J. of Animal and Poultry Production, Vol. 1 (2): 61 - 69, 2010

SOYMILK-BASED EXTENDER FOR CRYOPRESERVATION OF BOVINE SEMEN

El-Keraby, F. E.; K.T. Osman ; H. B. Ganah and E. M. El-Siefy
Anim. Prod. Res. Inst., Agric. Res. Center., Ministry of Agric.

ABSTRACT

The appraisal of incorporating whole soy-bean milk (SBM) instead of the traditional egg yolk (EY) in bovine semen extenders was investigated. Semen ejaculates from five clinically normal and sexually mature Holstein bulls were used. Two tris-based extenders titrated with citric acid (mono-hydrate) were used. Both extenders were similar in all ingredients except in that one of them contained 15% EY and the other contained 5% SBM. Efficiency of both extenders was evaluated on the basis of sperm viability during each step of semen freezing and after thawing. The initial bacterial counts in the raw EY & SBM, in the extenders, in the extended semen and in the post frozen / thawed semen were also determined .To confirm the laboratory results, an AI fertility trial using 548 Friesian cows was conducted and conception rates of the inseminates from both extenders were compared.

The post thaw sperm motility was significantly ($p< 0.05$) higher in SBM than in EY frozen/thawed semen. The recorded values for both estimates were 48.4±1.2 and 43.4±1.0%, respectively. Bacterial counts in the raw cryo-protectants, instant extenders, extended semen and in the post-frozen/ thawed semen were all in-favor of SBM extender ($p<0.001$).

The AI conception rate in the cows inseminated with SBM frozen semen was higher (64.3%) than that recorded for those inseminated with EY frozen semen (57.6 %).The difference was statistically significant ($p>0.05$).The results of this study showed that replacing the traditional EY by SBM in formulating the proposed extender resulted in a lower bacterial counts in the extended and post-thawed semen, better sperm post-thaw motility and higher AI conception rates. It is concluded that the use of whole SBM extenders (as free animal-source media) could be recommended for sanitary and efficient freezing of bovine semen.

Keywords: Bovine semen, Cryo-preservation, Extenders, Microbial contamination, Soymilk, Lecithin, Egg yolk Sperm viability, Conception rates.

INTRODUCTION

Artificial insemination (AI) is the most efficient tool ever devised for improving productivity and reproductivity of cattle. Freezability of bovine semen is affected by multi-various factors. Among them, are: diluent composition, type and concentration of the cryo-protectant used, method of adding the cryo-protectant, equilibration period of spermatozoa, packaging system of diluted semen, freezing rate and thawing regimen adopted (Foote, 1970). The two main challenges in the laboratory settings during freezing of semen are pathogenicity of semen/extender media and viability of the frozen spermatozoa (Aires *et al.,* 2003 and Fukui *et al,* 2008).

Egg yolk and/or milk have long been used as fundamental constituents in almost all bovine semen extenders used. In the recent years there has been frequent argument against incorporating substances of animal-origin in formulating semen extenders (Bousseau *et al.*, 1998; Aires *et al.*, 2003 and

Aboagla and Terada, 2004). These authors mentioned that the presence of EY in the freezing media may interfere with the integrity of the freezing process of spermatozoa. They related that to the wide variability of these substances in their composition, source and degree of bacterial or mycoplasma contamination. Such contamination could be a possible cause of indo-toxins capable of impairing fertilizing capacity of spermatozoa (Bousseau *et al.,* 1998). It has also been presumed that addition of EY to semen extenders may adversely affect the acrosomal integrity of spermatozoa (Aboagla and Terada, 2004). The accumulative results of these studies have raised a lot of questions waiting for answers. Of them, are: 1- Does successful freezing of bovine semen necessitate the presence of the conventional EY and /or milk in the freezing media? 2- What are the probable drawbacks of them? 3- In the light of the recent debates on the bio-safety aptitude of animal-origin substances, could the plant-origin sources be the other alternative choice for safer application of AI? 4- What are the most efficient substitutes to be used?

