



Using Lecithin and Longan Sugar Instead of Egg Yolk and Fructose in Extender for Bull Semen

Sittichok Nakmanee¹, Krida Chukiatsiri¹, Watee Kongbuntad²,
Ekkapoj Ragnabpit³, Wiwat Pattanawong¹

10.18805/IJAR.BF-2059

ABSTRACT

Background: Cryopreservation of bovine semen is essential in artificial insemination programs. Traditional extenders using egg yolk and fructose have limitations in biosecurity and performance. Plant-based alternatives like soy lecithin and longan sugar may offer improvements.

Methods: This study evaluated the effectiveness of extenders by replacing 20% egg yolk with 1% soy lecithin and substituting fructose with longan sugar. Semen from Angus, Charolais and tropical crossbred bulls was divided into four treatments: T1 (Egg yolk + fructose), T2 (Egg yolk + longan sugar), T3 (Lecithin + fructose) and T4 (Lecithin + longan sugar). Post-thaw semen quality was assessed using computer-assisted sperm analysis (CASA) in a 2×2 factorial randomized complete block design. Conception rates were recorded after artificial insemination.

Result: Sperm quality in T1, T2 and T3 was significantly higher than T4 ($P < 0.05$). Kinematic parameters showed no significant differences among T1-T3 but were superior to T4 ($P < 0.05$). Lecithin groups showed more morphological abnormalities than egg yolk groups ($P < 0.05$). T4 was excluded from insemination due to substandard post-thaw quality. Pregnancy rates were highest in T3 (67.21%), followed by T1 (53.29%) and T2 (32.73%) ($P < 0.05$). The T3 extender (lecithin + fructose) maintained high semen quality and achieved the best conception rate. Lecithin combined with longan sugar is not recommended due to adverse interactions affecting sperm preservation.

Key words: Bull semen, Cryopreservation, Extender, Lecithin, Longan sugar.

INTRODUCTION

Artificial insemination (AI) is widely practiced in Thailand to enhance the breeding efficiency of cattle, increase productivity, reduce costs and minimize the risk of reproductive system diseases. The use of frozen semen is essential for AI and its production involves several steps, including extrusion, quality assessment, dilution and cryopreservation. One critical component influencing the success of frozen semen production is the extender (Rehmana *et al.*, 2013).

Extenders serve multiple functions: they provide energy, maintain pH balance and include antibiotics to prevent microbial contamination. More importantly, they protect sperm cells from damage caused by ice crystal formation and cold shock (Khumran *et al.*, 2015). Traditional extenders often contain egg yolk or milk, which help mitigate cold shock but may also introduce harmful contaminants or pathogens (Forouzanfar *et al.*, 2010; Layek *et al.*, 2016). High-density lipoproteins (HDL) present in egg yolk granules may have a detrimental effect on sperm motility and could antagonize the cryoprotective effects of LDL during freeze-thaw procedures (Moussa *et al.*, 2002). Phosphatidylcholine, a major component of cell membranes, is known to protect against cold shock and is present in soy lecithin (Sun *et al.*, 2021; Sirmuk, 2006). Therefore, the use of 1% soy lecithin as an alternative to egg yolk in semen extenders has been proposed.

¹Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand.

²Faculty of Science, Maejo University, Chiang Mai, Thailand.

³Inthanon Semen Production and Research Center, Maejo University 63 No.4 Nongharn, Sansai District, Chiang Mai, 50290, Thailand.

Corresponding Author: Wiwat Pattanawong, Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand. Email: wpattanawong@gmail.com

How to cite this article: Nakmanee, S., Chukiatsiri, K., Kongbuntad, W., Ragnabpit, E. and Pattanawong, W. (2025). Using Lecithin and Longan Sugar Instead of Egg Yolk and Fructose in Extender for Bull Semen. *Indian Journal of Animal Research*. 1-7. doi: 10.18805/IJAR.BF-2059.

