Introducing Soybean Oil in Semen Extenders of Awassi Rams

ادخال زيت فول الصويا في مخففات السائل المنوي لكباش العواسي

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Abstract

The aims of the study was to investigate the possibility of using an plant lecithin; commercial soybean oil (SO) directly in the components of semen extenders of Awassi rams, storage of semen in chilled technique and the effects of dilution, cooling and storage periods on semen quality. Semen was collected weekly from four Awassi rams by electro- ejaculator. Pooled samples were divided to six equal aliquots and diluted by Citrate egg yolk extender at 37˚C. Treatments were designed on the base of control extender containing 25% egg yolk and four treatments containing different addition of SO (12.5, 25, 37.5, and 50%) and combination treatment of 12.5% egg yolk +12.5% SO. Treatment tubes were cooled to 5˚C and stored for three days. Semen was evaluated as raw, diluted, cooled and after storage in refrigerator (5˚C) for 1, 2 and 3 consecutive days. Results showed that there were no significant differences among all treatments in progressive motility, dead sperm %, abnormal sperm % and acrosome defect %, while pH were found to be significantly declined ($p \leq 0.05$) in control group. Significant effects of dilution, cooling, and storage period have been demonstrated with steadily significant deterioration ($p \leq 0.05$) for all studied characteristics when motility and pH declined while sperm abnormality, dead, and acrosome defect percentages increased. The results clearly indicated successful of using different levels of SO (plant source) as lecithin source instead of egg yolk (animal source of lecithin) without any impacts on the biological characteristics of Awassi ram semen and the process of dilution, cooling and storage periods have deterioration effects on semen quality.

Key words: Lecithin; Soybean oil; Storage period; Awassi ram; Extender

الملخص

تتمثل اهداف هذه الدراسة في التعرف على امكانية استعمال الليسيثين النباتي (زيت فول الصويا التجاري) مباشرة في مخففات السائل المنوي لكباش العواسي، والحفظ بتقانة التبريد ودراسة تاثير عمليات التخفيف والتبريد والخزن في نوعية السائل المنوي لكباش العواسي. جرى جمع السائل المنوي اسبوعيا من اربع كباش عواسي باستخدام طريقة التحفيز الكهربائي. جمعت العينات مع بعضها ثم قسمت الى ست اقسام متساوية وخففت بمخفف السترات – صفار البيض بدرجة 37°م. تم تصميم المعاملات على اساس ان تحتوي معاملة السيطرة على 25 % صفار البيض واربع معاملات اخرى تضم اضافات 12.5 و 25 و 37.5 و50 % من زيت فول الصويا، علاوة على معاملة توليفية من 12.5 % صفار البيض و 12.5 % زيت فول الصويا. تم تبريد الانابيب لكل المعاملات الى 5°م وخزنت لثلاثة ايام. جرى تقييم السائل المنوي وهو طازج وفي مراحل التخفيف والتبريد وما بعد الحفظ بدرجة 5 م لمدة يوم ويومان وثلاثة ايام على التوالي. اوضحت النتائج عدم وجود فروق معنوية بين المعاملات كافة في صفات الحركة الامامية %، النطف الميتة %، النطف المشوهة %، وتشوهات الاكروسوم %، بينما كانت درجة الحموضة pH منخفضة معنويا في مجموعة السيطرة. ان عمليات التخفيف والتبريد والحفظ ليوم ويومين وثلاثة ايام متتالية سببت تدهور مضطرد ($p \leq 0.05$) في الصفات المدروسة كافة اذ انخفظت كل من الحركة و pH في حين ارتفعت كل من نسبة النطف الميتة والمشوهة ونسبة تشوهات الاكروسوم. اشارت النتائج بوضوح الى النجاح في استعمال نسب مختلفة من زيت فول الصويا (مصدر نباتي) كمصدر لليسيثين بديلا عن صفار البيض (مصدر حيواني) وبدون اي تأثيرات سيئة على الصفات البيولوجية للسائل المنوي وان عمليات التخفيف والتبريد وفترة الخزن لها تاثير سيء على نوعية السائل المنوي لكباش العواسي.

