



## Effect of Duck Egg Yolk on Cryopreservation and Fertility of Egyptian Buffalo Bull Semen

**Mohamed E. El-Sharawy**

Fac. of Agric., Kafrelsheikh Univ., 33516,  
Egypt. e-mail/elsharawy78@yahoo.com

**El-Shenawy M. El-Seify**

Anim. Prod. Research Institute,  
Agric. Research Center, Giza, Egypt.

**Ahmed M. Hussien**

Anim. Prod. Research Institute, Agric.  
Research Center, Giza, Egypt.

**Mohamed El-Sherbieny**

Anim. Prod. Research Institute, Agric. Research Center, Giza,  
Egypt.

**Ibrahim S. El-Shamaa**

Fac. of Agric., Kafrelsheikh Univ., 33516,  
Egypt.

**Abstract:** The objective of this study was to compare the effectiveness of different duck egg yolk (DEY) concentrations (10, 15 and 20% DEY) with chicken egg yolk (20% CHEY) on the cryopreservation of Egyptian buffalo spermatozoa following dilution, equilibration and freezing-thawing processes. For this purpose, one ejaculate of semen from each of three Egyptian buffalo bulls was collected twice each week for 4 weeks with artificial vagina (42°C). Pooled ejaculates were divided into four parts and were diluted in Tric citric acid glycerol extender containing either 10 or 15 or 20% DEY or 20% CHEY at 37°C. Extended semen was equilibrated for 4h at 5°C and then was filled in 0.5 ml straws and frozen in liquid nitrogen. Thawing of semen was performed at 37°C for 30s. Progressive sperm motility, live sperm and plasma membrane integrity (%) after different stages of cryopreservation were assayed. The results of post-thaw sperm motility for all extenders containing DEY showed significantly ( $P < 0.05$ ) higher values (52.1, 59.6 and 52.5% for 10, 15 and 20% DEY, respectively) compared to 47.9% for control extender (20% CHEY). Also, post equilibration and post-thaw live sperm and plasma membrane integrity were significantly ( $P < 0.05$ ) higher in 15% DEY extender as compared with control extender (20% CHEY), the values being (78.4 vs. 70.7% and 85.8 vs. 79.8%) and (72.3 vs. 55.0 % and 80.7 vs. 69.1 %) respectively. Using a post-thawing semen containing 15% DEY yielded comparatively highest conception rate (65.8%) followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%). In conclusion, DEY compared to chicken egg yolk in extender improves the frozen-thawed quality of Egyptian buffalo bull spermatozoa and fertility rate.

**Keywords:** Duck Egg Yolk, Cryopreservation, Buffalo Bull Semen, Fertility.

### I. INTRODUCTION

Cryopreservation of domestic animal spermatozoa has been widely used for artificial insemination and egg yolk is one of the most commonly used cryoprotectants during the freezing- thawing process. Currently, egg yolk is a common component of most semen cryopreservation extenders for domestic animals. It has been shown to have a beneficial effect on sperm cryopreservation as a protectant of the plasma membrane and acrosome against cooled shock (Amirat, et al., 2004). Egg yolk is essential component for semen cryopreservation that has a cryoprotective role, but the egg yolk from different avian species utilized as cryoprotectant has shown improved the quality of cryopreserved semen (Anand, Yadav, & Shukla,

2014).

Recently several investigators have substituted chicken egg yolk with egg yolk from other a vain species as a component of media used for the cryopreservation of spermatozoa livestock (Bathgate, Maxwell, & Evans, 2006), (Webb, PAS Codi, Burris, Harmon, & Baker, 2011). Results obtained from these trials have been conflicting. Egg yolk from the duck proved superior to that of chicken for the cryopreservation of both stallion, and buffalo spermatozoa (Clulow, Maxwell, Evans, & Morris. L., 2007), (Andrabi, Ansari, Ullah, Anwar, Mehmood, & Akhter, 2008). In contrast opposite results, Su et al., (Su, et al., 2008) obtained when a similar study was conducted using bull spermatozoa. It is suggested that the differences in the protective effect with EY of different avian species are attributed to the differences in biochemical composition of the yolks in terms of their fatty acids, phospholipids and cholesterol contents. In stallion, Humes and Webb (Humes & Webb, 2006) reported that chukar (Alectorischukar) egg yolk yielded higher post-thaw motility than chicken yolk for cryopreservation of stallion spermatozoa. Ibrahim et al., (Ibrahim, El-Bawab, & El-Amrawi, 2012) Showed that DEY at level of 2.5% in diluents could improve bucks semen quality preserved at 5°C rather than CEY 2.5%.