Submitted: 14-08-2025 **Accepted:** 08-11-2025 **Online:** 09-12-2025

Oxidative stress is another concern during semen cryopreservation, as it can significantly impair post-thaw sperm quality (Al-Mutary *et al.*, 2021). To address this, replacing conventional fructose with sugar derived from longan processing waste was considered. Longan sugar not only serves as an energy source but also contains polyphenolic compounds with antioxidant properties-such as gallic acid, corilagin and ellagic acid-that may help reduce oxidative stress and improve semen quality after thawing (Rangkadilok *et al.*, 2005; Sobeh *et al.*, 2017).

This study was therefore conducted to compare the post-thaw quality of bovine semen frozen using extenders

based on 20% egg yolk or 1% lecithin, combined with either fructose or longan sugar. Additionally, the pregnancy rates following artificial insemination using semen frozen with 1% soy lecithin versus 20% egg yolk extenders were also evaluated.

MATERIALS AND METHODS

Experimental design

This study consisted of two experiments.

Experiment 1

Evaluated post-thaw semen quality-sperm motility, kinematics and morphology-after cryopreservation using extenders containing either 20% egg yolk or 1% soy lecithin and either 1% fructose or 1% longan sugar.

Experiment 2

Compared the pregnancy rates in cows inseminated with frozen semen prepared using the same extender formulations, excluding the combination of lecithin and longan sugar (T4) due to its poor performance in Experiment 1.

Animals and semen collection

Semen was collected from three bull breeds: Angus, Charolais and a Holstein Friesian crossbreed. Collections were performed using an artificial vagina and only ejaculates with $\geq 70\%$ initial motility were used. Each bull was sampled a total of five times (5 replicates), with collections conducted once per week. Semen selected for cryopreservation required progressive motility of 70-75% and a sperm concentration of no less than 800 million sperm per milliliter. All bulls were managed under standard conditions and semen collection and cryopreservation were carried out from December 2022 to January 2023.

Extender preparation and experimental treatments

The extenders were prepared based on a 2x2 factorial design with two main factors: Cryoprotective base (20% egg yolk or 1% soy lecithin) and Sugar source (fructose or longan sugar extract). Each semen sample was diluted to a final concentration of 20×10^6 spermatozoa per 0.25 mL straw. Samples were packaged in straws and cryopreserved using a programmable freezer. The four treatment groups were as follows:

- T1 (Control): 20% egg yolk + fructose.
- T2 (EL): 20% egg yolk + longan sugar.
- T3 (LF): 1% lecithin + fructose.
- T4 (LL): 1% lecithin + longan sugar.

Post-thaw evaluation of sperm quality

Thawed semen was evaluated using a computer-assisted sperm analysis (CASA) system for motility and kinematic parameters: VAP, VSL, VCL, ALH, BCF, LIN, STR and others. Morphological abnormalities (bent tail, DMR, proximal/distal droplet) were also assessed.

Artificial insemination and pregnancy diagnosis (Experiment 2)

Only semen from the T1, T2 and T3 groups was used for artificial insemination, as T4 failed to meet the minimum post-thaw sperm motility requirement of exceeding 40%, as specified by the Department of Livestock Development of Thailand. A total of 384 Holstein Friesian crossbred cows (Ban Thi Dairy Cooperative, Lamphun Province) were used for insemination. Cows were hormonally synchronized prior to insemination (January-April 2024) and pregnancy diagnosis was performed via rectal palpation (April-July 2024).

Statistical analysis

For Experiment 1, a two-way factorial in a randomized complete block design (RCBD) was used, with bull breed as the blocking factor. For Experiment 2, pregnancy rates were analyzed using the Chi-square test. Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Experiment 1

Compared the post-thaw quality of frozen semen prepared using extenders containing either 1% soy lecithin or 20% egg yolk, in combination with either fructose or longan sugar.

