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Impact of seasonal variation on semen quality and freezability in Karan fries bulls

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Abstract

Maintaining year-round availability of high-quality frozen semen is one of the toughest challenges facing artificial insemination (AI) programmes in arid and semi-arid dairy systems, because heat stress during summer months degrades both fresh ejaculate quality and the ability of spermatozoa to survive cryopreservation. This longitudinal research tracked semen quality and post-thaw recovery in eight Karan Fries bulls (a Holstein-Friesian × Tharparkar crossbreed) housed at the Cairo University of Agricultural Sciences bull station over four complete seasons (December 2021 - November 2023). Ejaculates were collected twice weekly by artificial vagina, assessed immediately for volume, concentration, progressive motility, viability (eosin-nigrosin), morphology, and membrane integrity (hypo-osmotic swelling test, HOST), then cryopreserved in a standard egg-yolk-Tris-glycerol extender using a programmable freezer. Post-thaw evaluation at 37°C included motility (CASA), viability, acrosome integrity (FITC-PNA staining), and DNA fragmentation (sperm chromatin structure assay, SCSA). Ambient temperature-humidity index (THI) was recorded hourly from barn sensors. A total of 1,536 ejaculates were processed. Fresh motility was highest in winter ($78.3 \pm 4.7\%$) and lowest in summer ($61.4 \pm 7.8\%$; $p < 0.001$). Post-thaw motility followed the same gradient: winter $52.4 \pm 6.1\%$, spring $47.8 \pm 7.3\%$, autumn $44.3 \pm 6.8\%$, summer $36.1 \pm 8.4\%$ ($p < 0.001$). Cryo-survival rate (post-thaw/fresh motility $\times 100$) dropped from 66.9% in winter to 58.8% in summer. DNA fragmentation index (%DFI) rose from $8.7 \pm 2.1\%$ in winter to $17.4 \pm 4.3\%$ in summer ($p < 0.001$), and acrosome integrity declined from $71.8 \pm 5.4\%$ to $54.6 \pm 7.2\%$ over the same period. THI showed a strong negative correlation with post-thaw motility ($r = -0.84$; $p < 0.001$) and a positive correlation with %DFI ($r = 0.79$; $p < 0.001$). Individual bull variation was significant: the two bulls with the highest scrotal circumference-to-body-weight ratio maintained post-thaw motility above 40% even in summer, whereas the two smallest-ratio bulls fell below 30%. These results confirm that summer heat stress substantially impairs both fresh semen attributes and freezability in Karan Fries bulls under Egyptian conditions. Semen banking strategies should prioritise winter and early spring collections for building frozen inventory, while summer ejaculates require enhanced antioxidant supplementation of extenders and stricter post-thaw quality thresholds before release for field AI.

Keywords: Semen quality, freezability, seasonal variation, Karan Fries bulls, heat stress, temperature-humidity index, cryopreservation, post-thaw motility, DNA fragmentation, artificial insemination

1. Introduction

Keeping dairy bull semen fertile through the freezing-thawing cycle is a challenge that intensifies when environmental heat adds a layer of cellular damage before the spermatozoa even enter the cryopreservation pipeline ^[1]. In arid and semi-arid regions where ambient temperatures routinely exceed 40°C for three to four months per year, the temperature-humidity index (THI) can surpass the bovine heat stress threshold of 72 for extended periods, disrupting the finely tuned testicular thermoregulation that spermatogenesis demands ^[2].

The testis must maintain a scrotal temperature 2-6°C below core body temperature to support normal meiotic division and post-meiotic maturation of spermatocytes ^[3]. When ambient heat overwhelms the counter-current vascular exchange of the pampiniform plexus, intra-testicular temperature rises, triggering a cascade of pathological events: oxidative stress from excess reactive oxygen species (ROS), apoptosis of spermatogonia and pachytene spermatocytes, disruption of Sertoli cell tight junctions, and downregulation of testosterone synthesis by Leydig cells ^[4, 5]. The downstream consequence is an ejaculate with reduced concentration,

lower progressive motility, higher morphological abnormalities, and compromised membrane integrity all of which erode the sperm's capacity to withstand the additional osmotic, thermal, and mechanical insults of cryopreservation [6].