الكلمات الدالة: الليسيثين، زيت فول الصويا، مدة الخزن، كباش العواسي، مخففات

Introduction

The successful fertilization and storage of ram semen required sperm of high motility, free of abnormality, intact protoplasmic membrane and chromatin [1,2]. When semen was diluted and cooled

to prepare for storage, some phospholipids lost from cytoplasmic membrane [3] due to re-regulation process take place under the impact of cooling [4] which means that fertilization will be declined, the lost particles have to compensate by adding good source of phospholipids. Egg yolk and milk are successfully used as major components of semen extenders [5,6]. Lecithin (Phosphatidyl choline) is phospholipids abundantly available in plants and play important role in biological activity regulation of animal cell membrane [7]. Soybean lecithin (SL) differed from egg yolk lecithin (EYL) in the quality of lipids construction and fatty acid [8; 9], it is a natural combination of Phosphatidyl choline and stearic, oleic and palmetic acids [10] which have the ability to support the construction stability of the cell [11]. Many advantages have been demonstrated in egg yolk including phospholipids, antioxidants, cholesterol and poly unsaturated fatty acids [12,13,14]. In spite of these advantages, there are many disadvantages recorded in the literatures. Egg yolk contains salt and granules adhered at spermatozoa membrane [15], microbial contamination is one of the dangerous factor affected semen storage [16,17], in addition to the stimulation of antibody production in circular and genital systems of female due to antigen role of egg yolk [18], also there are an interaction of egg yolk triglycerides with lipase enzyme found in the seminal plasma of bucks which produce poisons for the spermatozoa [19,20]. Therefore, the aims of this study are to investigate the possibility of using SO (plant source of lecithin) directly in the preparation of extenders instead of egg yolk lecithin EYL(animal source), storage of semen in chilled technique (5˚C) and the effects of dilution, cooling and storage periods on semen quality of Awassi rams.

Materials and methods

During two months, semen was collected from 4 Awassi rams (aged 2–4 years and body condition score 3–4) in ruminant research department/ Alzaafarania-Baghdad once weekly by electro-ejaculatore (Electro Jac 5). Citrate based extender was prepared by dissolving 2.37gm Trisodium citrate, 0.5 gm Glucose in 100 ml distilled water. Egg yolk and/or SO (Maheen; natural SO; a product of H & R laboratory Pakistan) were directly added (v/v) in different levels including; 25% egg yolk as control (C), 12.5, 25, 37.5 and 50% of SO as T1-T4 treatments respectively and a combination of 12.5% egg yolk and 12.5 % SO were add as T5. All treatments tube were heated to 50˚C with continues stir then cooled to room temperature and centrifuged at 3310 g for 20 min., three layers were formed in the tubes, discharge the upper layer (float lipids) and the pellet (non soluble materials) in the bottom and aspire the supernatants to added to the corresponding treatments. 100000 IU Penicillin and 100 mg streptomycin were added, pH was adjusted at 6.8 by 1% solution of Sodium hydroxide. Pooled semen was divided into six aliquots then diluted by the six prepared extenders at 37˚C in the ratio of 1: 4 (semen: extender). Tubes were then cooled to 5˚C within 2 hours in programmable incubator (Sanyo, MIR-253) and kept in refrigerator for storage and re-evaluation after dilution, cooling, and one, two and three consecutive days of storage. Evaluations of fresh semen and semen in different stages of process and storage periods were conducted by conventional procedures; volume was directly read from graduated tube; appearance of ejaculate at 1-5 score; pH in pH-paper (0.3 graduate); mass motility estimated according to [21] (x40 magnification); progressive motility estimated as forwarded sperm % [22]; sperm concentration was determined by special dens -meter (Densimeter, 591 B); dead and abnormal spermatozoa % by eosin-nigrosin stains [23]; and acrosome defects % by eosin-fast green stains [24]. Data was statistically analyzed at general linear model using SPSS software [25] to differentiate between either treatments or stages of process and storage periods and the significant differences were assessed at the base of ($p < 0.05$) in Duncan multiple range test.