Egg yolk (EY) is frequently used as a cryoprotective agent in mammalian semen diluents, and showed to be highly effective for the maintenance of sperm fertility in different species (Sansone, Nastri, & Fabbrocini, 2000), (Garde, Soler, Cassinello, Crespo, & Malo, 2003). The ultimate goal of laboratory evaluation of semen for use in AI is to predict the fertility achievable with the use of that semen. Thus, important factor of success in AI is semen quality represented in the original ejaculate (Saacke, 1983). The freezability has been widely used to indicate the ability of a sperm to with stand the stress of freeze thaw process. The freezability of bovine sperm could be evaluated through a wide variety of semen tests. This includes the assessment of one or more of semen traits which have been traditionally classified as viability or morphology related (Saacke, 1983), (Barth, 1992). In general, initial sperm viability has been positively correlated with sperm freezability and fertility (Barth, 1992), (Richardson, Donald, & Mackinnon, 1992) .

Fertility rate is considered to be the best parameter to assess the quality of frozen thawed semen (G., 1997). The

successful artificial insemination requires that a sufficient number of viable, fertile sperm are delivered at the site of fertilization in appropriate time (Amirat, et al., 2004).

The objective of the current study was to determine if substitution of chicken egg yolk with different concentration of duck egg yolk would improve post-thaw motility, percentage of plasma membrane integrity and fertility of Egyptian buffalo bull spermatozoa.

## II. MATERIALS AND METHODS

### A. Animals and semen collection

The experiment was carried out at the international livestock Management Training Center (ILMTC), Sakha Station belonging to the Animal Production Research Institute, Ministry of Agriculture, Egypt. Semen was collected from three adult and healthy Egyptian buffalo bulls (*B. bubalis*) of similar age group maintained under uniform managemental conditions. Semen was collected by artificial vagina at 42°C twice weekly for a period of 4 weeks (2 ejaculates x 3 bulls x 4weeks, replicates; n = 8). Ejaculates possessing more than 70% visual motility on each day collection were pooled in order to have sufficient semen for a replicate and to eliminate the bull effect. The semen was given a holding time of 10 min at 37°C in water bath before dilution. The pooled semen was diluted with the Tric extender containing 3.025 Tris (hydroxymethyl amino methane), 1.675 g citric acid, 0.75 g glucose, 7 ml glycerol, 0.005g streptomycin, 0.25g lincospectin and 20 ml chicken egg yolk or 10; 15, 20 ml duck egg yolk and completed with bi-distilled water up to 100 ml. after dilution, the semen was cooled and equilibrated for 4h at 5°C. Semen was then filled in 0.25 ml French straws using a semen filling machine. Straws were then plunged into liquid nitrogen (-196°C) and stored. After 24h storage, semen straws were thawed at 37°C for at least 30 sec. in water bath and then incubated at same temperature for 6 h to assess post thaw quality.

### B. Semen Evaluation:

Progressive sperm motility was estimated by adding one drop of semen after dilution, equilibration and freezing and thawing to a test tube containing 2 ml warm physiological saline (0.9% NaCl) and suspended in 30 °C water bath. The mixture was gently shaken and a drop of semen was taken from the test tube with a warm Pasteur pipette and placed on a worm slide. The drop was covered by a wormed cover slide and immediately examined under the 40x objective. The samples were graded according to the percentages of spermatozoa moving forward motion across the field of vision with normal vigorous swimming motion.

Live spermatozoa, immediately after dilution, equilibration and freezing and thawing a smear was made from a drop of semen stained by eosin negrosin mixture prepared as described by (Hancock, 1951) then dried by warm air. The percentage of live spermatozoa was calculated from total number 100 spermatozoa counted in different microscopic fields under magnification of (600x) and using hand telly counter.

The plasma membrane integrity of the tail of buffalo bull spermatozoa was evaluated using the hypotonic swelling test (HOST) as described by (Jeyendran, Van Der Ven, Perez-Pelaez, Crabo, & Zaneveld, 1984) after dilution, equilibration and freezing and thawing semen. The 100 µl semen was added to 1 ml of a hypo-osmotic solution prepared with 25mM Tri-sodium citrate dehydrate and 75 mM fructose in 100 ml distilled water. After incubation for 60 min at 37°C, sperm swelling was assessed by placing 15 µl of well-mixed sample on a warm slide 37°C which was covered with a cover glass before being observed under light microscopy at 400X magnification. Viable spermatozoa had coiled tails after HOST. At least 200 spermatozoa per slide were observed. The spermatozoa were classified as positive or negative based on the presence or absence of coiled tail.

