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INNOVATIVE IDEAS FOR RAM SEMEN FREEZING
a. EFFECT OF EXTENDER COMPOSITION, ADDITION METHOD OF EXTENDER AND TEMPERATURE AT ADDITION EXTENDER ON RAM SEMEN FREEZABILITY

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ABSTRACT: Semen was collected from four adult Ossimi rams using an artificial vagina to study the effects of the extender addition either on one-step or two-step technique, with three temperatures at extender addition to raw semen at (25°C, 15°C and 5°C) on ram semen freezability in liquid Nitrogen (-196°C). Two new extenders were used, Tris-based extender (A-extender) and Tris-based extender containing 35% skim milk (B-extender). The final glycerol level was 14.5% in two extenders, and the dilution rate was 1:6 (v/v). The equilibration period was 3 hours. The frozen semen was thawed at 42°C for 35 seconds. Sperm motility and livability were determined to evaluate semen at various freezing and thawing stages.

A-extender yielded the best post-thaw motility and livability (44.4 and 43.8 %, respectively) compared with B-extender, which recorded 33.1 and 32.7% values for motility and livability, respectively.

One-step method yielded better values ($P<0.01$) of post-thaw motility and livability as 40.4 and 40.0 %, respectively, compared with the two-step method, which revealed 37.2 and 36.5% values, respectively. Addition of extender at 15°C yielded better results ($P<0.01$) of motility and livability than addition at 25°C and significantly better than 5°C.

It could be advisable to use A-extender added to raw ram semen in one step at 15°C to improve semen freezability.

Keywords: Ram semen, Freezing, Extender, Glycerol, Addition method

INTRODUCTION

Several factors affect ram semen freezability, including the composition of the extender used to dilute semen before freezing, the glycerol level in the extender, and the extender addition method (Salamon and Maxwell, 2000). Although many studies on extenders have been carried out, none have shown an extender to be effective in protecting spermatozoa during the cryopreservation process (Ari et al., 2011).

Egg yolk-based extenders are commonly used in frozen semen. Sanson *et al.* (2000) reported that most workers use egg yolk concentration around 20%. According to Amirat *et al.* (2010) and Layek *et al. (2016), the percentage of motile spermatozoa after freezing and thawing was similar when egg yolk concentration ranged from 15% to 30%, while it was significantly lower at egg yolk concentrations* above and below this range.

Skim milk was used extensively by several investigators for liquid and frozen ram semen, mostly combined with fructose, sodium citrate, or Tris (Nebar, 1989; and Küçük *et al.*, 2014). Rahman *et al.* (2018) found that the skim milk-based extender was superior to the Tris-based extender regarding ram semen preservation. The efficiency of Tris and milk extenders over egg yolk-citrate and egg yolk glucose to maintain ram spermatozoa was reported by Aya *et al.* (2022). On the other hand, Nebar (1989) and Tuncer et al. (2010) reported that Tris-based extenders maintained the progressive motility and acrosome integrity of ram frozen spermatozoa over skim milk-based extenders.

Glycerol represents the most common cryoprotective agent (Salamon and Maxwell, 2000; Keskin *et al.,* 2020). It is added to the semen diluents in order to protect the sperm against the lethal effect of freezing, as well as influencing the revival of spermatozoa after thawing the frozen semen (Bane *et al.,* 2004). The cryoprotective effects of glycerol are most evident at higher concentrations but have to be balanced against glycerol toxicity (Curry, 2000). Salamon and Maxwell (2000) reviewed that most investigators suggested that the optimal glycerol concentration would be 6–8%.

The extender can be added to semen in a one-step or two-step method. Evans and Maxwell (1987) postulated that the one–step method of glycerolation of ram semen for frozen storage is a practical, successful, and widely used method. Kukovics *et al.,* 2011 and Vishal Mehta *et al.,* 2020 reported that a single diluent of ram semen at 30°C with extender containing glycerol was as effective as compared with a two-step dilution when glycerol was added at 5°C. However, Mattos *et al.* (1982) and Morrier *et al.* (2002) found that the addition of glycerol either immediately after collection of semen (one-step) or at 5°C (two-step) did not affect the subsequent quality of frozen-thawed ram sperm, assessed by motility and viability.

