Peertechz

LIFE SCIENCES GROUP

INTERNATIONAL JOURNAL OF
Veterinary Science and Research

ISSN: 2640-7604 DOI: https://dx.doi.org/10.17352/ijvsr

El-Battawy KA*

Department of Animal Reproduction and Artificial Insemination, National Research Center, Egypt

Received: 11 June, 2019
Accepted: 09 July, 2019
Published: 10 July, 2019

***Corresponding author:** El-Battawy KA, Department of Animal Reproduction and Artificial Insemination, National Research Center, Egypt,
E-mail: ekhairi@gmx.at

https://www.peertechz.com

Check for updates

Research Article

Preservation of goat semen at 5°C with emphasis on its freezability and the impact of melatonin

Abstract

Evaluation of goat semen, extended in Cornell University 16 extender, was used to study the effects of melatonin of various concentrations on sperm motility (SM) %, alive sperm (AS) % and sperm abnormalities (SA) % of extended goat semen stored at 5°C for seven days were studied. Melatonin at the doses of 0.0, 10.0, 15.0 and 20.0 µg/ 100 x 10^6 sperm was added to the extended semen and the samples were examined daily for the previous parameters. Additionally, the influence of melatonin on post-thawing motility was assessed.

The results of the present investigation elaborated that melatonin particularly the high concentration (20.0 µg) significantly improved SM%, reduced dead sperm % and improved post-thaw sperm motility. In conclusion, the addition of melatonin (particularly 20.0 µg) induced remarkable and profound physiological actions on goat semen quality during storage for long time at 5°C and improved its freezability during preservation.

Introduction

The success of an artificial insemination (AI) program depends on the proper management of semen collection, storage and use. Goat semen has been stored at temperatures ranging from 2 to 15°C and mostly at 5°C using various diluents such as sodium citrate-yolk, sodium citrate-fructose-yolk, milk (whole, skimmed or reconstituted) with or without egg yolk, Spermasol, Neoseminan, saline [1]. It was found that tris-buffer extenders were effective for dilution and storage of goat, sheep and rabbit semen [2-4].

Nishikawa et al., [5], stored goat spermatozoa in Neoseminan diluent at 4°C for 8-15 days and maintained their fertilizing capacity for 6 days. Moreover, Eppleston et al., [6], demonstrated that goat spermatozoa have retained their fertilizing capacity for at least 8 days of storage at 5°C in Tris- Fructose-citric acid-yolk diluent. 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. Also, El-Chahidi [7-9], elaborated that the use of different combinations of electrolytes mixture and glycine improved the motility and viability of ram spermatozoa for long time. Moreover, the use of glycine and glucose containing extenders maintain high fertility over a period of several days in bull [10].

Melatonin (N-acetyle-5-Methoxytryptamine) is a hormone produced mainly by the pineal gland besides other tissues like retina [11]. It neutralizes free radicals thus it prevents cell damage. Mammdouh et al., [12,13], recorded that the addition of melatonin to liquid bull semen improved its storage and preservation for 6 days. Unfortunately, no available literature was found concerning the addition of melatonin to goat semen. Thus the objective of the present study is to:

1. Study the effect of some extenders on viability of goat semen during preservation at 5°C.
2. Study the physiological influence of melatonin on the quality of goat semen preserved in liquid or frozen condition.

Materials and Methods

This investigation was carried out at private goat farm at Alexandria desert road.

A-experimental animals

Six male Zaraibi goats aged 19 month approximately and weighed 30-40 kg were used. Each buck was fed one Kg balanced concentrate and berseem hayad libidium. Water was offered to those animals ad libidium all the day using manual water trough system.

Citation: El-Battawy KA (2019) Preservation of goat semen at 5°C with emphasis on its freezability and the impact of melatonin. Int J Vet Sci Res 5(2): 035-038. DOI: http://dx.doi.org/10.17352/ijvsr.000039

B-experimental materials

Melatonin was imported from Twinlab Specialty Corporation, Ronkon Koma, New York, USA. While, the other chemical reagents used for the preparation of extenders were purchased from Sigma-Aldrich Co., Deisenhofen, Germany.

C-semen collection and evaluation. At 1000 rpm for ten minutes

Semen was collected twice weekly by means of an artificial vagina using an anestrous doe as a teaser for a period of two months. After collections, the ejaculates were transferred to the laboratory of the farm within 2-3 minutes where they were centrifuged to remove the seminal plasma and consequently to avoid the harmful effect of seminal goat enzyme. Semen was kept in water bath at 30°C for evaluation by means of conventional methods.