### C. Fertility trail

A group of 58 buffalo-cows were artificially inseminated with randomly frozen- thawed semen extended with Tris 20% CHEY and 62, 76 and 54 buffalo-cows were randomly AI with frozen semen extended with 10%, 15% and 20% DEY, respectively. Each female was inseminated with a single frozen-thawed straw at detected estrus using the recto-vaginal technique. Pregnancy rate was performed per-rectum at two months after AI.

### D. Statistical analysis

Results were statistically analyzed according to SAS system (1985). The differences among means were tested using Duncan's new multiple range test (Duncan, 1955). The data on in vivo fertility rates were analyzed using Chi-square test.

## III. RESULTS AND DISCUSSION

### A- Progressive sperm motility (%)

The Result of progressive motility of buffalo bull spermatozoa at different stage of cryopreservation in different extenders shown in (Fig.1). Post dilution sperm motility did not differ among extender containing 20% CHEY (control) and extenders containing 10% DEY and 15% DEY, the values being 76.7+ 1.12, 74.6+ 0.74 and 77.1+ 0.14, respectively (Fig.1). This shows that DEY has no beneficial effects over CHEY immediately after dilution of semen. Moreover, increasing DEY to 20% significantly decreased sperm motility to 72.1+ 0.97 when compared with other extenders. Similarly, post equilibration percentage of sperm motility did not differ among extenders containing 20% CHEY, 10%. DEY and 20% DEY, the values being 67.6%, 65.8% and 66.7%, respectively, while extender containing 15% DEY had a higher ( $P < 0.05$ ) percentage of sperm motility, being 73.8 + 0.65. However, when post-thaw sperm motility after 24h storage in liquid nitrogen was considered, all extenders containing DEY showed significantly higher values (52.1, 59.6 and 52.5% for 10, 15 and 20% DEY, respectively) compared to 47.9% for control extender containing 20% CHEY ( $P < 0.05$ ). We attempted to optimize the concentration of duck egg yolk in extender.

The progressive sperm motility showed that the 15%

DEY in extender was the best concentration to provide the best cryoprotective action for Egyptian buffalo sperm among other three concentrations of tested. These findings are supported by those (Clulow, Maxwell, Evans, & Morris. L., 2007), who demonstrated that the motility parameters of stallion sperm are improved when the semen is frozen in extender supplemented with duck egg yolk rather than chicken egg yolk and (Waheed, Nazir, Najib, Hafez, Younis, & Sajid, 2012), who recorded improved sperm motility parameters when the stallion and Nili-Ravi buffalo semen were frozen in extenders containing DEY as compared to CEY. Also, Andrabet *al.* (Andrabi, Ansari, Ullah, Anwar, Mehmood, & Akhter, 2008) found the highest forward motility of buffalo bull spermatozoa at 6 h post-thaw in DEY extender compared to those having egg yolk from other avian species including guinea fowl, indigenous hen and commercial chicken. Similarly, duck egg yolk provided better sperm quality of rams in terms of motility, viability, abnormal sperm and membrane integrity than other avian egg yolks except chucker egg yolk in the study of (Kulaksiz, Cigdem, Ergun, & A., 2010).

The improvement in the post-thaw semen motility of Egyptian buffalo bull due to replacement of 20% CHEY with different concentration of DEY in the extender can be attributed to differences in the composition of egg yolk from the two a vain species (Waheed, Nazir, Najib, Hafez, Younis, & Sajid, 2012).

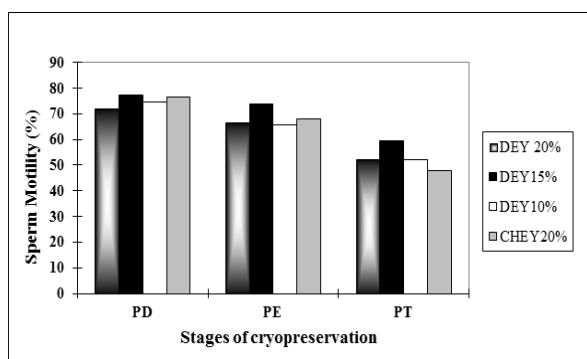


Figure (1): Progressive motility of buffalo bull spermatozoa at different stage of cryopreservation in different extenders. Different extenders DEY: Duck Egg Yolk, CHEY: Chicken Egg Yolk, Stages of cryopreservation PD: Post-dilution, PE: Post Equilibration, PT: Post Thawing.