There is no agreement among investigators concerning the superiority of specific diluents and a particular method for adding them to the ram's semen to maintain the motility and livability of ram sperm during freezing and thawing stages.

Therefore, the present study aimed to find new diluents containing a high percentage of glycerol and to find a new method of adding extender to rams' semen to make it less harmful to the motility and livability of the spermatozoa during freezing and thawing stages.

MATERIALS AND METHODS

Semen was collected twice a week from 4 healthy adult *Ossimi* rams (2-4 years) by artificial vagina at the Experimental Farm, Faculty of Agriculture, Menoufia University, Egypt. The experimental work lasted twenty-one months, from October 2002 until June 2004. Semen was evaluated from the rams for two weeks prior to the initiation of the experiment. Rams were fed on a balanced ration to meet the NRC requirements for adult rams (NRC, 1987).

Immediately after semen collection, every ejaculate was evaluated for color, motility, and sperm concentration. Ejaculates having 80% or more progressive motility and sperm concentration of more than $3x10^9$/ml were pooled together and gently mixed. Two different semen extenders were used in those experiments. Each semen extender was divided into two equal fractions: fraction I and fraction II. Fraction I was left un-glycerolated, while glycerol was added to fraction II at 29%. It is worth noting that several trials were conducted to arrive at the composition of the extenders used, which contain a high percentage of glycerol. The composition of fraction I of those extenders is shown in the following table.

Table 1: Composition of fraction I of semen extenders

Component	Tris–based extender (A)	Skim milk–Tris–based extender (B)
Tris*, g	2.422	1.574
Citric acid, g	1.340	0.871
Na_3 – Citrate, g	0.145	0.094
Fructose, g	0.750	0.111
Egg yolk, ml	20.0	13.0
Skim milk, ml	-	35

- * Tris: Hydroxymethyl aminomethane.
- Skim milk was heated to 92°*C* for 10 minutes and then used after cooling to room temperature as Flipse et al. (1954) described.
- Distilled water was added to 100 mL of A-extender and B-extender.
- Antibiotics were added as 75 mg penicillin plus 50 mg streptomycin to A-extender and B-extender.

Experiment I

The pooled semen sample was split into four aliquots to study the effect of the addition method of two extenders, either in a one-step or a two-step technique, on ram semen freezability as follows:

a- Two-step extender addition

Two parts of aliquot semen were partially diluted by adding three volumes of fraction I to one of the selected extenders, and gently mixed with the semen, so that the preliminary dilution rate was 1:3 (v/v). The temperature of both semen and fraction I was 30-32°C at such partial dilution. The partially diluted semen was gradually cooled to 4-5°C over 1.5 h. Semen extender was divided into two equal fractions; fraction I being free of glycerol, and fraction II being glycerolated. Glycerol was added to fraction II at a level of 29%. Glycerolation was carried out at 5°C by adding an equal volume of the appropriate glycerolated extender (fraction II) to dilute semen by unglycerolated extender (fraction I) in three parts over an interval of 15 minutes. The final glycerol concentration after adding fraction II was 14.5%, and the final dilution rate was 1:6 (volume/volume).

b- One-step extender addition

Fraction I of each extender was added to the same proportion of fraction II, so the final glycerol level in each extender was 14.5%. The undiluted semen in the two residual aliquots semen was cooled from 30-32°C to 4-5°C over two hours. Then the glycerolated extenders were added to the pre-cooled pooled raw semen at 5°C. The dilution rate was 1:6 (volume/volume). In one-step or two-step methods, after the addition of the glycerolated extender, the equilibration period started and remained at 4-5°C for three hours.

Experiment II

Depending on the results of the previous experiment, extenders A and B were used and added in only a one-step rather than a two-step procedure. However, the current experiment aimed to study the effect of extender addition to semen at three different temperatures on ram semen freezability as follows:

1- Pooled semen was cooled from 30-32°C to 25°C for 15 minutes before gently mixing with the extender at the same temperature. The diluted semen was then cooled to 5°C for an additional 1.75 hours before remaining at 4-5°C for 1.25 hours to complete the 3.0 hour equilibration process.
2- After cooling the pooled semen from 30-32°C to 15°C for half an hour, the extenders were added and mixed at the same temperature. The diluted semen was further cooled to 5°C over 1.5 hours before remaining at 4-5°C for 1.5 hours to complete a 3-hour equilibration period.
3- Both of the extenders and pooled semen were cooled from 30-32°C to 4-5°C over 2 hours. They were then mixed and held at 4-5°C for three hours to equilibrate. The dilution rate was 1:6 (v/v), and the glycerol concentration was 14.5%.

