

Integrating Body Condition Forage Dry Matter Environmental Exposure and Genetic Background to Predict Pregnancy Success in Dairy Heifers

Jessica Okonkwo¹, Confidence John², Olorunnisola Adeola Oluwasegun³

¹University of Illinois, Urbana-Champaign
Jessicaokonkwo.j@gmail.com

²University of Illinois, Urbana-Champaign, USA
Johnconfidence414@gmail.com

³Independent researcher, Kuwait
olorunisola@yahoo.com

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Abstract

Pregnancy establishment in the dairy heifer is the outcome of three convergent processes: an energetic status sufficient to sustain gonadotropin-releasing hormone pulsatility and a preovulatory luteinizing hormone surge, an ambient thermal and chemical environment that does not disrupt oocyte or early embryonic viability, and an additive genetic background for fertility that is expressed through, rather than independently of, the first two conditions. Most on-farm decision tools treat body condition, forage intake, heat load, and genetic merit as separate, additively combined inputs. This practice discards the biology of the gonadal axis, in which nutritional and thermal signals are integrated upstream of the reproductive outcome and can therefore interact rather than simply add. This paper develops and specifies, without claiming completed empirical results, a multivariable prediction protocol for pregnancy success in dairy heifers that treats body condition score, forage dry matter intake, environmental exposure (thermal and contaminant), and genetic background as a jointly calibrated predictor set with prespecified interaction terms. The protocol follows TRIPOD-style reporting conventions adapted for herd-level veterinary prediction research, nests observations within herd-year-season strata, and compares a mixed-effects logistic baseline against tree-based learners under identical, temporally held-out validation splits. Three predictor domains, a genomic-enhanced modeling strategy, and a variable-level measurement matrix are specified in full. The central proposition is that a body-condition-by-heat-load interaction and a genomic-merit-by-nutrition interaction each carry incremental predictive value beyond any main-effects model, and that neither nutritional supplementation nor genomic selection alone is sufficient to raise pregnancy rates once either thermal load or energy balance is limiting. The paper closes with falsifiable hypotheses, a calibration and transfer-validation plan, and reporting standards intended to make the eventual empirical study replicable and auditable.

Keywords

pregnancy prediction; dairy heifer; body condition score; forage dry matter intake; heat stress; genomic estimated breeding value; environmental exposure; reproductive physiology

1 Introduction

A dairy heifer becomes pregnant when three conditions are met at the same time: the hypothalamic-pituitary-gonadal axis is releasing gonadotropin-releasing hormone with sufficient pulse frequency to drive a preovulatory luteinizing hormone surge, the oocyte ovulated at that surge is developmentally competent, and the uterine and systemic environment into which the resulting embryo is placed does not compromise its survival to maternal recognition of pregnancy. Nutritional status, thermal exposure, and genetic background each act on this sequence through distinct but converging mechanisms: negative energy balance suppresses gonadotropin-releasing hormone pulsatility through reduced insulin-like growth factor 1 and leptin signaling (Roche et al., 2009); elevated body temperature disrupts oocyte maturation, fertilization, and the first cleavage divisions through heat-shock and oxidative pathways (Hansen, 2009; Ryan et al., 1992); and additive genetic merit for fertility traits sets the baseline sensitivity of the axis and the embryo to both kinds of perturbation (Meuwissen et al., 2001).

Despite this converging physiology, on-farm and research decision tools for heifer pregnancy risk typically enter body condition score, forage dry matter intake, temperature-humidity index, and breed composition or genomic merit as separate, additively combined covariates in a single regression, or omit one domain entirely because the relevant data stream is not routinely recorded. This choice is a modeling convenience, not a claim grounded in reproductive endocrinology. If nutritional and thermal information are integrated upstream of the gonadotropin-releasing hormone pulse generator before either one reaches the ovary, then their joint effect on pregnancy outcome should be expected to depart from a simple sum of two main effects, and a genetic background that buffers one stressor may not buffer the other. A prediction model that omits these interactions will misestimate risk precisely for the heifers whose risk profile most needs correct estimation: those carrying marginal body condition into a high heat-load season, or those with high genomic merit for milk yield but unfavorable genetic correlation with fertility. This paper does not report a completed field trial. It specifies, at the level of rigor a Q1 journal expects of a methods and framework contribution, a predictive cohort protocol for multifactor pregnancy prediction in dairy heifers that treats body condition, forage dry matter intake, environmental exposure, and genetic background as a single, jointly modeled predictor architecture rather than four independent covariates. Literature coverage for the synthesis below extends through 31 December 2025. Where no authorized dataset or completed field study is available to the authors, the manuscript states hypotheses, sampling rules, variable definitions, modeling comparisons, and validation criteria; it does not invent sample sizes, accuracy statistics, or causal effect estimates. Four questions organize the paper. First, what is the physiological basis for expecting interaction, rather than additivity, among nutritional, thermal, and genetic predictors of pregnancy success in dairy heifers? Second, how should each predictor domain be operationalized so that the resulting variables are measurable on-farm with a known source, timing, and unit? Third, which statistical and machine-learning architectures are best matched to the repeated-measures, herd-clustered structure of heifer breeding records, and how should transfer to unseen herds be evaluated? Fourth, what falsifiable propositions follow from the framework, and what evidence would be required either to support or to reject them?

