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A Comparison of Landrace Boars Sperm Quality in Tris-Egg Yolk Dilution with Different Carbohydrate Supplements

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Abstract | This study aims to improve boar sperm viability during storage by adding various carbohydrates (sucrose, lactose, and fructose) to Tris-egg yolk diluents , providing additional energy substrates that may extend sperm viability and motility. Good-quality sperm samples, with motility levels of ≥70% and abnormalities <20%, were diluted in a TEy control solution, with additional treatments including sucrose (TS), lactose (TL), fructose (TF), and combinations of the carbohydrates (TSF, TLF). The diluted samples were stored at 18-20°C and evaluated at 12-hour intervals until motility decreased to 40%. Statistical analyses were performed using ANOVA and Duncan's test. The addition of sucrose to the Tris-egg yolk (TS) diluent significantly (P<0.05) enhanced sperm motility (51%) and viability (58%) until the 60th hour. While the TS group showed enhanced longevity, there were no significant differences between TS, TL, TF, and TSF groups (P>0.05). The survival rate of sperm in the TS diluent was notably higher than the control, TL, TF, and TLF groups (P<0.05) but did not significantly differ from the TSF group (P>0.05). The TS diluent also exhibited the lowest rate of sperm abnormalities, significantly differing from all other diluents (P<0.05). Overall, the addition of sucrose to the Tris-egg yolk diluent emerged as the most effective method for maintaining sperm quality in landrace boars, with implications for improving sperm preservation protocols.

Keywords | Landrace boar, Sperm preservation, Sperm motility, Sperm viability, Tris-egg yolk diluent, Carbohydrate supplementation

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INTRODUCTION

One of the most significant challenges in the preservation of boar sperm is its limited viability during in vitro storage, which often renders the sperm unusable over extended periods (Waberski *et al*., 2019; Pezo *et al*., 2019). This limitation is largely due to the insufficient energy reserves needed by spermatozoa to maintain optimal viability and motility. Previous studies have demonstrated the efficacy of Tris-egg yolk (TEy) as a diluent capable of meeting

the energy requirements of sperm (Yeste, 2017; Namula *et al*., 2019). However, the sperm quality achieved with TEy is not ideal, as the primary energy source for sperm motility (glucose) is present in limited quantities in egg yolk, potentially restricting the kinematic potential of the sperm.

In order to address this limitation, this study investigates the use of additional carbohydrate sources, including sucrose, lactose, and fructose, as supplemental energy substrates in the TEy diluent. Each carbohydrate may offer distinct metabolic advantages, potentially improving sperm viability by meeting its energy needs more comprehensively. The TEy diluent also acts as a buffer, maintaining an optimal pH range for sperm and protecting against the harmful effects of cold storage (Amtiran *et al*., 2020; Dongkot *et al*., 2022). It is anticipated that the incorporation of these three carbohydrate types will ensure the provision of adequate energy for sperm survival, potentially enhancing boar sperm longevity and function during preservation.

The results of previous studies have shown promising results for the addition of carbohydrate such as sucrose, lactose, and fructose to diluents for various species, including poultry and cattle. For instance, sucrose has been found to improve fertility, motility, membrane integrity, acrosome integrity, and mitochondrial function in chicken sperm (Thananurak *et al*., 2019) and to enhance motility and viability in Holstein bull sperm while reducing abnormal morphology (Al-Badrany *et al*., 2020). Similarly, lactose has improved the quality of liquid-stored semen in Angus cattle by providing energy and protecting the sperm plasma membrane from cold shock (Leo *et al*., 2023). Additionally, fructose, a monosaccharide known to produce intracellular ATP, has been shown to enhance bovine sperm motility (Pappa *et al*., 2019; Kayina *et al*., 2020), indicating potential for similar effects in porcine sperm (Sengupta *et al*., 2020).

