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A simulation-based assessment of longitudinal and distributed-lag models for seasonal reproductive responses in three indigenous goat ecotypes

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Abstract

This study used a fully synthetic dataset to evaluate an analysis framework for a future longitudinal investigation of seasonal reproductive variation in indigenous Namibian goats. No live animals were screened or enrolled, and no specimens or environmental observations were collected. A single reproducible simulation generated 36 virtual bucks (12 Ovambo, 12 Kavango and 12 Caprivi), 12 monthly records per buck, correlated semen, endocrine and oxidative outcomes, measurement error and pre-specified missingness. The data-generating model used beta distributions for percentage outcomes, log-normal distributions for positively skewed outcomes and Gaussian mixed models for approximately symmetric outcomes, with buck-level random effects, first-order temporal autocorrelation and correlated reproductive, endocrine and oxidative latent factors. Seasonal and heat-lag parameters were calibrated as scenario inputs, not estimated biological truths. In the resulting synthetic dataset, model-adjusted progressive motility was 13.3 percentage points lower in the hot-dry than in the cool-dry scenario (95% confidence interval [CI]: -15.4 to -11.2; $p < 0.001$). This simulated reduction was 15.2, 14.0 and 10.6 percentage points for virtual Ovambo, Kavango and Caprivi bucks, respectively (interaction $p = 0.004$). Simulated testosterone levels were lower during the hot-dry period (geometric-mean ratio 0.63; 95% CI: 0.57-0.69), while malondialdehyde was higher and antioxidant indices were lower. Under the pre-specified lag coefficients, the largest modeled association with progressive motility occurred for heat exposure 36-49 days before collection. These outputs show that the proposed data structure and models can recover imposed seasonal, ecotype and lag patterns. They do not provide evidence of actual seasonal effects, ecotype differences, fertility or heat tolerance in Namibian goats. Multiyear, multisite empirical validation is required.

1. Introduction

Indigenous goats support meat and milk production, household income and food security in smallholder and semi-arid systems. Their capacity to remain productive under variable feed, water and climatic conditions makes them important animal genetic resources (Peacock 2005; Mdladla et al. 2018). Population-level adaptation, however, does not imply that reproductive function is constant throughout the year. Male fertility is especially important because a single buck may influence the conception rate and genetic structure of an entire herd. Seasonal assessment is therefore relevant to breeding-soundness evaluation, artificial insemination, semen preservation and conservation of locally adapted populations (Ranjan et al. 2021). Male goat reproduction is influenced by photoperiod, temperature, humidity, rainfall, nutrition, body condition and disease pressure. Photoperiod acts through melatonin and the hypothalamic-pituitary-gonadal axis, whereas high thermal load can disrupt scrotal heat dissipation, steroidogenesis and germ-cell development. Nutrition may modify these responses through energy, protein, and mineral availability. The relative importance these factors varies with latitude, breed, and production system, contributing to reported seasonal differences in libido, testicular size, testosterone and semen quality (Martínez-Rodríguez et al. 2021; Khan et al. 2025; Golher et al. 2018; Barkawi et al. 2006; Talebi et al. 2009).

The timing of exposure matters when semen measurements are interpreted. The seminiferous epithelial cycle in goats lasts approximately 10.6 days and spermatogenesis requires about 48 days before epididymal maturation (França et al. 1999). Heat exposure several weeks before collection could therefore influence ejaculated spermatozoa, whereas collection-day conditions may be more relevant to acute endocrine or thermoregulatory responses. Repeated observations combined with lagged temperature-humidity exposure provide a way to distinguish these temporal patterns. Semen quality is multidimensional. Volume, concentration and total sperm output describe production; whereas motility, kinematics, viability, morphology, membrane and acrosomal integrity, mitochondrial function and DNA integrity describe complementary aspects of functional potential. Seasonal changes in abnormalities may occur even when conventional measurements change little (Barbas et al. 2024). Computer-assisted sperm analysis (CASA) improves objectivity, but chamber depth, dilution, temperature, loading and analysis time materially affect reported values (Del Gallego et al. 2017).

Testosterone supports spermatogenesis, accessory-gland function and libido. Seasonal changes in circulating testosterone concentrations may accompany changes in testicular dimensions, sexual activity and semen output (Martínez-Rodríguez et al. 2021; Barkawi et al. 2006; Talebi et al. 2009). Oxidative stress supplies a further plausible pathway: excessive reactive oxygen species can promote lipid peroxidation, mitochondrial dysfunction, impaired motility and DNA

damage, and spermatozoa are vulnerable because their membranes contain abundant polyunsaturated fatty acids and their cytoplasmic antioxidant capacity is limited (Sengupta et al. 2024; Aitken and Roman 2008). The work presented in this manuscript is exclusively a simulation study, with the complete data-generating process described explicitly and all results identified as synthetic outputs. The aim was to determine whether a pre-specified longitudinal analysis could recover imposed seasonal contrasts, ecotype-by-season heterogeneity, correlated endocrine and oxidative patterns, and biologically motivated heat-lag effects. The primary estimand was the model-adjusted hot-dry versus cool-dry difference in progressive motility in one synthetic realisation. All numerical effects are scenario parameters or outputs; none are estimates from live goats.