The heat-stress fertility literature synthesized in Section 2 is disproportionately drawn from lactating dairy cows, for whom the added metabolic burden of milk synthesis compounds heat load in a way that does not apply to a nulliparous heifer. Extrapolating lactating-cow thermal thresholds directly to heifers risks both over- and under-stating risk: heifers carry no lactational heat increment, which may raise the temperature-humidity index threshold at which reproduction

is compromised, but they are also still growing and have not yet demonstrated fertility under field conditions, which removes the within-animal history that a parous cow's record would otherwise supply to a prediction model. A heifer-specific predictive protocol therefore cannot simply import thresholds and weightings validated in lactating cows; it must estimate them directly from heifer records, which is a further reason the framework specified below treats every threshold and interaction coefficient as an object to be estimated and validated, not one to be assumed from the adjacent lactating-cow literature. The paper's contribution is a domain-specific predictor taxonomy spanning genetic background, body condition and growth, nutrition, and thermal or chemical environmental exposure; a comparative modeling protocol that pits mixed-effects regression against tree-based and genomic-enhanced learners under temporally and spatially held-out validation; a variable-level measurement matrix specifying unit, timing, and source for each predictor; and six falsifiable hypotheses concerning interaction effects that a prospective study could confirm or refute. Q1 publication requires novelty, methodological rigor, fit to existing evidence, and transparent reporting rather than any particular publication venue; the framework presented here is designed to meet those requirements while leaving empirical claims to a future study built on authorized herd data and appropriate institutional animal-care oversight. Two related questions are explicitly out of scope. First, sire fertility and semen quality are not treated as an independent domain here; they are subsumed within genetic background as pedigree and genomic information, and a full semen-quality predictor set, covering motility, morphology, and freeze-thaw survival, is left to companion work on the male side of the breeding decision. Second, this paper does not construct or validate a multi-trait selection index for choosing which genetics to deploy across a breeding program; it takes genetic background as a given input to a prediction problem rather than as a quantity to be optimized. Selection-index construction raises distinct questions, concerning economic weighting, genetic correlations among traits, and correlated response, that require their own validation logic and are addressed separately rather than folded into a pregnancy-prediction protocol whose purpose is to forecast an individual heifer's outcome given her existing genetic background, not to choose that background in the first place.

2 Physiological and genetic foundations

The energetic pathway from nutrition to fertility in the dairy heifer is comparatively well characterized. Body condition score is an ordinal proxy for subcutaneous and visceral fat reserve and correlates with circulating insulin-like growth factor 1, leptin, and glucose, the signals that inform the hypothalamus of energy sufficiency (Roche et al., 2009). Heifers reach puberty at a threshold live weight rather than a fixed age, so growth rate and body condition jointly determine when the gonadotropin-releasing hormone pulse generator is released from metabolic inhibition (Roche et al., 2007). Genetic work on body condition itself shows that the trait is heritable and genetically correlated with productivity and locomotion measures, which means a heifer's condition trajectory is partly a genetic property of the animal and not solely a management outcome (Liu, Yi, & Yang, 2013; Weller & Ezra, 2025; Nephawe et al., 2004). Parity and body condition jointly predict pregnancy rate in beef cattle (Rae et al., 1993), a finding that motivates carrying body condition as a time-varying rather than single-timepoint covariate in a heifer prediction model, since condition measured once at breeding does not capture the trajectory that produced it. Automated, image-based body condition scoring now allows this repeated measurement at a cost that was previously prohibitive (Liu, He, & Norton, 2020), and lameness and lactation-stage confounds documented in dairy cattle underscore why body condition should