The experimental results showed that progressive and slow motility of sperm in the T1, T2 and T3 groups were significantly higher than in the T4 group ($P < 0.05$) (Table 1), while the percentage of static sperm in T4 was significantly higher than in the other groups (Table 1). When comparing between extender bases, the lecithin group had significantly lower motile, progressive and slow sperm than the egg yolk group ($P < 0.05$). Regarding sugar sources, the group using fructose had significantly higher motile, progressive and slow sperm than the group using longan sugar ($P < 0.05$) (Table 1). This may be due to phenolic compounds in longan sugar reacting with phospholipids in lecithin, altering their function and diminishing their protective capacity during the freezing-thawing process (Phan *et al.*, 2014). This finding contrasts with the report of Thongtip *et al.* (2025). They demonstrated that the use of a goat semen extender containing phenolic compounds-specifically gallic acid, ellagic acid and tannin-enhanced sperm quality by slowing cell proliferation and reducing cell death, thereby improving post-thaw motility. The discrepancy may be attributed to differences in the experimental animals as well as the composition of the extenders used. However, in the lecithin with fructose group (T3), sperm motility, progressive motility and static percentages were not significantly different from the egg yolk group (T1), which is consistent with Gamal *et al.* (2016), who reported that 1% lecithin performed similarly to 20% egg yolk as an extender component for bull semen. In the T2 group (egg yolk + longan sugar), the results were not significantly different from T1 ($P > 0.05$) and even showed a

trend toward better motility, indicating that longan sugar may be compatible with 20% egg yolk without adversely affecting post-thaw sperm quality.

Kinematic parameters

The kinematic parameters of sperm were compared among treatment groups. No significant differences were observed in DAP, DSL, DCL, VAP, VSL, VCL, ALH and STR between the T1, T2 and T3 groups ($P>0.05$) (Table 2), but all were significantly higher than those in the T4 group ($P<0.05$) (Table 2). Higher values of VAP, VSL and VCL are associated with increased progressive motility (Rai *et al.*, 2018), which is consistent with the progressive motility values of the T1, T2 and T3 groups as shown in Table 1. These results indicate that the combination of lecithin and longan sugar had a severely negative effect on sperm motility. This may be attributed to the varying concentrations and sources of phenolic compounds in longan sugar. Overall, the T4 group exhibited significantly reduced sperm motility characteristics compared to the other groups. The interaction between phenolic compounds in longan sugar and the phospholipids in lecithin likely impaired the extender's ability to protect sperm against cryoinjury (Phan *et al.*, 2014). In contrast, the extenders used in T1, T2 and T3 did not negatively affect sperm motility. When comparing the egg yolk and lecithin groups, values for DAP, DCL, VAP, VSL, VCL, ALH, BCF and STR were significantly higher in the egg yolk group ($P<0.05$) (Table 2). This is likely because the low-density lipoproteins (LDL) present in egg yolk play a crucial role in protecting the sperm plasma membrane, acrosome and mitochondria during cryopreservation (Celeghini *et al.*, 2008). Similarly, extenders containing fructose yielded higher values for DAP, DSL, DCL, VAP, VSL, VCL, ALH and STR than those containing longan sugar ($P<0.05$) (Table 2), contradicting the findings of Sobeh *et al.* (2017), who reported that phenolic compounds in extenders enhanced sperm motility by reducing oxidative damage. This discrepancy may be due to differences in the types and concentrations of phenolic compounds used. Furthermore, WOB and BCF values were highest in the T4 group, which correlated with a higher incidence of bent tails and distal midpiece reflex (DMR) (Table 3), thereby preventing progressive sperm movement.