2. Materials and Methods

2.1 Study type, scope and ethical status

This was a computer simulation study. No live animals, owners, farms, biological specimens, clinical records or identifiable data were involved. Animal-research ethics approval and owner consent were therefore not applicable. References to animal handling and laboratory techniques below define a target protocol and measurement model for a possible future empirical study; they do not describe procedures performed in the present study.

2.2 Simulation design, estimand and reproducibility

The simulation followed the ADEMP structure: aims, data-generating mechanisms, estimands, methods and performance reporting (Morris et al. 2019). It generated one synthetic longitudinal cohort rather than a Monte Carlo comparison of competing estimators. The pseudorandom-number seed was fixed at 20260907. The virtual buck was the experimental unit, and monthly records were nested within each buck. The pre-specified primary estimand was the marginal difference in progressive motility between hot-dry and cool-dry periods, averaged across ecotypes. The primary interaction estimand compared this seasonal contrast across the three ecotypes. Secondary outcomes were semen volume, sperm concentration, total sperm output, viability, morphology, membrane integrity, testosterone, malondialdehyde and total antioxidant capacity. Other CASA measures, antioxidant enzymes, lag windows and mediation models were exploratory.

2.3 Virtual cohort and calendar

A balanced cohort of 36 virtual bucks was generated directly: 12 Ovambo, 12 Kavango and 12 Caprivi. There was no simulated recruitment from real herds and no actual screening. Each virtual buck had 12 scheduled monthly records. Seasons were coded a priori as cool-dry (May-August), hot-dry (September-November) and wet (December-April). Calendar inputs were calibrated to a hot semi-arid Nkurenkuru scenario (17.62° S, 18.60° E; approximately 1,095 m above sea level), with seasonal mean temperature inputs of 18.7, 31.8 and 27.4°C and relative-humidity inputs of 34.2%, 27.6% and 67.8%, respectively. These are simulation settings rather than measurements from a study site.

2.4 Environmental data-generating model

Daily mean temperature and relative humidity were generated from seasonal means plus a sinusoidal annual component and a bivariate first-order autoregressive innovation. Innovation standard deviations were 2.8 °C and 8 percentage points; the within-day temperature-humidity innovation correlation was -0.35, and the temporal autocorrelation was 0.72. Values were truncated to 8-42°C and 10-95% relative humidity. Rainfall was generated from a zero-inflated gamma distribution: the daily probability of rain was 0.03, 0.05 and 0.32 in cool-dry, hot-dry and wet periods, and positive amounts followed Gamma(shape = 1.8, scale = 8.4 mm). Photoperiod was a deterministic sinusoid ranging from 11.2 to 12.8 hours. Temperature-humidity index (THI) was computed as THI = (1.8T + 32) – (0.55-0.0055 RH) (1.8T-26), where T is temperature in °C and RH is relative humidity in percentage. Excess heat was the daily integral above THI 78 and was accumulated over 0-7, 8-21, 22-35, 36-49 and 50-63 days before each virtual collection.

2.5 Baseline animal variables

Age was drawn from ecotype-specific truncated normal distributions over the range of 2-5 years. Body weight, body-condition score and scrotal circumference were generated jointly from a Gaussian copula, then transformed to the marginal distributions in Table 1. The target correlations were 0.40 between body weight and body condition, 0.45

Table 1. Prespecified data-generating distributions and calibration parameters

Component	Distribution	Parameters or constraints
Age (years)	Truncated normal [2,5]	Means: 3.5, 3.3, 3.4; SDs: 0.9, 1.0, 0.8
Body weight (kg)	Normal, truncated [28,60]	Means: 46.2, 42.5, 39.8; SDs: 5.4, 4.8, 4.6
Body-condition score	Normal, truncated [1,5]	Means: 3.1, 3.0, 2.9; SDs: 0.4, 0.4, 0.3
Scrotal circumference (cm)	Normal, truncated [20,32]	Means: 26.7, 25.9, 25.3; SDs: 1.5, 1.4, 1.3
Percentage semen outcomes	Beta mixed model	Mean from logit predictor; precision $\phi = 85$
Volume and velocity outcomes	Gaussian mixed model	Outcome-specific SD; physiologic truncation
Concentration, testosterone, MDA, antioxidants	Log-normal mixed model	Geometric mean from log predictor; CV = 8%-18%
Repeated observations	Random intercept + AR(1)	Buck SD outcome-specific; residual $\rho = 0.45$
Cross-outcome dependence	Three correlated latent factors	Corr(Q,T) = 0.45; Corr(Q,O) = -0.55; Corr(T,O) = -0.35

Ecotype-specific values are ordered Ovambo, Kavango and Caprivi. Q is the semen-quality factor, T the endocrine factor and O the oxidative-stress factor. All parameters are simulation inputs

between body weight and scrotal circumference, and 0.30 between body condition and scrotal circumference. A Bernoulli variable represented previous breeding use. Visit-level body condition included a +0.18 wet-period and -0.05 hot-dry shift, plus a buck random intercept and AR(1) residual.