In experiments I and II, after an equilibration period, the diluted semen was packed in 0.5 ml French plastic straws, which were sealed using polyvinyl powder. Three straws for each extender were prepared, labeled, and coded. The cold straws were frozen by reducing their temperature to -16.5°C over 4 minutes; the preliminary freezing rate is shown in Table 2. The preliminary freezing rate was accomplished using an easy and simple method: immersing the straws in a beaker filled with ethyl alcohol at the same temperature as the straws, which contained a thermometer. The preliminary freezing rate was controlled by sequentially raising and lowering the beaker within a trough filled with liquid nitrogen until it reached -16.5°C. The straws were dipped in liquid nitrogen (-196°C) and stored for 24 hours. The frozen semen was thawed in a water bath at 42°C for 35 seconds.

Table 2: Preliminary freezing rate of the filled straws in ethyl alcohol and time spent reaching the subzero temperature of 16.5 °C

Temp., °C	+4.0	-4.0	-7.0	-9.0	-10.5	-12.0	-13.5	-15.0	-16.5
Time, min	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0

The percentages of sperm motility and livability were determined to evaluate semen at the following stages during semen processing.

Statistical analysis

All the processing steps mentioned above and the previous evaluations were replicated eight times. The results were statistically analyzed according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Experiment I

Data listed in Table 3 indicated that the type of extender significantly (P<0.01) affected the percentage of sperm motility and livability after thawing, and there were no significant differences between the two extenders at all stages before freezing. In this respect, the Tris-egg yolk-based extender (A-extender) yielded significantly (P<0.01) higher post-thawing motility and livability. Values in this concern were (44.4 and 43.8%, respectively), while the results after extension with skim milk-Tris-egg yolk-based extender (B-extender) were (33.1 and 32.7%, respectively).

Data in Table 3 indicated that the addition method of extender on one-step or two-step techniques significantly (P<0.01) affected sperm motility and livability at all stages of semen processing. In this respect, the one-step method yielded greater values of the overall average of post-thaw motility and livability, which were 40.4 and 40.0 %, respectively, compared with the two-step method, which yielded 37.2 and 36.5%, respectively. The loss in motility and livability was lower for the one-step method compared with the two-step method at the pre-freezing (stages 1 and 2), and the post-thawing stage.

Salamon (1976a) and Evans and Maxwell (1987) found that a one-step method of glycerolation of ram semen for frozen storage is a practical, successful, and widely used. Early investigators (Entwistle and Martin, 1972; and Fiser and Fairfull, 1989) found that the single addition of the glycerolated fraction before freezing at 5°C was better than the gradual addition in divided portions. This was in agreement with our results. Mattos *et al.* (1982) and Morrier *et al.* (2002) found that the addition of glycerol either at once after collection of semen (one-step) or at 5°C (two-step) did not affect the motility and viability of frozen-thawed ram sperm. In this concern, Hill *et al.* (1959) and Feredean and Bragaru (1964) reported that gradually adding the glycerol-containing diluent at 2-5°C on a two-step dilution was better than one step. This disagreed with the present study. This may be due to differences with the extender components and glycerol ratio. Since a high glycerol level (14.5%) was used in the present study, and the interaction between extender and method of glycerol addition has already been found to be significant (Table 5). In addition to that, in the one-step method, the extender was added to raw semen at 5°C.

Table (3): Effect of extender type and extender addition method on percentage of motility and livability of ram spermatozoa at various freezing and thawing stages.

