J. Agric. Sci. Mansoura Univ., 32 (5): 3347 - 3358, 2007

EFFECT OF SEPHADEX FILTRATION OF RAM SEMEN ON ITS FREEZABILITY AND FERTILITY

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ABSTRACT

Semen of 10 healthy mature crossbred (½ FR) rams was frozen in Tris-egg yolk glycerol extender before and after filtration through sephadex column G25-150 to investigate the effect of filtration on freezability and fertility outcomes during different seasons of the year. Filtration of semen through sephadex column resulted in significantly ($P < 0.05$) better maintenance of sperm progressive motility, live sperm count and sperm morphology compared to unfiltrated semen. There was significant ($P < 0.05$) decrease in sperm cell concentration x 10^9/ml after filtration (2.05 ± 0.5) over the unfiltered semen (2.68 ± 0.08). Progressive motility of filtered frozen-thawed spermatozoa was significantly ($P < 0.05$) improved to progressive motility of unfiltered frozen semen (60.10 v.s 43.88%). Conception rate was higher for ewes inseminated cervically with filtered frozen-thawed semen (63.4%) than those inseminated with unfiltered frozen semen (52.3%) at synchronized estrus with GnRH-PG-GnRH protocol. However, conception rate at observed estrus for ewes inseminated cervically with filtered frozen semen was ($P < 0.05$) better in comparison with those inseminated with unfiltered frozen semen (95 v.s 75%). These results concluded that removal of dead and abnormal spermatozoa by filtration of ram semen through sephadex column resulting in highest fertility after cervical artificial insemination.

Keywords: Ram, Semen, Filtration, frezability, fertility.

INTRODUCTION

At semen production centers, it is desirable to start with high quality semen, since a high proportion of viable spermatozoa are killed from time of semen collection to insemination. The presence, of dead and abnormal spermatozoa in neat ram semen leads to the production of hydrogen peroxide which has a lethal (negative toxic) effect on the normal spermatozoa in semen and consequently reduces fertility (Lindermann *et al.,* 1982). Most spermatozoa are also lost during semen freezing and thawing processes (Maxwell *et al.,* 1999). In natural mating, cervical mucus differentially select motile spermatozoa and acts as a barrier to non-motile ones (Saacke, 1984). This cervical selection is bypassed in artificial insemination. The filtration of semen through sephadex column removes most of these dead and abnormal spermatozoa and, thus, improves its quality in buffalo (Kumar *et al.,* 1999) and goat semen (El-Saidy, 2000).

The present study was conducted to investigate the effects of filtration of ram semen through sephadex column G25-150 on motility, livability and abnormality of spermatozoa both before and after freezing throughout one year and fertility outcomes after cervical artificial insemination (CAI) at synchronized and natural estrus cycles compared with natural mating of ewes.

MATERIALS AND METHODS

1. Animals and management:

Ten healthy crossbred rams (½ Finnish Landrace x ½ Rahmani), 2-3 years old and weighing 68 kg, were used in the present study. They were in regular use at Sakha Experimental Station. The animals were fed ad libitum on berseem (*Trifolium alexandrinum*) and 1 kg feed mixture during winter period, however, they were fed on 1 kg concentrate feed mixture plus one kg berseem hay during summer period. Animals had free accen to fresh water all the day.