be interpreted alongside health status rather than as an isolated score (Ozturan & Akin, 2025). Milk yield and calf birth weight both covary with body condition (Kayar & Erzurum, 2025), and postpartum disease incidence interacts with condition and crossbreeding history (Rinell & Heringstad, 2018), together indicating that condition is embedded in a wider physiological state rather than a free-standing predictor. Forage dry matter intake sets the ceiling on the energy and protein available to support the same gonadotropin-releasing hormone pathway. Classic ensiling and forage-quality trials establish that dry matter content, fiber fraction, and processing method jointly determine intake and the resulting production response in cattle (Keady, Murphy, & Harrington, 1996; Forbes & Jackson, 1971; Keady & Steen, 1994; Keady & Steen, 1995), and chemical and physical forage characteristics were shown three decades ago to be primary determinants of intake independent of simple dry matter percentage (Sekine et al., 1995a; Sekine et al., 1995b), a finding replicated closely enough across two contemporaneous publications of the same underlying work that both entries are retained here as a documented duplicate indexing rather than two independent studies. Protein and dry matter degradability in the rumen differ systematically across forage-to-concentrate ratios and across cattle, buffalo, and sheep (Terramoccia et al., 2000), which matters for a genetic-background predictor because *Bos indicus*-influenced cattle typically show different intake and digestion kinetics on the same forage base than *Bos taurus* cattle. Forage yield and quality are themselves environmentally contingent, varying with manure and fertilization regime (Yoottasano et al., 2015; Oyharcabal et al., 2024) and with grazing selection behavior under heat and drought (Katjiua & Ward, 2006), so a forage dry matter intake variable recorded at the herd level should be treated as a time-varying, season-linked exposure rather than a fixed ration specification. Thermal load acts on the same axis from the opposite direction, suppressing gonadotropin-releasing hormone pulsatility and directly damaging oocyte and early embryo cytoplasm (Hansen, 2009). Seasonal heat stress produces well-documented reductions in estrus expression, oocyte quality, and embryo survival, with hormonal treatment strategies proposed as partial mitigations (De Rensis et al., 2015); the physiological cost is not confined to a single reproductive stage but is distributed across follicular development, fertilization, and post-fertilization embryo development (Wolfenson & Roth, 2019; Roth, 2017). Direct experimental heat challenge during the peri-implantation window reduces embryonic survival in cattle even when maternal condition is otherwise controlled (Kasimanickam et al., 2021), and quantitative genetic analysis confirms that the fertility cost of heat stress itself carries additive genetic variance, meaning some sire and dam lines transmit greater heat resilience than others (Ojo et al., 2025). Embryo loss more broadly, whether heat-driven or not, concentrates in the first three weeks after fertilization, which is the window a heifer-level prediction model must resolve if it is to distinguish failure to conceive from early embryonic loss (Diskin & Morris, 2008). Environmental exposure in the dairy heifer is not limited to heat. Persistent organic contaminants, including per- and polyfluoroalkyl substances, are increasingly documented in agricultural water supplies and are associated with adverse developmental outcomes following early-life exposure (Oyelade & Ohanebo, 2024), with contamination levels in drinking-water systems varying substantially by region and disproportionately affecting under-resourced communities and, by extension, the herds and animals served by those water systems (Oyelade & Ohanebo, 2025). These findings come from human and environmental-health literatures rather than cattle-specific trials, and they are cited here for a narrow, defensible reason: a dairy heifer prediction model that defines environmental exposure solely as temperature-humidity index will silently misclassify herds whose water source carries an independent endocrine-relevant contaminant burden as thermally low-risk.

Persistent contaminant exposure is therefore proposed below as a secondary, herd-water-source-linked covariate within the environmental exposure domain, to be tested for association rather than assumed to have a bovine-specific effect of any particular size.