2.6 Target measurement protocol and measurement-error model

A future empirical protocol was assumed to collect semen between 08:00 and 10:00 AM after three days of sexual rest and evaluate fresh samples within 15 minutes. CASA settings were fixed at a 20 µm chamber depth, 37 °C, 60 frames/s, at least five fields and 500 accepted tracks; progressive spermatozoa were defined by straightness ≥70% and average-path velocity >25 µm/s (Del Gallego et al. 2017). Viability and morphology were conceptualized as duplicate counts of 200 cells each, membrane integrity as a 200-cell hypo-osmotic swelling assay, acrosomal integrity as a 200-cell fluorescein-labelled peanut-agglutinin assay, mitochondrial potential as 10,000 gated events and DNA fragmentation as a dispersion assay based on 300-cells. These quantities were not measured; the specifications determined binomial or continuous measurement error in the synthetic variables. The units and definitions of CASA variables are given in Table 2.

Testosterone was represented on the scale of a competitive ELISA with an assumed 0.083 ng/mL lower sensitivity and 7.4% inter-assay coefficient of variation. Malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione were assigned log-normal analytical errors of 6%, 6%, 8%, 8%, 8%, and 7%, respectively. Volume, concentration, and CASA measurement errors were set at 5%, 7%, and 2.5 percentage points, respectively. Errors were independent of ecotype and season after conditioning on the latent true value. Laboratory methods mentioned, such as Diaz De Leon and Borges (2020), Kielkopf et al. (2020), and Munteanu and Apetrei (2021) are proposed assays for future validation.

2.7 Outcome means, seasonal effects and nonlinear heat relationships

Each outcome model contained an ecotype main effect, a seasonal term, an ecotype-by-season interaction, age, body condition, scrotal circumference, a buck random intercept, an outcome-family latent factor and an AR(1) residual. Seasonal marginal targets were set before data generation and corresponded to the values later reported in Table 4 and Table 6. Thus, for example, the intended progressive-motility means were 76.4%, 63.1%, and 71.8% for cool-dry, hot-dry, and wet periods, respectively. The imposed hot-dry declines by ecotype were 15.2, 14.0, and 10.6 percentage points. These values were calibration targets, not biological estimates discovered from the data.

Heat effects were superimposed on within-season daily deviations. For progressive motility, coefficients for a one-standard-deviation increase in excess heat across the five lag windows were -0.3, -0.8, -1.8, -3.1 and -1.2 percentage points, respectively. A hinge function above THI 76 contributed -0.72 percentage points per THI unit plus a quadratic term of -0.025 per squared unit. For testosterone, the largest coefficient was assigned to 0-7 days (-0.12 on the log scale per heat SD); for MDA it was assigned to 8-21 days (+0.11); and for TAC negative coefficients were assigned to both 0-7 and 22-35 days. These deliberately imposed coefficients created the lag patterns evaluated by the fitted models.

2.8 Correlation structure

Cross-outcome dependence was generated with three standard-normal latent factors: semen quality (Q), endocrine status (T) and oxidative stress (O). Their correlation matrix had diagonal elements 1.00 and off-diagonal correlations Corr(Q,T) = 0.45, Corr(Q,O) = -0.55, and Corr(T,O) = -0.35. Within the semen family, residual correlations were 0.65 for progressive motility with viability, 0.55 with membrane integrity and 0.45 with normal morphology. Within the oxidative family, MDA correlated -0.45 with each antioxidant measure, whereas pairwise antioxidant correlations were 0.55. Positive-definiteness was checked before sampling. This structure produced biologically coherent but explicitly assumed covariation.

2.9 Missingness and analytic sample construction

Missingness was generated after outcomes. Two clinical visits were designated as missing using Bernoulli draws calibrated to an overall probability of 0.5%. Conditional on a completed virtual visit, the first-ejaculate success probability was 0.995; semen-quality exclusion probability was 0.007; testosterone invalidity probability was 0.005; and the probability of insufficient volume for oxidative assays was 0.012. These draws were independent of unobserved outcome values conditional on ecotype, month and a simulated recent-illness indicator. The fixed seed yielded 430 clinical records, 425 valid semen records, 428 testosterone records and 423 oxidative profiles. The counts therefore describe the generated dataset only, not animals or specimens actually encountered.

2.10 Statistical analysis

Analyses were pre-specified for R 4.5.1 using lme4, nlme, mgcv, dlnm, emmeans, mice, and boot. Gaussian identity-link mixed models were used for approximately symmetric outcomes. Positively skewed variables were log-transformed and reported as geometric means or ratios. Percentage outcomes were represented as proportions and fitted using beta mixed models after the Smithson-Verkuilen adjustment. Models included ecotype, season, their interaction, age, body condition, scrotal circumference, recent illness, pen and source-herd terms, a buck random intercept and, when supported, a random month slope. An

Table 2. Definitions and reporting units for simulated CASA variables

Abbreviation	Unit	Definition
TMOT	%	Total motile spermatozoa
PMOT	%	Progressively motile spermatozoa
VCL	µm/s	Curvilinear velocity
VSL	µm/s	Straight-line velocity
VAP	µm/s	Average-path velocity
LIN	%	100 × VSL/VCL
STR	%	100 × VSL/VAP
WOB	%	100 × VAP/VCL
ALH	µm	Amplitude of lateral head displacement
BCF	Hz	Beat-cross frequency

These definitions parameterise synthetic variables; they are not records from an instrument

AR(1) residual represented within-buck temporal dependence.