Bathgate et al. (Bathgate, Maxwell, & Evans, 2006) reported that duck egg yolk have more monounsaturated fatty acids than chicken egg yolk. When unsaturated fatty acids integrated into plasma membrane of spermatozoa, the temperature at which plasma membrane undergoes phase transition from fluid mosaic state into gel (solid) state upon cooling will be lowered. Also, Choi et al. (Choi, Song, & Oh, 2001) found that DEY contained higher levels of cholesterol than CEY. Highest amount of cholesterol was observed in ostrich compared to partridge, duck and chicken EYs (16.29), (13.93), (10.81) and (13.91) mg/g of yolk, respectively (Kaźmierska,

Jarosz, Korzeniowska, Trziszka, & Dobrzański, 2005). Whereas, duck EY has more monounsaturated fatty acid than chicken EY (Bathgate, Maxwell, & Evans, 2006) and both duck and chicken EYs have a different ratio of the fatty acids comprising the total yolk lipids (Surai, Speake, Noble, & Mezes, 1999). The importance of cholesterol is that it protects spermatozoa against cold shock (Ladbrooke, Williams, & Chapman, 1968).

#### *B- Live spermatozoa and plasma membrane integrity.*

According to the results, 15% DEY had the best cryoprotective effect in terms of the highest live spermatozoa and plasma membrane integrity compared to the other three extenders evaluated at different stages of cryopreservation (Fig.2 and Fig.3). Post extension live sperm percentage and plasma membrane integrity did not differ between extender containing 15% DEY and control (20% CHEY) extender (82.3 vs. 81.0% and 89.0 vs. 88.41%) respectively. However, Post equilibration and post-thaw live sperm and plasma membrane integrity were significantly ( $P < 0.05$ ) higher in 15% DEY extender as compared with extender containing 20% CHEY, the values being (78.4 vs. 70.7% and 85.8 vs. 79.8%) and (72.3 vs. 55.0 % and 80.7 vs. 69.1 %) respectively, (Fig.2 and Fig.3).

The present findings are supported by (Waheed, Nazir, Najib, Hafez, Younis, & Sajid, 2012) who recorded improved post-thaw livability when the Nili-Ravi buffalo semen was frozen in extender containing DEY as compared to CEY, the values were 7.03+ 0.10 and 6.5 + 0.15h, respectively ( $P < 0.05$ ). Kulaksiz et al. (Kulaksiz, Cigdem, Ergun, & A., 2010) found that karayaka ram frozen semen extended in chicken egg yolk recorder lower percentages regarding sperm motility (35+ 1.6 vs. 47+ 2.5%), viability (50+ 3.4 vs. 58+ 2.6%) and membrane integrity (44+ 3.3 vs. 49.2+ 5.0%) as compared to DEY extender.

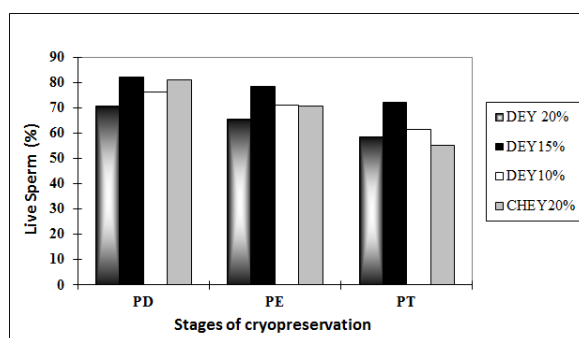


Figure (2): Live spermatozoa % at different stage of cryopreservation in different extenders.-Different Extenders DEY: Duck Egg Yolk, CHEY: Chicken Egg Yolk, Stages of cryopreservation PD: Post-dilution, PE: Post Equilibration, PT: Post Frozen –thawing.

