



Review Article

Egg Yolk Diversity as a Natural Cryoprotectant in Livestock Semen Technology: A Review

Ivan YULIO ¹, Nurcholidah SOLIHATI ¹, Rangga SETIAWAN ¹

¹ Faculty of Animal Husbandry, Department of Production, Animal Husbandry Science Program, Padjadjaran University, Jatinangor, 45363, Indonesia

(*) Corresponding author:

Ivan YULIO

E-mail: ivan22001@mail.unpad.ac.id

Received: 07-09-2026 | Accepted: 70-10-2026 | Published: 10-10-2026



Copyright © 2026 by the author(s). This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. See credit lines of images or other third-party material in this article for license information.

The wide application of egg yolk in the development of livestock semen technology as a natural cryoprotectant has been well researched since it helps to protect spermatozoa from damage associated with freezing and thawing processes. During cryopreservation, spermatozoa usually become less potent as a result of cell membrane injury, oxidative damage, and abnormalities in the shape, resulting in negative fertility outcomes. Egg yolk, especially its low-density lipoprotein component, has been proven to contribute significantly to the stability of spermatozoa cell membrane, motility maintenance, and viability. The biochemistry of avian egg yolk, coming from chickens, ducks, or quails, affects their effectiveness in use as a semen extender. The current paper is aimed at reviewing new information about how egg yolk contributes to increasing the quality of spermatozoa, including motility, viability, and abnormality rates. It becomes clear that effective concentrations of egg yolk are likely to lie within the range of 10–20%. Alternative avian egg yolk sources, including those coming from quails and ducks, demonstrate high efficiency due to increased levels of antioxidants and lipids.

Keywords: Egg Yolk Diversity, Cryoprotectant, Semen Cryopreservation, Livestock Reproduction.

INTRODUCTION

Cryopreservation has really changed things for livestock reproduction. It gives us a way to store and share material from all around the world for breeding or conservation. Cryopreservation is not perfect. Sperm get damaged a lot during the freezing and thawing process. Ice crystals. This causes problems for the sperm. The pressure inside the sperm changes and this can be very bad for them. Also, the sperm experience something called stress, which is not good for them. The membranes of the sperm just cannot handle all of these problems. As a result the sperm are not as good as they used to be. This affects fertility.

Researchers have found that this is a problem in studies, such as the ones done by Larbi *et al.* (2024) and Sharafi *et al.* (2022). To deal with these problems people use something called cryoprotectants. One of the popular cryoprotectants is egg yolk. Egg yolk acts like a shield that protects the sperm from the cold. The egg yolk has something called low-density lipoprotein which helps the sperm in ways. It helps keep the membranes of the sperm stable reduces the loss of lipids and protects the sperm from shock. This is what Aramli *et al.* (2024). When we add egg yolk to the sperm it helps them to keep moving. Their membranes stay intact. The parts of the sperm that generate energy, called mitochondria are still working well after the sperm have been thawed. This is true for types of livestock.

The composition of egg yolk can vary there is a risk of contamination and the results can be unpredictable. That is why researchers are still looking for options or ways to improve what we already have (Selige *et al.*, 2021). There is a growing interest in using egg yolks from types of birds such as chickens, ducks and quails. Each type of bird has its unique mix of lipids, phospholipids and antioxidants. These differences could affect how well each type of egg yolk protects the sperm during freezing and thawing. It is important to study these differences not to find the best cryoprotectant

but to develop specific protocols for different animals or reproductive technologies (Aramli *et al.*, 2024; Zhang *et al.*, 2026).