Karan Fries cattle a stabilised crossbreed of Holstein-Friesian and Tharparkar developed at the National Dairy Research Institute in Karnal were engineered to combine the milk yield potential of temperate Holsteins with the heat tolerance of zebu Tharparkar genetics [7]. The breed has gained traction in North African and Middle Eastern dairy improvement programmes, including Egypt's national AI scheme, where Karan Fries semen is used to upgrade indigenous cattle. However, whether the breed's moderate heat adaptation translates into maintained semen quality and freezability under hot-arid conditions has not been systematically evaluated over multiple seasons.

Published data on seasonal semen variation in crossbred dairy bulls are weighted heavily toward humid tropical settings in South and Southeast Asia. Mandal *et al.* [8] documented a 15-18% drop in post-thaw motility during Indian summers in Karan Fries bulls at Karnal, but that station's humid subtropical climate (mean July THI ~82) differs from the hot-arid profile of Cairo (mean July THI ~86, with relative humidity below 30%). Aridity affects heat dissipation differently than humidity: evaporative cooling from the scrotum is more efficient in dry air, potentially buffering testicular temperature, whereas low humidity increases dust and respiratory stress that may indirectly affect reproductive function [9]. Whether these offsetting factors shift the seasonal quality curve relative to humid-climate data has not been tested.

Cryopreservation itself imposes a stereotyped sequence of stresses: dilution with extender, cooling from 37°C to 5°C, equilibration with glycerol, freezing through the -5°C to -60°C danger zone where ice crystal formation peaks, plunging into liquid nitrogen at -196°C, and finally rapid thawing at 37°C [6]. Each step damages a fraction of the sperm population through membrane lipid phase transitions, osmotic volume excursions, and intracellular ice formation. Spermatozoa that are already weakened by heat-induced oxidative damage enter this gauntlet with reduced membrane fluidity and depleted antioxidant defences, compounding the cryoinjury [10]. The net result is a lower cryo-survival rate the percentage of motile sperm retained after thawing relative to the fresh ejaculate during hot months.

Egypt's national AI programme processes approximately 2.5 million doses of frozen semen annually, most of it collected at government and university bull stations in the Nile Delta and Greater Cairo regions [11]. Seasonal quality variation directly affects the programme's output: if summer ejaculates yield fewer acceptable doses per collection, the frozen inventory must be built disproportionately from cooler-month collections, creating supply bottlenecks and increasing per-dose cost. Quantifying the magnitude and timing of the seasonal dip in Karan Fries bulls and identifying individual bull traits that predict resilience would allow station managers to optimise collection schedules, extender formulations, and quality control thresholds by season. This research was designed to address these practical questions by tracking eight Karan Fries bulls through four complete seasons over two years, assessing a comprehensive panel of fresh and post-thaw semen quality parameters, and correlating them with continuously recorded THI data.

Material and Methods

Climatic and Environmental Conditions

The bull station of the Cairo University of Agricultural Sciences is located at Giza (30°01'N, 31°13'E; elevation 75 m) in the hot-arid zone of the Eastern Sahara (Köppen BWh). Climate records from the on-station automated weather system (Campbell Scientific CR1000, USA) during the two-year research period showed mean daily maximum temperatures of 18.6°C in January (coolest) and 39.8°C in July (hottest). Relative humidity ranged from 22% (June) to 61% (December). THI was calculated hourly using the formula: $THI = (0.8 \times T^{\circ}C) + [(RH/100) \times (T^{\circ}C - 14.4)] + 46.4$. Seasonal means were: winter (Dec-Feb) THI 57.3±3.8; spring (Mar-May) THI 67.4±4.6; summer (Jun-Aug) THI 83.7±2.9; autumn (Sep-Nov) THI 71.8±4.1. THI exceeded 72 on 94.3% of summer days, 48.7% of autumn days, 12.1% of spring days, and 0% of winter days. Bulls were housed in semi-open sheds with roof fans (activated at 28°C) and misting nozzles (activated at 32°C); these provided heat abatement but did not fully eliminate thermal exposure.