Because all virtual bucks shared one annual environmental sequence, monthly records were not treated as independent environmental replications. Month was included as a common-exposure effect and uncertainty for environmental contrasts was evaluated with a wild cluster bootstrap at the month level. A cyclic spline represented calendar time in distributed-lag models. Benjamini-Hochberg correction controlled the false-discovery rate at 5% within the pre-specified CASA and oxidative families. Sensitivity analyses included observations assigned recent illness, changed seasonal coding, used robust estimation and multiple imputation for missing values. Two-sided $p < 0.05$ was used, but p-values quantify model behaviour in the generated dataset and do not support population inference.

3. Results

Every number in this section is a simulation output from one seeded synthetic realisation. The terms 'virtual buck', 'generated record' and 'simulated estimate' are used deliberately. No count represents a live animal, clinical encounter, specimen or assay.

3.1 Simulated dataset yield and baseline profile

The generator created 36 virtual bucks and 432 scheduled monthly records. After the pre-specified missingness mechanism, the synthetic dataset contained 430 clinical records, 425 valid first-ejaculate records, 428 testosterone values, and 423 oxidative profiles (Fig. 1). There was no empirical screening or animal exclusion. The realized missing-data proportions did not materially differ by ecotype or season in this synthetic run (Table 3).

3.2 Simulated environmental sequence

The generated calendar reproduced the intended seasonal contrast (Fig. 2). Mean temperature inputs were 18.7°C in cool-dry, 31.8°C in hot-dry and 27.4°C in wet months, while mean humidity inputs were 34.2%, 27.6% and 67.8%. The realized annual rainfall total was 527 mm, concentrated in the wet period. Mean THI was higher in hot-dry and wet months than in cool-dry months. These values demonstrate the environmental exposure structure imposed on all virtual bucks and are not meteorological observations.

3.3 Simulated semen quantity and functional quality

The fitted model recovered the imposed seasonal pattern in semen quantity and quality (Table 4). The simulated hot-dry versus cool-dry difference in ejaculate volume was -0.26 mL (95% CI: -0.32 to -0.20), while the sperm-concentration ratio was 0.77 (95% CI: 0.71-0.84).

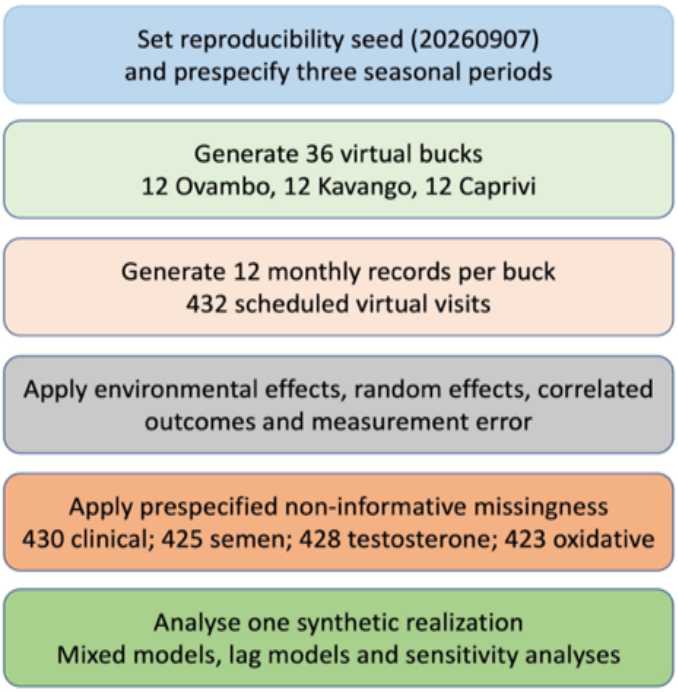

Fig. 1. *Synthetic cohort generation and analysis workflow. All counts are outputs of the prespecified generator; no animals or specimens were involved*

Synthetic total sperm output was 40.5% lower in the hot-dry period. These values should be interpreted as recovery of the calibration targets. For the primary synthetic outcome, adjusted progressive motility was 76.4% in cool-dry, 63.1% in hot-dry and 71.8% in wet months. The simulated hot-dry versus cool-dry contrast was -13.3 percentage points (95% CI: -15.4 to -11.2; $p < 0.001$). The imposed ecotype heterogeneity was also recovered: declines were 15.2 percentage points for Ovambo, 14.0 for Kavango, and 10.6 for Caprivi virtual bucks (interaction $p = 0.004$; Table 5 and Fig. 3). These are not comparative heat-tolerance estimates.