2. Semen processing, filtration and freezing:

Semen was collected using an artificial vagina from each ram once weekly for one year starting on January 1st, to December 31, 2005. At least 2 false mounts were given prior to semen collection. Immediately after collection ejaculate volume, sperm cell concentration/ml, percentage of sperm motility and percentages of live and abnormal spermatozoa were determined. The obtained data are given in Table (1). Semen was transferred to water bath at 37°C. Semen of all 10 rams was pooled to increase the volume. The pooled semen was, then, divided into 2 parts. The first part was kept unfiltered and the second part was filtered through the sephadex column (G25-150, Pharmacia Fine Chemicals AB, Uppsala, Sweden). The filtered semen was also evaluated for the percentages of sperm motility, live and abnormal spermatozoa and sperm cell concentration/ml semen. Slurry of Sephadex G25-150 (20%, w/v) was prepared (Chauhan *et al.,* 1993) in 3% sodium citrate and the columns of 0.7 cm highest were fitted to 5 ml disposable plastic syringes. A small amount of glass wool fiber was placed at the bottom of syringe to prevent the leakage of sephadex slurry. One ml fresh semen (undiluted) was applied at the top of each column and the filtrate was collected in a sterilized graduated test tube. Three ml of the 3% sodium citrate buffer was added into each column to complete the filtration of semen. Equal volume of buffer was also added to the control semen samples (unfiltered). After filtration, the concentration of spermatozoa in filtered and non-filtered semen was adjusted to 300 x 10^6/ml. Filtered and unfiltered semen were diluted with the Tris-egg yolk extender at a rate of 1: 4. The chemical components of the extender were: 6.05 g Tris, 3.35 g citric acid, 20 ml egg yolk, 7 ml glycerol, 1.25 g fructose, 10000 IU penicillin and 10 000 µg streptomycin and completed with distilled water to 100 ml. Both semen samples were placed in a water bath at 37°C, then placed into a refrigerator at 5°C for 4 hrs, for equilibrium. The cooled semen was frozen in pellet form (0.30 ml/pellet) on a special plate with holes engraved in the surface and freezed at -79°C to -196°C by immersion in liquid nitrogen (Evans and Maxell, 1987) until used for post-thaw quality assessment. Frozen 2-3 pellets were thawed at 40°C for 10 sec. in a water bath and kept for 2-3 minutes before use for insemination.

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3. Cervical artificial insemination experiments:

Filtered and unfiltered frozen-thawed semen were used for cervical artificial insemination at synchronized (126 ewes) and natural estrus (40 ewes) during January, May and September months. Forty, 46 and 40 crossbred ewes (½ Finnish Landrace x ½ Rahmani) were synchronized by Ovsynch protocol and fixed time AI (GnRH-PG-GnRH at 0-7 and 9 days, respectively) during January, May and September months, respectively. However, 40 crossbred ewes natural came in heat after detection of estrus twice daily were used as control without any hormonal treatment. The Ovsynch protocol was applied as follows: ewes received i.m. injection of 0.004 mg Buserelin (GnRH analogue, Receptal, Intervet International B.V. Boxmeer, Holland) on day 0, followed 7 days later by i.m. injection of 0.7 ml $PGF_{2\alpha}$ (Estrumate). A second dose of 0.004 mg Receptal (GnRH) was given on day 9 and artificial insemination of treated ewes was carried out 24 h later (Pursely *et al.,* 1995 and 1997).

4. Natural service:

Natural service was performed at the onset of detected estrus for 68, 58 and 79 crossbred ewes during January, May and September months, respectively.

5. Statistical analysis:

The data were subjected to statistical analysis using general linear models procedure adapted by SPSS for Windows (2003) for user's guide with one-way ANOVA. Ducnan test within program SPSS was done to determine the degree of significance between the means.

RESULTS AND DISCUSSION

Most of the mean values for the semen physical characteristics throughout one year, presented in Table 1, lie within one range reported by previous studies (Ibrahim, 1997; Taha *et al.,* 2000; El-Saidy, 2004 and El-Sharawy, 2005).

Sephadex is a bead forming gel prepared by cross-linking dextran with epichlorophydrin. The gel is extremely hydrophilic due to presence of a large number of hydroxyl groups and it swells up in water and electrolyte solution (Kumar *et al.,* 1999). Sephadex column could retain dead or immotile spermatozoa and it is possible to obtain 95-98% motile spermatozoa (Graham *et al.,* 1976).

In this study, filtration of ram semen through sephadex column resulted in significant ($P < 0.05$) increase in sperm motility and live sperm count (Table 2) and decreased ($P < 0.05$) significantly sperm abnormalities and sperm cell concentrations (Table, 3). There was 19.9 and 13.2% increase in sperm motility and live sperm count, respectively.

Table (2): Mean values (± SE) of sperm motility and live sperm percentage in filtered and unfiltered semen throughout one year.