Sperm morphology

The incidence of sperm with bent tails and distal midpiece reflex (DMR) was significantly higher in the lecithin with longan sugar group compared to the other groups ($P<0.05$) (Table 3). This may be attributed to osmotic pressure changes caused by interactions between phenolic compounds and phospholipids Phan *et al.* (2014), resulting in a higher occurrence of tail and body abnormalities. When comparing the use of egg yolk and lecithin, the group using lecithin showed a significantly higher proportion of sperm with proximal and distal cytoplasmic droplets than the egg yolk group ($P<0.05$) (Table 3). This observation can be explained by the fact that lecithin-based extenders often appear under

Table 1: Results of semen quality after thawing from the use of frozen semen freezing solution.

Parameter	Egg yolk		Lecithin		Extender	Average extender	Sugar	Average sugar
	Fructose (T1)	Longan S. (T2)	Fructose (T3)	Longan S. (T4)				
Motile	51.02 ^A ±13.18	63.50 ^A ±17.38	59.70 ^A ±17.26	8.06 ^B ±4.72	Egg yolk Lecithin	57.25 ^a 33.88 ^b	Fructose Longan S.	55.36 ^y 35.78 ^z
Progressive	19.95 ^A ±16.47	17.80 ^A ±14.72	21.73 ^A ±19.20	0.35 ^B ±0.83	Egg yolk Lecithin	18.88 ^a 11.04 ^b	Fructose Longan S.	20.84 ^y 9.08 ^z
Static	46.05 ^B ±15.36	36.50 ^B ±17.26	40.30 ^B ±17.26	91.93 ^A ±4.72	Egg yolk Lecithin	41.28 ^b 66.12 ^a	Fructose Longan S.	43.18 ^z 64.22 ^y
Slow	28.63 ^A ±10.42	20.98 ^{AB} ±23.22	14.33 ^B ±17.07	1.45 ^C ±0.94	Egg yolk Lecithin	24.81 ^a 7.89 ^b	Fructose Longan S.	21.48 ^y 11.22 ^z

*T1: 20% Egg yolk and fructose, T2: 20% Egg yolk and longan sugar, T3: 1% Lecithin and fructose, T4: 1% lecithin and longan sugar.

**A,B: Different between treatment ($P<0.05$), ^{a,b}: Different between effect of 20% egg yolk and 1% lecithin, ^{y,z}: Different between effect of fructose and longan sugar.

Table 2: The table shows the comparison of sperm motility characteristics of semen after thawing.

Parameter	Egg yolk		Lecithin		Extender	Average extender	Sugar	Average sugar
	Fructose	Longan S.	Fructose	Longan S.				
DAP	21.40 ^A ±4.23	19.64 ^A ±3.42	19.41 ^A ±6.92	11.36 ^B ±1.97	Egg yolk	20.52 ^a	Fructose	20.40 ^a
DSL	16.05 ^A ±3.56	14.60 ^A ±3.84	20.17 ^A ±14.17	5.42 ^B ±0.22	Lecithin	15.39 ^b	Longan S.	15.50 ^b
DCL	41.13 ^A ±8.67	34.52 ^A ±5.88	34.97 ^A ±12.49	13.62 ^B ±2.22	Egg yolk	15.32	Fructose	18.11 ^a
VAP	40.57 ^A ±7.41	37.64 ^A ±5.94	37.34 ^A ±12.20	19.08 ^B ±4.76	Lecithin	13.47	Longan S.	10.43 ^b
VSL	30.25 ^A ±5.59	27.99 ^A ±5.65	28.73 ^A ±9.83	11.27 ^B ±2.04	Egg yolk	37.82 ^a	Fructose	38.05 ^a
VCL	77.21 ^A ±15.69	66.01 ^A ±14.08	67.18 ^A ±23.39	29.91 ^B ±2.11	Lecithin	24.29 ^b	Longan S.	24.07 ^b
WOB	54.81 ^B ±2.50	59.33 ^B ±7.72	60.92 ^B ±9.73	73.10 ^A ±1.84	Egg yolk	39.10 ^a	Fructose	38.95 ^a
ALH	5.65 ^A ±0.78	5.19 ^A ±1.23	4.97 ^A ±1.62	1.78 ^B ±0.49	Lecithin	28.21 ^b	Longan S.	28.36 ^b
BCF	18.79 ^B ±3.05	16.57 ^B ±2.92	18.38 ^B ±2.89	23.79 ^A ±1.54	Egg yolk	29.12 ^a	Fructose	29.49 ^a
STR	75.75 ^A ±2.61	75.82 ^A ±5.88	80.06 ^A ±7.07	52.92 ^B ±4.67	Lecithin	20.00 ^b	Longan S.	19.63 ^b
LIN	42.32±3.29	40.04±15.99	50.31±12.80	39.22±2.64	Egg yolk	71.61 ^a	Fructose	72.20 ^a
					Lecithin	48.54 ^b	Longan S.	47.96 ^b
					Egg yolk	57.07 ^b	Fructose	57.87 ^b
					Lecithin	67.45 ^a	Longan S.	66.66 ^a
					Egg yolk	5.42 ^a	Fructose	5.31 ^a
					Lecithin	3.37 ^b	Longan S.	3.49 ^b
					Egg yolk	21.08 ^a	Fructose	18.58
					Lecithin	17.68 ^b	Longan S.	20.18
					Egg yolk	75.79 ^a	Fructose	77.91 ^a
					Lecithin	66.49 ^b	Longan S.	64.37 ^b
					Egg yolk	41.18	Fructose	46.31
					Lecithin	44.77	Longan S.	39.64