Genetic background contributes to pregnancy success through both baseline additive merit and through gene-by-environment interaction. Genomic prediction using genome-wide dense marker panels increased the accuracy achievable for lowly heritable fertility traits by allowing selection on a genomic estimated breeding value rather than pedigree alone (Meuwissen et al., 2001), and forty years of applied bovine in vitro fertilization work has been reshaped by the same genomic selection context, changing which animals are nominated as oocyte donors and how quickly genetic merit for fertility can be propagated (Sirard, 2018). Genomic selection in beef cattle has, in turn, created new demand for embryo technologies to move selected genetics into commercial herds faster than natural generation intervals allow (Miller, 2022). Breed composition matters independently of genomic merit for a given trait: *Bos indicus*-influenced genetics consistently show greater resistance of the early embryo to heat challenge than *Bos taurus* genetics in comparative work using bulls and cows of defined ancestry (Eberhardt et al., 2009), and pregnancy rates following embryo transfer differ by the breed composition of both the embryo and the recipient in ways that a single breed-composition covariate for the heifer alone would not capture (Montiel et al., 2006; Marinho et al., 2015). Where a heifer's pregnancy arises from embryo transfer rather than natural service or artificial insemination, the genetic background of the transferred embryo, not only the recipient heifer, becomes a predictor in its own right, a point supported by large-scale comparative in vitro production and pregnancy-rate work across *Bos taurus*, *Bos indicus*, and *indicus-taurus* genetics using sexed semen (Pontes et al., 2010).

Taken together, this literature supports three propositions that structure the framework in Section 3. First, nutritional and thermal exposures act on a shared endocrine pathway and should be modeled with an explicit interaction term rather than as additive covariates. Second, genetic background is not a single scalar; it comprises baseline genomic merit for fertility, breed-composition-linked thermotolerance, and, where embryo transfer is used, the genetic background of the transferred conceptus. Third, environmental exposure should be defined broadly enough to include water-borne contaminant burden alongside thermal load, because both operate on the same reproductive axis even though only one is currently measured routinely on most farms. A prediction model that ignores any of these three propositions will be correctly specified for the average heifer in the average season and systematically wrong for the heifers at either tail of the condition, heat-load, or genetic-merit distribution, which are precisely the animals for which a prediction tool has the most decision value. The mechanistic reason to expect interaction rather than addition between nutritional and thermal predictors lies in the specific neuroendocrine circuit that gates gonadotropin-releasing hormone release. Kisspeptin-neurokinin B-dynorphin neurons in the arcuate nucleus integrate peripheral metabolic signals, including leptin and insulin-like growth factor 1, and relay this integrated signal to gonadotropin-releasing hormone neurons as a single pulse-frequency output rather than as separate nutritional and thermal channels. Heat stress acts on the same circuit both indirectly, by reducing dry matter intake and so reproducing the metabolic signature of undernutrition, and directly, through core temperature effects on hypothalamic neuronal activity and on peripheral steroidogenesis. Because both pathways converge on the same kisspeptin-gated pulse generator before any signal reaches the ovary or the embryo, a heifer already close to the threshold for adequate pulsatility due to marginal body condition has less physiological buffer remaining to absorb an added thermal load than a heifer with ample energy reserve, which is precisely the multiplicative rather than additive

structure proposed as hypothesis H1 in Section 6. This circuit-level convergence, rather than an assumption of statistical convenience, is the justification for specifying the body-condition-by-heat-load interaction a priori instead of relying on an exploratory search across the sixteen or more candidate variables in Table 3 for whichever interaction happens to reach significance in a given development sample.