Month	1- Extended sperm motility		% Change	2- Live sperm, %		% Change
	Extended unfiltered	Extended filtered		Extended unfiltered	Extended filtered	
Jan.	78.50 ± 0.53 bB	91.50 ± 0.53 bA	16.6cd	77.30 ± 0.54 bcB	85.40 ± 0.60 cA	10.5de
Feb.	77.00 ± 0.56 bB	92.75 ± 0.57 abA	20.5bc	79.95 ± 0.57 aB	87.70 ± 0.89 abcA	9.97e
Mar.	77.00 ± 0.76 bB	93.00 ± 0.56 abA	20.8b	77.05 ± 0.84 bcB	88.10 ± 0.62 abcA	14.3bc
Winter	77.50 ± 0.37 B	92.42 ± 0.33 A	19.3	78.03 ± 0.41 B	87.07 ± 0.43 A	11.6b
Apr.	77.50 ± 0.57 bB	93.50 ± 0.53 aA	20.7b	78.30 ± 0.59 abcB	89.05 ± 0.72 abA	13.7bc
May	77.75 ± 0.57 bB	93.00 ± 0.56 abA	19.6bc	78.35 ± 0.65 abcB	87.05 ± 0.86 abcA	11.1de
Jun.	78.00 ± 0.56 bB	93.50 ± 0.53 aA	19.9bc	77.35 ± 0.74 bcB	86.40 ± 1.05 bcA	11.7de
Spring	77.75 ± 0.32 B	93.33 ± 0.31 A	20.0	78.00 ± 0.38 B	87.50 ± 0.52 A	12.2b
Jul.	77.50 ± 0.57 bB	93.50 ± 0.53 aA	20.7b	78.70 ± 0.62 abB	88.05 ± 1.12 abcA	11.9ee
Aug.	77.85 ± 0.57 bB	93.50 ± 0.53 aA	20.3bc	77.10 ± 0.68 bcB	87.00 ± 0.87 abcA	12.8cd
Sept.	78.50 ± 0.73 bB	93.25 ± 0.55 aA	18.8bc	76.20 ± 0.67 cdB	86.85 ± 0.85 abcA	13.98bc
Summer	77.92 ± 0.36 B	93.42 ± 0.30 A	19.9	77.33 ± 0.39 B	87.30 ± 0.54 A	12.9b
Oct.	81.50 ± 0.90 aB	93.50 ± 0.53 aA	14.7d	77.60 ± 0.67 abcB	89.25 ± 0.71 abA	15.0bc
Nov.	76.75 ± 0.75 bB	93.25 ± 0.55 aA	21.5b	77.30 ± 0.83 bcB	89.5 ± 0.94 aA	15.9ab
Dec.	74.00 ± 0.69 cB	92.50 ± 0.57 abA	25.0a	74.85 ± 1.02 dB	88.30 ± 1.00 abA	17.97a
Autumn	77.42 ± 0.60 B	93.08 ± 0.32 A	20.2	76.58 ± 0.51 B	89.03 ± 0.51 A	16.3a
Overall mean	77.65 ± 0.21B	93.06 ± 0.16A	19.9	77.49 ± 0.21B	87.73 ± 0.26A	13.21

a and b: Means in the same column with different superscripts differ significantly (P < 0.05).
A and B: Means in the same row with different superscripts differ significantly (P < 0.05).

Filtration also resulted in 62.6 and 23.5% reduction in total sperm abnormalities and sperm cell concentrations, respectively. These results are, more or less, similar to the findings of Vyas *et al.* (1991, in bull semen), Kumar *et al.* (1999, in buffalo semen) and El-Saidy (2000) in goat semen. This increase in the number of live-motile spermatozoa and normal morphology might be due to retention of dead, immotile or damaged spermatozoa in the sephadex column. Agglomeration of dead spermatozoa because of physico-chemical reaction with sephadex particles (Graham *et al.,* 1976) or increased stickness of spermatozoa after their death (Baker and Degan, 1972) might be responsible for their retention. Sephadex column represents a pack of beds which provide a zig-zag path to the moving spermatozoa and the immotile or damaged spermatozoa could not traverse this zig-zag path (Kumar *et al.,* 1999). According to another hypothesis, there is leakage of different macromolecules from abnormal spermatozoa which might bind to sephadex particles leading to their retention (Lodhi and Carbo, 1984).