Stages of semen processing	Effect of extender		Step of glycerolation	
	A	B	one-step	Two-step
Sperm motility (%) :				
Glycerolated (G)	79.4	78.2	82.5	75.0 **
Equilibration (E)	74.4	74.7	79.4	69.7 **
Post thawing (PT)	44.4	33.1 **	40.4	37.2 **
Sperm livability (%) :				
Glycerolated (G)	79.4	78.0	82.8	74.5 **
Equilibration (E)	73.9	74.1	78.6	69.5 **
Post thawing (PT)	43.8	32.7 **	40.0	36.5 **

Initial motility: 90.6 % Initial livability: 91.0 %

** Significant (P<0.01). n.s: not significant.

Data in Table 4 indicated that the reduction of sperm motility and livability was affected at all stages of the process, but at variable rates, after semen extension by A and B-extenders. In this respect, the most significant reduction of sperm motility and livability occurred in stage 3, while the lower reduction occurred in stage 2. The reduction in stage 3 was 3 times greater than the reduction that occurred in stage 1. The obtained results in Table 4 indicated that the post-thaw recovery rate of motility and livability was the best for A-extender (49.0 and 48.1%, respectively), compared with B-extender (36.5 and 35.9%, respectively), as a proportion of the initial motility and livability in fresh semen. This rate was 59.7 and 59.3% respectively, for A-extenders compared with 44.3 and 44.1%, and for B-extenders, as a proportion of the motility and livability after equilibration.

Table 4: Reduction of motility and livability of ram spermatozoa during freezing.

Stages of semen processing	One-step method		Two-step method	
	A-extender	B-extender	A-extender	B-extender
Reduction of motility (%):				
(I - G)	11.2	12.4	8.1	15.6
(G-E)	5.0	3.5	3.2	5.3
(E-PT)	30.0	41.6	39.0	32.5
Total	46.2	57.5	50.2	53.4
Recovery rate % of:				
Initial	49.0	36.5	44.6	41.1
Equilibration	59.7	44.3	50.9	53.4
Reduction in livability (%):				
(I-G)	11.6	13.0	8.2	16.5
(G-E)	5.5	3.9	4.2	5.0
(E-PT)	30.1	41.4	38.6	33.0
Total	47.2	57.4	51.0	54.6
Recovery rate % of:				
Initial	48.1	35.9	44.0	40.1
Equilibration	59.3	44.1	51.0	52.5

Initial motility: 90.6 % Initial livability: 91.0 %

Initial motility and livability (I). After glycerolation (G). After equilibration (E). Post-thawing (PT).

Results listed in Table 5 illustrated that the interaction effect of extender type and method of extender addition on sperm motility and livability was only significant ($P<0.01$) at post-thawing. In contrast, such an interaction effect was insignificant either after glycerolation or after equilibration. The results further indicated that A-extender was significantly ($P<0.01$) effective in this respect than B-extender. Moreover, the corresponding difference between extenders was much more pronounced when the extender addition was added using the two-step method than after the one-step extender addition.

Our findings agreed with Paulenz *et al.* (2002), who found that ram spermatozoa diluted in Tris-based extender showed higher sperm motility and membrane integrity than those diluted in skim milk-based extender. Akhtar *et al.* (1990) found that the Tris–based extender was superior to milk diluent as determined by post–thawing motility, which agreed with the present study. An opposite trend was reported by Galli *et al.* (1993), who found that milk–based extenders were better than Tris–based extenders in terms of post–thawing sperm motility.

The present study used skim milk combined with Tris–citrate buffer; the extender contained a high level of glycerol 14.5%, and there were differences in freezing methods, which may explain the conflict between the present finding and that of Galli *et al.* (1993).

Table 5: Interaction effect between extender type and addition method of extender on the percentage of motility and livability of ram spermatozoa at the various stages of freezing and thawing.

Stages of semen processing	One-step method		Two-step method	
	A-extender	B-extender	A-extender	B-extender
Sperm motility (%):				
Glycerolated (G)	81.3	83.8	77.5	72.5
Equilibration (E)	77.5	81.3	71.3	68.1
Post thawing (PT)	46.3	34.4 **	42.5	31.8 **
Sperm livability (%):				
Glycerolated (G)	81.7	84.0	77.0	72.0
Equilibration (E)	76.7	80.4	71.1	67.8
Post thawing (PT)	45.6	34.4 **	41.9	31.6 **

Initial motility: 90.6 % Initial livability: 91.0 %

** Significant (P<0.01). n.s: not significant.

Salamon (1968), Kukovics *et al.* (2011), and Vishal Mehta *et al.* (2020) reported that a single diluent of ram semen at 30°C with an extender containing glycerol was as effective as the two-step dilution when glycerol was added at 5°C. This agrees with our study results, where we added the extender in one step, but at a temperature of 5°C to the raw semen.