Quite a bit of efforts aimed at controlling the possible drawbacks hovering the sanitary use of EY and milk in semen extenders (Bousseau *et al.,* 1998 and Aires *et al.,* 2003). These efforts came out with a series of commercial sterilized extenders available in the market today (Biociphos plus, IMV, France and Andro Med, Mini-tub, Germany). These sterilized extenders contained soy-lecithin extract instead of the traditional EY and /or milk.

This study was conducted to evaluate the impact of using whole SBM (as a safer plant-source ingredient) instead of the traditional EY in bovine semen extenders. Besides, the fact that soymilk is cheaper and more available than the soy-lecithin extract should also be regarded.

MATERIALS AND METHODS

This work was conducted at the International Livestock Management Training Centre – Sakha (ILMTC), Animal Production Research Institute (APRI), Agriculture Research Centre, Ministry of Agriculture, Egypt.

Soy-bean milk preparation

Ten grams of Soybean grains was washed, soaked in 100 ml distilled water and boiled for 30 min. After boiling, the water was discarded, the whole soybean grains washed again and finally cooled down with 50 ml distilled water containing 0.25% $NaHCo_3$. The grains were then grounded in a blender for 5 min and the slurry cooled. Soymilk was extracted by filtration through a clean cotton cloth, centrifuged and boiled again for 10 minutes. The slurry was allowed again to cool down. Then, antibiotics were added at the rate of 0.25 g Lincospectin and 0.005 g streptomycin/100 ml of the slurry. After all, the SBM extender was ready for use.

Semen collection and evaluation

Five sexually mature Holstein bulls were used in this study. All bulls were clinically normal and free from venereal diseases. The semen was collected by means of the artificial vagina and immediately transferred to the lab. The ejaculated semen was incubated in a 37°C water bath and evaluated

according to the standard measures applied on the center. Specimens with ≥ 70% initial sperm motility and ≥ 800×10^6 sperm/ml were only used for further freezing.

Semen extension

Two comparable extenders (EY and SBM) were formulated. Both extenders were similar in osmotic pressure (330 m osmol) and pH (6.8). They were also similar in their all contents except in the type of cryo-protectant used. One extender contained 15% EY (served as control), while, the other contained 5% whole SBM and zero EY. The composition of each 100 ml of each extender also contained 3.025 g Tris (hidroxymethyle) amino methane, 1.675 g citric acid (mono-hydrate), 7 ml glycerol, 0.75 g glucose, 0.25 g lincospectin and 0.005 g streptomycin. All contents were thoroughly dissolved in bi-distilled water (up-to 100 ml).

The semen was extended in a one-step manner (Osman, 1996). Split ejaculates were prepared and extended separately with each extender, so that, both extenders were represented in each ejaculate from each bull. The extension rate was determined on the basis of ejaculate volume, sperm progressive motility, sperm concentration/ml and the packaging-unit capacity (0.25 ml German straws). Calculations were made, so that, each insemination dose contained 20×10^6 motile spermatozoa/straw before freezing.

Semen freezing

In a conventional manner, the straws were labeled, loaded with extended semen (allowing one cm air-space column in each straw) and finally sealed with polyvenile alcohol powder. The loading and sealing of the straws were performed at 37°C. The sealed straws were immediately dumped into a 37°C water bath. The water bath containing the straws was transferred to a 5°C refrigerator and left there for 4 hours as an equilibration period. By the end of the 4-hours equilibration period, the straws were removed from the water bath, dried out and spread horizontally onto a straw rack. The rack holding the straws was then transferred into a liquid nitrogen (LN) processing container and located horizontally in static nitrogen vapor 4 cm above the surface of LN. After ten minutes of exposure to the vapor, the straws were completely dipped into the LN (at −196°C). The post-thaw motility of the frozen spermatozoa representing both extenders was evaluated. The frozen spermatozoa were thawed at the rate of 37°C for 20 second. Five straws from each treatment from each bull were assessed for sperm post-thaw motility and the overall means recorded.

Bacteriological procedure

Bacterial counts were performed in the raw SBM, raw EY, raw semen, instant SBM extender, instant EY extender and in the diluted semen in both extenders. Bacterial counts were assessed in the frozen/thawed semen and also 4 hours after thawing.