Data in Table (2) indicated that the improvement of sperm motility and live sperm count was highest in autumn (20.2 and 16.3%, respectively), however, it was lowest in winter (19.3 and 11.6%, respectively). El-Saidy (2000), however, found that rates of change in live sperm percentage in goat semen due to filtration were 7.4, 4.0, 36.7 and 44.7% in summer, autumn, winter and spring season, respectively. Irrespective the high rate of reduction in sperm abnormalities (65%) and sperm cell concentrations (26.3%) due to filtration in spring and summer season (Table, 3) were contrasted with the finding of El-Saidy (2000) who found low rates of change in sperm

abnormality and sperm cell concentration (4.9 and 29.7%) in spring and summer season respectively. However, Panghal and Tuli (1999) and Panghal *et al.* (2002) found that sperm abnormalities remained unchanged in filtered (10.48%) and non filtered buffalo semen (10.45%).

Table (3): Mean values (± SE) of total sperm abnormalities and sperm cell concentration x 10^9/ml in filtered and unfiltered semen throughout one year.

Month	3- Abnormal sperm %		% Change	4- Sperm cell concentration		% Change
	Extended unfiltered	Extended filtered		Extended unfiltered	Extended filtered	
Jan.	10.60 ± 0.33 aA	3.60 ± 0.44 abB	-66.0	3.08 ± 0.08 aA	2.41 ± 0.12 aB	-21.8
Feb.	10.45 ± 0.37 abA	4.10 ± 0.39 abB	-60.8	2.76 ± 0.10 bcA	2.25 ± 0.06 abB	-18.5
Mar.	9.35 ± 0.45 abcA	4.00 ± 0.36 abB	-57.2	2.78 ± 0.12 bcA	2.2 ± 0.09 abB	-20.9
Winter	10.13 ± 0.23 A	3.90 ± 0.23 B	-61.5	2.87 ± 0.01 A	2.29 ± 0.07 B	-20.2
Apr.	9.40 ± 0.50 abcA	2.95 ± 0.34 bB	-68.7	2.11 ± 0.03 dA	1.51 ± 0.04 eB	-28.4
May	9.65 ± 0.47 abcA	3.70 ± 0.40 abB	-61.7	2.85 ± 0.09 abA	2.15 ± 0.10 bcB	-24.6-
Jun.	9.00 ± 0.49 bcdA	3.15 ± 0.38 bB	-65.0	2.75 ± 0.03 bcA	2.04 ± 0.01 bcd	-25.8
Spring	9.35 ± 0.28 A	3.27 ± 0.22 B	-65.0	2.57 ± 0.02 A	1.90 ± 0.002 B	-24.4
July	9.95 ± 0.54 abcA	3.45 ± 0.34 abB	-65.3	2.72 ± 0.09 bcA	1.98 ± 0.09 cdB	-27.2
Aug.	10.85 ± 0.44 aA	4.60 ± 0.39 aB	-57.6	2.56 ± 0.05 bA	1.88 ± 0.04 dB	-26.6
Sep.	8.85 ± 0.55 cdA	3.35 ± 0.36 bB	-62.2	2.58 ± 0.07 bcA	1.95 ± 0.03 cdB	-24.4
Summer	9.88 ± 0.31 A	3.80 ± 0.22 B	-61.5	2.62 ± 0.01 A	1.9 ± 0.03 B	-26.3
Oct.	8.65 ± 0.52 cdA	3.15 ± 0.34 bB	-63.6	2.64 ± 0.04 bcA	2.09 ± 0.06 bcdB	-20.8
Nov.	8.60 ± 0.54 cdA	3.25 ± 0.51 bB	-62.2	2.67 ± 0.07 bcA	2.01 ± 0.009 bcdB	-24.7
Dec.	7.80 ± 0.54 A	3.10 ± 0.34 bB	-60.3	2.71 ± 0.13 bcA	2.08 ± 0.12 bcdB	-23.2
Autumn	8.35 ± 0.31 bA	3.17 ± 0.23 B	-62.0	2.67 ± 0.06 A	2.06 ± 0.03 B	-22.9
Overall mean	9.43 ± 0.15A	3.53 ± 0.11B	-62.6	2.68 ± 0.08A	2.05 ± 0.05B	-23.5

a and b: Means in the same column with different superscripts differ significantly (P < 0.05).

A and B: Means in the same row with different superscripts differ significantly (P < 0.05).