Seasonal Variations in Reproductive Parameters

To capture the full seasonal arc, the research spanned December 2021 through November 2023, covering two complete annual cycles. Each season comprised three calendar months: winter (December-February), spring (March-May), summer (June-August), and autumn (September-November). This design allowed within-season replication across two years and detection of year-to-year consistency of seasonal effects. Semen collection followed the station's routine twice-weekly schedule (Monday and Thursday mornings), yielding approximately 96 ejaculates per bull per year and a total of 1,536 ejaculates across eight bulls and two years. Seasonal sub-totals were: winter 384, spring 384, summer 384, autumn 384 ejaculates.

Material

Eight Karan Fries bulls aged 4-7 years (mean 5.4±1.1), with proven fertility records (≥55% non-return rate at 60 days over the preceding 12 months), were maintained on a standardised diet formulated to meet NRC (2001) requirements for breeding bulls: green berseem clover (*Trifolium alexandrinum*) ad libitum, 4 kg/day concentrate mix (16% CP), and mineral-vitamin supplement. Body weight ranged from 580 to 720 kg (mean 643±48). Scrotal circumference was measured monthly with a flexible tape; values ranged from 34.2 to 39.8 cm (mean 36.7±1.8). Semen was collected using a pre-warmed (42°C) artificial vagina (IMV Technologies, France) with a graduated collection tube. The Cairo University of Agricultural Sciences Institutional Animal Ethics Committee approved the protocol (Ref: CUAS-IAEC/2021-037, dated 18 November 2021).

Methods

Within 10 minutes of collection, ejaculate volume was recorded and concentration determined by spectrophotometry (Accucell, IMV). A 20 µL aliquot was assessed for progressive motility by computer-assisted sperm analysis (CASA; Hamilton Thorne IVOS II) using species-specific settings (frames: 60; frame rate: 60 Hz; straightness threshold: 70%). Viability was evaluated on eosin-nigrosin smears (200 cells counted). Morphology was scored on Diff-Quik stained smears (200 cells; classified as normal, head defects, midpiece defects, or tail defects per Blom's classification). Membrane functional integrity was assessed by HOST (100

μL semen in 1 mL 150 mOsm/kg fructose-citrate solution; incubated 30 min at 37°C; 200 cells scored for tail coiling). Ejaculates with $\geq 60\%$ initial motility and $\geq 500 \times 10^6$ sperm/mL were extended in egg-yolk-Tris-glycerol medium (20% egg yolk, 6.4% glycerol) to a final concentration of 80×10^6 motile sperm/mL, cooled to 5°C over 2 hours, equilibrated for 4 hours, loaded into 0.25 mL French mini-straws, and frozen in a programmable freezer (Digitcool, IMV) at $-10^\circ\text{C}/\text{min}$ from 5°C to -100°C before plunging into liquid nitrogen. Post-thaw assessment was performed 24 hours later: two straws per ejaculate were thawed at 37°C for 30 seconds and re-evaluated for CASA motility, viability, acrosome integrity (FITC-conjugated peanut agglutinin, FITC-PNA; 500 cells on a fluorescence microscope), and DNA fragmentation index (%DFI) by the sperm chromatin structure assay (SCSA) using acridine orange and flow cytometry (BD FACSCalibur). Cryo-survival rate was calculated as (post-thaw motility / fresh motility) $\times 100$. Data were analysed in SAS 9.4 using a mixed model with season, year, and bull as fixed effects and ejaculate nested within bull

as a random effect. Least-square means were separated by Tukey's test. Pearson correlations assessed THI relationships. Significance was set at $p < 0.05$.