The medium used for the determination of viable bacterial counts was prepared by dissolving 23 g of dehydrated nutrient agar (Gilco lab USA) in 1000 ml distilled water and heating to boiling point. The pH was titrated to 7.0 and the medium autoclaved at 121°C under 15 Hg/inch pressure for 20 minutes. After cooling down to 50-55°C, 10% fresh de-fibrinated blood was

added and subsequently poured in sterilized petri dishes. To assure the sterility of the media, Petri dishes were kept in an incubator at 37°C for 24 hours and the viable bacterial counts were determined in all samples using the spread plate method (Harry and Paul, 1981). Equal volumes (0.5 ml) of each of the raw ingredients (EY, SBM or semen) were added into a test tube containing 4.5 ml Phosphate Buffer Saline in a step-wise manner so that, dilution rates of 1:10, 1:1000 and 1:10,000 dilution were obtained. Using a double set of petri dishes for each dilution, half a ml from each sample was spread on the nutrient agar plates.

The presumptive coliform bacterial counts were assessed using Maconkey agar media. The presumptive staphylococcus aureus was counted on Baired Paker agar media with sheep blood for appearance of haemolysis.

All plates were then incubated at 37°C for a period of 24 hours and the number of colonies arose counted by colony counter. The number of bacteria present in each Petri dish was calculated by multiplying the number of the colonies by the dilution rate at which the colonies were developed. The final results were expressed as CFU/ml according to Qureshi *et al.*, (1993).

Field study

The results of conception rates representing the two extenders were relied-upon as an emphasizing criterion for the efficiency of freezing procedure. Five-hundred forty eight mature Holstein cows were randomly inseminated with the semen frozen in both extenders. The cows were inseminated at the detected estrus using the recto-vaginal technique. The AI pregnancy rates-to the first insemination were recorded. Pregnancy diagnosis was performed per-rectum two months after insemination. Pregnancy rates were calculated in percentage by averaging the number of cows conceived with the number of cows inseminated.

Statistical analysis

The data were subjected to statistical analysis using SAS computer program. The overall means ± SE for all parameters studied were computed. The analysis of variance was also computed using the general linear models procedure of SAS (GLM/SAS, 1998). When treatment effects were significant, the individual means were compared by Duncan's Multiple Range Test (Duncan, 1955). The χ^2 test was used to determine the statistical significance between the traits expressed as percentages.

RESULTS AND DISCUSSION

Bacterial count

The overall mean bacterial counts in the raw EY, raw SBM, raw semen, as well as, in the instant EY and SBM extenders are shown in table 1.The coliform and staph aureus counts were significantly higher ($p< 0.001$) in the raw EY than in the raw SBM. The counts for both types of bacteria in the raw SBM were found to be zero. The raw collected semen showed higher bacterial counts than that found in the raw EY. The differences in this trait between the individual bulls were statistically significant ($p<0.001$).The overall mean bacterial counts recorded for the five bulls were 7333 ± 271.6, 5817 ±

913.8, 15167 ± 833.3, 2917 ± 800.2 and 16333 ± 666.7 CFU/ ml, respectively. These results agreed with the early findings of Bush *et al.* (1950) who reported wide variations in bacterial counts in both raw and diluted semen from individual bulls. On the other hand, the current bacterial counts were generally lower than those recorded by Qureshi *et al.* (1993). Variations in this trait could be attributed to the differences in the hygiene and sanitation measures applied on the different farms. The probable differences in the inherited sensitivity of bulls to bacterial infections may be regarded as another factor.

Table 1: Overall mean bacterial counts ± S.E (CFU/ml) in raw EY, raw SBM, raw semen and in the instant EY and SBM extenders.