This study thus seeks to explore the effects of these carbohydrates as additives in the preservation of landrace boar sperm. By incorporating these carbohydrates into TEy, we hypothesize that the resulting energy support will improve sperm viability, motility, and overall preservation outcomes. This study aims to improve boar sperm viability during storage by adding various carbohydrates (sucrose, lactose, and fructose) to Tris-egg yolk diluents , providing additional energy substrates that may extend sperm viability and motility.

MATHERIALS AND METHODS

Ethical Approval and Experimental Animals

The study was conducted at Happy Farm Tilong East Nusa Tenggara. This study used 6 landrace boars kept according to the SOP to maintain males. All boars were given feed and vitamin. This study used an experimental method. All procedures were approved by the Animal Ethics Commission at the Faculty of Animal Husbandry, Marine and Fisheries, Nusa Cendana University (075/1.KT/KEP-PKP/IV/2024).

Research Method

The study utilized a completely randomized design, with six types of diluents randomly assigned to the experimental units (boar sperm samples). Each diluent condition was replicated 5 times, resulting in a total of 30 experimental units. To ensure randomization, each boar was assigned a diluent type using a computer-generated randomization sequence, which helped avoid selection bias. Only healthy, reproductively mature Landrace boars with no recent history of illness or reproductive abnormalities were selected for this study to ensure consistent sperm quality across treatments. Additionally, semen samples from each boar were standardized in terms of initial motility and concentration before the start of the experiment, ensuring that baseline characteristics were consistent across diluent groups. The diluents used were as follows: Tris-egg-yolk (TEy), TEy+sucrose, TEy+lactose, TEy+sucrose+fructose, TEy+sucrose+fructose, Tey+lactose+fructose.

Preparation of Diluent

The preparation of semen diluent is shown in Table 1.

Table 1: The diluent preparation.

Ingredient composition	Diluent					
	T	TS	TL	TF	TSF	TLF
Tris-hydroxymethyl aminomethane (g)	3,03	3,03	3,03	3,03	3,03	3,03
Citric acid monohydrate(g)	1,78	1,78	1,78	1,78	1,78	1,78
Fructose (g)	0	0	0	1,25	1,25	1,25
Sucrose (%)	0	0,2	0	0	0,2	0
Lactose (%)	0	0	0,4	0	0	0,4
Aquadest (mL)	100	100	100	100	100	100
Kuning telur (%, v/v)	20	20	20	20	20	20
Penisilin (IU/mL)	1000	1000	1000	1000	1000	1000
Streptomisin (µg/mL)	1000	1000	1000	1000	1000	1000

Collection, Evaluation and Preservation of Sperm

The semen utilized in this study was collected from six Landrace boars using the hand-gloving method, a widely accepted technique for boar semen collection. Semen was collected in the early morning to ensure that the boars were rested and that collection timing was consistent across replicates. Collections were performed in a controlled environment, with a stable temperature and minimal distractions to reduce stress levels. The semen were subjected to a comprehensive examination encompassing macroscopic and microscopic analyses. These assessments included evaluations

of volume, pH, consistency, color, sperm motility, viability, the presence of abnormalities, and sperm concentration. A binocular microscope with 400x magnification was used for microscopic examination. Following examination, the fresh semen was divided equally into six tubes, with one of the six treatment diluents added to each tube at a ratio of 1:2 (semen to diluent) (Thananurak *et al*., 2019; Al-Badrany *et al*., 2020). The diluted semen was then stored at 18-20°C and examined after dilution and at 12-hour intervals until motility reached 40% of the standard use of boar liquid semen (SNI, 2023).

Evaluation of the Semen Quality during Preservation

The quality of the semen was evaluated based on the sperm motility, viability, the presence of abnormalities, and the survival of the sperm. The assessment of sperm motility was conducted by dripping the sperm onto a microscope slide, covering it with a coverslip, and observing it under a microscope at 400x magnification. The mean motility value for the five fields of view was recorded as the final motility value. The viability of the sperm was determined through the utilization of nigrosine-eosin staining. A drop of sperm was placed on a microscope slide, stained with nigrosine-eosin, and immediately dried at 37°C. Observations were conducted on 200 sperm across ten fields of view. Each spermatozoon was classified as either live (i.e., unstained) or dead (i.e., stained). The scoring of sperm abnormalities is analogous to sperm viability, except only sperm with aberrant shapes are included in the count. In contrast, the viability of the sperm is calculated based on the time the sperm can survive until their motility drops to 40%.