D-Semen extender

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

1. TGGY was prepared according to Roca et al., [4], for rabbit semen. It consisted of Tris-hydorxymethyl amino methane (3.801 g), glucose (0.6 g), citric acid monohydrate (2.166 g), glycerol (6.7 ml), the egg yolk and antibiotics were added as previously mentioned.s

2. TFGY formulated according to Foote [14], for bull semen. It consisted of Tris-hydorxymethyl amino methane (3.028 g), fructose (1.25 g), citric acid monohydrate (1.675 g), glycerol (6.7 ml), the egg yolk and antibiotics were added as previously mentioned.

3. Cornell University (CU-16) extender was prepared according to Shannon [10], for bull semen. It consisted of trisodium citrate dihydrate (1.45 g), glucose (1.25 g), glycine (0.94 g) the egg yolk and antibiotics were added as previously mentioned.

4. Extender 14 was prepared according to Shannon [10], for bull semen. It consisted of trisodium citrate dihydrate (2.00 g), glucose (0.30 g), glycine (1.00 g) the egg yolk and antibiotics were added as previously mentioned.

E-semen processing and experimental design

Only ejaculates of >70% initial motility and 2000 x 106 sperm cells/ ml were used in the following experiments:

Experiment I: This experiment was designed to find out the impact of melatonin on the viability of chilled goat semen in CU-16 extender. Semen samples were divided and diluted (1: 4 according to Evans and Maxwell, [3], at 30°C with semen extenders. 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 [15].

Melatonin was added to the extended semen at the rate of 0.0 µg (control sample), 10, 15 and 20 µg/100x106 sperm [13].

Experiment 2: This experiment was designed to find out the impact of melatonin on the viability of chilled goat semen in CU-16 extender as it achieved the best results in comparison with the rest of used extenders. Semen samples were processed as previously mentioned in experiment I, then melatonin was added to the extended semen at the rate of 0.0 µg (control sample), 10, 15 and 20 µg/100x10^6 sperm [13]. After daily examination, the samples were centrifuged at 3000 rpm for 20 minutes to get supernatant for immediate daily determination of acid phosphatase (AcP) according to Moss [16].

Experiment 3: This experiment was designed to investigate influence of melatonin (0.0, 10, 15 and 20 µg) on freezability of goat semen exte d in TGGY, TFGY, CU-16 and 14 extenders. Semen samples were split and diluted 1 : 4 at 30 °C. The diluted semen was cooled and loaded into 0.25 ml straws at 5°C. The straws were placed horizontally on freezing racks and lowered into liquid nitrogen vapour inside small tank containing 10 liters of liquid nitrogen at a height of 2.0 cm above the level of liquid nitrogen, for 15 minutes. The straws were then immersed gradually (within 2-3 minutes) in liquid nitrogen and transferred into liquid nitrogen storage container. After few weeks, frozen goat semen was thawed in a water bath at 40°C for 30 seconds. The thawed semen was emptied in pre-warmed tubes and incubated in water bath at 30°C for assessment of sperm motility [17].

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

Table 1 declared the significant (P<0.0001) effect of melatonin on sperm motility %. Furthermore, both Table 2 and Table 3 showed the influence of melatonin on both AS % and SA %.

The high concentration of melatonin (20 µg) had significantly (P<0.0001) increased the motility and the live sperm in comparison with the other concentrations, during

Table 1: The effect of melatonin on the sperm motility % stored at 5°C for 7 days using CU-16 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 µg	15 µg	20 µg	
1	67.63 ± 2.75	69.53 ± 2.03	74.61 ± 2.47	75.70 ± 1.38	71.86A
2	60.92 ± 1.60	64.47 ± 1.73	68.44 ± 1.96	70.47 ± 1.09	66.07B
3	56.06 ± 1.50	60.86 ± 0.86	62.66 ± 1.72	66.41 ± 1.93	61.50C
4	53.02 ± 1.44	58.40 ± 0.93	60.86 ± 0.86	63.52 ± 1.47	58.95D
5	49.34 ± 1.88	56.06 ± 1.50	58.40 ± 0.93	60.92 ± 1.60	56.18E
6	47.16 ± 1.38	54.52 ± 1.44	56.83 ± 1.28	58.40 ± 0.93	54.23F
7	43.56 ± 1.44	52.25 ± 0.85	54.52 ± 1.44	57.63 ± 1.50	51.49G
Overall mean	53.96D	59.44C	62.33B	64.72A	

Mean ± SE.; LSD for days 2.201 (P<0.05); LSD for treatments 1.664 (P<0.05).