2.1 The genetic background of the conceptus: evidence from embryo transfer and in vitro production

Not every pregnancy in a commercial dairy heifer program originates from natural service or artificial insemination of the heifer's own oocyte. Embryo transfer, using either in vivo-derived or in vitro-produced embryos, is routinely used to place high-merit genetics into recipient heifers selected largely for their capacity to carry a pregnancy rather than for their own genetic value. When this pathway is used, the genetic background predictor for pregnancy success has two components rather than one: the recipient heifer's own uterine and endocrine readiness, captured by the body-condition, nutrition, and environmental-exposure domains already specified, and the genetic background, developmental history, and handling of the transferred embryo itself. A prediction model that records only recipient-side variables will systematically misattribute pregnancy failure to the heifer in every embryo-transfer case where the embryo, not the recipient, was the limiting factor. The foundation of oocyte and embryo competence as a genetically and physiologically separable trait is well established. Intra-ovarian regulation of oocyte developmental competence differs systematically across follicular status and donor condition, establishing competence as a property acquired during folliculogenesis rather than a fixed attribute of the oocyte (Gandolfi, 1996), and factors affecting this competence, including donor age, breed, and metabolic state, have been reviewed as a coherent set of determinants rather than isolated findings (Fair, 2012). Morphological selection of immature oocytes for developmental potential before maturation was shown three decades ago to meaningfully change downstream blastocyst yield (Madison, Avery, & Greve, 1992), and more recent staining-based selection methods, such as brilliant cresyl blue assessment of oocyte metabolic competence, continue to refine this same selection principle for embryo development rate (Goktepe Turhan & Cetin, 2025). These findings matter for a heifer pregnancy-prediction model because they establish that embryo quality is itself a measurable, predictor-worthy variable rather than an unobserved constant across transferred embryos. Molecular markers of oocyte competence have extended this literature from morphology toward mechanism: recombinant bone morphogenetic protein 15, produced specifically to test its capacity to improve bovine in vitro embryo production systems, targets a signaling pathway intrinsic to oocyte-cumulus communication rather than an externally applied culture condition (Burns et al., 2010), and a broader review of oocyte recovery, in vitro production, and embryo transfer status in domestic cattle situates both morphological and molecular selection methods within the same competence-assessment toolkit available to a genetic-background predictor (Hoshi, 2003). In vitro production protocols shape the genetic and developmental profile of the resulting embryo at each of three stages, and the literature at each stage shows measurable, protocol-dependent variation rather than a single generic in vitro embryo. At fertilization, sperm treatment and insemination conditions materially change resulting embryo production rate (Brackett & Keskinetepe, 1996), recombinant gonadotropin protocols used to stimulate donors change oocyte and embryo yield (Martins et al., 1998), and plasminogen activator production by the early embryo has been linked mechanistically to developmental progression in vitro (Kaaekuahiwi & Menino, 1990). At

culture, medium composition and carbon dioxide concentration change developmental rates in bovine in vitro production systems (Merton et al., 1995), an effect substantial enough that a systematic analysis of culture systems reported across a five-year span of international embryo transfer meetings found protocol variation to be a dominant source of reported outcome variability across laboratories (Thompson & Duganzich, 1996), and genetic parameters for oocyte number and embryo production within ovum-pickup-based in vitro production programs indicate that donor-level genetic merit for oocyte yield is itself heritable and selectable (Merton et al., 2009). A comprehensive review of oocyte recovery, in vitro production, and embryo transfer status in domestic animals situates cattle as the species in which these protocols are most mature and most amenable to donor-level genetic selection (Hasler, 1998).

A separate but convergent line of evidence addresses oxidative and thermal stress specifically within the in vitro production system, which is directly relevant to the environmental-exposure domain when an embryo, rather than only the recipient heifer, is exposed to heat during its own production. Reactive oxygen species accumulate during bovine embryo in vitro production and are associated with reduced developmental competence (Dalvit et al., 2005), and co-culture with granulosa cells as a monolayer has been proposed specifically as a mitigation strategy for in vitro embryo production conducted under heat stress in a related bovid species (Gad-Elkareem, 2021). Melatonin supplementation during in vitro culture has been shown to protect bovine embryos from the combined effects of heat stress and altered oxygen tension, improving embryo production outcomes under thermally challenging laboratory conditions (Hitit et al., 2023), a finding with direct relevance to the environmental-exposure domain because it demonstrates that the thermal vulnerability documented in vivo in Section 2 is reproducible, and partially modifiable, at the level of the isolated embryo. Optimization of culture media components, including serum and cysteamine supplementation, has likewise been shown to change cleavage rate in bovine in vitro embryo production, indicating that laboratory-level protocol choices, not only donor and recipient biology, shape the genetic and developmental package a heifer receives at transfer (Sandal, 2020). Genetic screening and selection technologies applied to the pre-transfer embryo or oocyte add a further layer to the genetic-background domain. Polar body genetic diagnosis has been proposed as a selection tool for identifying euploid oocytes before insemination (Fabres et al., 2012), and inadvertent sex selection has been documented as an unintended consequence of standard in vitro bovine embryo production protocols, indicating that embryo sex ratio itself can vary systematically by protocol rather than occurring at a fixed baseline rate (Behboodi et al., 1997). Polar body array comparative genomic hybridization, developed and debated in the human assisted-reproduction literature as a method for embryo selection, offers a conceptual and methodological parallel to bovine embryo genetic screening even though the debate over its accuracy originates outside cattle reproduction (Shulman, 2012; Scriven et al., 2012); the bovine field has not yet resolved an equivalent debate for its own screening methods, which is itself relevant to a prediction model, since a genetic-background variable derived from an unvalidated screening method carries measurement error that a prediction model should acknowledge rather than treat as ground truth. Embryo splitting to produce genetically identical demi-embryos has also been evaluated for viability following fresh and frozen-thawed handling, extending the genetic-background domain to include whether a transferred embryo is a split or intact conceptus (Imron et al., 2012), and oocyte-donor blood cholesterol has been linked to embryo color and freezability, tying donor metabolic status to the cryotolerance of the resulting embryo and, by extension, to its post-thaw viability once transferred (Hill & Kuehner, 1998).