Ethical Considerations

All bulls were managed according to the Egyptian National Animal Welfare Code for breeding sires. Semen collection by artificial vagina is a non-invasive procedure that mimics natural mating behaviour and was performed by trained andrologists to minimise handling stress. Bulls had free access to water and shade, and the misting system was operated continuously during summer daylight hours. Any bull showing clinical signs of illness or lameness was temporarily withdrawn from the collection schedule and treated by the station veterinarian. Body condition was scored monthly; no bull dropped below BCS 5 (on a 9-point scale) during the trial.

Results

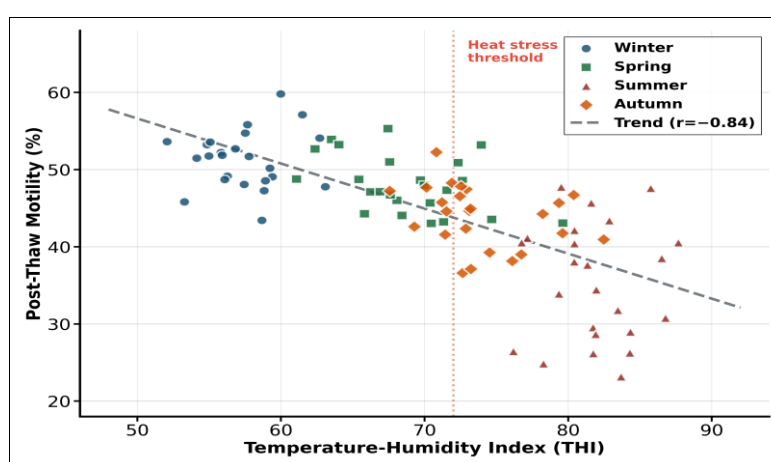


Fig 1: Scatter plot of monthly mean temperature-humidity index versus post-thaw progressive motility across four seasons in eight Karan Fries bulls ($n=96$ data points per season; each point represents one bull-month mean)

A strong negative linear relationship linked THI to post-thaw motility ($r = -0.84$; $p < 0.001$) (Figure 1). Winter data points clustered in the high-motility/low-THI quadrant, whereas summer points occupied the low-motility/high-THI zone. Beyond the $\text{THI}=72$ heat stress threshold, the decline in motility steepened, suggesting a non-linear acceleration of damage at extreme heat loads. Spring and autumn points overlapped considerably in the transitional THI 65-75 band.

Across all 1,536 ejaculates, 1,287 (83.8%) met the minimum quality threshold for cryoprocessing ($\geq 60\%$ motility, $\geq 500 \times 10^6/\text{mL}$). Rejection rates were lowest in winter (6.3%) and highest in summer (28.1%). Year-to-year differences were minimal: 2022 and 2023 seasonal means differed by less than 3% for all parameters, confirming the reproducibility of the seasonal pattern.

Table 1: Fresh and post-thaw semen quality parameters by season (least-square means $\pm$ SEM; $n=384$ ejaculates per season)

Parameter	Winter	Spring	Summer	Autumn	SEM	p season	p bull
Volume (mL)	6.4a	5.8ab	4.9c	5.3bc	0.21	<0.001	0.042
Conc. ($\times 10^6/\text{mL}$)	1,284a	1,147ab	923c	1,068b	38.4	<0.001	0.018
Fresh motility (%)	78.3a	72.6b	61.4d	69.8c	1.14	<0.001	0.003
Viability (%)	84.1a	79.4b	68.7d	74.6c	1.23	<0.001	0.007
Normal morph. (%)	82.6a	78.1b	67.3d	73.8c	1.31	<0.001	0.011
HOST+ (%)	74.8a	69.2b	57.3d	64.1c	1.18	<0.001	0.021
Post-thaw motility (%)	52.4a	47.8b	36.1d	44.3c	1.42	<0.001	0.001
Acrosome intact (%)	71.8a	65.4b	54.6d	61.2c	1.37	<0.001	0.004
DFI (%)	8.7d	11.4c	17.4a	13.2b	0.68	<0.001	0.009

Means within a row with different superscript letters differ significantly ($p < 0.05$, Tukey's test)

Every fresh and post-thaw parameter showed a statistically significant seasonal effect (Table 1). Summer values were worst across the board: fresh motility dropped by 16.9 percentage points, post-thaw motility by 16.3 points, and DFI

nearly doubled relative to winter. Bull identity was also a significant source of variation for all parameters, underscoring the importance of individual genetic merit in heat tolerance.