Statistical Analysis

The data on fresh and frozen semen quality were analyzed using a descriptive method. The obtained results presented the mean ± standard error for all parameters. A one-way ANOVA was applied to the data. The Duncan's multiple range test was used as a post-hoc analysis to determine specific differences between treatment groups. All data was analyzed using SPSS software 26 version (IBM® Corp., Armonk, NY).

RESULTS AND DISCUSSION

Sperm Motility and Viability

The motility and viability of sperm from fresh semen of landrace boars from five collections exhibit favorable characteristics. The semen was suitable for dilution and maintenance in an in vitro environment. The motility of the sperm from the five collections exhibited a range of 80-85%, with an average value of 84% (Table 2), while the viability of the sperm was higher, at 92-96%, with an average value of 93.40% (Table 3). The dilution process did not result in any alterations to sperm motility or viability (as observed at the 0-hour mark), indicating that sperm can adapt to the conditions of the dilution treatment employed in this study.

Table 2: Motility of landrace boars sperm in Tris-egg yolk diluent supplemented with different types of carbohydrates.

ST (hours)		Treatment %						P-value
		T	TS	TL	TF	TSF	TLF	
0	Means	84,00^{A}	84,00^{A}	84,00^{A}	84,00^{A}	84,00^{A}	84,00^{A}	1,000
	SD	2,24	2,24	2,24	2,24	2,24	2,24	
12	Means	76,00^{B}	84,00^{A}	74,00^{B}	82,00^{A}	81,00^{A}	74,00^{B}	0,000
	SD	4,18	2,24	5,48	2,74	4,18	2,24	
24	Means	78,00^{BC}	61,00^{A}	74,00^{C}	77,00^{A}	68,00^{A}	78,00^{B}	0,000
	SD	4,47	4,18	4,18	4,47	2,74	4,47	
36	Means	52,00^{B}	68,00^{A}	55,00^{B}	65,00^{A}	67,00^{A}	60,00^{AB}	0,002
	SD	2,74	2,74	5	3,54	13,51	3,54	
48	Means	38,00^{C}	64,00^{A}	40,00^{C}	54,00^{AB}	55,00^{AB}	46,00^{BC}	0,000
	SD	4,47	2,24	7,07	4,18	15,00	9,62	
60	Means	19,00^{B}	51,00^{A}	31,00^{B}	34,00^{AB}	36,00^{AB}	29,00^{B}	0,037
	SD	11,4	6,52	8,22	4,18	26,79	13,42	

Description; **ST:** Storage time; **T:** Tris-yolk egg; **TS:** Tris-yolk egg + sucrose; **TL:** Tris-yolk egg + lactose; **TF:** Tris-yolk egg + fructose; **TSF:** Tris-yolk egg + sucrose + fructose; **TLF:** Tris-yolk egg + lactose + fructose; Different superscripts on the same line indicate significant differences (P<0.05).

Table 3: Viability of landrace boars sperm in Tris-egg yolk diluent supplemented with different types of carbohydrates.

ST (hours)		Treatment %						P-value
		T	TS	TL	TF	TSF	TLF	
0	Means	93,40^{A}	93,40^{A}	93,40^{A}	93,40^{A}	93,40^{A}	93,40^{A}	1,000
	SD	1,67	1,67	1,67	1,67	1,67	1,67	
12	Means	79,80^{C}	89,60^{A}	84,00^{BC}	85,70^{AB}	84,90^{AB}	80,80^{BC}	0,006
	SD	5,96	1,82	4,3	3,73	2,97	2,59	
24	Means	70,10^{C}	85,20^{A}	72,50^{BC}	79,40^{AB}	79,70^{AB}	74,40^{BC}	0,001
	SD	6,11	2,59	8,76	4,16	4,24	2,3	
36	Means	55,20^{C}	75,00^{A}	63,40^{BC}	67,50^{AB}	71,20^{AB}	64,00^{BC}	0,001
	SD	2,08	3,87	1,39	3,32	14,41	3,08	
48	Means	39,80^{D}	71,00^{A}	48,00^{CD}	65,30^{AB}	67,20^{AB}	55,60^{BC}	0,000
	SD	5,23	2,24	5,2	7,5	15,09	4,62	
60	Means	23,70^{C}	58,00^{A}	36,30^{BC}	42,40^{AB}	42,60^{AB}	39,00^{BC}	0,013
	SD	11,47	5,43	13,9	6,53	23,79	6,52	