Item	Bacterial Counts (CFU)/ml				
	Raw EY	Raw SBM	Raw semen	Instant EY extender	Instant SBM extender
Coliform	41.5±4.5 ^{b}	0.0±0.0 ^{d}	1202±177 ^{a}	3.0±0.4 ^{c}	0.0±0.0 ^{d}
St. aureus	29.0±2.4 ^{b}	0.0±0.0 ^{c}	96.1±13.6 ^{a}	0.0±0.0 ^{c}	0.0±0.0 ^{c}
Total Count	1362.0±112 ^{b}	0.0±0.0 ^{d}	9493±1031 ^{a}	27.4±3.1 ^{c}	0.0±0.0 ^{d}

Means with different superscripts in the same row are significantly different (p<0.001). Means with similar superscripts in the same row are not statistically different.

The overall mean total bacterial count in the instant EY extender was significantly higher than that of the instant SBM extender (p<0.001). It was worth mentioning that the antibiotics of the EY-based extender did not totally suppress bacterial contamination in the instant extender. These results came in complete agreement with those reported by Qureshi *et al.* (1993) and Bousseau *et al.* (1998) who detected one or more types of bacteria (including coliform, staph aureus or mycoplasma) in each of raw semen, fresh EY, fresh milk and instant extenders.

The overall mean bacterial counts in the extended semen during freezing and after thawing are shown in Table 2. The total bacterial count in the EY-extended semen (at zero time) was significantly (p<0.001) higher than that recorded for SBM-extended semen (201.0 ±13.9 vs. 69.2±4.8 CFU/ml, respectively).Similar trends were also found for both extenders (four hours after extension at 5°C) and four hours post-thawing (at 35°C). The differences between both extenders in the total bacterial counts, as well as, in the counts of coliform bacteria were statistically significant (p<0.001) favoring the SBM extender. It was also shown that staph aureus bacteria were totally absent in the extended semen during all steps of extension, freezing and after thawing. No bacterial contamination with coliform was detected in SBM-extended semen after 4 hours of chilled incubation and after thawing. It was also noticed that the antibiotics of EY-extender did not totally suppress bacterial contamination of the extended semen, both, during chilling and after thawing. In agreement to these results, Bousseau *et al.* (1998) reported that the soymilk-lecithin containing extender was superior to that contained EY with regard to bacterial contamination of both extended & frozen/post-thawed semen. Similar trends were also reported by Wagtendonk

et al. (2000); Aires *et al.* (2003) and Fukui *et al.* (2008) using soymilk-lecithin extract instead of EY in formulating bovine semen extenders.

Table 2: Overall mean bacterial counts ± S.E (CFU) in extended semen with EY or SBM extenders.

Item	Extended Semen Bacterial Counts(CFU/ml)					
	At zero time		After four hrs at 5°C		Four hrs after thawing	
	EY	SBM	EY	SBM	EY	SBM
Coliform	10.4 ± 1.0 [a]	9.0 ± 0.9 [a]	7.2 ± 0.7 [b]	0.0±0.0 [c]	5.4 ± 0.4 [b]	0.0±0.0 [c]
Staph aureus	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ±0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ±0.0 [a]
Total count	201.0±13.9 [d]	69.2±4.8 [b]	92.0±6.3 [c]	30.0±1.9 [a]	68.0±3.5 [b]	42.3±2.0 [a]

Means with different superscripts in the same raw are significantly different (p < 0.001).

Sperm post-thaw motility

The impact of extenders and of individual bulls on the percent post-thaw motility is shown in Table 3. The differences in this trait among the individual bulls were statistically significant (p<0.05). However, this finding was not new and previously reported by the vast majority of workers (Foote,1970.; Bhandari et al., 1982 and Richardson et al., 1992). They attributed such variation to the differences in the genetic make-up of the individual bulls.

The overall mean percent post-thaw motility of spermatozoa frozen in SBM-extender (48.4±1.0%) was higher than that frozen in EY-extender (43.4±1.0%). The differences were statistically significant (p<0.05).

Table 3: Sperm post-thaw motility as affected by extender and individual bulls.

Extender	Bull No.					Overall mean
	1	2	3	4	5	
EY	38.0±1.2	47.0±1.2	52.0±1.2	42.0±1.2	38.0±1.2	43.4 ± 1.0 [b]
SBM	42.0±1.2	53.0±1.2	56.0±1.0	47.0±1.2	44.0±1.0	48.4 ± 1.2 [a]
Mean±SE	40.0±1.1 [c]	50.0±1.2 [a]	54.0±1.2 [a]	44.5±1.0 [b]	41.0±1.1 [c]	45.9±1.0

Means with different superscripts in bottom row are significantly different (p<0.05).
Means with different superscripts in the right row are statistically significant (p<0.05).