According to Bathgate et al. (Bathgate, Maxwell, & Evans, 2006), the basic components of the yolks from chicken and duck eggs did not differ, but the ratios of fatty acids and phospholipids classes were different. Yolk from duck eggs had more monounsaturated fatty acid than yolk

from chicken eggs. Moreover, yolk from duck eggs contained more phosphatidylcholine than CEY. This supports the idea that low density lipoproteins present in the egg yolk stick to the sperm plasma membrane during freeze-thaw process, preventing loss of phospholipids through improving membrane tolerance for the freezing process (Parks & Graham, 1992). Spermatozoa subjected to cryopreservation are most sensitive to a rapid reduction in temperature i.e. cooling rate especially from 25 to 5°C (Watson, 1981), this produces cold shock, a membrane transition phase behavior is exhibited by biological membranes (Morris & Clark, 1987). Cold shock results in a loss of selective permeability, integrity of the plasma membrane and release of intracellular enzymes (Ortman, Rodriguez, & Martin, 1994). On contrast, Su et al. (Su, et al., 2008) found that frozen-thawed bull sperm progressive motility and sperm viability were significantly higher in extender containing chicken egg yolk as compared to DEY, the values being 48.9 vs. 37.1 and 53.3 vs. 42.6%, respectively. It is suggested that the improvement or decline in post-thaw quality of mammalian spermatozoa with EY of different avian species in freezing extender is attributed to the differences in biochemical composition of the yolk (Trimeche, Anton, Renard, Gandemer, & Tainturier, 1997), (Bathgate, Maxwell, & Evans, 2006), (El-Sheshtawy, E., Alaa, & El-Natat, 2010).

Egg yolk during sperm cryopreservation act as protectant of the plasma membrane and acrosome against temperature related injury in association with the other components (Amirat, et al., 2004). It is believed that the beneficial role of EY in sperm cryopreservation can be attributed to phospholipids, cholesterol and low density lipoproteins (LDL) contents which afford successful protection to the sperm against cold shock and the lipid-phase transition effect during the freeze-thaw process (Lanz & Komarek), (Moussa, Martinet, Trimeche, Tainturier, & Anton, 2002). These findings have opened up new opportunity to improve cyopreservation efficiency by replacing hen egg yolk with egg yolk from different avian species (domestic chicken, goose, turkey, duck, Japanese quail and chucker) in the extender and to study its effect on semen cryopreservation (Anand, Yadav, & Shukla, 2014).

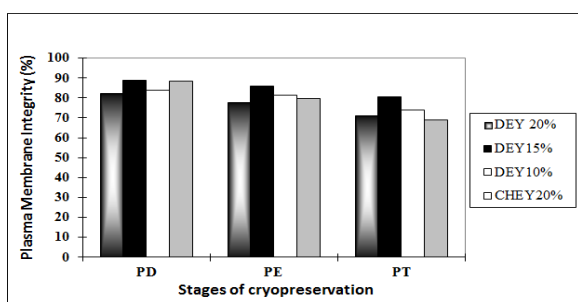


Figure (3): Plasma membrane of buffalo bull spermatozoa at different stage of cryopreservation in different extenders. Different Extenders DEY: Duck Egg Yolk, CHEY: Chicken Egg Yolk, Stages of cryopreservation PD: Post-dilution, PE: Post Equilibration, PT: Post Frozen-thawing.

### C: Fertility of frozen buffalo Semen.

A total of 58, 62, 76 and 54 buffalo females were artificially inseminated with frozen-thawed semen extended in 20% CHEY, 10% DEY, 15% DEY and 20% DEY, respectively, (Fig.4). High conception rate was recorded with the use frozen-thawed semen containing 15% DEY, being 65.8% followed by 20% DEY (59.3%), 20% CHEY (58.6%) and 10% DEY (58.1%), but the differences were not significant.

Fertility is the only single measure, ever used for accurate evaluation of any artificial insemination program. It should be mentioned that achieved more 50% in the current study is satisfactory as compared to the previous studies using various freezing and thawing techniques. The fertility rate is considered to be the best parameter to assess the quality of frozen thawed semen (G., 1997).

The conception rate in this study for DEY 15% (65.8%) was higher than the results in Egyptian buffalo semen as follow: Osman (Osman, 1996) found that conception rate of frozen semen was 60.4%. Also our results were rather than El-Amrawi, (El-Amrawi, 1997) who examined the fertility of semen thawed by different procedures and found that the best fertility rate (64.5%) for semen thawed at 35°C for 60sec. In another study, El-Siefy (El-Siefy, 2004) reported that the overall mean conception rate of the artificially inseminated buffalo cow was 61.4%. In our previous studies, we reported that fertility rates for Egyptian buffalo bulls were higher with semen cryopreserved in extender containing 12% LDLs compared with the control (egg yolk 20%) (72.7% vs. 50%, respectively) (El-Sharawy, El-Shamaa, Ibrahim, & El-Seify, Using of Low Density Lipoproteins and Glutamine to Improve Frozen Buffalo Bull Semen and Fertility, 2012a (Abstract).) - (Mostafa, et al., 2014). According to Vale, (G., 1997) suggested that a pregnancy rate higher than 50% can be regard as a good result after insemination with frozen- thawed semen.