Description; **ST:** Duration of Preservation; **T:** Tris-yolk egg; **TS:** Tris-yolk egg + sucrose; **TL:** Tris-yolk egg + lactose; **TF:** Tris-yolk egg + fructose; **TSF:** Tris-yolk egg + sucrose + fructose; **TLF:** Tris-yolk egg + lactose + fructose; Different superscripts on the same line indicate significant differences (P<0.05).

Following a 12-hour storage period, there was a notable decline in sperm motility and viability across most diluents, except for TS diluent, which demonstrated no reduction in motility. The highest percentage of sperm motility and viability was observed in the case of TS diluent. The results demonstrated a statistically significant difference between this group and T, TL, and TLF diluents ($P < 0.05$) but no significant difference when compared to TF and TSF diluents ($P > 0.05$). The superiority of TS diluent was maintained in the sperm until the 60th hour of preservation.

During the sperm preservation process, T diluent resulted in the lowest levels of sperm motility and viability. However, observation after the 60th hour of preservation demonstrated no statistically significant difference in motility between TL, TF, TSF, and TLF diluents ($P>0.05$). Conversely, a statistically significant difference was observed between the TS and the diluents mentioned above ($P<0.05$). These findings suggest that adding fructose or lactose, or a combination of sucrose with fructose and lactose, to TEy diluent does not result in a more favorable outcome than diluent TEy alone. This finding contradicts the initial hypothesis that adding various carbohydrates to the TEy diluent would enhance sperm motility during preservation. The unexpected lack of improvement with additional carbohydrates may be due to their combined solute concentration, which can lead to increased osmotic pressure (Blandin *et al*., 2020). This, in turn, could offset any potential benefits of added carbohydrates, suggesting that the solution's tonicity may play a more critical role than carbohydrate diversity alone.

Regarding the accessibility of the energy substrates present in the diluent, it has been demonstrated that the greater the quantity of carbohydrates introduced to the diluent, the greater the energy availability (Chandel, 2021). The sperm can utilize carbohydrates to enhance their motility and viability. However, the availability of this high-energy content may not have an optimal effect on sperm longevity, as the concentration of solutes from the various carbohydrates added to the diluent solution also increases simultaneously. An excessive increase in solute concentration can result in an elevation of osmotic pressure (Mughal *et al*., 2018), thereby rendering the dilution solution hypertonic. The release of water from the sperm cell to the surrounding hypertonic solution can result in cell shrinkage. The beneficial effect of carbohydrate addition to the TEy diluent appeared to be counterbalanced by increased solute concentration, as evidenced by osmotic pressure levels that approached or exceeded 300 mOsm/kg in the TL, TF, TSF, and TLF diluents. This high osmotic pressure likely contributed to hypertonicity, leading to the observed cell shrinkage and decreased motility over time. In contrast, the TS diluent demonstrated osmotic pressure closer to an optimal range (around 295 mOsm/kg), which may explain its relatively stable motility rates.

As indicated by Namula *et al*. (2019), an increase in solution concentration (hypertonic) has been observed to result in a reduction in the viability, progressive motility, and integrity of the plasma membrane of boar sperm. Similarly, dilution solutions with low osmotic pressure have been demonstrated to impair the motility of buffalo sperm. The findings of Mughal *et al*. (2013), (2018) indicated that a reduction in osmotic pressure to 255-275 mOsm/kg led to a decline in buffalo sperma motility, with the optimal sperm motility being observed at an osmotic pressure of 295 mOsm/kg. An osmotic pressure of 255 mOsm/kg resulted in sperm motility of 37.0%, significantly lower than the osmotic pressure of 295 mOsm/kg, which reached 50.3%.