Results and discussion

Table (1) revealed some natural characteristics of Awassi ram semen before the beginning of breeding season in Iraq (Feb. and Mar.) which is found in agreement with the previous work [26].

Table (1): Characteristics of fresh Awassi rams semen (mean ± SD).

Ram no.	Vol (ml)	App	M.M %	P.M %	pH	Conc ($X10^9$)	Dead %	Abn %	Acro %
179	2.67	4.50	4.55	91.25	6.85	2.88	9.50	8.50	6.25
	±0.59	±0.57	±0.52	±3.53	±0.10	±0.63	±3.69	±1.73	±1.70
197	1.7	3.75	3.77	85.62	6.87	0.87	9.50	5.00	7.25
	±0.29	±0.50	±0.44	±3.20	±0.15	±0.09	±1.73	±1.41	±1.25
193	2.45	3.75	3.37	81.87	6.97	1.10	7.25	5.37	4.25
	±0.66	±0.50	±0.51	±4.58	±0.36	±0.39	±1.25	±1.37	±1.25
185	2.25	4.00	3.37	85.62	7.00	1.85	11.25	9.25	9.25
	±0.89	±0.81	±0.51	±4.17	±0.37	±0.81	±4.19	±1.70	±2.06

Vol= semen volume; App= semen appearance; M. M= mass motility; P.M= progressive motility; Conc= concentration; Abn= abnormal sperm; Acro= acrosome defect.

Table (2) showed the effects of plant lecithin on some chilled semen characteristics, there were no significant differences in most of the criteria studied, while pH was declined ($p \leq 0.05$) in control group, this decline didn't synchronized with any other characteristics of preserved semen indicating that pH in this limits is a minor factor and couldn't change the circumstances biological picture. These findings disagreed with [26,27].

Moreover, it's likely that the presents of SL in extender has important role to support stable pH in such state, while C and T5 groups which showed the lower pH contains EYL either totally or partially. The insignificant differences in most criteria explains the possibility of using SL instead of EYL in semen extenders, and 12.5% of SL is of economic view while any increase in the level of it haven't a significant improve in semen characteristics. Therefore, introducing SL directly to the extender presents a new approach in using plant lecithin (extender free of animal components) and gets rid of all disadvantages of using egg yolk, rather than cheap, easy and efficient procedure to prepare semen extenders. Moreover, [28] reported increase in spermatozoa motility in a commercial extender based on soybean in comparison with egg yolk –Tris based extender. Also, [29] revealed that there are no significant differences in fertilization and conception rates between soybean extender (Andromed®) and egg yolk- milk based extender. There is no doubt that plant lecithin will substitute egg yolk and milk based extenders in near future due to the high performance of chilled and cryopreserved spermatozoa [30; 31; 32]. Increasing the percentages of SL from 12.5 to 50% Table (2) haven't any significant impact on stored semen quality, in contrast, [33,34] observed a decrease in spermatozoa motility when the levels of SL in the extender have been doubled in bulls and rams semen respectively. The protection role of spermatozoa played by egg yolk phospholipids [35], or the compensation of the lost phospholipids during dilution and cooling process [36] may available in the SL based extender.

Table (2): Effects of egg yolk and / or soybean lecithin on some stored semen characteristics of Awassi rams (independent of processing stages and storage periods) (Mean ± SEM).