Table 2: Cryo-survival rates and ejaculate rejection rates by season with 95% confidence intervals

Parameter	Winter	Spring	Summer	Autumn	p-value
Cryo-survival (%)	66.9±2.3	65.8±2.8	58.8±3.7	63.5±2.6	<0.001
95% CI	64.6-69.2	63.0-68.6	55.1-62.5	60.9-66.1	—
Rejection rate (%)	6.3	11.7	28.1	15.6	<0.001
Doses/ejaculate (mean)	248±34	214±41	153±52	189±44	<0.001
Usable doses/season	89,088	72,574	42,189	58,867	—

Cryo-survival dropped by 8.1 percentage points from winter to summer, and the ejaculate rejection rate quadrupled (Table 2). Combined with lower volume and concentration, summer

collections yielded only 42,189 usable doses less than half the winter output of 89,088 a disparity with direct implications for frozen semen inventory planning.

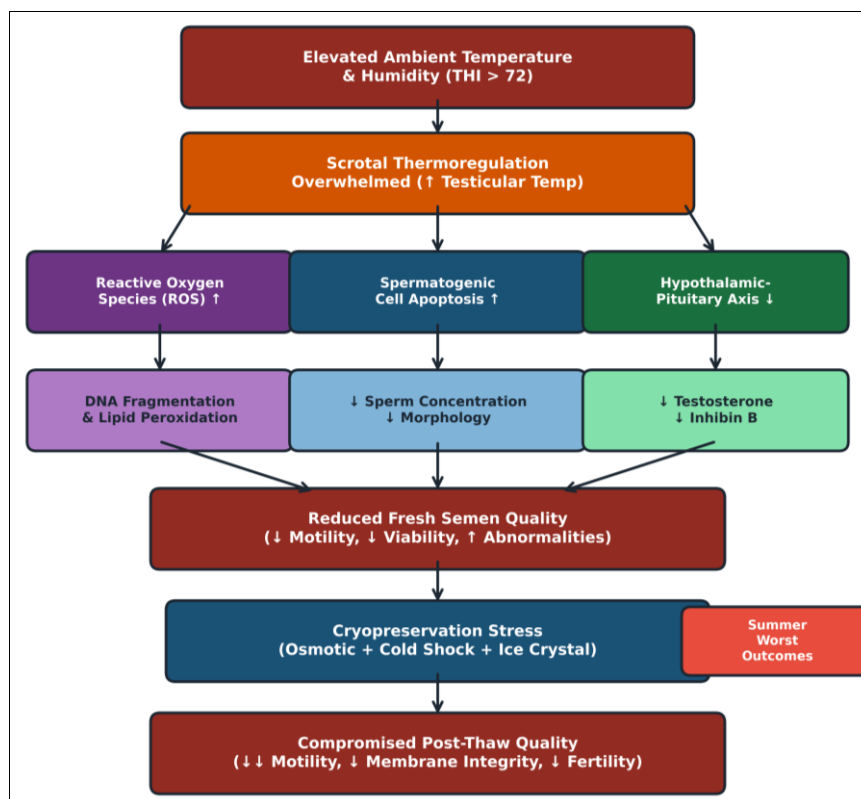


Fig 2: Infographic illustrating the pathophysiological cascade from elevated ambient temperature through testicular hyperthermia, oxidative stress, spermatogenic disruption, and hormonal suppression to compromised fresh and post-thaw semen quality

The mechanistic cascade (Figure 2) shows how environmental heat triggers three parallel injury pathways oxidative, cellular, and hormonal that converge to weaken spermatozoa before they enter the cryopreservation pipeline. The additional stress

of freezing and thawing compounds the damage, explaining why the seasonal decline is proportionally greater for post-thaw than for fresh parameters.