The present work was done to assess the possibility of improving ram semen quality by filtration through sephadex columns. The present results are in accordance with those reported for other farm animal species Nevertheless, an interesting point is the high rate of reduction in sperm cell concentration following filtration (23.5%), which represents 89% of dead and abnormal sperm retained in the filter. This may be attributed to trapping damaged or abnormal acrosomes in the filter (El-Saidy, 2000). However, with the enhancement of sperm livability, motility and normal morphology compensated for part of this loss and led to increase the total number of live spermatozoa recovered in the filtered semen.

The average values of progressive motility at different stages of freezing of unfiltered and filtered semen are presented in Table 4. There was a gradual decline in progressive sperm motility from initial extension to 24 hrs. post-freezing of filtered and unfiltered ram semen. Filtration of semen through sephadex column resulted in 9.22 and 37.7% increase of progressive motility before and after freezing and storage of semen in liquid nitrogen. This may possible be due to the higher number of motile, live and normal morphology spermatozoa present in the filtered semen.

The results of present study are in accordance with the finding of Goyal (1993) who reported that buffalo semen frozen after filtration through sephadex G-15 column could survive for longer time after thawing and incubation at 37°C as compared to semen frozen without filtration. Ayoub *et al.* (1996) obtained 72, 54 and 63% post-thaw sperm motility after filtration goat semen in sephadex G-15, glass cotton and glass wool filtration, respectively. Panghal and Tuli (1999) demonstrated significant improvement in progressive motility (41%), live spermatozoa (34%) and intact acrosome (37%) between extension and 7 days post-freezing of sephadex filtered semen over the unfiltered semen. Also, Kumar *et al.* (1999) reported that, filtration of buffalo semen through sephadex column resulted in a significant increase in sperm motility being 9.58 and 5.83% in fresh and post-freeze thawed semen. However, Ahmad *et al.* (2003) found that the mean percentage of spermatozoal motility in both filtered and unfiltered semen was not effected by dilution and equilibration but declined significantly after freezing.

Analysis of data in Table (4), showed that highest rate of increase (13.03%) in equilibrated sperm motility was recorded in spring season followed by autumn (9.23%) and summer (8.17%), however, the lowest value was recorded in winter (6.84%), the differences were statistically ($P < 0.05$) significant. Also, the rate of increase of frozen thaw motility was higher in winter (42.1%) compared to those of spring, summer and autumn (37.5, 37.6 and 32.8%, respectively).

Table (4): Mean values (± SE) of sperm motility post equilibrate and freeze thawed filtered and unfiltered ram semen throughout one year.

Month	1- Equilibrated sperm motility		% Change	2- Frozen thawed motility		% Change
	Unfiltered	Filtered		Unfiltered	Filtered	
Jan.	73.00 ± 0.57 bcB	78.25 ± 0.746cfB	7.18cd	46.00 ± 0.88 abC	65.25 ± 0.97 aC	40.4bc
Feb.	73.00 ± 0.76 bcdeB	77.25 ± 1.20 fgB	5.82d	48.50 ± 1.26 aC	63.25 ± 1.60 abcC	30.4cd
Mar.	71.25 ± 0.50 deB	76.50 ± 0.90 gB	7.37cd	39.00 ± 0.69 fC	62.25 ± 1.50 abcC	59.6a
Winter	72.42 ± 0.39 B	77.33 ± 0.56 B	6.84b	44.50 ± 0.73 C	63.58 ± 0.78 C	42.1a
Apr.	73.50 ± 0.73 beB	86.50 ± 1.30 aB	17.69a	42.75 ± 1.10 cdeC	60.75 ± 1.50 bcC	42.1bc
May	73.00 ± 0.56 beB	82.00 ± 0.76 bcdB	12.33b	42.50 ± 1.10 deC	59.75 ± 0.92 cC	40.6bc
Jun.	74.25 ± 0.75 bcB	81.00 ± 1.00 cdeB	9.09bcd	47.50 ± 1.10 abC	62.00 ± 1.40 abcC	30.5cd
Spring	73.58 ± 0.40 B	83.17 ± 0.66 B	13.03a	44.25 ± 0.67 C	60.83 ± 0.75 C	37.5ab
Jul.	74.00 ± 0.78 bcB	78.00 ± 0.56 fgB	5.41d	40.50 ± 0.72 efC	52.25 ± 0.57 dC	29.0d
Aug.	74.25 ± 1.11 bcB	81.00 ± 0.86 cdeB	9.09bcd	32.50 ± 0.57 gC	48.00 ± 1.20 eC	47.7b
Sept.	75.00 ± 0.73 bB	82.50 ± 0.57 bcB	10.0bcd	46.75 ± 0.98 abC	64.50 ± 0.80 abC	37.96bcd
Summer	74.42 ± 0.51 B	80.50 ± 0.46 B	8.17b	39.92 ± 0.88 C	54.92 ± 1.05 C	37.6ab
Oct.	79.00 ± 1.43 a	84.50 ± 1.10 abB	6.96cd	45.50 ± 0.88 abcC	60.00 ± 1.30 cC	32.2cd
Nov.	72.25 ± 0.57 cde	79.25 ± 0.83 dgB	9.69bcd	48.00 ± 1.010 aC	62.00 ± 1.20 abcC	29.2d
Dec.	71.00 ± 0.46 eB	79.00 ± 1.20 efgB	11.27bc	44.50 ± 1.10 bcdC	61.25 ± 1.70 bcC	37.6bcd
Autumn	74.08 ± 0.70 B	80.92 ± 0.69 B	9.23b	46.00 ± 0.62 C	61.08 ± 0.82 C	32.8b
Overall mean	73.76 ± 0.25B	80.40 ± 0.32B	9.22	43.88 ± 0.39C	60.10 ± 0.48C	37.7