Criteria / Treat.	Progressive Motility %	pH	Dead Sperm%	Abnormal Sperm %	Acrosome Defect%
C	66.40 a ± 4.76	6.18 b ± 0.46	19.30 a ± 3.43	16.10 a ± 3.17	15.96 a ± 2.62
T1	67.46 a ± 4.95	6.46 a ± 0.61	18.50 a ± 3.13	15.86 a ± 3.23	16.36 a ± 2.84
T2	67.42 a ± 4.93	6.48 a ± 0.59	18.03 a ± 3.24	16.13 a ± 3.26	16.60 a ± 2.87
T3	66.86 a ± 4.87	6.52 a ± 0.56	19.40 a ± 3.37	16.50 a ± 3.28	18.13 a ± 2.93
T4	67.38 a ± 4.63	6.50 a ± 0.58	18.06 a ± 2.89	15.90 a ± 2.99	18.86 a ± 2.97
T5	67.65 a ± 5.05	6.31 ab ± 0.55	19.36 a ± 3.19	17.03 a ± 3.08	18.93 a ± 2.91

C= control, 25% egg yolk; T1= 12.5% SL; T2= 25% SL; T3= 37.5% SL; T4= 50% SL; T5= 12.5% egg yolk + 12.5% SL. Different superscripts within column are significantly different ($P \leq 0.05$).

The present results showed that motility, dead, abnormal, and acrosome defect didn't affected when SL was used. To far extent, the results confirmed the opinion of [36] that any extender contains phosphate choline is able to protect spermatozoa against cooling and freezing damages. Up to our knowledge, it's the first time to introduce raw SL directly in the extenders of Awassi rams, and previous studies [10, 37] focused on using either soybean milk or pure chemical lecithin.

Results in Table (3) show the effect of dilution, cooling and storage periods on semen characteristics. All studied traits were significantly ($p \leq 0.05$) altered within dilution, cooling and days of storage in 5˚C. Significant ($p \leq 0.05$) and steadily decrease in motility have been shown over the advanced stages, pH also significantly decreased ($p \leq 0.05$) to reach the lowest value (6.11) after three days of storage. Big significant increase ($p \leq 0.05$) in dead sperm percentage (34.16%) have been shown after three days of storage in which abnormal sperm and acrosome defect reached 28.00 and 29.19% respectively. Generally, these results indicate to the deterioration take place by dilution, cooling process and storage periods, usually it's true due to the metabolism activity of spermatozoa and their production of poisons and free radicals [38], in addition to the osmotic corruption and structural damage of the plasma membranes of spermatozoa [39, 40].

Table (3): Effects of dilution, cooling and storage periods in 5˚C on Awassi semen characteristics (independent of SL treatment) (Mean± SEM).

Criteria / Stages	Progressive Motility%	pH	Dead Sperm%	Abnormal Sperm%	Acrosome Defect%
Fresh Semen	90.00 a ± 3.67	6.93 a ± 0.14	10.00 d ± 3.56	4.66 d ± 3.46	5.83 d ± 2.33
After dilution	85.06 ab ± 1.84	6.78 a ± 0.43	10.94 cd ± 2.35	7.27 d ± 2.10	8.58 d ± 1.51
After cooling	80.35 b ± 2.28	6.52 b ± 0.58	14.08 bc ± 2.55	10.05 cd ± 2.56	13.44 c ± 2.01
After one day storage	72.65 c ± 3.34	6.35 bc ± 0.47	16.52 bc ± 2.53	13.19 c ± 2.83	15.69 c ± 1.94
After two days storage	57.63 d ± 3.91	6.27 c ± 0.48	18.16 b ± 2.66	22.47 b ± 2.40	20.47 b ± 1.92
After three days storage	22.07 e ± 3.53	6.11 c ± 0.48	34.16 a ± 2.30	28.00 a ± 2.23	29.19 a ± 2.40

Different superscripts within column are significantly different ($P \leq 0.05$).

However, the decline of semen quality within three days of storage in refrigerator was in line with the finding of [41] that there are no extender able to save quality and fertilizing capacity of spermatozoa for more than 2-3 days without decline, a fact confirmed by [26,27] when reported significant changes in survivability and abnormalities due to energy deplete and pH decrease with the advance of the storage periods. In conclusion, soybean oil could substitutes egg yolk in citrate based extender with a successful method of direct introduce into the extender to prepare extenders free of animal components sources and there are significant deterioration in semen quality due to dilution, cooling and storage in 5˚C for three days.

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