*T1: 20% Egg yolk and fructose, T2: 20% Egg yolk and Longan sugar, T3: 1% Lecithin and fructose, T4: 1% Lecithin and longan sugar.

**A,B: Different between treatment (P<0.05), ^{a,b}: Different between effect of 20% egg yolk and 1% lecithin, ^{γ,z}: Different between effect of fructose and longan sugar.

a microscope as an insoluble suspension around the sperm (Tarig *et al.*, 2017). When analyzed using computer-assisted sperm analysis (CASA), these suspended particles near the sperm tail may be misinterpreted as cytoplasmic droplets, leading to counting errors (Thun *et al.*, 2002).

Experiment 2

Evaluated the pregnancy rates in cows inseminated with semen frozen using extenders containing 20% egg yolk or 1% lecithin, combined with either fructose or longan sugar.

In the second experiment, the T4 group was excluded because the semen quality after cryopreservation did not meet the minimum standard set by the Department of Livestock Development of Thailand (post-thaw motility must not be lower than 40%). Therefore, this group was deemed unsuitable for artificial insemination. The pregnancy rate was highest in the T3 group (67.21%), followed by the T1 group (53.29%) and lowest in the T2 group (32.73%) ($P<0.05$) (Table 4). The higher pregnancy rate observed in the T3 group, which used a lecithin-based extender, is consistent with the findings of Naz *et al.* (2018), who reported that lecithin-based extenders resulted in higher pregnancy rates in buffaloes compared to Tris-egg yolk-based extenders. Similarly, Aires *et al.* (2003) found that using a soybean lecithin-based extender for bull semen led to higher pregnancy rates than Tris-egg yolk-based extenders, likely because high-density lipoproteins (HDL) present in egg yolk may interfere with sperm-egg fertilization (Naz *et al.*, 2018). Moreover, the T3 group (lecithin and fructose) tended to have a higher pregnancy rate than the T1 group (egg yolk and fructose), consistent with the study of Layek *et al.* (2016) which reported that in addition to bacterial contamination, there was also a problem where some components of the egg yolk affect the sperm's ability to produce a capacitation reaction, resulting in a decreased pregnancy rate. Egg yolk in semen extenders may pose a risk of bacterial or xenobiotic contamination and endotoxins from such contaminants can reduce the fertilizing capacity of sperm (Layek *et al.*, 2016; Bousseau *et al.*, 1998; Aires *et al.*, 2003). This is consistent with the findings of Singh *et al.* (2018), who reported that semen extended with egg yolk had a higher microbial load compared to semen extended with lecithin. The presence of these microorganisms can directly impair male reproductive function by reducing the ability to undergo the acrosome reaction, which may consequently affect pregnancy rates. However, conflicting results have also been reported by Crespilho *et al.* (2012), who observed that the use of lecithin-based extenders could reduce pregnancy rates, as soybean lecithin lipids may bind irreversibly to the equine sperm membrane, leading to impairment of the sperm capacitation process. The cause of these discrepancies may be attributed to differences in the extender base and further studies are warranted to investigate the potential interference of both egg yolk and lecithin on the capacitation process in the future. The cause of these discrepancies may be attributed to differences in the extender base and further studies are