3.4 Simulated CASA kinematics and sperm-function measures

In the synthetic CASA family, total motility decreased from 84.1% in cool-dry to 72.8% in hot-dry months. VCL, VSL, and VAP during hot-dry period were lower than their corresponding cool-dry values by 18.6, 9.4, and 12.1 µm/s. Simulated linearity and straightness were lower, while ALH was slightly higher. After correction across nine exploratory CASA outcomes, all seasonal terms except BCF remained

Table 3. Baseline characteristics generated for the virtual cohort

Characteristics	Ovambo	Kavango	Caprivi	Overall
Number of virtual bucks	12	12	12	36
Age (years)	3.5 ± 0.9	3.3 ± 1.0	3.4 ± 0.8	3.4 ± 0.9
Body weight (kg)	46.2 ± 5.4	42.5 ± 4.8	39.8 ± 4.6	42.8 ± 5.4
Body-condition score	3.1 ± 0.4	3.0 ± 0.4	2.9 ± 0.3	3.0 ± 0.4
Scrotal circumference (cm)	26.7 ± 1.5	25.9 ± 1.4	25.3 ± 1.3	26.0 ± 1.5
Previous breeding use, n (%)	10 (83.3)	9 (75.0)	8 (66.7)	27 (75.0)

Values are synthetic means ± standard deviations or synthetic counts (percentages)

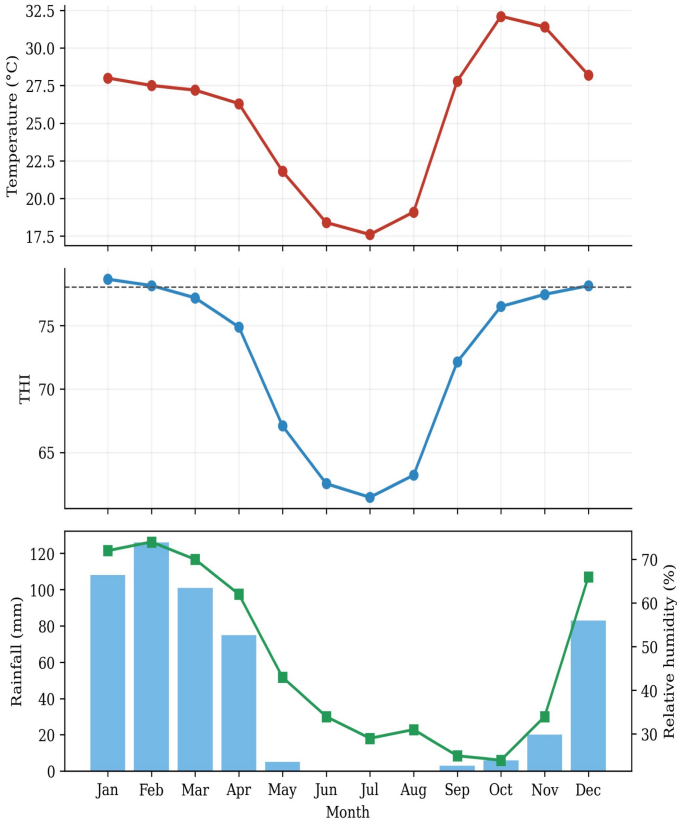

Fig. 2. Generated monthly environmental sequence used in the simulation. THI, temperature-humidity index. Values are synthetic inputs and outputs, not observations from a weather station

significant (BCF q = 0.083). The generated DNA fragmentation index was 9.8%, 19.4% and 13.1% in cool-dry, hot-dry and wet periods, respectively. These patterns were encoded to test multivariate reporting and are not evidence about sperm function in any ecotype.

3.5 Simulated testosterone and oxidative profile

The endocrine and oxidative models recovered their imposed seasonal directions (Table 6). Synthetic testosterone was 37% lower in hot-dry than cool-dry months (geometric-mean ratio 0.63; 95% CI: 0.57-0.69). The significant interaction effect (p = 0.018) reflected a smaller imposed proportional decline for virtual Caprivi bucks (Fig. 4). Simulated MDA increased from 1.84 to 3.42 nmol/mg protein, while TAC and antioxidant enzyme activities decreased. All six oxidative-family seasonal terms remained significant after false-discovery-rate correction (q ≤ 0.009). No assay was performed.

3.6 Simulated heat-lag and multivariable associations

The fitted exposure-response model recovered the imposed hinge above THI 76 (Fig. 5). Under the generator, a five-unit increase in THI above 76 corresponded to a 4.8-percentage-point reduction in progressive motility (95% CI: -6.0 to -3.6). The largest assigned motility coefficient was in the 36-49-day window; virtual records in the highest versus the lowest heat-exposure quartiles differed by 9.6 percentage points (95% CI: -12.1 to -7.1). Synthetic testosterone responded most strongly in the preceding 0-7 days, whereas MDA responded most strongly at 8-21 days (Table 7). These are expected recoveries of the chosen lag coefficients, not a discovery of biologically critical windows.