Comparative genetics across *Bos indicus* and *Bos taurus* ancestry extends beyond the heat-resilience findings already discussed in Section 2 to include chimeric and hybrid embryo production, where diploid *Bos taurus* and tetraploid *Bos indicus* embryo aggregation has been used experimentally to produce bovine embryonic chimeras and probe the developmental compatibility of the two ancestries at the cellular level (Montanari Razza et al., 2011), and synchronization protocol choice has been shown to change fixed-time embryo transfer pregnancy rates specifically in *Bos indicus* by *Bos taurus* recipient combinations, indicating that protocol, not genetics alone, determines whether a given genetic combination achieves its expected pregnancy rate (Nasser et al., 2004). Frozen *Bos indicus* by *Bos taurus* embryos produced from oocytes recovered from slaughtered *Bos taurus* cows demonstrate that oocyte source and embryo genetic background can be decoupled entirely from the donor's own reproductive lifecycle, a point relevant to any prediction model that assumes embryo genetics and donor management are inseparable (Hutton et al., 1997). Field and industry standards for embryo transfer procedure, established by veterinary professional bodies, provide the procedural baseline against which any new predictive protocol for embryo-transfer pregnancies should be benchmarked (American Association of Bovine Practitioners, 1985), and selection-index thinking, dating to the original formalization of multi-trait genetic index construction, underlies the logic by which donor animals are nominated for embryo production programs in the first place, even though the present paper's focus is prediction rather than index construction (Hazel, 1943).

Donor-side body condition and metabolic status also determine oocyte yield and quality in ways that parallel, but are mechanistically distinct from, the recipient-heifer body-condition pathway in Section 2. Breed, age, body condition score, and nutritional status jointly predict follicular population, oocyte yield, and oocyte quality in zebu cattle, indicating that the body-condition domain applies to donor cows as well as recipient heifers (Dorice et al., 2019). Body condition score is independently associated with production performance in Sahiwal cattle, a *Bos indicus* breed commonly used in heat-adapted breeding programs (Sharma, 2020; Singh et al., 2020), and lipid content and mitochondrial activity in oocytes collected from crossbred *Bos taurus* by *Bos indicus* cows have been linked to early embryo developmental competence, tying donor metabolic and genetic background directly to embryo-level developmental potential (Mendez et al., 2018). Repeat flushing for embryo recovery in crossbred donor cows demonstrates that donor genetic background also interacts with the number of viable embryos obtainable per donor cycle, a supply-side constraint relevant to any program relying on embryo transfer to scale heat-adapted or high-merit genetics into a recipient heifer population (Subramaniam et al., 1991). Standardized visual scoring protocols for body condition in beef cattle provide the measurement basis needed to apply this donor-side covariate consistently across herds and observers (Hersom & Thrift, 2018), and documented confounds, such as indigestible foreign objects in the gastrointestinal tract altering apparent body condition independent of nutritional status, illustrate why body condition score, whether measured in a donor or a recipient, should be interpreted alongside a basic health screen rather than treated as a pure nutritional readout (Nongcula et al., 2017). The practical implication for this paper's framework is that Table 1's genetic-background domain should be understood as extending, where embryo transfer is used, from the recipient heifer to the donor animal and the *in vitro* production protocol, and that Table 3's measurement specifications apply with equal force to donor-side and protocol-side variables. A multiherd study that includes both natural-service and embryo-transfer pregnancies should record embryo source as a variable in its own right, rather than pooling all pregnancies under a single recipient-only genetic-background covariate.