In agreement to these results, the post-thaw motility of bovine spermatozoa frozen in soymilk-lecithin extenders was reported to be superior to that frozen in EY-based extenders (Bousseau *et al.*, 1998; Wagtendonk *et al.*, 2000 and Aires *et al.*, 2003). It was also found that addition of EY to semen extenders may adversely affect the acrosomal integrity of post-thawed spermatozoa of several species including sheep (Watson, 1981); buffalo (Kumar *et al.*, 1993) and goat (Aboagla and Terada, 2004). The results of these studies came out with a dramatic variability in the adverse effect of EY on sperm post-thaw motility and acrosome integrity. This variability could possibly be attributed to the wide variations in the EY content of low-density lipoproteins responsible for the protection of plasma membranes of spermatozoa during freezing (Watson, 1981 and Bousseau *et al.*, 1998). However, the implications encountered with the use of EY in formulating semen extenders are many and interrelated. Variability in composition, difficulty to standardize and high vulnerability to microbial contamination are the main EY drawbacks that interfere with sperm viability and acrosome

integrity before and after freezing (Wagtendonk *et al.*, 2000; Aires *et al.*, 2003 and Fukui *et al.*, 2008). In addition, the fact that EY of semen extenders can increase the incidence of sperm acrosome reaction (resulting in reduced sperm post-thaw motility and acrosome integrity) should also be considered (Aboagla and Terada, 2004).

Pregnancy rates

The results of 548 inseminations representing both extenders are shown in Table 4. As shown from the table, the overall pregnancy rate-to the 1st insemination was (60%). It could also be seen that pregnancy rate for SBM-frozen semen was higher (64.3%) than that recorded for EY-frozen semen (57.6%). The difference was statistically significant ($p<0.05$). The current AI field results came in a partial agreement with the findings of Wagtendonk *et al* (2000). Meanwhile, they were almost similar to those reported by *Aires et al. (2003)* using two synthetic tris-based extenders containing either soymilk-lecithin extract or the traditional EY. In the same vein, Fukui *et al.* (2008) concluded that EY could be safely replaced by soymilk-lecithin extract in formulating ram semen extenders without reducing fertility of the frozen/thawed semen.

It was early reported that glycerol of semen extenders may interact with or possibly cross-link the hydrophilic ends of lecithin molecules of EY, thus potentiating the cryo-protective effect on spermatozoa (Buckingham and Staehelin, 1969). More recently, soybean-lecithin extracts of ready-to use extenders were found to contain a considerable content of EY-like phospholipids (Bousseau *et al.*, 1998 and Anel *et al.*, 2006). Yet, the exact role of lecithin/glycerol interaction and of phospholipids in protecting sperm plasma membranes during freezing has not been fully understood. However, it could be concluded that the current proposed extender can provide sufficient protection for spermatozoa during freezing, thus rendering their fertilizing capacity normal and satisfactorily matching to that achieved with EY-based extenders.

Table 4: Pregnancy rate-to the first insemination with frozen semen using EY or SBM based extender

Type of Extender	Number of cows		First insemination Pregnancy rates
	Inseminated	Pregnant	
SBM	199	128	64.3 % [a]
EY	349	201	57.6 % [b]
Total	548	329	60.0%

Percentages with different superscripts in the right column are significantly different ($p<0.05$)

The current results have demonstrated that the whole-SBM extender is superior to that of EY in all parameters studied, including, hygienisity, sperm-post-thaw motility and fertility. To the best of our knowledge, this is the first attempt to introduce the whole-SBM extender as a safer and efficient extender for freezing bovine semen. It is also expected that the use of whole-SBM would be more economic as compared to the commercial soymilk-extract. Such that, the current proposed extender could be recommended for use in freezing bovine semen.