The lipid layer from egg yolk provides natural cryoprotection by stabilizing sperm plasma membrane and preventing cold shock damage during cryopreservation. It is thought that low density lipoprotein (LDL) plays a role in the cryoprotection mechanism. LDL constitute ~85% of the plasma fraction in egg yolk, and is the active ingredient of egg yolk. LDL is typically included at 4–12% concentrations (or 15–20% whole egg yolk in semen extenders) (Mahiddine and Kim, 2021). It acts by binding to seminal plasma proteins, forming a lipid membrane layer around the sperm, and replacing lipids lost during freezing and thawing (Chang *et al.*, 2025), thereby maintaining the membrane integrity, reducing oxidative damage and keeping the acrosome function intact. After thawing sperm quality on egg yolk based extenders typically shows motility 40–65%, viability 50–75%, abnormalities 15–30% (Mahiddine and Kim, 2021; Strzeżek and Reksa, 2022). Chicken egg yolk is commonly used for its stable composition; the phospholipids and cholesterol aid in maintaining membrane fluidity. When added to semen extenders at approximately 20%, it can result in post-thaw motility of 45–60%, viability 55–70%, and abnormalities below 20–25% although variability and biosecurity remain an issue (Chang *et al.*, 2025; Strzeżek and Reksa, 2022). Quail egg yolk demonstrates greater antioxidant potential and enhances sperm movement, yielding motility of 55–70%, viability 60–72%, and abnormalities 15–25% when added at 5–10% (Łukaszewicz *et al.*, 2023; Strzeżek and Reksa, 2022). Higher lipid content also enhances the cryoprotective capacity of duck and indigenous chicken egg yolk which can offer comparable or better post-thaw sperm quality and affordability (Yendraliza and Rahman, 2023).

This review focuses on the types of egg yolk cryoprotectants used in livestock semen technology. It looks at the science behind how they work compares types of egg yolks from birds and explores the latest improvements and

challenges. By looking at research the goal is to establish a solid foundation for better, safer and more reliable ways to preserve semen for animal reproduction. Cryopreservation and semen preservation are very important for livestock reproduction and egg yolk cryoprotectants play a role, in this.

METHODS

This study was performed as a narrative review of latest literature on diversification of egg yolk as natural cryoprotectant in livestock semen technology including its mechanism, biochemical components, and effects on sperm motility, viability, and abnormality. Literature searched in the databases including PubMed, ScienceDirect, Scopus, and Google Scholar using the keywords related to egg yolk and cryopreservation for research published between 2016 and 2025 that has the evidence based data and has published as peer review. Criteria to select appropriate papers were as; it was on the use of egg yolk as an extender to preserve semen with quantitative measurements of sperm quality parameters in livestock animals; the publication status must be peer review; non-peer-reviewed, duplicate, or poorly designed publications were not included. The data was categorized based on egg yolk types, concentrations and species to determine the relation with the measured sperm parameters, and the relevant information was synthesized for a better understanding of the role of egg yolk to improve cryopreservation.

DISCUSSION

Summary of studies investigating the sources of egg yolk, their concentration and the impact on various sperm quality parameters is given in Table 1. From the table, it can be observed that there is difference among the sources of egg yolk regarding their effect on motility, viability and abnormality.

Egg yolks of several types are now routinely used as semen extenders in livestock since it protects the spermatozoa during cooling and freezing. Chicken egg yolk is mostly used in bulls (10-30% concentration) to preserve the spermatozoa (Ardiana *et al.*, 2024; Ferdinand *et al.*, 2014). Omega chicken egg yolk is considered better to extend ram semen since its contains lipoproteins and antioxidants which is protect the spermatozoa (Yimer *et al.*, 2014). Duck, quail, pigeon, ostrich, turkey and guinea fowl egg yolks have also shown some protective effects in preserving semen of different species (Achi *et al.*, 2017; Naz *et al.*, 2019). Oven-dried chicken yolk provides a poorer protection as some protein may be denatured (Ardiana *et al.*, 2024). Types of egg yolk and its concentration affect the preservation of semen; higher concentration is generally from 10% to 20%.