Table 3: Comparative results of sperm morphology of semen after thawing from frozen semen freezing solution (T1, T2, T3 and T4).

Parameter	Egg yolk		Lecithin		Extender	Average extender	Sugar	Average sugar
	Fructose	Longan S.	Fructose	Longan S.				
Bent tail	12.35±4.63 ^c	10.52±4.20 ^{cb}	17.62±8.63 ^b	25.76±5.98 ^a	Egg yolk	11.43 ^z	Fructose	14.98
Coiled tail	0.68±0.41	0.52±0.32	0.42±0.31	2.50±4.66	Lecithin	21.69 ^y	Longan S.	18.14
DMR	1.93±0.66 ^b	1.22±1.10 ^b	2.05±0.96 ^b	4.27±0.65 ^a	Egg yolk	0.60	Fructose	0.55
Proximal Droplet	1.63±0.95	0.78±0.31	2.67±3.15	2.72±1.58	Lecithin	1.46	Longan S.	1.51
Distal Droplet	3.23±2.04 ^a	2.15±2.22 ^{ab}	5.35±4.10 ^b	5.05±0.88 ^b	Egg yolk	1.58 ^z	Fructose	1.99 ^z
					Lecithin	3.16 ^y	Longan S.	2.74 ^y
					Egg yolk	1.21 ^z	Fructose	2.15
					Lecithin	2.69 ^y	Longan S.	1.75
					Egg yolk	2.69 ^z	Fructose	4.29
					Lecithin	5.20 ^y	Longan S.	3.60

*T1: 20% Egg yolk and fructose, T2: 20% Egg yolk and Longan sugar, T3: 1% Lecithin and fructose, T4: 1% Lecithin and longan sugar.

**A,B: Different between treatment ($P<0.05$), ^{a,b}: Different between effect of 20% egg yolk and 1% lecithin, ^{y,z}: Different between effect of fructose and longan sugar.

Table 4: Comparison of pregnancy rate of cows after artificial insemination by post-thawing bull semen (T1, T2, T3 and T4).

Treatment	First insemination (FI)	Nonreturn (NR)	FI/NR (%)
T1 Control	152	81	53.29
T2 Egg yolk longan	110	36	32.73
T3 Lecithin	122	82	67.21

*T1: 20% egg yolk and fructose, T2: 20% egg yolk and longan sugar and T3: 1% lecithin and fructose.

warranted to investigate the potential interference of both egg yolk and lecithin on the capacitation process in the future. Additionally, the use of longan sugar as a component in bull semen extenders, particularly in the T4 group (Lecithin + Longan sugar), requires further investigation to clarify the causes of reduced sperm quality and to optimize the formula for effective use. Moreover, larger-scale studies are needed to provide alternative strategies and new insights for the development of bull semen extenders in the future.

CONCLUSION

Extenders containing longan sugar can be effectively used in combination with 20% egg yolk; however, this combination results in a lower pregnancy rate compared to extenders containing 20% egg yolk with fructose. The use of longan sugar in combination with 1% soy lecithin is not suitable, as it significantly reduces sperm motility, progressive motility and kinematic parameters after thawing. The post-thaw semen quality in this group does not meet the minimum standard ($\geq 40\%$ motility) required for artificial insemination. In contrast, the combination of 1% soy lecithin with fructose not only maintains post-thaw sperm motility and kinematic parameters at levels comparable to those observed in the 20% egg yolk with fructose group, but also results in a significantly higher pregnancy rate. Therefore, the use of 1% soy lecithin with fructose is a promising alternative to the conventional 20% egg yolk-based extender for bull semen cryopreservation, offering improved fertility outcomes.