3.7 Simulated sensitivity analyses

Sensitivity analyses were directionally consistent within this synthetic realisation. Including records assigned to recent illness changed the hot-dry versus cool-dry motility contrast from -13.3 to -13.1 points. Multiple imputation yielded -13.2 points (95% CI: -15.3 to -11.1), and robust estimation retained both the seasonal term and ecotype-by-season interaction. Alternative lag windows moved the maximum fitted

Table 4. Model-adjusted synthetic semen-quality estimates by season

Outcome	Cool-dry	Hot-dry	Wet	Ecotype x Season p
Ejaculate volume (mL)	1.12 (1.06-1.18)	0.86 (0.80-0.92)	1.03 (0.97-1.09)	0.084
Sperm concentration (10^9/mL)	3.21 (3.05-3.37)	2.47 (2.32-2.62)	2.92 (2.77-3.07)	0.061
Total sperm output (10^9)	3.58 (3.37-3.79)	2.13 (1.94-2.32)	2.99 (2.80-3.18)	0.032
Progressive motility (%)	76.4 (74.8-78.0)	63.1 (61.4-64.8)	71.8 (70.2-73.4)	0.004
Viability (%)	81.7 (80.1-83.3)	68.9 (67.1-70.7)	77.5 (75.9-79.1)	0.017
Normal morphology (%)	87.2 (85.8-88.6)	76.8 (75.2-78.4)	83.6 (82.2-85.0)	0.043
Membrane integrity (%)	79.6 (78.0-81.2)	65.4 (63.6-67.2)	74.3 (72.7-75.9)	0.021

Values are simulated adjusted means (95% CI). Synthetic first-ejaculate counts were 142, 106 and 177 for cool-dry, hot-dry, and wet periods (total n = 425). Overall season p < 0.001 for every listed outcome. No cell is based on an animal measurement

Table 5. Simulated progressive-motility estimates by ecotype and season

Ecotype	Cool-dry	Hot-dry	Wet
Ovambo	77.6 (74.8-80.4)	62.4 (59.3-65.5)	71.9 (69.1-74.7)
Kavango	75.8 (73.0-78.6)	61.8 (58.7-64.9)	71.0 (68.2-73.8)
Caprivi	75.9 (73.1-78.7)	65.3 (62.3-68.3)	72.5 (69.7-75.3)

Values are simulated model-adjusted percentages (95% CI). Ecotype-by-season interaction p = 0.004

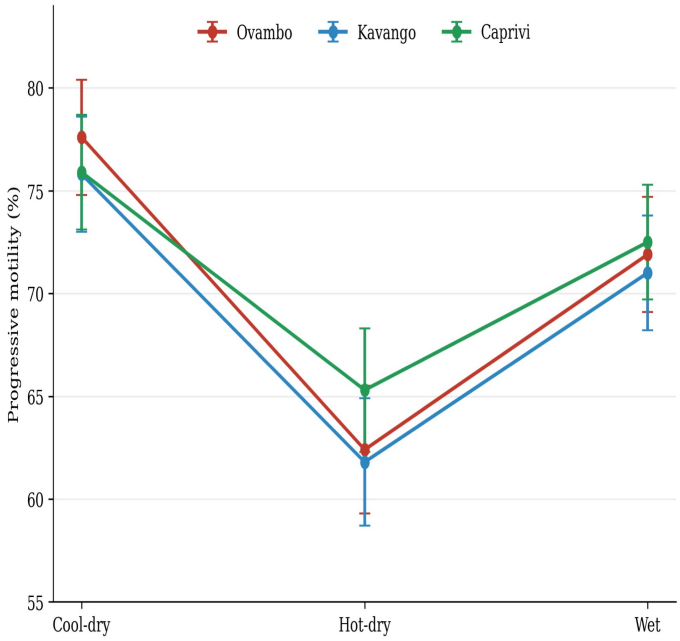

Fig. 3. Model-adjusted synthetic progressive-motility estimates with 95% confidence intervals. Lines connect seasonal scenario outputs and must not be interpreted as observed biological trajectories

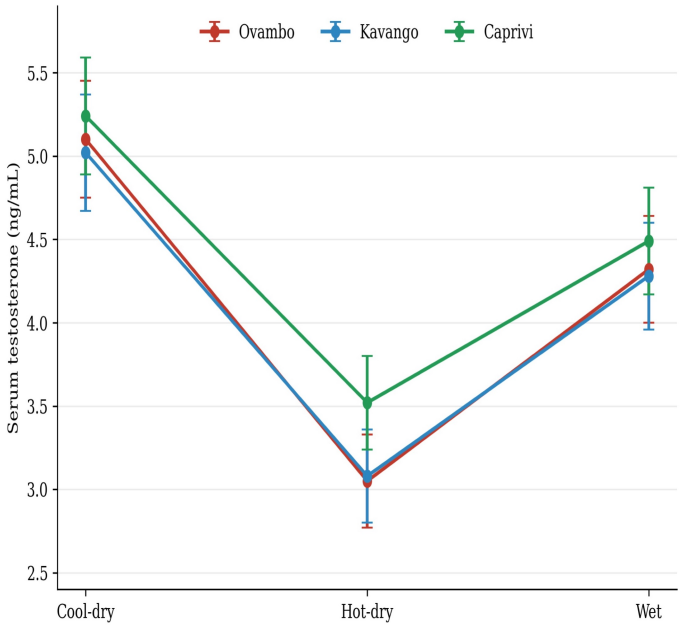

Fig. 4. Model-adjusted synthetic testosterone concentrations with 95% confidence intervals. The ecotype differences are imposed scenario features, not empirical estimates

association by about one week. These checks test computational stability under the imposed scenario; they do not create independent years or environmental replication.