DIFFERENT TYPE OF AVIAN EGG YOLK EFFECT ON SPERM MOTILITY

The type and concentration of egg yolk added as a semen extender influences sperm motility, as the yolk contains lipoproteins and antioxidants that can help in protecting the sperm membrane during cold storage and freezing. In bull semen chicken egg yolk added to the extender in different concentrations of 10-30% yielded motility between 75.70%-84.00% (Ferdinand *et al.*, 2014) while at 20% it yielded high variations from 33.90%-94.07% between different trials. Ram semen added with omega chicken yolk gave high motility of up to 79.8% at a concentration of 10% (Yimer *et al.*, 2014). Yolk from other species such as duck, quail, pigeon, turkey and guinea fowl can support sperm motility from other species, while ostrich yolk resulted in high motility of up to 95.26% (Naz *et al.*, 2019), while quail yolk yielded up to 85.00% motility (Achi *et al.*, 2017). The variations can be attributed to differences in the composition of lipids and protein components that affect the stability of the sperm membrane and the availability of energy for sperm movement.

Table 1. Comparison of Egg Yolk Types, Concentrations, and Effects on Livestock Sperm Quality

Egg Yolk	Livestock Species	Method	Concentration (%)	Motility (%)	Viability (%)	Abnormality (%)	Reference
Chicken	Bull	Chilled	10	84.00	-	-	Ferdinand et al., 2014
Chicken	Bull	Chilled	15	83.75	-	-	Ferdinand et al., 2014
Chicken	Bull	Chilled	20	80.00	-	-	Ferdinand et al., 2014
Chicken	Bull	Chilled	25	77.25	-	-	Ferdinand et al., 2014
Chicken	Bull	Chilled	30	75.70	-	-	Ferdinand et al., 2014
Chicken	Bull	Freezed	20	50.42	-	11.38	Jamil-ur rahman et al., 2011
Chicken	Bull	Freezed	20	45.19	51.27	6.17	Prastiya et al., 2021
Chicken	Bull	Freezed	20	63.27	65.29	9.01	Gunawan et al., 2025
Chicken	Bull	Freezed	20	50.00	-	12.10	Akhter et al., 2010
Chicken	Bull	Freezed	20	60.00	74.00	12.10	Khatimah et al., 2021
Chicken	Bull	Freezed	20	94.07	-	-	Naz et al., 2019
Chicken	Bull	Chilled	20	66.00	71.00	-	Achi et al., 2017
Chicken	Rooster	Freezed	15	59.41	65.57	-	Pitchayapip atkul et al., 2026
Chicken	Mithun	Freezed	20	41.20	-	-	Perumal, 2018
Omega Chicken	Ram	Freezed	2.5	63.20	-	9.20	Yimer et al., 2014

Omega Chicken	Ram	Freezed	5	78.7	-	2.90	Yimer et al., 2014
Omega Chicken	Ram	Freezed	10	79.8	-	7.00	Yimer et al., 2014
Duck	Bull	Freezed	20	57.00	70.00	13.00	Khatimah et al., 2021
Duck	Bull	Freezed	4	61.58	-	17.00	Saad et al., 2022
Duck	Bull	Freezed	6	63.25	-	15.41	Saad et al., 2022
Duck	Bull	Freezed	8	67.25	-	15.33	Saad et al., 2022
Duck	Bull	Freezed	10	70.25	-	13.16	Saad et al., 2022
Duck	Bull	Freezed	20	64.20	-	19.58	Saad et al., 2022
Duck	Rooster	Freezed	15	44.18	50.69	-	Pitchayapip atkul et al., 2026
Quail	Mithun	Freezed	15	47.52	-	-	Perumal, 2018
Quail	Rooster	Freezed	15	58.64	62.96	-	Pitchayapip atkul et al., 2026
Quail	Bull	Chilled	20	85.00	87.00	-	Achi et al., 2017
Pigeon	Bull	Freezed	5	39.70	-	16.04	Jamil-ur rahman et al., 2011
Pigeon	Bull	Freezed	10	37.92	-	14.17	Jamil-ur rahman et al., 2011
Pigeon	Bull	Freezed	15	41.40	-	12.13	Jamil-ur rahman et al., 2011
Pigeon	Bull	Freezed	20	51.67	-	8.58	Jamil-ur rahman et al., 2011
Pigeon	Bull	Freezed	20	52.50	-	12.00	Akhter et al., 2010