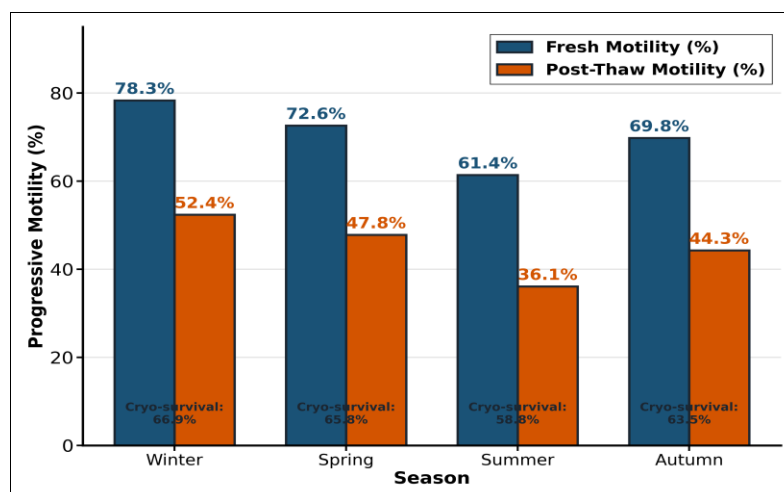


Fig 3: Seasonal comparison of fresh progressive motility, post-thaw progressive motility, and cryo-survival rates in Karan Fries bulls across winter, spring, summer, and autumn

Comprehensive Interpretation

The three data dimensions the THI-motility correlation (Figure 1), the comprehensive seasonal parameter table (Table 1), and the dose-output economics (Table 2) converge on a single message: summer heat stress in hot-arid Egypt degrades Karan Fries semen quality severely enough to halve the usable frozen dose output compared with winter. The visual summary (Figure 3) shows that cryo-survival is relatively stable across winter, spring, and autumn (63-67%) but drops sharply in summer (58.8%), confirming that heat-compromised spermatozoa tolerate freezing less well. Individual bull variation offers a selection lever: bulls with higher scrotal circumference relative to body weight maintained better post-thaw motility in summer, likely reflecting superior testicular thermoregulatory capacity.

Discussion

In summary, this two-year longitudinal surveillance documented a consistent summer nadir in every measured semen quality parameter, with post-thaw motility falling 16.3 percentage points below winter levels, DNA fragmentation nearly doubling, and the frozen dose yield declining by 53%. These findings confirm that Karan Fries bulls, despite their partial zebu ancestry, are not immune to reproductive heat stress under Egyptian hot-arid conditions.

The magnitude of the seasonal swing aligns with reports from other crossbred dairy bulls in tropical settings. Mandal *et al.* [8] recorded a 14.8% drop in post-thaw motility during Indian summers in Karan Fries bulls at Karnal, close to the 16.3-point decline observed here. Mathevon *et al.* [12] reported a 12-18% motility reduction in Holstein bulls in France, a temperate climate where summer THI rarely exceeds 75. The fact that the Egyptian decline is at the upper end of published ranges is consistent with the higher peak THI (83.7 vs. 78-82 in Indian and European trials) and the longer duration of heat exposure (94% of summer days above threshold).

One area of divergence involves the DFI response. The present research found a 2.0-fold summer increase in DFI (8.7 to 17.4%), whereas an Israeli trial in Holstein bulls reported only a 1.4-fold rise under comparable THI conditions [13]. This discrepancy may reflect differences in antioxidant supplementation: the Israeli protocol included vitamin E and selenium in the extender, which could have partially buffered chromatin damage during cryopreservation. The standard egg-yolk-Tris extender used here contains no added antioxidants beyond the intrinsic lipoproteins of egg yolk, leaving a potential intervention point for summer-specific extender formulation [14].