4. Discussion

This study makes a narrower and methodologically defensible contribution: it demonstrates a transparent simulation framework for planning a future longitudinal investigation of seasonal male-goat reproduction. The results do not describe animals that were screened, specimens that were processed or effects observed in Namibia. Instead, they show how one seeded synthetic dataset behaves when known seasonal, ecotype, correlation, and lag parameters are imposed. This distinction is essential because a simulation can evaluate workflow coherence and estimator recovery, but it cannot establish biological prevalence, causation or population differences (Morris et al. 2019). The simulated decline in motility during the hot-dry scenario is directionally consistent with reports from empirical goat literature but its magnitude remains an input-dependent output. Earlier field studies reported seasonal changes in semen quality, testicular activity or testosterone in goat bucks (Martínez-Rodríguez et al. 2021; Khan et al. 2025; Barkawi et al. 2006; Talebi et al. 2009). Differences among those studies also show why a numerical expectation should not be directly inferred from the present scenario. Latitude, photoperiod, nutrition, husbandry, collection frequency, breed and laboratory methods differ, and these factors may change both the direction and size of a seasonal contrast.

The ecotype-by-season interaction illustrates how heterogeneous trajectories could be estimated in a balanced repeated-measures design. The smaller simulated decline assigned to Caprivi bucks is not evidence of heat tolerance or genetic superiority. Nonetheless, including such an interaction in the planned model is scientifically useful because locally

Table 6. Model-adjusted synthetic testosterone and oxidative-status estimates by season

Outcome	Cool-dry	Hot-dry	Wet	Ecotype x Season p
Serum testosterone (ng/mL)	5.12 (4.78-5.48)	3.21 (2.98-3.46)	4.36 (4.05-4.69)	0.018
MDA (nmol/mg protein)	1.84 (1.69-1.99)	3.42 (3.25-3.59)	2.51 (2.35-2.67)	0.026
TAC (μmol Fe^{2+} equivalents/L)	968 (934-1002)	715 (684-746)	842 (810-874)	0.039
SOD (U/mg protein)	42.6 (40.8-44.4)	31.9 (30.2-33.6)	38.8 (37.1-40.5)	0.052
Catalase (U/mg protein)	18.4 (17.5-19.3)	12.6 (11.8-13.4)	15.7 (14.9-16.5)	0.044
GPx (U/mg protein)	29.2 (27.9-30.5)	20.8 (19.6-22.0)	25.5 (24.3-26.7)	0.063
Reduced glutathione (μmol/g)	8.4 (8.0-8.8)	5.8 (5.4-6.2)	7.2 (6.8-7.6)	0.049
Oxidative-stress index	-0.62 (-0.75 to -0.49)	0.81 (0.67-0.95)	0.03 (-0.10 to 0.16)	0.031

Values are simulated adjusted means (95% CI); testosterone is a geometric mean. Oxidative-profile counts were 142, 106 and 175 by season (total n = 423). MDA, malondialdehyde; TAC, total antioxidant capacity; SOD, superoxide dismutase; GPx, glutathione peroxidase

Table 7. Mixed-effects estimates from the synthetic progressive-motility model

Predictor	Estimate	SE	95% CI	p value
Heat load, per 100 index-hours	-1.42	0.22	-1.85 to -0.99	<0.001
Kavango versus Ovambo	0.60	0.69	-0.75 to 1.95	0.382
Caprivi versus Ovambo	2.10	0.76	0.61 to 3.59	0.006
Heat load × Kavango	0.16	0.09	-0.02 to 0.34	0.078
Heat load × Caprivi	0.39	0.10	0.19 to 0.59	<0.001
Testosterone, per 1 ng/mL	1.32	0.25	0.83 to 1.81	<0.001
MDA, per 1 nmol/mg protein	-2.08	0.41	-2.88 to -1.28	<0.001
TAC, per 100 µmol/L	1.14	0.31	0.53 to 1.75	<0.001
Body-condition score, per point	1.76	0.72	0.35 to 3.17	0.015

The dependent variable is simulated progressive motility in percentage points. Estimates describe one generated dataset. The model also included age, scrotal circumference, season, recent illness, pen, source herd, month and cyclic calendar time

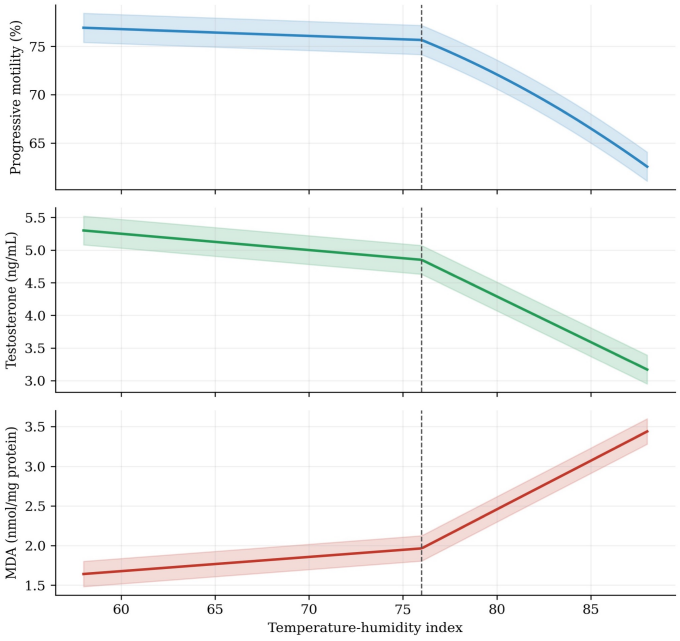

Fig. 5. *Pre-specified nonlinear exposure-response functions recovered from the synthetic dataset. Curves are adjusted simulation outputs, shaded areas are model-based 95% confidence bands and the dashed line marks the imposed THI hinge at 76*