Ostrich	Bull	Freezed	10	92.96	-	-	Naz et al., 2019
Ostrich	Bull	Freezed	15	95.26	-	-	Naz et al., 2019
Ostrich	Bull	Freezed	20	91.91	-	-	Naz et al., 2019
Turkey	Rooster	Freezed	15	45.51	56.75	-	Pitchayapip atkul et al., 2026
Turkey	Mithun	Freezed	15	44.76	-	-	Perumal, 2018
Turkey	Bull	Chilled	20	46.00	54.00	-	Achi et al., 2017
Guinea Fowl	Bull	Chilled	20	59.00	72.00	-	Achi et al., 2017
Guinea Fowl	Bull	Freezed	20	60.50	-	10.00	Akhter et al., 2010

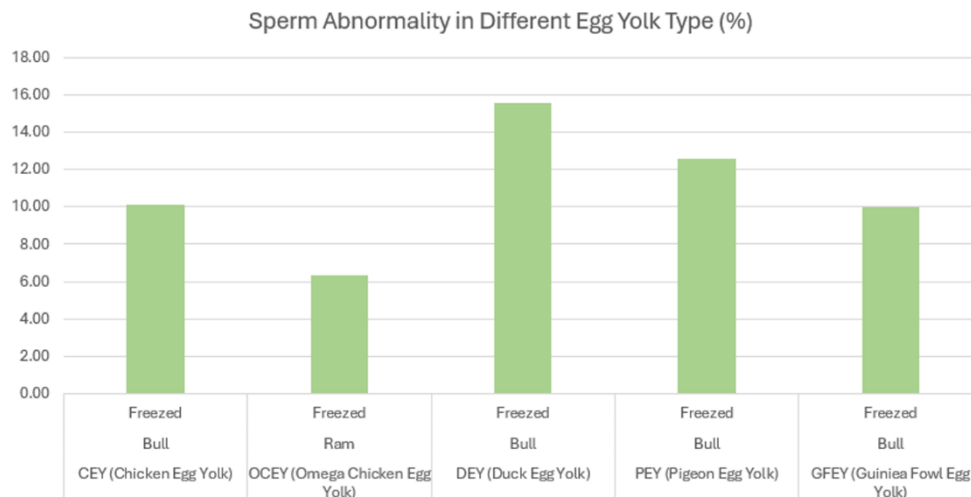


Figure 1. Comparison of Different Egg Yolk Type Effect on Livestock Sperm Motility

Figure 2. Comparison of Different Egg Yolk Type Effect on Livestock Sperm Viability