ACKNOWLEDGEMENT

The authors wish to express their appreciation to Dr. Azza M. El-Baz, senior researcher of microbiology, International Livestock Management Training Center, Sakha, Animal Production Research Institute, for her valuable contribution in the microbiological investigations of this work.

REFFERENCES

Aboagla, E. M. and Terada, T. (2004). Effects of egg-yolk during the freezing step of cryopreservation of the viability of goat spermatozoa. Theriogenology, 62: 1160 – 1172.

Aires, V. A.; Hinsch, K.D.; Mueller-Schloesser F.; Bonger K.; Mueller Shoedder S. and Hinsch, E. (2003). In vitro and In vivo comparison of egg yolk-based and soybean Lecithin-based extenders for cryopreservation of bovine semen. Theriogenology, 60: 269 – 279.

Anel, L.; Alvarez, M.; Martinez-Pastor F.; Garcia-Macias V.; Anel, E. and de Paze. (2006). Improvement strategies in ovine artificial insemination. Reprod. Dom. Anim., 41:30-42.

Bhandari, N.; Chavhan, R.A. and Mathew, A. (1982). Note on the effect of rate of freezing on the Freezability of bull spermatozoa . Ind. J. Anim. Sci., 52: 812.

Bousseau, S.; J. P.; Brillard, B.; Marquant-le Guienne, B.; Guérin, A. Camus and M. Lechat (1998). Comparison of bacteriological qualities of various egg yolk sources and the in vitro and in vivo fertilizing potential of bovine semen frozen in egg yolk or lecithin based diluents. Theriogenology, 50: 699-706.

Bush, L. J.; Ludwick,T.M.; Fergupon, L.G. and F. Ely (1950). The effect of bacteria on fertility of bovine semen. J. Dairy Sci., 33: 633.

Duncan, D. B. (1955). Multiple range and multiple F-test. Biometrics, 11:1-42.

Foote, R.H. (1970) Influence of extender, extension rate and glycerolizing technique on on fertility of frozen bull semen. J. Dairy Sci, 53:1478.

Fukui, Y.; Kohno,H.; Togary,T.; Hiwasa, M. and Okabe,B (2008). Fertility after artificial insemination using a soybean-based semen extender. J. Rrproduction and Development, 54,4 :286-289.

Harry, W.S.L. and Paul, J.V. (1981). Microbes. In: Action Laboratory Manual of Microbiology., 3rd ed. pp 37-40. Freeman and Co. Son Fran. London.

Kumar, S.; Sahni, K. L. and Mohan, G. (1993). Effect of different extender formulations on acrosomal maintenance of buffalo spermatozoa frozen in milk, tris, and sodium-citrate dilutors. Ind. J. Anim. Sci., 63:1233-1239.

Osman, K.T. (1996). Some reproductive aspects of Egyptian buffalo bulls. Ph.D. Thesis, Fac. Agric. Ain Shams Univ. Cairo, Egypt.

Qureshi, Z. I.; Ala-Ud-Din.; Lodhi, L.A.;Sahahid, R.U. and Naz, N.A. (1993). Comparative effect of different levels of gentamycin in viable bacterial count of cow bull semen. J. of Islamic Academy of Sci, 6:2, 114 – 117.

Richardson,G.F.; Donald, A.W. and MacKinnon, C.E. (1992). Comparison of different techniques to determine the percentage of intact acrosomes in frozen thawed bull semen. Therio., 38: 557.

SAS (1998). SAS users guide: Statistics SAS Instit. Inc., Cary., N.C.
Wagtendonk-de leeuw A.M.V; Haring, R.M.; Kaal-Lansbergen L.M. and den Das, J.H. (2000). Fertility results using bovine semen cryopreserved with extenders based on egg yolk and soy bean extract. Theriogenology, 54: 57-67.
Watson, P.F. (1981). The role of lipid and protein in the protection of ram spermatozoa at 5°C by egg yolk lipoprotein. J. Reprod. Fert. 2:337-340.

إستخدام مخفف لبن الصويا فى تجميد السائل المنوي للأبقار

فكرى السيد القربى، خالد توفيق عثمان، هانى بكر جناح و الشناوى محمد الصيفي
معهد بحوث الانتاج الحيوانى - مركز البحوث الزراعية - وزارة الزراعة - الدقي .