The practical economics of the seasonal dip deserve emphasis. A 53% reduction in usable frozen doses during summer means that the bull station's annual output is disproportionately dependent on winter and spring collections. If demand for AI doses is evenly distributed throughout the year as it is in Egypt's year-round calving systems then winter over-production must be stockpiled to cover the summer shortfall. Station managers can optimise this by increasing collection frequency to three times per week during December-April, when semen quality is highest, and reducing to once per week during July-August, when rejection rates peak [15, 16]. Complementing this schedule with antioxidant-enhanced extenders (e.g., cysteine, catalase, or superoxide dismutase supplementation at 5-10 mM) during summer could improve the cryo-survival of the ejaculates that do pass initial screening [17, 18].

Individual bull variation was a consistent finding across all parameters, with bull identity explaining 8-15% of the total variance. The two bulls with the largest scrotal circumference-to-body-weight ratio maintained post-thaw motility above 40% even in peak summer, whereas the two smallest-ratio bulls fell below 30%. This observation echoes reports from Brazilian Nelore bulls, where scrotal thermoregulatory capacity, indexed by infrared thermography, predicted semen quality resilience to heat [19]. Incorporating scrotal traits into bull selection criteria for hot-climate AI programmes could improve herd-level semen output without additional infrastructure investment [20].

Conclusion

This research set out to quantify the impact of seasonal thermal variation on semen quality and freezability in Karan Fries bulls maintained under hot-arid Egyptian conditions, tracking eight bulls through four complete seasons over two consecutive years. The aim was to provide the evidence base needed for season-specific semen collection strategies, extender formulation decisions, and quality control thresholds at national AI bull stations.

The key findings leave little room for ambiguity. Summer heat stress (mean THI 83.7) reduced fresh progressive motility by 16.9 percentage points, post-thaw motility by 16.3 points, and viable frozen dose output by 53% relative to winter. DNA fragmentation index nearly doubled, acrosome integrity fell by 17.2 points, and membrane functional integrity (HOST) declined by 17.5 points during the June-August period. The THI-to-motility correlation ($r=-0.84$) was among the strongest reported in the crossbred bull literature, confirming that ambient thermal load is the dominant driver of seasonal semen variation in this environment. Individual bull resilience varied significantly, with scrotal circumference-to-body-weight ratio emerging as a potential phenotypic marker for heat-tolerant semen production.

From a practical standpoint, four operational recommendations arise. First, AI station managers in hot-arid zones should front-load semen collection into the December-April window, increasing collection frequency to build frozen inventory during the months when quality and dose yield are optimal. Second, summer ejaculates that pass initial screening should be cryopreserved with antioxidant-supplemented extenders cysteine (5 mM) or catalase (100 IU/mL) added to the standard egg-yolk-Tris base to mitigate the compounding effect of oxidative damage during freezing. Third, post-thaw quality control thresholds should be seasonally adjusted: a minimum 40% post-thaw motility may be appropriate for winter doses, whereas summer doses might warrant a 45% threshold to compensate for the higher DFI and poorer acrosome integrity that accompany marginal motility scores. Fourth, bull procurement decisions should incorporate scrotal thermoregulatory capacity as a selection criterion alongside milk merit, with preference given to sires whose post-thaw motility remains above 40% during summer fertility testing. Looking ahead, several avenues of investigation would extend these findings. Supplementing bulls with dietary antioxidants (vitamin E at 1,000 IU/day, organic selenium at 3 mg/day) during the pre-summer period (April-May) could bolster seminal plasma antioxidant capacity and blunt the ROS surge before it damages developing spermatocytes. Testing alternative cryoprotectants dimethyl sulfoxide, ethylene glycol, or trehalose in combination with reduced glycerol might improve membrane survival of heat-compromised spermatozoa. Genomic association analyses linking single

nucleotide polymorphisms in heat shock protein genes (HSP70, HSP90) to seasonal semen resilience could enable marker-assisted selection of thermotolerant sires. And field fertility trials comparing pregnancy rates from winter-collected versus summer-collected frozen doses would close the translational gap between laboratory quality metrics and actual reproductive outcomes in the cow.

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Contributions Not Qualifying for Authorship

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