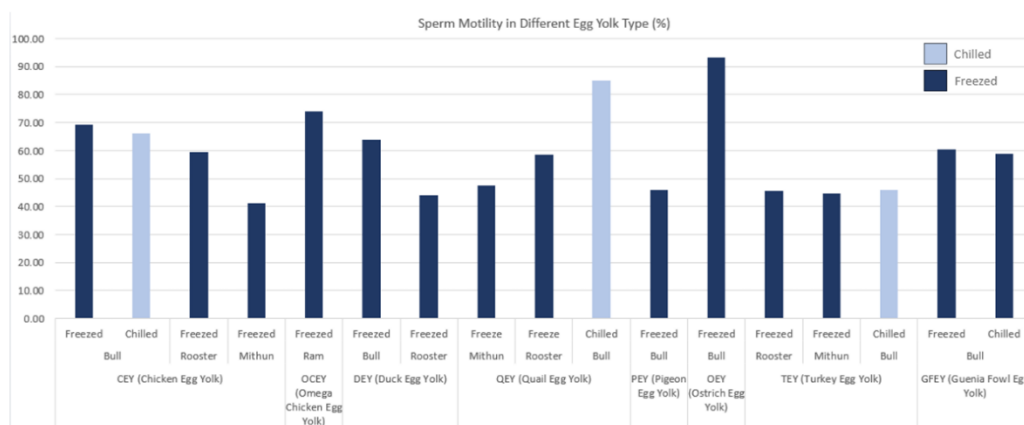
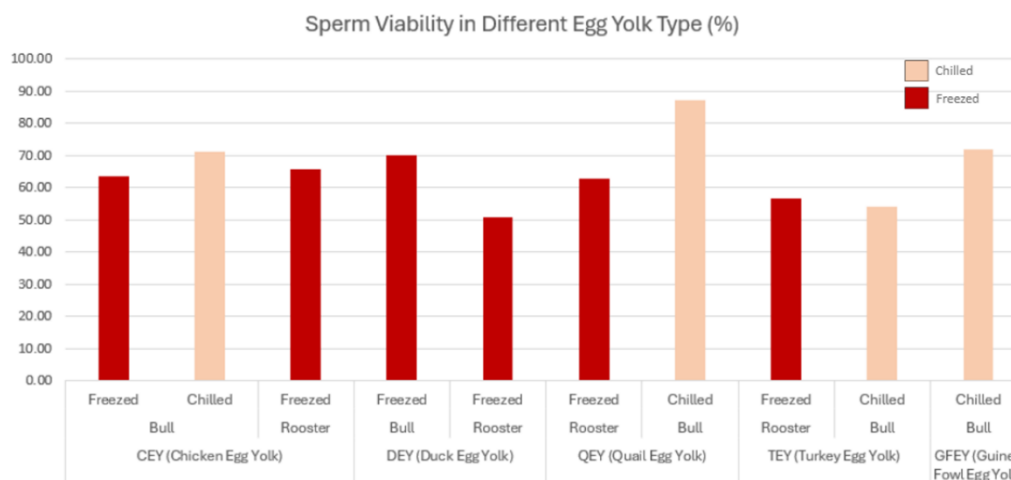


Figure 3. Comparison of Different Egg Yolk Type Effect on Livestock Sperm Viability



Different motility percentages among types of egg yolk are mainly due to different lipid composition, phospholipid content,

antioxidant properties and fatty acid profile of each yolk type and that affect the sperm membrane against chilling and freezing

(Ferdinand *et al.*, 2014). Chicken egg yolk provided a quite good protection due to a high concentration of LDL, however the percentage values of motility were greatly varying between different studies, from 45.19% to 94.07%, depending on concentration, extender composition and cryopreservation process (Naz *et al.*, 2019; Prastiya *et al.*, 2021). In addition, Ferdinand *et al.* (2014) observed that with increasing of chicken egg yolk concentration from 10% to 30%, motility reduced from 84.00% to 75.70%. Therefore, too high concentration might increase the viscosity and hinder the movement of sperm cells. Duck and Quail egg yolk were relatively high, with values from 80 to 90%, due to their high phospholipid and unsaturated fatty acid concentrations and that will help sperm membrane stable when freezing (Achi *et al.*, 2017; Saad *et al.*, 2022). Ostrich egg yolk yielded highest motility percentage 95.26% at 15% concentration. Its lipid profile with highly composed lipoprotein is the main reason of this result (Naz *et al.*, 2019). Meanwhile, pigeon, turkey and guinea fowl egg yolk provided low percentage values of motility, probably the chemical compositions of their egg yolk are less suitable to give cryoprotection to sperm cells.

DIFFERENT TYPE OF AVIAN EGG YOLK EFFECT ON SPERM VIABILITY

Egg yolk concentration and type play a major role in maintaining sperm viability by stabilizing the plasma membrane against cold shock and oxidation. In the case of bulls, different levels of chicken egg yolk (20%) have resulted in different levels of viability (32.10%-79.00%) dependent on the condition the semen was collected under and the experiment performed (Achi *et al.*, 2017; Ardiana *et al.*, 2024; Khatimah *et al.*, 2021). The effect of the concentration of omega-enriched chicken egg yolk (10%) has shown to maintain high levels of ram viability due to its lipoprotein and antioxidant properties, with in the case of goats having 20% chicken egg yolk at an efficiency of 64.60%. High levels of sperm viability have also

been shown using duck and quail egg yolk (Larbi *et al.*, 2024; Pitchayapipatkul *et al.*, 2026; Wahjuningsih *et al.*, 2019).