a and b: Means in the same column with different superscripts differ significantly ($P < 0.05$).

A and B: Means in the same row with different superscripts differ significantly ($P < 0.05$).

Table 5 describes the effect of fixed time artificial insemination and natural insemination at observed estrus in crossbred ewes receiving the Ovsynch

protocol on conception rate. Overall conception rate for ewes inseminated at observed estrus was significantly (P < 0.05) higher compared to ewes artificially inseminated at fixed time (80.4 v.s 58.0%). The present conception rate in the synchronized estrus ewes with ovsynch protocol (58%) was lower than 88% of Beck *et al.* (1996) and 80% El-Saidy *et al.* (2005) in crossbred ewes. However, it was higher than the finding of El-Shamaa *et al.* (2003) in ewes treated during the end breeding season (50%). Also, it is worth noting that rate of conception during the breeding seasons (September month) was (P < 0.05) higher in both treated and control ewes (75 and 90.8%, respectively) than January (late breeding season) 42.5 and 77.9%, respectively and May month (anestrus period) 56.5 and 66.2%, respectively. Aboul-Ela *et al.* (2004) using ovsynch protocol in goats, found that rates of fertility for both application of pre-determined time based insemination or insemination based on the overt signs of estrus in September (90.9 v.s 27.6%), early August (40 v.s 10%) and in late August (60 v.s 50%) indicate that advantage of applying pre-determined time based insemination when this GnRH hormonal protocol is used.

Table (5): Overall effect of estrous synchronization on conception rate.

Type of estrus	No. of ewes	Month of breeding			Conceived ewes			Overall mean
		Jan.	May	Sept.	Jan.	May	Sept.	
Synchronized	126	40	46	40	17 42.5 bcB	26 56.5 ab	30 75 a	73 58.0 B
Natural	245	68	68	109	53 77.9 abA	45 66.2 bc	99 90.8 a	197 80.4 A

In the same row means with the small letters are not significantly different at P<0.05.
In the same column means with the Capital letters are not significantly different at P<0.05.

To determine whether differences in type of semen and site of insemination at synchronized or observed estrus would be reflected in conception rate, field experiments were conducted using CAI with filtered and unfiltered-frozen semen at fixed time and observed estrus and vaginal natural mating involving 126, 40 and 205 crossbred ewes, respectively (Table 6). At synchronized estrus ewes, filtered frozen semen increased the rate of conception (63.5%) after cervical artificial insemination as compared to ewes conceived with unfiltered frozen semen (52.4%) but difference was not significant. Moreover, conception rate was significantly (P < 0.05) higher for ewes inseminated with filtered-than unfiltered-frozen spermatozoa at observed estrus (95 v.s 75%, respectively). These findings are, similar or more to the findings of Sallam (1999) and El-Sharawy (2005). Maxwell (1984) and Salamon and Maxwell (1995) found that cervical artificial insemination with frozen semen results in low fertility, about 10%, due to the slow transport of the frozen-thawed espermatozoa and their short survival in the female reproductive tract (Lightfoot and Salamon, 1970 a and b).