أجرى هذا البحث لدراسة تأثير استبدال صفار البيض كأحد المكونات التقليدية لمخففات السائل المنوى البقرى- بلبن فول الصويا الكامل- بغرض خفض حد التلوث البكتيرى للسائل المنوى المجمد- من جهه ، وتلافيا للتأثيرات المحتملة للمكونات ذات الأصل الحيوانى- من جهة أخري.

وقد أجريت هذه الدراسة بمركز تدريب رعاية الحيوان بسخا - بأستخدام خمسه طلائق فريزيان ناضجة جنسيا، وسليمه من الناحية الصحية - حيث تم جمع السائل المنوى منها، وتقييمه وتخفيفه بأحد مخففى: صفار البيض (١٥%) او لبن فول صويا الكامل (٥%). هذا، وقد تم اجراء عد بكتيري لكل من صفار البيض الخام ، لبن الصويا الخام ، السائل المنوى الخام ، مخفف صفار البيض ، ومخفف لبن الصويا. كذلك فقد تم اجراء عد بكتيرى للسائل المنوى المخفف بكل من المخففين خلال مراحل التجميد ، ومابعد الاسالة . كذلك، فقد تم تقدير الحركة التقدمية للحيوانات المنوية بكل من المخففين خلال مرحلة ما بعد الاسالة. ولتأكيد المشاهدات المعملية- فقد أجريت دراسة حقلية تم من خلالها تلقيح عدد ٥٤٨ بقرة فريزيان اصطناعيا بأستخدام السائل المنوى المجمد الممثل لكل من المخففين.

وقد أظهرت نتائج الدراسة- إرتفاعا معنويا ($p<001$) فى المحتوى البكتيرى لكل من صفار البيض الخام ، والسائل المنوى الخام- مقارنه بالمحتوى البكتيرى للبن فول الصويا الخام- حيث وصل العد البكتيرى للاخير الى الصفر تقريبا. كذلك، فقد ارتفع المحتوى البكتيرى للسائل المنوى المخفف بصفار البيض الي 92.0±6.3 CFU/ml مقارنة بالسائل المنوى المخفف بلبن الصويا الكامل (±0.7 CFU/ml 7.2).

أظهرت الحيوانات المنوية المجمدة باستخدام مخفف لبن الصويا الكامل- تفوقا فى معدلات الحركة التقدمية للحيوانات المنوية المسالة- مقارنة بتلك المجمدة بمخفف صفار البيض (٤٨,٤ ±١,٤ مقابل ٤٣,٤ ±١,٠ %) على الترتيب.

تفوقت معدلات الحمل للسائل المنوى المجمد باستخدام مخفف لبن الصويا علي تلك المتحصل عليها باستخدام مخفف صفار البيض (٦٤,٣ مقابل ٥٧,٦%- علي الترتيب) ، وكانت الاختلافات معنوية ($p<0.05$).

هذا، وقد خلصت الدراسة إلى إمكانية استخدام لبن الصويا الكامل (ذو الأصل النباتى) كبديل لصفار البيض (ذو الأصل الحيوانى) فى مخففات السائل المنوى للأبقار- حيث أظهر المخفف المقترح تفوقا على المخفف المحتوى على صغار البيض فى جميع الصفات المدروسة- متضمنة الحركة التقدمية للحيوانات المنوية المسالة ، معدلات الحمل لأول تلقيحة ، انخفاض المحتوى البكتيرى للمخفف ، علاوة على الإنخفاض المتوقع للتكلفة مقارنه بالمخففات التجارية الأخري المحتوية على مستخلصات لبن الصويا ، وكذلك- تلافيا للاختلافات في محتويات مكونات المخفف التقليدية ذات الأصل الحيواني التي قد تتداخل مع كفائة المخفف.

قام بتحكيم البحث

أ.د / مصطفى عبد الحليم الحرايرى — كلية الزراعة - جامعة المنصورة
أ.د / مصطفى قطب البنا — مركز البحوث الزراعية