Shannon (1964) and El-Chahidi (1973) who reported that the addition of glycine to the extender improved long time storage of bull and sheep semen respectively. On the contrary Dessouky et al. (1970) found that the glycine containing extenders were less efficient in storage of rams semen Paleg et al. (1981) attributed the beneficial effect of glycine to its ability to retard thermal denaturation of enzymes thus it maintains the enzyme structure and function via its protective action.

It was found that melatonin supplementation produced a significant increase in sperm motility and alive sperm percentages especially at the last four days of incubation in all extenders. These results go parrellel with Mamdouh et al. (1996) who reported the same results on addition of melatonin to liquid bull semen. On the contrary, the current results were in disagreement with Bornmann et al. (1989) who concluded that seminal plasma melatonin play no important role in sperm motility. The impact of melatonin on sperm motility and alive sperm percentages may be due to melatonin increases ATP ase levels (Chen et al., 1994). The increase of ATPase is correlated with an increase in ATP which is the mai energy source used by the sperm flagellum to initiate and activate forward motility (Burger et al,1991). Melatonin stimulates cellular influx of Ca^{+2} into sperm cells enhancing their motility (Delgadillo et al,1994).

Melatonin could be a potent cyclic AMP (cAMP) stimulator (Yung et al,1995). cAMP stimulates sperm motility via its direct action on the axoneme of the tail (Lindamann ,1978) or indirectly through acting on the cell membrane as secondary messenger (Garbers and Kopf,1980).

Also, it was obvious that melatonin induced a significant decrease in the sperm abnormalities percentages and a significant reduction in non prostatic acid phoshatase. These results were in accord with Abdine (1993) who recorded that melatonin treatment of Cambridge rams, in vivo, lowered the sperm dead percentage and decreased the abnormal sperms. Additionally, our results were coincided with Poeggeler et al. (1993) who reported that the number of abnormal and dead sperms were reduced after addition of melatonin. Moreover, the current results were in a harmony with those results obtained by Mamdouh et al. (1996) who concluded that melatonin decreased significantly sperm abnormalities and acid phosphatase level. The impact of melatonin on sperm abnormalities and acid phosphatase could be ascribed to melatonin can pass the cell membrane and protects DNA from free radical damage effect through its potent antioxidant and antiaging effects on the cells (Poeggeler et al., 1993).Melatonin lowers the levels of acid phosphatase enzyme when is considered as an indicator of cellular death or damage (Moss and Henderson, 1993).

With regard to the effect of melatonin on post-thawing motility, it was evident that it produced a significant increase. These results were in agreement with Kaya et al. (2001) who concluded that melatonin administration to rams, in vivo, improved post-thawed sperm viability as well as the intact acrosome rates. The beneficial effect of melatonin on post-thawed motility could be attributed to its decreasing effect on the phosphatase enzyme release and leakage from sperm cells during cryopreservation.

In conclusion, the use of CU-16 and 14 extenders was better than Tris extenders for goat semen preservation. Moreover the inclusion of melatonin

(particularly 15.0 and 20.0 g) improved the extended goat semen quality and its freezability.

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