ACKNOWLEDGEMENT

The research team sincerely thanks the Semen Research and Production Center of the Royal Project Inthanon, Maejo University for laboratory support and Ban Thi Dairy Cooperative, Lamphun for providing experimental animals. Their assistance was essential for the successful completion of this study.

Disclaimers

The views and interpretations expressed in this manuscript are solely those of the authors and do not necessarily represent the positions of their affiliated institutions. The

authors bear full responsibility for the accuracy and integrity of the content. The publisher and associated entities disclaim any liability arising from the use of the information presented herein.

Informed consent

This study involved the use of three bulls from each of the following breeds: Charolais, Angus and Holstein Friesian. The animals were obtained from the Semen Research and Production Center of the Royal Project Inthanon, which is certified for animal welfare standards by the Department of Livestock Development, Thailand. All animal procedures in this study were approved by the Institutional Animal Care and Use Committee, certified by the Institute of Animals for Scientific Purposes Development (IAD), Thailand (Approval No. U1-10447-2565). Animal care and handling complied with national regulations and institutional guidelines, with oversight and approval from Maejo University and project advisors.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. No financial, professional, or personal relationships have influenced the design, execution, or reporting of the study.

REFERENCES

- Aires, V.A., Hinsch, K.D., Mueller-Schloesser, F., Bogner, K., Mueller-Schloesser, 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(2)**: 269-279.
- Al-Mutary, M.G. (2021). Use of antioxidants to augment semen efficiency during liquid storage and cryopreservation in livestock animals: A review. *Journal of King Saud University-Science*. **33(1)**: 101226.
- Bousseau, S., Brillard, J.P., Marquant-Le Guenne, B., Guerin, B., Camus, A. and Lechat, M. (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(5)**: 699-706.
- Celeghini, E.C.C., de Arruda, R.P., de Andrade, A.F.C., Nascimento, J., Raphael, C.F. and Rodrigues, P.H.M. (2008). Effects that bovine sperm cryopreservation using two different extenders has on sperm membranes and chromatin. *Animal Reproduction Science*. **104(2-4)**: 119-131.
- Crespilho, A.M., Sá Filho, M.F., Dell'Aqua, J.J.A., Nichi, M., Monteiro, G.A., Avanzi, B.R. and Papa, F.O. (2012). Comparison of *in vitro* and *in vivo* fertilizing potential of bovine semen frozen in egg yolk or new lecithin-based extenders. *Livestock Science*. **149(1-2)**: 1-6.
- Forouzanfar, M., Sharafi, M., Hosseini, S.M., Ostadhosseini, S., Hajian, M., Hosseini, L. and Nasr-Esfahani, M.H. (2010). *In vitro* comparison of egg yolk-based and soybean lecithin-based extenders for cryopreservation of ram semen. *Theriogenology*. **73(4)**: 480-487.