Conversely, sperm motility up to the 60th hour of preservation was markedly superior in TS diluent compared to T diluent, suggesting that sucrose in TEy diluent provides key metabolic benefits. Sucrose may enhance sperm motility due to its ability to act as a sustained energy source. Following the cleavage of its glycosidic bond into glucose and fructose, sucrose can facilitate ATP production, which is essential for maintaining motility (Folch *et al*., 2021). Furthermore, the gradual breakdown of sucrose may contribute to more stable energy availability over time, reducing the risk of rapid depletion that could occur with simpler monosaccharides (Rizakalla, 2010).

Sperm Viability

The period during which sperm survive in vitro storage until the percentage of motility drops to 40% is the sperm survival duration (SD). This value is a benchmark for the minimum percentage of sperm motility that semen samples must exhibit for artificial insemination in cattle. The survival rate of sperm is presented in Table 4.

Table 4: Survival of landrace boars sperm in Tris-egg yolk diluent supplemented with different types of carbohydrates.

Treatment	Survival Duration (hour)	
	Means	SD
T	46.4000 ^{D}	3.05
TS	65.80 ^{A}	3.49
TL	49.20 ^{CD}	6.57
TF	55.60 ^{BC}	2.51
TSF	58.80 ^{AB}	10.85
TLF	53.68 ^{BCD}	6.79
P-value	0.001	

Description; **ST:** Storage time; **T:** Tris-yolk egg; **TS:** Tris-yolk egg + sucrose; **TL:** Tris-yolk egg + lactose; **TF:** Tris-yolk egg + fructose; **TSF:** Tris-yolk egg + sucrose + fructose; **TLF:** Tris-yolk egg + lactose + fructose; Different superscripts on the same line indicate significant differences ($P<0.05$).

The highest sperm survival rate in Landrace boars was observed with diluent TS, which exhibited a statistically significant difference from T, TL, TF, and TLF diluents ($P < 0.05$) but not from TSF diluent ($P > 0.05$). The elevated sperm SD in the TS diluent was found to correlate closely with the high percentage of sperm motility observed in this diluent compared to the other treatments. This result indicates that adding sucrose to T diluent benefits sperm motility, contributing to the high sperm survival rate observed in the in vitro environment.

Sucrose is a disaccharide (Moiset *et al*., 2014), consisting of one unit of fructose and one unit of α-glucose, with the chemical formula C12H22O11. A glycosidic bond is formed between the C1 atom of glucose and the C2 atom of fructose, thereby linking the two sugars together. These two monosaccharides are most commonly involved in energy production through glycolysis and the Krebs cycle. The conversion of these two monosaccharides into energy occurs in the form of adenosine triphosphate (ATP). This result is corroborated by Surachman *et al*. (2009) and Mesang *et al*. (2007), who reported that sucrose could be utilized as an energy source for sperm metabolism during in vitro storage.

The availability of an adequate energy substrate during the preservation process is crucial for optimal cell metabolism. If the energy substrate within the diluent is insufficient to meet the needs of the cells, the sperm cells will undergo a more rapid death process. Accordingly, the incorporation of energy substrates, such as carbohydrates, in an optimal quantity into the sperm diluent can prolong the percentage of motility and the number of live sperm and facilitate storage at low temperatures (Surachman *et al*., 2009; Shi *et al*., 2018). The energy derived from the sucrose degradation process can be utilized to synthesize biomolecules, including proteins, to maintain the functionality of cell organelles and the viability and motility of sperm.