Variations in sperm viability between different types of egg yolks are mainly influenced by the capacities of each type of yolk to prevent sperm plasma membrane from cold shock, osmotic pressure and lipid peroxidation throughout the storage period. Chicken egg yolks generally result in high viability values due to presence of large amounts of LDL, phospholipids and antioxidants that protect membrane integrity. It leads to the viability of bull semen during freezing varying between 51.27 and 74.00% (Khatimah *et al.*, 2021; Prastiya *et al.*, 2021). Duck egg yolk also exhibits fairly high viability up to 70.00% of frozen bull semen. It could be correlated to the fact that duck egg yolks possess more phospholipids and unsaturated fatty acids which enhance membrane fluidity and provide better cryoprotection (Khatimah *et al.*, 2021). Quail egg yolk demonstrates the best result of 87.00% in chilled bull semen (Achi *et al.*, 2017), potentially ascribed to strong antioxidative activities and balanced lipid profiles which inhibit oxidative damage. In contrary, turkey and duck egg yolks used for freezing rooster semen yield low viability at 56.75 and 50.69% (Pitchayapipatkul *et al.*, 2026), suggesting that the effectiveness of egg yolks is also dependent on species-specific sperm properties and their similarity with yolk lipids. In sum, difference of viability in different egg yolks is determined greatly by lipids composition and content, preservation method and sperm properties.

DIFFERENT TYPE OF AVIAN EGG YOLK EFFECT ON SPERM ABNORMALITY

The prevalence of sperm abnormality was dependent on the type and level of egg yolk concentration used as semen extender. Egg yolk enhances semen membrane and decreases the occurrence of sperm damage in cooling or freezing processes. In bulls, abnormality varied

from 6.17 to 37.40% with the addition of 20% of chicken egg yolk depending on the different researchers showing the contribution of semen handling and egg yolk type in the rate of sperm abnormality (Ardiana *et al.*, 2024; Prastiya *et al.*, 2021; Rahman and Pang, 2020). Different abnormalities found on duck and goat semen extended with chicken egg yolk were also variable depending on the level and protection to membrane (Khatimah *et al.*, 2021; Wahjuningsih *et al.*, 2019). While oven dried chicken egg yolk showed a higher rate of sperm abnormality.

Differences in abnormal sperm percentage among various types of egg yolk is primarily due to differences in lipid content, antioxidant capacity, and ability to protect membrane during chilling and freezing process. Chicken egg yolk, gave lower value of abnormality, the contents of LDL and phospholipid in chicken egg yolk were high enough to provide adequate protection against cold shock and oxidative damage to sperm morphology in bull frozen semen which had values of abnormality between 6.17 and 12.10% (Akhter *et al.*, 2010; Prastiya *et al.*, 2021). Duck egg yolk had high values of abnormal sperm, ranged from 13.00 and 19.58% (Khatimah *et al.*, 2021; Saad *et al.*, 2022) may be because the composition of fatty acid was different and also there was excessive amount of lipids could disrupt the stability of membrane during freezing. Pigeon egg yolk had the trend that the value of abnormal sperm decreased with increasing the concentration of pigeon egg yolk, ranged from 16.04 at 5% concentration to 8.58% at 20% concentration (Jamil-ur-rahman *et al.*, 2011), the reason is high concentration gave better protection of membrane. Omega chicken egg yolk resulted in low value of abnormal sperm, 2.90% at 5% concentration in ram frozen semen (Yimer *et al.*, 2014) the presence of

omega fatty acids may improve membrane fluidity and increase antioxidant protection. Therefore, the abnormality values were influenced by the content of phospholipid and fatty acids, antioxidant activity, concentration of egg yolk used and how well egg yolk's component fit with the sperm membrane's characteristic during cryopreservation process.