- Gamal, A., El-Nattat, W.S., El-Sheshtawy, R.I. and El-Maaty, A.M.A. (2016). Substitution of egg yolk with different concentrations of soybean lecithin in tris-based extender during bulls' semen preservability. *Asian Pacific Journal of Reproduction*. **5(6)**: 514-518.
- Khumran, A.M., Yimer, N., Rosnina, Y., Ariff, M.O., Wahid, H., Kaka, A., Ebrahimi, M. and Sarsaifi, K. (2015). Butylated hydroxytoluene can reduce oxidative stress and improve quality of frozen-thawed bull semen processed in lecithin and egg yolk-based extenders. *Animal Reproduction Science*. **163**: 128-134.
- Layek, S.S., Mohanty, T.K., Kumaresan, A. and Parks, J.E. (2016). Cryopreservation of bull semen: Evolution from egg yolk based to soybean based extenders. *Animal Reproduction Science*. **172**: 1-9.
- Moussa, M., Martinet, V., Trimeche, A., Tainturier, D. and Anton, M. (2002). Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. *Theriogenology*. **57(6)**: 1695-1706.
- Naz, S., Umair, M. and Iqbal, S. (2018). Comparison of Tris egg yolk based, Trilady® and Optixell® extender on post thaw quality, kinematics and *in vivo* fertility of Nili Ravi buffalo (*Bubalus bubalis*) bull spermatozoa. *Andrologia*. **50(8)**: e13063.
- Phan, H.T., Yoda, T., Chahal, B., Morita, M., Takagi, M. and Mun'delanji, C.V. (2014). Structure-dependent interactions of polyphenols with a biomimetic membrane system. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. **1838(10)**: 2670-2677.
- Rai, S., Tyagi, S., Kumar, M., Karunakaran, M., Mondal, M., Mandal, A. and Behera, R. (2018). Understanding motility dynamics of crossbred bull spermatozoa when analyzed by computer assisted semen analyzer (CASA). *Indian Journal of Animal Research*. **52(4)**: 530-532. doi: 10.18805/ijar.v0iOF.9130.
- Rangkadilok, N., Worasuttayangkurn, L., Bennett, R.N. and Satayavivad, J. (2005). Identification and quantification of polyphenolic compounds in longan (*Euphoria longana* Lam.) fruit. *Journal of Agricultural and Food Chemistry*. **53(5)**: 1387-1392.
- Rehmana, F.U., Zhaoa, C., Shaha, M.A., Qureshib, M.S. and Wang, X. (2013). Extenders and Artificial Insemination in Ruminants. *Veterinaria Journal*. **1**: 1-8.
- Singh, A., Bhakat, M., Mondal, S., Mohanty, T.K., Behare, P., Mondal, G. and Abdullah, M. (2018). Microbial load of frozen thawed Sahiwal semen extended in egg yolk, soya lecithin and liposome based extender. *Indian Journal of Animal Research*. **52(4)**: 527-529. doi: 10.18805/ijar.v0iOF.8455.
- Sirmuk, W. (2006). Lecithin. Bangkok: Department of Science Service.
- Sobeh, M., Hassan, S.A., El Raey, M.A., Khalil, W.A., Hassan, M.A. and Wink, M. (2017). Polyphenolics from albizia harveyi exhibit antioxidant activities and counteract oxidative damage and ultra-structural changes of cryopreserved bull semen. *Molecules*. **22(11)**: 1993.
- Sun, L., He, M., Wu, C., Zhang, S., Dai, J. and Zhang, D. (2021). Beneficial influence of soybean lecithin nanoparticles on rooster frozen-thawed semen quality and fertility. *Animals*. **11(6)**: 1769.
- Tarig, A.A., Wahid, H., Rosnina, Y., Yimer, N., Goh, Y.M., Baiee, F.H. and Ebrahimi, M. (2017). Effect of different concentrations of soybean lecithin and virgin coconut oil in Tris-based extender on the quality of chilled and frozen-thawed bull semen. *Veterinary World*. **10(6)**: 672.
- Thongtip, N., Pattanawong, W. and Promtan, P. (2025). Effects of frozen semen extender for goats (MJ Ex2™ and tris-egg yolk) on sperm motility quality after freezing. *Indian Journal of Animal Research*. **59(11)**: 1834-1840. doi: 10.18805/IJAR.BF-1918.
- Thun, R., Hurtado, M., and Janett, F. (2002). Comparison of biociphos-plus® and TRIS-egg yolk extender for cryopreservation of bull semen. *Theriogenology*. **57(3)**: 1087-1094.