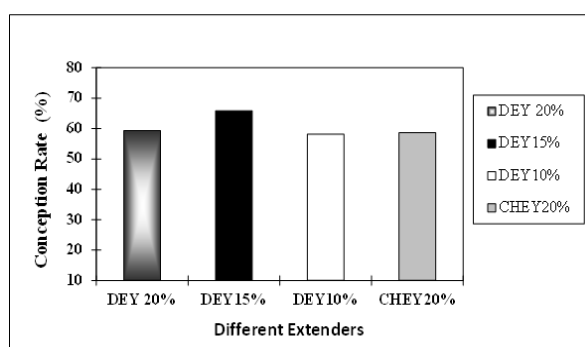


Figure (4): Conception rate % of buffalo-cows inseminated with frozen semen cryopreserved in different extenders. -Different Extenders DEY: Duck Egg Yolk, CHEY: Chicken Egg Yolk.

## IV. CONCLUSION

In conclusion, our results showed that 15% duck egg yolk provided the best cryoprotective action to Egyptian buffalo bull sperm between the two avian egg yolks during

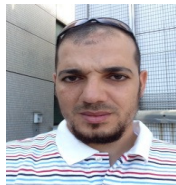
the freezing-thawing process in terms of progressive motility, live spermatozoa, plasma membrane integrity and fertility rate. This conclusion is based on sperm characteristics and a full fertility trail which confirmed the beneficial effects of the inclusion of duck egg yolk in Egyptian buffalo semen cryopreservation protocols.