Experiment II

Based on the results of experiment I, which revealed that the one-step addition method of extender was better than the two-step addition method, experiment II was designated to study the effect of three different temperatures at extender addition (25°C, 15°C, and 5°C) and the effect of extender composition on ram semen freezability using the one-step technique.

Data listed in Tables (6 and 7) indicated that the overall average of sperm motility and livability were significantly (P<0.01), lowest at all stages of semen processing, when the extender addition was conducted at 25°C after a 15 min pre-cooling period. The difference between the addition temperature at 15°C and 5°C was insignificant, although the first protocol was slightly better in sustaining the motility and livability of ram spermatozoa.

Recovery rate of motile spermatozoa was the greatest when the extender was added at 15°C protocol and the minimum when it was added at 25°C, concerning the extender effect. The tabulated data indicate that the A-extender was better than the B-extender. The difference was significant (P<0.01) for post-thawing motility and livability of ram spermatozoa and insignificant for glycerolated and equilibrated (Table 6). Post-thawing motility and livability for A-extender were higher (P<0.01) than those of B-extender, regardless of the temperature of extender addition. Total pre-freezing motility reduction at three different temperatures (25°C, 15°C, and 5°C) was 34.0, 7.7, and 10.9%, respectively. An opposite trend was noticed at stage 3, where the values were 32.5, 40.4, and 40.3% respectively (Table 7).

Table 6: Effect of extender and temperature of extender addition on ram sperm motility and livability.

Stages of semen processing	Effect of extender		Temperature at dilution		
	A	B	25°C	15°C	5°C
Sperm motility (%):					
Glycerolated (G)	77.9	77.1	63.8 ^{b}	85.9 ^{a}	82.9 ^{a}
Equilibration (E)	72.9	73.2	56.6 ^{b}	82.9 ^{a}	79.7 ^{a}
Post thawing (PT)	40.4 ^{A}	30.2 ^{B}	24.1 ^{b}	42.5 ^{a}	39.4 ^{a}
Sperm livability (%):					
Glycerolated (G)	77.7	77.2	64.1 ^{b}	85.7 ^{a}	82.7 ^{a}
Equilibration (E)	71.7	72.4	55.4 ^{b}	81.8 ^{a}	79.1 ^{a}
Post thawing (PT)	39.9	29.9**	24.9 ^{b}	41.7 ^{a}	38.1 ^{a}

Initial motility: 90.6 % Initial livability: 90.3 %

A, B: Values within the same row with different superscripts significantly differ (P<0.01).

a, b: Values within the same row with different superscripts significantly differ (P<0.01).

Table 7: Reduction of motility and livability of ram spermatozoa during freezing.

Stages of semen processing	Extender		The temperature at the addition		
	A	B	25°C	15°C	5°C
Reduction of motility (%):					
(I - G)	12.7	13.5	26.8	4.7	7.7
(G-E)	5.0	3.9	7.2	3.0	3.2
(E-PT)	32.5	43.0	32.5	40.4	40.3
Total	50.2	60.4	66.5	48.1	51.2
Recovery rate % of:					
Initial	44.6	33.3	26.6	46.9	43.5
Equilibration	55.4	41.3	42.6	51.3	49.4
Reduction in livability (%):					
(I - G)	12.6	13.1	26.2	4.6	7.6
(G-E)	6.0	4.8	8.7	3.9	3.6
(E-PT)	31.8	42.5	30.5	40.1	41.0
Total	50.4	60.4	65.4	48.6	52.2
Recovery rate % of:					
Initial	44.2	33.1	27.6	46.2	42.2
Equilibration	55.6	41.3	44.9	51.0	48.2

Initial motility: 90.6 % Initial livability: 90.3 %

Initial motility and livability (I). After glycerolation (G). After equilibration (E). Post-thawing (PT).