Citation: El-Battawy KA (2019) Preservation of goat semen at 5°C with emphasis on its freezability and the impact of melatonin. Int J Vet Sci Res 5(2): 035-038. DOI: http://dx.doi.org/10.17352/ijvsr.000039

the storage duration. Moreover, melatonin (20 µg) resulted in a significant (P<0.0001) decrease in the sperm abnormalities.

Regarding the effect of melatonin on the post-thawed sperm motility, only there was a significant (P<0.0001) difference 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 4).

Discussion

This investigation elaborates to effect of various extenders on preservation of extended goat semen, impact of melatonin on sperm motility %, alive sperm %, sperm abnormalities % as well as freezability of that semen. 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 [3], Roca et al., [4] and Chehadeh et al., [2], who reported that Tris-buffer extenders were the best diluents for sheep, rabbit and goat respectively. Regarding the storage of goat semen in glycine containing extenders (CU-16 and 14), our results are in agreement with Shannon [10] and El-Chahidi [9], who reported that the inclusion of glycine in extender improved and allowed long time storage of bull and sheep semen respectively. On the contrary Dessouky et al., [18], found that the glycine containing extenders were less efficient in storage of rams semen Paleg et al., [19], 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.

Table 2: The effect of melatonin on the alive sperm % stored at 5°C for 7 days using CU- 16 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 µg	15 µg	20 µg	
1	87.25 ± 3.79	90.25 ± 2.75	95.00 ± 3.00	96.75 ± 1.25	92.31A
2	78.25 ± 2.39	82.75 ± 2.14	88.50 ± 2.72	91.25 ± 1.75	85.19B
3	71.25 ± 2.14	78.25 ± 1.25	80.50 ± 2.18	85.75 ± 2.59	78.94C
4	66.25 ± 2.32	74.50 ± 1.44	78.25 ± 1.25	81.75 ± 1.84	75.19D
5	63.25 ± 3.30	71.25 ± 2.14	74.50 ± 1.44	78.25 ± 2.39	71.81E
6	57.25 ± 2.14	68.75 ± 2.14	72.25 ± 1.84	74.50 ± 1.44	68.19F
7	51.25 ± 2.75	65.50 ± 1.44	68.75 ± 2.14	73.50 ± 2.18	64.75G
Overall mean	67.82D	75.89C	79.68B	83.11A	

Mean ± SE.; LSD for days 3.146 (P<0.05); LSD for treatments 2.378 (P<0.05).

Table 3: The effect of melatonin on the sperm abnormalities % stored at 5°C for 7 days using CU-16 extender.

Storage time (days)	Control	Treatment with different melatonin concentrations			Overall mean
		10 µg	15 µg	20 µg	
1	10.00 ± 0.71	8.25 ± 0.25	7.25 ± 0.25	6.00 ± 0.41	7.88F
2	11.75 ± 0.48	9.75 ± 0.48	8.75 ± 0.48	7.00 ± 0.41	9.31E
3	13.00 ± 0.58	10.75 ± 0.25	10.50 ± 0.65	9.50 ± 0.65	10.94D
4	13.25 ± 0.48	11.75 ± 0.25	11.00 ± 0.41	10.75 ± 0.63	11.69C
5	14.00 ± 0.41	12.25 ± 0.25	11.50 ± 0.65	11.50 ± 0.29	12.31BC
6	14.75 ± 0.48	12.75 ± 0.25	12.25 ± 0.48	12.00 ± 0.41	12.94AB
7	15.50 ± 0.29	13.25 ± 0.25	12.75 ± 0.48	12.25 ± 0.48	13.44A
Overall mean	13.18A	11.25B	10.57C	9.86D	

Mean ± SE.; LSD for days 0.637 (P<0.05); LSD for treatments 0.482 (P<0.05).

Table 4: 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 µg (Control)	36.99 ± 1.44	34.68 ± 1.99	36.73 ± 4.22	39.09 ± 3.25	36.87A
10 µg	34.74 ± 0.88	38.43 ± 2.51	39.15 ± 3.16	40.66 ± 1.88	38.24A
15 µg	42.12 ± 1.18	43.56 ± 1.86	42.12 ± 1.18	44.28 ± 1.37	43.02B
20 µg	45.72 ± 1.37	46.44 ± 0.83	46.44 ± 0.83	47.15 ± 0.72	46.44C

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

It was observed that addition of melatonin resulted in a significant increase in sperm motility and alive sperm percentages in all types of extenders. The effects of melatonin as an additive were profound and clear in high concentrations and at the last four days of incubation. These results are in accord with Mamdouh et al., [13], who reported the same results on addition of melatonin to liquid bull semen. On the other hand, our results were in disagreement with Bornmann et al., [20], who concluded that seminal plasma melatonin play no important role in sperm motility. The influence of melatonin on sperm motility and alive sperm percentages may be ascribed to one and/or all of the following physiological mechanisms:

I- Melatonin increases ATP ase levels [21]. The increase of ATPase is correlated with an increase in ATP which is the main energy source used by the sperm flagellum to initiate and activate forward motility [22].