adapted populations may differ in morphology, metabolism and stress response. Genetic diversity among southern African indigenous goats supports the general possibility of local adaptation (Mdladla et al. 2018), and coat characteristics have been related to heat stress resilience, testicular haemodynamics, hormones and semen quality in goats (El-Sherbiny et al. 2023). A real ecotype claim would require representative sampling, defensible ancestry classification, multisite replication and direct measurement of individual heat exposure. The imposed oxidative profile provides a coherent test of joint biomarker analysis. Higher MDA levels with lower TAC and antioxidant enzyme activities are compatible with lipid peroxidation and weakened antioxidant defence, mechanisms widely discussed in male reproduction (Sengupta et al. 2024; Aitken and Roman 2008). However, biomarkers are not interchangeable, and their correlations were selected by the generator. In an empirical study, pre-analytical handling, storage, protein normalization, kit lot, assay precision and laboratory batch could alter apparent patterns. Individual markers should therefore be reported alongside a composite index, with blinded duplicate assays and balanced laboratory batches across seasons and ecotypes.

The lag structure is biologically motivated but remains imposed. Goat spermatogenesis takes approximately 48 days, with subsequent epididymal maturation, so exposure occurring 36-49 days before collection is a reasonable candidate window (França et al. 1999). The simulated model was designed to recover that window. It cannot prove that this is the dominant critical period in Namibian goats. Empirical estimation would require continuous environmental monitoring, more frequent semen collection, independent years and careful control of nutrition, illness, management, and photoperiod. Flexible lag models should also be constrained to avoid overfitting when only 12 monthly time points are available. CASA and sperm-function outputs require equally cautious interpretation. CASA improves repeatability but remains sensitive to chamber depth, dilution, temperature, loading and analysis delay (Del Gallego et al. 2017). The settings specified here make the proposed future protocol auditable; they do not validate a device or classification threshold. Similarly, conventional semen indices indicate reproductive potential rather than pregnancy or kidding performance. Earlier work has shown that seasonal abnormalities may not be captured by a single conventional measure (Barbas et al. 2024), reinforcing the need for morphology, membrane, mitochondrial and DNA measures. Yet no laboratory panel can substitute for post-thaw recovery and field-fertility outcomes when the goal is to make breeding recommendations (Ranjan et al. 2021). A central design limitation is shared environmental exposure. All virtual bucks experienced the same calendar sequence. Buck-level random effects correctly address repeated measurements within animals, but they cannot create independent seasons or weather replicates. Month-level resampling reduces false precision only within the simulated sequence. A future study should span at least two, preferably three, years and more than one site. That design would help distinguish heat, humidity, rainfall, forage and photoperiod, which are strongly correlated within a single annual cycle.

The simulation also exposes choices that should be pre-specified

before empirical data are collected. One primary outcome and one primary seasonal contrast should be identified; ecotype interactions and lag surfaces should be limited; assay batches should be blocked across groups; and missingness rules should be documented. Power calculations should include within-buck correlation, common environmental exposure and attrition. Publishing the generator, seed, synthetic data and analysis script will allow readers to determine which findings were imposed and which arose from sampling variation. The main strengths are explicit distributional assumptions, transparent correlation and error structures, clear separation of target protocol from performed work, and consistent labelling of synthetic results. Limitations are equally important: one calibrated scenario cannot assess robustness across alternative biological truths; several covariance and measurement-error values are assumptions; model-based confidence intervals may appear more precise than the design warrants; and no external validation exists. Accordingly, the manuscript supports protocol refinement and computational checking only. It does not support animal selection, semen collection, conservation or breeding season recommendations.

5. Conclusions

The revised study is an explicitly defined simulation, not an empirical animal reproduction experiment. In one seeded synthetic realisation, the analysis recovered imposed hot-dry reductions in progressive motility, testosterone and antioxidant status, the imposed smaller decline among virtual Caprivi bucks and a pre-specified 36-49-day motility lag. These outputs demonstrate that the proposed data structure and statistical workflow can represent seasonal, ecotype and delayed exposure patterns. They do not demonstrate actual biological effects, fertility, cryotolerance, ecotype superiority or a reproducible Namibian seasonal pattern. Multiyear, multisite research involving real animals, validated laboratory procedures, direct environmental monitoring and field-fertility outcomes is required before practical recommendations can be made.

Declarations

Funding: This simulation study received no external funding

Conflict of interest: The author declare no conflicts of interest

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Data Availability: The complete data-generating specification is reported in this manuscript. The corresponding author will provide the synthetic dataset, data dictionary, fixed seed, and analysis code as part of the final reproducibility package upon reasonable request

Ethics approval: Not applicable

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