CONCLUSION

Different species of egg yolks have varied biological cryoprotective function by preventing decrease of motility and viability of sperm, and maintaining structural stability of sperm in freeze thaw process. Varied proportions of lipid contents, antioxidants and LDL amount in different avian's egg yolk, result in different efficiencies. It's reported that suitable amount of egg yolks varies from 10% to 20%. In spite of widely usage of chicken egg yolk, quail egg yolk and duck egg yolk appear to show better results in some sperm parameters. It is important to choose an appropriate type of egg yolks and correct formulation to avoid cryoinjury, reduce the number of abnormality of sperm and promote fertility.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

ACKNOWLEDGMENTS

This study was supported by the Faculty of Animal Husbandry, Padjadjaran University.

REFERENCES

1. Achi JN, Barje P, Rekwot P, Achi NP, Alphonsus C. National Animal Production Research Institute Ahmadu Bello University P.M.B 1096, Shika-Zaria, Kaduna State,

- Nigeria. *J. Anim. Prod. Res.* 2017;29(1):31–40.
2. Akhter S, Rakha BA, Murtaza S, Andrabi H, Ansari MS. Comparison of egg yolks from three avian species in extender for cryopreservation of Sahiwal bull epididymal spermatozoa. *Anim. Sci. Pap. Rep.* 2010;29(2):131–138.
3. Aramli MS, Moghanlou S, Pourahad Anzabi M. A brief review of the methodology and cryoprotectants in selected fish and mammalian species. *Reprod. Domest. Anim.* 2024;59. doi:10.1111/rda.14575
4. Ardiana D, Yusuf M, Zulkharnaim Z. Effect of Egg Yolk Powder as An Alternative Extender on The Quality of Bali Bull Spermatozoa. Hasanuddin J. *Anim. Sci.* 2024;6(1):60–67. doi:10.20956/hajas.v6i1.26956
5. Chang F, Zhang B, Liu H, Fan H, Xie R, Li J, Hu Q, Ruan C. Effect of Centrifuged Chicken Egg Yolk on the Cryopreservation of Boar Semen. *Animals.* 2025;15:1–12. doi:10.3390/ani15040599
6. Ferdinand N, Ngwa TD, Augustave K, Pougoue B, Dieudonné H, Willington BO, Alex TCD, Pierre K, Joseph T. Effect of Egg Yolk Concentration in Semen Extender, pH Adjustment of Extender and Semen Cooling Methods on Bovine Semen Characteristics. *Global Vet.* 2014;12(3):292–298. doi:10.5829/idosi.gv.2014.12.03.8236
7. Gunawan M, Wayan N, Karja K, Setiadi MA. Development and evaluation of soy lecithin-derived nanoliposomes as a plant-based alternative to egg-yolk extender for Ongole-grade bull semen cryopreservation. *Vet. World.* 2025;18:3433–3446. doi:10.14202/vetworld.2025.3433-3446
8. Jamil-ur-rahman H, Ahmad N, Waheed S, Ahmad M, Younis M, Ahmad T. Effects of Different Levels of Pigeon Egg Yolk in Extenders on the Post-Thaw Semen Quality of Sahiwal Bulls. *Pak. Vet. J.* 2012;32(3):315–318.
9. Khatimah K, Yusuf M, Utami RF, Toleng AL, Masturi, Sahiruddin, Masir U. Comparison of chicken-egg yolk and duck-egg yolk in tris-citric acid as extender to maintain the quality of post-thawing Bali bull semen. *IOP Conf. Ser.: Earth Environ. Sci.* 2021;788(1):012148. doi:10.1088/1755-1315/788/1/012148
10. Larbi A, Li C, Quan G. An updated review on the application of proteomics to explore sperm cryoinjury mechanisms in livestock animals. *Anim. Reprod. Sci.* 2024;263:107441. doi:10.1016/j.anireprosci.2024.107441
11. Łukaszewicz E, Jerysz A, Kowalczyk A. Effect of freeze-dried quail egg white and yolk addition to semen extender on viability of rooster sperm stored for 6 h at 4 °C. *Reprod. Domest. Anim.* 2023;58(3):450–458. doi:10.1111/rda.14307
12. Mahiddine FY, Kim MJ. Overview on the Antioxidants, Egg Yolk Alternatives, and Mesenchymal Stem Cells and Derivatives Used in Canine Sperm Cryopreservation. *Animals.* 2021;11(7):1930. doi:10.3390/ani11071930
13. Naz S, Umair M, Iqbal S. Ostrich egg yolk improves post thaw quality and in vivo fertility of Nili Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. *Theriogenology.* 2019;126:140–144. doi:10.1016/j.theriogenology.2018.12.018
14. Perumal P. Effect of egg yolk of different avian species on semen quality parameters in mithun. *Indian J. Anim. Sci.* 2018;88(10):1152–1155.
15. Pitchayapipatkul J, Niyomdecha A, Pitchayapipatkul S, Inthamat NI, Lona MI. Effect of the different avian species egg yolk in freezing extender on frozen-thawed Betong rooster (*Gallus domesticus*) semen quality. *IOP Conf. Ser.: Earth Environ. Sci.* 2026;1592:012021. doi:10.1088/1755-1315/1592/1/012021
16. Prastiya RA, Munir MM, Nugroho AP. The Protective Impacts of α -tocopherol Supplementation on the Semen Quality of Sapera Goat Preserved at 4 o C. *Trop. Anim. Sci. J.* 2021;44(3):261–266. doi:10.5398/tasj.2021.44.3.261
17. Rahman MS, Pang MG. New Biological Insights on X and Y Chromosome-Bearing Spermatozoa. *Front. Cell Dev. Biol.* 2020;7:388. doi:10.3389/fcell.2019.00388
18. Saad MF, Hussein YS, Soliman SH, Eliraqy EZ, Mark C. Study on Low Density Lipoprotein of Duck Egg Yolk as Cryoprotectants of Holstein Bulls Semen Cryopreservation. *J. Anim. Poult. Prod.* 2022;13(10):137–142. doi:10.21608/jappmu.2022.161086.1055