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The results strongly indicate, that satisfactory fertility can be consistently obtained after cervical insemination of ewes with filtered frozen-thawed semen, provided that strategies are employed to filtration of spermatozoa to removal dead and abnormal spermatozoa from ejaculates to maximize the genetic potential of the sire.

In addition, to achieve maximum benefit from synchronization programs, ewes observed in estrus prior to timed artificial insemination (TAI) should be inseminated on observed estrus and ewes not observed in estrus should be inseminated on TAI.

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تأثير الترشيح بالسفادكس للسائل المنوى للكباش على قابليته للتجميد والخصوبة

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تم تجميد السائل المنوى لعشر كباش ناضحة (خليط ½ فنلندى رحمانى) فى مخفف التريس - صفار البيض والجليسرول قبل وبعد الترشيح من خلال عمود السفادكس ٢٥-١٥٠ لمعرفة تأثير الترشيح على القابلية للتجميد والخصوبة أثناء فصول السنة المختلفة. أظهرت النتائج أن ترشيح السائل المنوى فى عمود السفادكس ادى لتحسن معنوى فى الحركة التقدميه للحيوانات المنوية ، إعداد الحيوانات المنوية الحية والحيوانات المنويه الطبيعيه بالمقارنة بالسائل المنوى الغير مرشح. ولقد كان هناك نقص معنوى فى تركيز الحيوانات المنوية بعد الترشيح (٢,٠٥ ± ٠,٥ × ٩١٠ مل) عن السائل المنوى الغير مرشح (٢,٦٨ ± ٠,٨ × ٩١٠ مل).

الحركة التقدميه للسائل المنوى المرشح والمجمد كانت احسن معنويا بالمقارنة بالحركة التقدميه للسائل الحيوى الغير مرشح المجمد (٦٠,١ مقابل ٤٣,٨٨%).

نسبة الحمل فى النعاج الملقحه داخل عنق الرحم بالسائل المنوى المرشح المجمد كانت اعلى (٦٣,٤%) وذلك بالمقارنة بالنعاج الملقحه داخل عنق الرحم بالسائل المنوى الغير مرشح والمجمد (٥٢,٣%) والمنظم بها الشياع باستخدام برتوكول GnRH-PG-GnRH. كما كانت نسبة الحمل فى النعاج التى اكتشف بها الشياع والملقحه داخل عنق الرحم بالسائل المنوى المفلتر المجمد احسن معنويا مقارنة بتلك الملقحه باستخدام السائل المنوى الغير مفلتر المجمد (٩٥ مقابل ٧٥%). أوضحت النتائج ان ازالة الحيوانات المنويه الميته والغير الطبيعيه بترشيح السائل المنوى للكباش باستخدام عمود السفادكس ادى الى ارتفاع نسبة الخصوبة بعد التلقيح الصناعى بعنق الرحم.

Table (1): Initial physical characteristics of crossbred rams semen (1/2 F.R) throughout one year.