Sucrose also protects the plasma membrane of sperm during preservation or cryopreservation (Arando *et al*., 2017; Pezo *et al*., 2020). The cell membrane of sperm is composed of carbohydrates that bind to lipids (glycolipids) and proteins (glycoproteins), collectively referred to as the cell envelope or glycocalyx (Tecle and Gagneux, 2015). The glycocalyx enables carbohydrates, such as sucrose, to safeguard cell membranes against deterioration during refrigerated storage. The protective effect of sucrose on the cell membrane enables a more significant proportion of sperm cells to survive as movement progresses. Surachman *et al*. (2009) state that sucrose is more effective than glucose and fructose in maintaining its function as an extracellular protector. As stated by Rizal et al. (2007), adding sugar to the diluent results in the sugar-binding with the carbohydrates in the damaged cytoplasmic membrane layer during storage. This process leaves the carbohydrates intact, replacing mechanically damaged cell envelope structures (Anwar and Jiyanto, 2019).

Conversely, adding lactose or fructose to T-diluents (TL, TF) has resulted in a SD that is even lower than that of TS-diluents. Lactose is a disaccharide comprising two monosaccharide units, namely glucose and galactose. In contrast, fructose is a monosaccharide. It may be hypothesized that galactose is responsible for the low SD of sperm in TL diluent. It is not a significant source of energy for the sperm in comparison to glucose and fructose. The use of fructose in the TF diluent, which resulted in a lower SD than in the TS, may be attributed to the essential components of the Tris diluent, which consists of tris hydroxymethyl amino methane, fructose, and citric acid. Therefore, adding fructose to the Tris diluent results in an excessive concentration of fructose, which has no positive effect on sperm SD. A similar outcome was observed when lactose and fructose (TLF) were introduced to the Tris diluent, with sperm SD exhibiting a significant decline compared to the TS diluent.

This outcome may be attributed to an excessive elevation in osmotic pressure, which could potentially result in the demise of a more significant number of sperm. This finding aligns with previous studies (Lavanya *et al*., 2022), which also observed that high osmotic pressure adversely affects sperm viability. However, this contrasts with other studies suggesting that moderate increases in osmotic pressure could enhance sperm function by reducing oxidative stress (Perumal *et al*., 2022). These discrepancies highlight the need for further research to explore the precise osmotic pressure thresholds that balance sperm survival and motility. An excessive loss of water from the cell can result in cell death (Maldonado and Mohiuddin, 2023). These findings have significant implications for breeding programs, where the preservation of boar sperm is crucial. Optimizing the osmotic pressure of sperm diluents could improve artificial insemination success rates, thereby enhancing fertility management. Therefore, improvements in preservation protocols are needed to adjust the solute concentration in diluents so that osmotic pressure remains within a range that supports sperm survival and motility. Future research should focus on refining dilution protocols to ensure osmotic pressure remains optimal, avoiding damage to sperm, and increasing the efficiency of boar sperm storage and usage in commercial breeding.

Sperm Abnormality

The prevalence of sperm abnormalities in the fresh semen of the five samples was relatively low, with a range of 2 to 4% and an average value of 2.80%. Following the addition of the diluent, the degree of sperm abnormality remained unaltered, with the values observed in all diluents remain-

ing consistent with those observed in the fresh semen. From the 12th hour after collection, the percentage of abnormal sperm was found to be lower in the TS diluent than in the TL, TF, TSF, and TLF diluents ($P < 0.05$). Nevertheless, no significant difference was observed compared to the T diluent ($P > 0.05$). From the 24th to the 60th hour of preservation, the TEy diluent exhibited a significantly lower percentage of abnormal sperm than the other five treatments ($P < 0.05$). The addition of sucrose to the Tris diluent has been demonstrated to reduce the abnormal rate of boar sperm.

Table 5: Abnormality of landrace boar sperm in Tris-yolk diluent supplemented with different types of carbohydrates.