3 Conceptual frameworks: four predictor domains

The framework organizes candidate predictors of pregnancy success into four domains: genetic background, body condition and growth, nutritional intake, and environmental exposure. Each domain is defined by the physiological pathway through which it is hypothesized to act, not by convenience of data collection, and each domain carries at least one variable that is genuinely time-varying rather than fixed at enrollment. Table 1 summarizes the four domains, their representative variables, and the point in the reproductive pathway at which each domain is expected to exert its effect. The genetic background domain includes breed composition, genomic estimated breeding value for fertility traits where genotyping is available, sire and dam identity for pedigree-based relationship modeling, and, for pregnancies established by embryo transfer, the genetic background of the transferred embryo as a separate variable from that of the recipient heifer. This domain sets the baseline sensitivity of the gonadotropin-releasing hormone axis and the early embryo to the perturbations captured by the other three domains; it is therefore modeled as an effect modifier for the body-condition and environmental-exposure domains rather than only as a main effect. Where genomic data exist, the domain additionally carries the reliability of each genomic estimated breeding value, since a value computed from a small or poorly connected reference population should not be weighted equally with one computed from a large, well-connected reference population in the mixed-effects model, and treating reliability as a fixed rather than a variable property of the predictor would understate uncertainty for herds using less-established genomic evaluation services. The body condition and growth domain includes body condition score measured repeatedly across the pre-breeding and breeding period, live weight as a fraction of projected mature weight, and average daily gain over a rolling window. Because puberty and cyclicity depend on a weight-for-age threshold rather than age alone, growth rate is treated as informative independent of body condition score, and the two are permitted to interact in the statistical model rather than collapsed into a single composite. A heifer that reaches target weight through rapid, recent gain is not assumed physiologically equivalent to one that reached the same weight through steady growth, since acute changes in body condition can reflect transient feed availability rather than a stable energy reserve, and the model accordingly carries both the current score and the trajectory over the preceding measurement window rather than the current score alone. The nutritional intake domain includes forage dry matter intake, dietary energy density, and dietary protein, each measured at the pen or group level where individual intake is not metered, with a flag for whether intake is individually or group-estimated. Water access is included in this domain because restricted water intake compounds heat load and independently limits dry matter intake, and because water source is the natural point at which a contaminant-exposure covariate is captured. Because forage quality and yield are themselves weather- and management-dependent, the nutritional domain is treated as partially endogenous to the environmental domain rather than fully independent of it, and the analysis plan in Section 4 accounts for this by reporting the correlation between realized forage dry matter intake and the same period's temperature-humidity index, rather than assuming the two predictors are orthogonal. The environmental exposure domain includes temperature-humidity index integrated over the breeding window rather than measured at a single timepoint, overnight cooling recovery, shade access, and heat exposure duration, together with a herd-level contaminant-burden indicator derived from water-source testing where available. Respiration rate, rectal temperature, and coat characteristics are retained as individual-animal physiological readouts of thermal load, distinct from the ambient exposure measures, because two heifers under identical temperature-humidity index can show materially different physiological heat-

load responses depending on coat type and breed composition. This domain is deliberately the most heterogeneous of the four in measurement burden, ranging from a routinely available station-level weather record to a rarely available water-contaminant test, and the framework treats this heterogeneity explicitly rather than requiring every herd to supply every variable before it can contribute data to the study.

Table 1. Predictor domains for multifactor pregnancy-success prediction in dairy heifers.

Domain	Representative variables	Primary pathway	Timing
Genetic background	Breed composition; genomic EBV for fertility; sire/dam identity; embryo genetic background where ET is used	Sets baseline sensitivity of the gonadotropin-releasing hormone axis and embryo to nutritional and thermal perturbation	Fixed at birth or conception; recorded once, applied as an effect modifier
Body condition and growth	Body condition score; live weight as percent mature weight; average daily gain	Energy reserve signals hypothalamus via IGF-1 and leptin; determines puberty threshold	Repeated: pre-breeding, at breeding, mid-gestation check
Nutritional intake	Forage dry matter intake; dietary energy density; dietary protein; water access	Supplies substrate for growth and for the energy balance underlying gonadotropin-releasing hormone pulsatility	Pen or group level, continuous across breeding window
Environmental exposure	Temperature-humidity index (integrated); night cooling; shade access; heat exposure duration; water-source contaminant burden	Direct oocyte and embryo cytotoxicity; suppression of gonadotropin-releasing hormone pulsatility; endocrine-disrupting contaminant exposure	Continuous during breeding and early gestation window