Season	Month	Ejaculate volume (ml)	Mass-motility (%)	Live sperm (%)	Abnormal sperm (%)	Sperm concentration x 10^9/ml	Sperm output x 10^9
Winter	January	0.860 ± 0.05 de	83.00 ± 0.82 ab	81.85 ± 1.17 bcd	10.05 ± 0.34 a	3.08 ± 0.07 a	2.67 ± 0.20 abc
	February	0.870 ± 0.045 de	83.25 ± 0.55 ab	86.33 ± 1.14 a	9.67 ± 0.35 ab	2.76 ± 0.10 bc	2.43 ± 0.19 bcd
	March	0.925 ± 0.074b-e	82.25 ± 0.59 b	82.48 ± 1.42a-d	8.78 ± 0.46 a-d	2.78 ± 0.12 bc	2.57 ± 0.25 a-d
Mean ± SE		0.885 ± 0.034c	82.80 ± 0.36 b	83.56 ± 0.75 a	9.50 ± 0.23 a	2.87 ± 0.06 a	2.55 ± 0.12 b
Spring	April	0.990 ± 0.090a-d	83.75 ± 0.50 ab	84.76 ± 1.24 abc	8.72 ± 0.49 a-d	2.11 ± 0.06 d	2.083 ± 0.20 d
	May	0.805 ± 0.053e	83.50 ± 0.53 ab	84.24 ± 1.11 abc	8.99 ± 0.44 abc	2.85 ± 0.08 ab	2.26 ± 0.15 cd
	June	1.060 ± 0.028abc	80.25 ± 0.85 c	79.68 ± 1.34 d	8.77 ± 0.49 a-d	2.75 ± 0.09 bc	2.91 ± 0.12 ab
Mean ± SE		0.952 ± 0.038 bc	82.50 ± 0.42 b	82.89 ± 0.76 a	8.83 ± 0.27 a	2.57 ± 0.06 b	2.42 ± 0.10 b
Summer	July	1.075 ± 0.036abc	84.00 ± 0.46 ab	85.34 ± 0.79 ab	9.20 ± 0.52 abc	2.72 ± 0.07 bc	2.92 ± 0.12 ab
	August	0.915 ± 0.055cde	85.00 ± 0.00 a	84.34 ± 0.78 abc	9.92 ± 0.41 a	2.56 ± 0.05 c	2.34 ± 0.14 cd
	September	1.05 ± 0.034abc	83.25 ± 0.55 ab	80.91 ± 1.01 cd	8.36 ± 0.54 bcd	2.58 ± 0.08 bc	2.69 ± 0.11 abc
Mean ± SE		1.013 ± 0.026b	84.08 ± 0.25 a	83.53 ± 0.55 a	9.16 ± 0.29 a	2.62 ± 0.04 b	2.65 ± 0.08 b
Autumn	October	1.155 ± 0.46a	85.00 ± 0.00 a	81.10 ± 1.07 cd	8.29 ± 0.51 bcd	2.64 ± 0.07 bc	3.07 ± 0.20 a
	November	1.155 ± 0.46 a	83.25 ± 0.55 ab	84.04 ± 1.46 abc	7.94 ± 0.50 cd	2.67 ± 0.09 bc	3.09 ± 0.17 a
	December	1.090 ± 0.040 ab	78.25 ± 1.30 d	79.23 ± 1.75 d	7.47 ± 0.56 d	2.71 ± 0.13 bc	2.97 ± 0.20 ab
Mean ± SE		1.133 ± 0.029a	82.18 ± 0.60 b	81.46 ± 0.86 a	7.90 ± 0.30 b	2.67 ± 0.06 b	3.04 ± 0.11 a
Overall mean		0.996 ± 0.170	82.89 ± 0.22	82.86 ± 0.37	8.85 ± 0.14	2.68 ± 0.03	2.67 ± 0.05

In the same column, means with the same small letter are not significantly different at P<0.05.

Table (6):Overall conception rate as affected by site of insemination and type of semen in synchronized and natural estrous ewes.

Site of insemination	Total No. of ewes	Frozen non filtered semen						Frozen filtered semen						Overall conception rate	
		Jan.		May		Sept.		Jan.		May		Sept.		Non	Filtered
		No.	Conceived	No.	Conceived	No.	Conceived	No.	Conceived	No.	Conceived	No.	Conce-ived	filtered semen	semen
A. Cervical AI at:															
a-Synchronized estrus															
No.	126	20	6	23	13	20	14	20	11	23	13	20	16	(33/63)	(40/63)
(%)			(60%) bc		(56.5%)abB		(70a)		(55b)		(56.5)Bbd		(80)a	(52.3)	63.4B
b- Natural heat															
No.	40	-	-	5	4	15	11	-	-	5	5	15	14	(15-20)	((19/20)
(%)					(80) A		(73.3)				(100) A		(93.3)	(75b)	(95%)aA

B- Vaginal natural mating at natural heat	205	Ewes conceived and conception rate during							
		Jan.		May		Sep.		Overall conception rate	
		No.	Conceived	No.	Conceived	No.	Conceived	No.	Conceived
No.		68	53	58	36	79	74	205	163
%			77.98 ab		62.1 b		93.7		79.5

In the same row means with the small letters are not significantly different at P<0.05.
In the same column means with the Capital letters are not significantly different at P<0.05.