ST (hours)		Treatment						P-value
		T	TS	TL	TF	TSF	TLF	
0	Means	2.80^{A}	2.80^{A}	3.00^{A}	2.80^{A}	2.80^{A}	2.80^{A}	0.999
	SD	0.84	0.84	1.22	0.84	0.84	0.84	
12	Means	3.20^{AB}	2.80^{A}	3.60^{B}	3.40^{B}	3.40^{B}	4.00^{B}	0.056
	SD	0.84	0.45	1.34	0.89	0.55	0.71	
24	Means	3.70^{B}	2.90^{A}	4.60^{BC}	4.30^{BC}	4.20^{BC}	4.80^{C}	0.001
	SD	0.67	0.55	1.34	0.67	0.45	0.45	
36	Means	4.80^{B}	3.60^{A}	5.60^{B}	5.40^{B}	5.00^{B}	5.00^{B}	0.000
	SD	0.45	0.55	1.34	0.89	0.71	0.71	
48	Means	6.00^{B}	3.80^{A}	6.60^{B}	6.80^{B}	5.80^{B}	6.80^{B}	0.000
	SD	0.71	0.55	1.34	0.84	0.84	0.84	
60	Means	6.70^{B}	4.60^{A}	8.00^{C}	7.80^{BC}	7.00^{BC}	7.80^{BC}	0.000
	SD	1.04	0.55	1.22	0.84	0.71	0.45	

Description; **ST:** Duration of Preservation; **T:** Tris-yolk egg; **TS:** Tris-yolk egg + sucrose; **TL:** Tris-yolk egg + lactose; **TF:** Tris-yolk egg + fructose; **TSF:** Tris-yolk egg + sucrose + fructose; **TLF:** Tris-yolk egg + lactose + fructose; Different superscripts on the same line indicate significant differences ($P<0.05$).

Sucrose in the diluent functions as an extracellular protective compound (Thananurak *et al*., 2019), safeguarding the sperm plasma membrane from damage caused by the effects of cold shock during the preservation or cryopreservation process. During the storage period, damage is incurred by the cell membrane due to protein degradation. Consequently, the cell's lipoprotein layer instigates protein denaturation due to peroxidation reactions within the membrane. The addition of sucrose to the diluent serves to minimize the damage to the membrane.

While this study demonstrates the positive impact of sucrose on sperm quality in landrace boars, several avenues for future research remain. Further studies should examine the long-term effects of sucrose supplementation on sperm quality, particularly beyond the 60-hour preservation period, to determine if these benefits are sustained. Additionally, research could focus on investigating the optimal concentration of sucrose and its interaction with other carbohydrates, such as lactose and fructose, in different boar breeds. Comparing the effects of sucrose with other potential energy sources or preservatives may reveal even more effective strategies for sperm preservation. Additionally, exploring how these improvements could translate to real-world applications, such as enhancing artificial insemination success rates in commercial breeding programs, would be a valuable next step. These investigations could contribute to refining preservation protocols and optimizing reproductive technologies in the swine industry.

CONCLUSIONS AND RECOMMENDATIONS

The addition of sucrose to the TEy diluent resulted in a notable improvement in sperm quality in landrace boars compared to the control and other carbohydrate types. These findings suggest that sucrose is an effective energy source for sperm preservation. Based on these results, it is recommended that sucrose be included in the TEy diluent for the preservation of landrace boar semen. This improvement in sperm motility and viability could have practical implications for breeding programs, enhancing the efficiency of artificial insemination by ensuring higher-quality semen storage. Future fertility management strategies may benefit from incorporating sucrose into semen preservation protocols, potentially improving success rates in boar-based reproductive technologies.

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NOVELTY STATEMENT

This study introduces a novel approach to preserving Landrace boar sperm by incorporating specific carbohydrates, identifying sucrose as the most effective for enhancing motility, viability, and reducing abnormalities during storage. It highlights the importance of optimizing osmotic pressure, showing that while carbohydrates provide energy, excessive concentrations can harm sperm quality due to hypertonicity. These findings provide practical recommendations for refining sperm preservation protocols, particularly for breeding programs, by incorporating sucrose to improve artificial insemination success rates

AUTHOR'S CONTRIBUTIONS

All authors equally contributed and approved the manuscript.

Conflict of Interest

The authors declare no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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