## REFERENCES

- [1] L. Amirat, D. Tainturier, L. Jeanneau, C. Thorin, O. Gerard, J. L. Courtens and M. Anton, " Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with Optidyl, a commercial egg yolk extender," *Theriogenology*, vol. 61, pp. 895-907, 2004.
- [2] M. Anand, S. Yadav and P. Shukla, "Cryoprotectant in semen extender: From egg yolk to low-density lipoprotein (LDL)," *Livestock Research International*, vol. 2, no. 3, pp. 48-53 (a review), 2014.
- [3] R. Bathgate, W. Maxwell and G. Evans, " Studies on the effect of supplementing boar cryopreservation media with different avian egg yolk types on in vitro post-thaw sperm quality," *Reproduction Domestic Animal*, vol. 41, pp. 68-73, 2006.
- [4] G. W. Webb, L. PAS Codi, S. E. Burris, R. Harmon and H. Baker, "Effects of Egg Yolk Source on the Cryopreservation of Stallion Spermatozoa," *Journal of Equine Veterinary Science*, vol. 31, pp. 66-173, 2011.
- [5] J. Clulow, W. Maxwell, G. Evans and Morris. L., " A comparison of duck and chicken egg yolk for cryopreservation of stallion sperm.," *Aust. Vet. J.*, vol. 85, pp. 232-245, 2007.
- [6] S. Andrabi, M. Ansari, N. Ullah, M. Anwar, A. Mehmood and S. Akhter, "Duck egg yolk in extender improves the freezability of buffalo bull spermatozoa," *Anim. Reprod. Sci.*, vol. 427, no. 104, p. 33, 2008.
- [7] L. Su, X. Li, J. Quan, S. Yang, Y. Li, X. He and X. Tang, "A comparison of the protective action of added egg yolks from five avian species to the cryopreservation of bull sperm," *Anim. Reprod. Sci.*, vol. 104, p. 212, 2008.
- [8] R. Humes and G. Webb, " Use of chicken or chukar egg yolk with two cryoprotectants for preservation of stallion semen," *Anim. Reprod. Sci.*, vol. 94, pp. 62-63 (Abstract), 2006.
- [9] M. A. Ibrahim, I. E. El-Bawab and G. A. El-Amrawi, "Evaluation of the protective effect of duck egg yolk during cooling of buck semen. Alex.," *J. Vet. Science*, vol. 37, no. 1, pp. 78-100, 2012.
- [10] G. Sansone, M. Nastri and A. Fabbrocini, "Storage of buffalo ( *Bubalus bubalis* ) semen. Animal Reproduction Science," *Animal Reproduction Science*, vol. 62, pp. 55-76, 2000.
- [11] J. J. Garde, A. J. Soler, J. Cassinello, C. Crespo and A. F. Malo, "Sperm cryopreservation in three species of endangered gazelles (*Gazellacuvieri*, *G. damamhor* and *G. dorcasneglecta*)," *Biology of Reproduction*, vol. 69, pp. 602-611, 2003.
- [12] R. Saacke, "Semen quality: importance of and influencing factors. In: Proceedings of 10th NAAB Tech. Conf. A. I. Reprod Milwaukee, WI, USA," in *National Association of Animal Breeders*, Columbia, USA, 1983.
- [13] A. D. Barth, "The relationship between sperm abnormalities and fertility," in *Proc. 14th Tech. Conf. AI Reprod. National Assoc. Anim. Breeders*, Milwaukee, Wisconsin, 1992.
- [14] G. F. Richardson, A. W. Donald and C. F. Mackinnon, "Comparison of different techniques to determine the percentage of intact acrosome in frozen thawed bull semen," *Theriogenology*, vol. 38, p. 557, 1992.
- [15] V. W. G., "News on reproduction biotechnology in males," in *Proceedings of the Vth World Buffalo Congress*, Caserta, Italy, 1997.
- [16] J. L. Hancock, "A staining technique for the study of temperature shock in semen," *Nature*, vol. 167, pp. 323-324, 1951.
- [17] R. S. Jeyendran, H. H. Van Der Ven, M. Perez-Pelaez, B. G. Crabo and L. J. D. Zaneveld, "Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics," *J. Reprod. Fertil*, 1984.
- [18] D. B. Duncan, "Multiple Range and Multiple F test," *Biometrics*, vol. 11, pp. 1-42, 1955.
- [19] S. Waheed, A. Nazir, U. Najib, J. Hafez, M. Younis and I. Sajid, "Evaluation of duck egg yolk for the cryopreservation of Nili-Ravi buffalo bull semen," *Animal Reproduction Science*, vol. 131, pp. 95-99, 2012.
- [20] R. Kulaksiz, C. Cigdem, A. Ergun and D. A., "The protective effect of egg yolk from different avian species during the cryopreservation of Karayaka ram semen," *Small Ruminant Research*, vol. 88, pp. 12-15, 2010.
- [21] S. Choi, K. T. Song and H. Oh, " Cholesterol contents and fatty acid composition of chukar, pheasant, guinea fowl and quail egg yolk.," *J. Anim. Sc., Asian- Australas.*, vol. 14, pp. 831-836, 2001.
- [22] M. Kaźmierska, B. Jarosz, M. Korzeniowska, T. Trziszka and Z. Dobrzański, "Comparative analysis of fatty acid profile and cholesterol content of egg yolks of different bird species," *Polish Journal of Food Nutrition Science*, vol. 14, pp. 69-73, 2005.
- [23] P. F. Surai, B. K. Speake, R. C. Noble and M. Mezes, "Species-specific differences in the fatty acid profiles of the lipids of the yolk and of the liver of the chick," *Journal of Science Food and Agriculture*, vol. 79, pp. 733-736, 1999.
- [24] B. D. Ladbrooke, R. M. Williams and D. Chapman, " Studies on lecithin-cholesterol-water interactions by differential scanning calorimetry and x-ray diffraction," *Biochim. Biophys. Acta.*, vol. 150, pp. 333-340, 1968.
- [25] J. E. Parks and J. K. Graham, " Effects of cryopreservation procedures on sperm membranes," *Theriogenology*, vol. 38, pp. 209-222, 1992.
- [26] P. F. Watson, "The roles of lipid and protein in the protection of ram spermatozoa at 5 °C by egg yolk lipoprotein.," *Journal of Reproduction and Fertility*, vol. 62, pp. 483-492, 1981.
- [27] G. J. Morris and A. Clark, *The effects of low temperatures on biological systems*. London, London: Edward Arnold. Pg., 1987, pp. 72-119.
- [28] K. Ortman, A. Rodriguez and H. Martin, "Membrane damage during dilution, cooling and freezing- thawing of boar spermatozoa packaged in plastic bags," *Veterinary Medicine A-Zbl Vet A – Physiolog*, vol. 41, pp. 73-47, 1994.
- [29] A. Trimeche, M. Anton, P. Renard, G. Gandemer and D. Tainturier, "Quail egg yolk: a novel cryoprotectant for the freeze preservation of Poitou jackass sperm," *Cryobiology*, vol. 34, p. 385-393, 1997.
- [30] R. I. El-Sheshtawy, G. A. E., A. M. Alaa and S. W. El-Natat, "Effect of Egg Yolk from Different Avian Species on Cryopreservability of Buffalo Semen. Global," *J. of Biotechnology and Biochemistry*, vol. 5, no. 4, pp. 211-215, 2010.
- [31] N. R. Lanz, B. W. Pickett and R. J. Komarek, "Effect of lipid additives on pre- and post-freeze survival of bovine spermatozoa," *J. Dairy Sci.*, vol. 48, pp. 1692, 1965.
- [32] M. Moussa, V. Martinet, A. Trimeche, D. Tainturier and M. Anton, "Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen," *Theriogenology*, vol. 57, pp. 1695-1706, 2002.
- [33] K. T. Osman, "Some reproductive aspects on Egyptian buffalo bulls," Ph. D. thesis, Fac. of Agric. Ain Shams Univ. Egypt., 1996.
- [34] G. A. El-Amrawi, "Effect of thawing time and Post-thaw temperature on fertility of buffalo spermatozoa frozen in straws," 13-16 Caserta, Italy, 1997.
- [35] E. M. El-Siefy, "Reproductive aspective on effect of selenium and vitamin E on semen quality and fertility of Egyptian buffalo bulls," Tanta Univ. Egypt, Ph.D. Sci. Thesis, Fac. of Agric., 2004.
- [36] M. E. El-Sharawy, I. S. El-Shamaa, M. A. Ibrahim and E. El-Seify, " Using of Low Density Lipoproteins and Glutamine to Improve Frozen Buffalo Bull Semen and Fertility," *Reproduction in Domestic Animals*, p. 444, 2012a (Abstract).
- [37] M. E. El-Sharawy, I. S. El-Shamaa, M. A. Ibrahim, I. M. Abd El-Razek and E. M. El-Seify, " Effect of Low Density Lipoproteins in Extender on Freezability and Fertility of Egyptian Buffalo Bull Semen.," *Scientific Papers, Animal Science*, Series D 55, 2012b.
- [38] I. S. El-Shamaa, E. M. El-Seify and a. M. E. E.-S. M. A. R. Ibrahim, "Effect of addition different glutamine levels in lowdensity lipoproteins extender on freezability and fertility of Egyptian buffalo bulls semen.," in *Proceeding of the 4th Scientific Conference of Animal Production Research Institute*, 12-13<sup>th</sup> Nov. Dokki, Giza, Egypt., PP 9-17, 2013.
- [39] M. AR. Ibrahim, S. Zamfirescu, A. Anghel, N. Dobrin, I. M. Abdelrazek, M. E. El-Sharawy and E. M. a. D. M. El-Seify,