II- Melatonin stimulates cellular influx of Ca+2 into sperm cells enhancing their motility [23].

III- Melatoni could be a potent cyclic AMP (cAMP) stimulator [24]. cAMP stimulates sperm motility via its direct action on the axoneme of the tail [25] or indirectly through acting on the cell membrane as secondary messenger [26].

Also, it was obvious that melatonin induced a significant decrease in the sperm abnormalities percentages and a significant reduction in seminal acid phoshatase. These results were in accord with Abdine [27], 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., [12], who reported that the number of abnormal and dead sperms was reduced after addition of melatonin. Moreover, the current results were in a harmony with those results obtained by Mamdouh et al., [13], who concluded that melatonin decreased significantly sperm abnormalities and acid phosphatase level. The impact of melatonin on sperm abnormalities and acid phosphatase could be accredited to one and/or the following physiological actions:

1- Melatonin can pass the cell membrane and protects DNA from free radical damage effect through its potent antioxidant and anti-aging effects on the cells [12].

Citation: El-Battawy KA (2019) Preservation of goat semen at 5°C with emphasis on its freezability and the impact of melatonin. Int J Vet Sci Res 5(2): 035-038.
DOI: http://dx.doi.org/10.17352/ijvsr.000039

2- Melatonin lowers the levels of acid phosphatase enzyme which is considered as an indicator of cellular death or damage (Moss and Henderson, 1993).

At last, it was clear that melatonin produced significant increase in post- thawing motility. These results were in agreement with Kaya et al., [28], 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 (leakage) from sperm cells during cryopreservation [29].

In conclusion, the addition of melatonin (particularly 20.0 µg/100 x 106 goat sperm) induced remarkable and profound physiological actions that improved the extended goat semen quality, its storage for long time at 5°C and improve its freezability.