19. Selige C, Quirino M, Schröter F, Aepli M, Schulze M. Effect of pasteurized egg yolk on the quality of cryopreserved boar semen. *Reprod. Domest. Anim.* 2021;56(8):1152–1155. doi:10.1111/rda.13977
20. Sharafi M, Borghei-Rad SM, Hezavehei M, Shahverdi A, Benson JD. Cryopreservation of Semen in Domestic Animals: A Review of Current Challenges, Applications, and Prospective Strategies. *Animals.* 2022;12(23):3271. doi:10.3390/ani12233271
21. Strzeżek R, Rekxa A. Effect of different egg yolk sources on dog semen quality following cryopreservation. *Pol. J. Vet. Sci.* 2022;25(1):187–189. doi:10.24425/pjvs.2022.140856
22. Wahjuningsih S, Ciptadi G, Nur Ihsan M, Rahayu. Supplementation of Moringa oleifera leaves' extract in Tris-egg yolk extender on the quality and fertility of cryopreserved Senduro goat sperm. *Livest. Res. Rural Dev.* 2019;31(12):185.
23. Yendraliza Y, Rahman YG. Cryopreservation of Simmental cattle semen with egg yolk from different avian species and level glycerol of different in tris diluent. *Livest. Anim. Res.* 2023;21(3):147. doi:10.20961/lar.v21i3.61255
24. Yimer N, Noraisyah A, Rosnina Y, Wahid H, Sarsaifi K, Hafizal A. Comparison of Cryopreservative Effect of Different Levels of Omega-3 Egg-Yolk in Citrate Extender on the Quality of Goat Spermatozoa. *Pak. Vet. J.* 2014;34(3):347–350.
25. Zhang M, Luo Z, Mao W, Zhang G, Gu P. Cryoprotectant effects of mangiferin on the quality of frozen-thawed boar sperm. *Front. Vet. Sci.* 2026;12:1706051. doi:10.3389/fvets.2025.1706051