"Advanced Studies on Improving Sheep Fertility by Using Artificial Means of Reproduction.," *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, vol. 14, no. 2, pp. 147-158, 2014.

### **AUTHORS PROFILES**



**Mohamed El-Sayed El-Sharawy, PhD.**, Assistant Professor, Animal Production Department, Faculty of Agriculture, Kafr El-Sheikh University.

E-mail: elsharawy78@yahoo.com

Author was born in Kafr El-Sheikh, Egypt, in April, 1978. He received the BSc. and MSc.

degrees in Agriculture studies (Animal Production) from Kafr El-Sheikh Faculty of Agriculture, Tanta University and PhD degrees from Kafr El-Sheikh University, Egypt, in 2000, 2005 and 2010 respectively.

Dr. El-Sharawy was Participated in FP7 Project PIRSES-GA-2011 "International Research Staff" under title "Advanced Studies on Improving Sheep Fertility by Using Artificial Means of Reproduction" University Ovidius Constanta, Romania during the period from 15 July to 10 October, 2012 and he has travel award for young scientists from developing countries at the 17<sup>th</sup> International Congress on Animal Reproduction (ICAR) at the Vancouver Convention Centre in Vancouver, British Columbia, Canada on July 29 - Aug. 2, 2012. His last publication titled by "In Vitro Maturation, Fertilization and Development of Prepubertal and Mature Buffalo Oocytes". Currently; he is a Visiting Researcher in Laboratory of Reproduction Physiology and Biotechnology (LRPB), Animal Production and marine Science Department, Faculty of Agriculture, Kyushu University and Current research about advanced studies in vitro and in vivo maturation (IVM), fertilization (IVF) and development oocytes in mouse.

**Dr. El-Shenawy M. El-Seify; Dr. Ahmed M. Hussien and Dr. Mohamed El-Sherbieny** were Senior Research Biotechnology Department, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

**Prof. Dr./Ibrahim S. El-Shamaa** Professor of Animal Production and Reproduction, Animal Production Department, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.