References

1. Lebouef B, Restall B, Salamon S (2000) Production and storage of goat semen for artificial insemination. Anim Reprod Sci 62: 113-141. Link: http://bit.ly/2XB1Qhi
2. Chehadeh RY, Ziada MS, Seida AAM, Ghallab AM (2001) Effect of adding biological fluids on quality and neat of chilled goat semen.Proc.13th. Ann. Congr. Egypt. Soc Anim Reprod Fert 171.
3. Evans G, Maxwell WMC (1987) Salamon's artificial insemination of sheep and goats. Butterworths, Sydney 194. Link: http://bit.ly/2S7M8nZ
4. Roca J, Martinez S, Vazquez JM, Lucas X, Parrilla I, et al. (2000) Viability and fertility of rabbit spermatozoa diluted in tris-buffer extenders and stored at 15°C. Anim Reprod Sci 64: 103-112. Link: http://bit.ly/2G2I7MW
5. Nishikawa Y, Tangai Y, Tachikawa M, Walde Y (1961) Experiments on conception with spermatozoa preserved with Neoseminan prepared exclusively for goat use. In: Proc. Silver Jubilee Lab. Anim. Husband., Kyoto Univ. 67-73.
6. Eppleston J, Pomars CC, Stogonov T, Maxwell WM (1994) *In vitro* and *in vivo* fertility of liquid stored goat spermatozoa. Proc Aust Soc Reprod Biol 26: 111.
7. Ahmed SI (1955) Effect of glycine on storage at ram semen. J Agric sci 46: 164-167. Link: http://bit.ly/2XzwF16
8. Schindler H, Amir D (1961) Longevity of ram sperms in various diluents and at different dilution rates. J Agric Sci 56: 183-189. Link: http://bit.ly/2XArXVN
9. El-Chahidi AA (1973) Evaluation and preservation of ram semen. M.V.Sc., thesis, Cairo Univ.
10. Shannon P (1964) The effect of diluents containing glycine, and glycine and glycerol, on the fertility of diluted bovine semen. J Agric Res 7: 357-363. Link: http://bit.ly/32dXr2E
11. Dubocovich ML, Shankar G, Mickel M (1989) 2^{125} iodomelatonin labels sites with identical pharmacological characteristics in chicken brain and chicken retina. Eur J Pharmacol 162: 298-299. Link: http://bit.ly/2L9igan
12. Poeggeler B, Reiter RJ, Tan DX, Chen LD, Manchester LC (1993) Melatonin hydroxyl radical mediated oxidative damage and aging: a hypothesis. J Pineal Res 14: 151-168. Link: http://bit.ly/2XArV07
13. Mamdouh M, Anwar GA, Megahed T, El-Deeb S, Shehata HS (1996) The effect of melatonin on the bull liquid semen and enzymatic release in seminal plasma. Assiut Vet J 35: 42-62.
14. Foote RH (1970) Fertility of bull semen at high extensive rates in Tris-buffered extenders. J Dairy Sci 53: 1475-1477. Link: http://bit.ly/2LIAnU0
15. Shaffer HE, Almquist JO (1948) Vital staining of bovine spermatozoa with an eosine-aniline blue staining mixture. J Dairy Sci 31: 677-678. Link: http://bit.ly/2JnX5Pq
16. Moss DW (1984) Acid phosphatase. In: "Methods of Enzymatic Analysis" ed. Bergmeyer HU, Verlag-Chemie, 3rd edition. 4: 92-106.
17. Waheed MM, Khalifa TAA, El-Shahat KH (2003) Effect of thiourea on motility and viability of chilled stored and frozen thawed goat spermatozoa. Vet Med J Giza 61: 49-57.
18. Dessouky F, Al-Hakim MK, Juna KH, Farhan SMA (1970) A comparative study on livability of Awassi sperms in different extenders. J Vet Med Assoc Egypt 30: 43-51. Link: http://bit.ly/2JsuwPS
19. Paleg LG, Douglas TJ, Van Daal A, Keech DB (1981) Poline and betaine protect enzymes against heat inactivation. Aust J Plant Physiol 8: 107-114.
20. Bornman MS, Oesthuizen JM, Barnard HC, Schulenburg GW, Boomker D, et al. (1989) Melatonin and Sperm Motility/Melatonin und Spermatozoenmotilität. Andrologia 21: 483-485. Link: http://bit.ly/32dIXB0
21. Chen L, Kumar P, Reiter RJ, Tan D, Manchester LC, et al. (1994) Melatonin prevents the suppression of cardiac Ca^{+2} stimulated ATPase activity induced by Alloxan. Am J Physiol 267: E57-E62. Link: http://bit.ly/32kBxLp
22. Burger B, VanderHorst G, Menkveld R, Maritz GS, de Villierse A, et al. (1991) Relationship between biochemical markers and fertilization *in vitro*. Presented at the Annual Reproductive Biology Work Seminar, Pretoria, South Africa.
23. Delgadillo LH, Tay FAA, King GB (1994) Effect of melatonin on microtubule assembly depend on hormone concentration: Role of melatonin as a calmodulin antagonist. J Pineal Res 17: 55-62. Link: http://bit.ly/2NIQNOJ
24. Yung LY, Tsim ST, Wong YH (1995) Stimulation of cAMP accumulation by the cloned Xenopus melatonin receptor through G1 AND G2 proteins. FFBS letters 372: 99-102. Link: http://bit.ly/2XD9xyF
25. Lindemann CB (1978) A cAMP-induced increase in the motility of dimembranted bull sperm models. Cell 13: 9-18. Link: http://bit.ly/2XzuFuS
26. Garbers DL, Kopf GS (1980) The regulation of spermatozoa by calcium and cyclic nucleotides. Adv Cyclic Nucleotide Res 13: 251-306. Link: http://bit.ly/2xNboqx
27. Abdine AM (1993) Study of prolificacy in Cambridge breed of sheep. Ph.D. Thesis, UCNW, UK.
28. Kaya A, Aksay M, Baspinar N, Yildiz C, Ataman MB (2001) Effect of melatonin implantation to sperm donor rams on post-thaw viability and acrosomal integrity of sperm cells in the breeding and non-breeding season. Reprod Domes Anim 36: 211-215. Link: http://bit.ly/2NHKydS
29. SAS (1988) User's Guide release 6.03 edition, SAS Institute Inc., Cary, NC, USA.

Citation: El-Battawy KA (2019) Preservation of goat semen at 5°C with emphasis on its freezability and the impact of melatonin. Int J Vet Sci Res 5(2): 035-038. DOI: http://dx.doi.org/10.17352/ijvsr.000039