According to Medeiros *et al.* (2002), a relatively high proportion (40-60%) of ram spermatozoa preserves their motility after freeze-thawing, but only about 20-30% remain biologically functional, which agrees with the present recovery rate with A-extender, but it is

greater with B-extender. Vishwanath and Shannon (2000) reported that the best sperm recovery after thawing is just over 50%, which agrees with the present recovery rate proportional to sperm motility in equilibrated semen. However, it is greater than the recovery rate proportional to the initial motility.

The present study agreed with Blackshaw (1955), who did not find differences in the revival of ram spermatozoa when glycerol was mixed with semen between 5°C and 15°C. Salamon and Maxwell (1995), Maxwell and Watson (1996), and Watson (2000) demonstrated that cold shock starts soon after the semen is cooled to room temperature. Fiser and Fairfull (1986) suggested that the temperature for adding the glycerolated portion must be approximately 15°C to avoid the cold shock on spermatozoa. This was noticed in our study, too.

Table 8: Interaction effect between extender and the temperature of extender addition on percentage of motility and livability of ram spermatozoa

Stages of semen processing	25°C		15°C		5°C	
	extender		extender		extender	
	A	B	A	B	A	B
Sperm motility (%):						
Glycerolation (G)	67.5^{c}	60.0^{d}	84.4^{b}	87.5^{a}	81.9^{b}	83.8^{ab}
Equilibration (E)	59.4^{c}	53.8^{d}	81.3^{a}	84.4^{a}	78.1^{b}	81.3^{a}
Post-thawing (PT)	27.5^{c}	20.6^{d}	48.1^{a}	36.9^{b}	45.6^{a}	33.1^{b}
sperm livability (%):						
Glycerolation (G)	67.0^{c}	61.1^{d}	84.3^{b}	87.1^{a}	81.9^{b}	83.5^{ab}
Equilibration (E)	57.9^{d}	52.8^{C}	80.1^{a}	83.4^{a}	77.2^{b}	80.9^{a}
Post- thawing (PT)	27.6^{c}	22.1^{d}	47.6^{a}	35.7^{b}	44.4^{a}	31.8^{b}

Initial motility: 90.6 % Initial livability: 90.3 %

a, b, c, d: Values within the same row with different superscripts significantly differ (P<0.01).

Table 8 indicated that the interaction effect between the extender and the temperature of extender addition on the motility and livability of ram spermatozoa was significantly greater for dilution with A-extender at the 25°C extender addition protocol. In contrast, a trend was noticed at 15°C and 5°C extender addition protocols. However, the differences were not significant.

Motility and livability percentage after glycerolation and equilibration indicated the superiority of B-extender when the extender addition was conducted at 15°C, with an opposite trend when the extender was added at 25°C. The total reduction in post-thawing sperm motility and livability with A-extender was lower than with dilution with B-extender. In contrast, the recovery rate showed the opposite trend.

Concerning the glycerol concentration in extenders, Fahey (1986) suggested that the glycerol concentration in the extenders is ultimately limited by its toxicity, which depends on cooling and freezing rates, diluent composition, and method of glycerol addition. Salamon and Maxwell (2000) reviewed that most investigators suggested that the optimal glycerol concentration would be within the 6–8% range. Alvarez *et al.* (2012) confirmed this opinion and stated that glycerol concentrations higher than 8% have a significant toxic effect on spermatozoa cryosurvival.

According to Curry (2000), glycerol's cryo-protective effects are evident at higher concentrations but must be balanced against its toxicity. Accordingly, our study aimed to achieve extenders that contain a high glycerol concentration, exceeding 8%, without toxic effects on sperm.

Most investigators use mainly hypertonic extenders. This has a harmful effect on the spermatozoa during pre-freezing stages, whether the addition method was one-step or two-step, and leads to a significantly increased osmotic pressure of the sperm during freezing.

In our study, extenders were used that had suitable osmolarity for sperm (295 mOsm), containing a much higher percentage of glycerol than 8%, but they contained a low percentage of fructose. This may explain the disappearance of glycerol toxicity during pre-freezing stages in the present study, when adding the extender in one step compared to adding it in two steps. In addition, increasing the concentration of glycerol and decreasing the concentration of sugar in the extender will lead to an increase in the amount of glycerol absorbed by the sperm. It can be used as an energy source and reduces the formation of intracellular ice crystals, which induces cryodamage inside the sperm. This agrees with Filipp *et al.* (2023), who found that the inhibition of extracellular ice by 1,4-CHD does not improve the results of ram sperm freezability, possibly due to the destruction of the plasma membrane, induced by intracellular ice. Furthermore, an increase in glycerol concentration in the extender will reduce the freezing point of the extender, resulting in a reduction in the dehydration cryodamage effect of sperm, too. It is possible that the new method of adding the extender to the cooled raw semen at 15°C, which was used in our experiments, led to the disappearance or at least a high reduction of the toxic effect of the high glycerol concentration (14.5%) on the spermatozoa. In this regard, this subject must be reconsidered and studied again using this new method to achieve the truth about the harmful effects of glycerol on sperm during pre-freezing stages.

CONCLUSION

Our discoveries resulted in tangible accomplishments. The novel extenders containing 14.5% glycerol and the technique of their incorporation into semen mitigated detrimental impacts on sperm, preserving elevated motility and viability following the equilibration phase. Motility diminished following the equilibration period upon the addition of the extender at 15°C, with A and B extenders yielding 9.3% and 6.2%, respectively, which constituted a highly satisfactory outcome. The significant reduction in spermatozoa motility and viability post-thawing, recorded at 40.4% and 40.1% respectively, was unsatisfactory. This led us to anticipate that the freezing rate, thawing method, or both employed in these experiments were inappropriate for these extenders. This prompted us to undertake the subsequent experiments.

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أفكار مبتكرة لتجميد السائل المنوي للأغنام

أ. تأثير تركيب المخفف وطريقة إضافة المخفف ودرجة حرارة إضافة المخفف على قابلية السائل المنوي للأغنام للتجميد

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الملخص العربي

تم جمع السائل المنوي من ٤ كباش أوسيمي ناضجة (٤-٢ سنوات) بالمهبل الصناعي وتم خلط عينات السائل المنوي واستخدمت الدراسة مخففان: مخفف ترس (المخفف أ) ومخفف ترس به ٣٥٪ لبن فرز (المخفف ب) لإجراء تجربتين:

التجربة الأولى تمت لدراسة تأثير طريقة إضافة المخفف إلى السائل المنوي على خطوة واحدة أوعلى خطوتين على قابلية السائل المنوي للكباش للتجميد. وتمت إضافة المخفف على خطوة واحدة عن طريق إضافة المخفف إلى السائل المنوي المبرد من ٣٢ °م إلى ٥ °م خلال ساعتين أما الإضافة على خطوتين تمت بإضافة نصف المخفف الخالي من الجلسرول إلى السائل المنوي على ٣٢ °م ثم التبريد إلى ٥°م خلال ساعة ونصف ثم إضافة الجزء من المخفف المحتوي على الجلسرول.

التجربة الثانية تمت لدراسة تأثير درجة حرارة إضافة المخفف (٢٥ °م و١٥ °م و٥ °م) على قابلية السائل المنوي للكباش للتجميد. وكانت النسبة النهائية للجلسرول في المخفف ١٤,٥٪ ومعدل التخفيف ١ : ٦ وأُجريت الموازنة لثلاث ساعات وبعد تجميد السائل المنوي في النيتروجين المسال (-١٩٦°م) تمت إسالته على درجة حرارة ٤٢°م لمدة ٣٥ ثانية وتم تقدير النسبة المئوية لحركة وحيوية الحيوانات المنوية خلال مراحل التجميد والإسالة.

كانت النسبة المئوية لحركة وحيوية الحيوانات المنوية أفضل معنوياً على مستوى (١ ٪) نتيجة إضافة المخفف على خطوة واحدة فكانت (٤٠,٤ و٤٠,٠ و١٨,١٪) على الترتيب، وكان المخفف أ أفضل من المخفف ب وكانت إضافة المخفف عند ١٥°م الأفضل والفروق بين الإضافة عند ١٥°م و٢٥°م معنوية (١ ٪) بينما الفروق بين الإضافة عند ١٥°م و٥ °م غير معنوية وكان التداخل بين المخفف وطريقة الإضافة معنوياً على مستوى (١ ٪).

الكلمات المفتاحية: الحيوانات المنوية، الأغنام، التجميد الموسع، الجلسيرول، طريقة الإضافة