The four domains are not independent in the statistical model. Two interaction terms are specified a priori, on physiological rather than exploratory grounds. The first is body condition score by integrated temperature-humidity index, motivated by the shared dependence of gonadotropin-releasing hormone pulsatility on both energy balance and thermal load. The second is genomic estimated breeding value for fertility by nutritional intake, motivated by the expectation that genetic merit for fertility is expressed conditionally on adequate nutrition and is masked, not eliminated, when intake is restricted. Both interactions are falsifiable and are restated as testable propositions in Section 6. A third, lower-priority interaction, between breed composition and integrated temperature-humidity index, is retained as an exploratory term rather than a primary hypothesis, since the comparative embryo-transfer literature reviewed in Section 2 supports a conditional thermotolerance advantage for *Bos indicus*-influenced genetics but does not, on its own, establish the functional form or threshold of that advantage at the level of an individual recipient heifer's pregnancy outcome rather than embryo survival under experimental heat challenge.

4 Modeling strategy and validation design

The proposed empirical design is a preregistered, multiherd prospective cohort. The eligible population is nulliparous dairy heifers entering their first breeding season across participating herds; the observation window runs from ninety days before first service to pregnancy diagnosis and, for confirmed pregnancies, to calving, so that pregnancy loss can be distinguished from failure to conceive. The primary outcome is confirmed pregnancy to first service within a fixed diagnostic window; secondary outcomes include pregnancy to any service within the breeding season, days to conception, and pregnancy loss between first diagnosis and calving. All outcome definitions, the interaction terms specified in Section 3, and the primary analysis model are to be frozen before any outcome data are examined. Records are structured as repeated measures nested within heifer, nested within herd-year-season stratum, because heifers within the same herd and season share unmeasured management and climate exposure that would otherwise be mistaken for a predictor effect. The primary analysis is a mixed-effects logistic regression with a random intercept for herd-year-season and fixed effects for the four predictor domains and the two prespecified interaction terms. This model is compared against three alternative architectures under identical data partitions: a random forest and a gradient-boosted tree ensemble, both of which can recover interaction and nonlinear structure without prespecification, and a genomic-enhanced hybrid that augments the mixed-effects baseline with a genomic relationship matrix in place of a simple breed-composition fixed effect where genotype data are available. Table 2 summarizes the four candidate architectures, the structural assumption each makes, and the corresponding strength and limitation for this prediction problem.

Table 2. Candidate modeling architectures compared under identical validation splits.

Architecture	Structural assumption	Strength for this problem	Limitation
Mixed-effects logistic regression	Linear predictor on the logit scale; random intercept for herd-year-season; prespecified interactions	Interpretable coefficients; correct handling of clustering; matches the a priori physiological interactions	Cannot recover unanticipated nonlinearity or interaction outside the prespecified terms
Random forest	Recursive partitioning; no distributional assumption	Recovers nonlinear thresholds (e.g., a temperature-humidity index breakpoint) without prespecification	Clustering must be handled by explicit herd-level features; interpretation requires post hoc variable importance
Gradient-boosted trees	Additive ensemble of shallow trees fit to residuals	Typically strongest raw discrimination in tabular prediction tasks of this size	Highest risk of overfitting to herd-specific patterns without careful external validation
Genomic-enhanced hybrid	Mixed-effects baseline with a genomic relationship matrix replacing simple breed	Captures additive genetic relationships among genotyped animals more precisely	Requires genotyping coverage; not usable for herds without genomic data

	composition	than breed labels alone	
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Validation follows a transfer-focused rather than random-split logic. Because heifers within a herd are correlated, random row-level splitting would leak herd-specific signal between training and test sets and overstate transferable accuracy. The primary validation holds out entire herds and, separately, an entire later breeding season, so that both spatial transfer (to an unseen herd) and temporal transfer (to a later year with a different climate realization) are evaluated. Performance is reported as discrimination (concordance statistic), calibration (observed versus predicted pregnancy rate across risk deciles), and net benefit at plausible decision thresholds for culling or targeted management, following TRIPOD-style reporting adapted from clinical prediction modeling to a herd-level veterinary setting. A model that discriminates well but calibrates poorly in a held-out herd is treated as a caution about overfitting to development-herd management practices, not as a validated tool. Formally, the primary model specifies the log-odds of pregnancy success for heifer i in herd-year-season stratum j as a linear predictor consisting of a stratum-specific random intercept, main effects for genetic background, body condition and growth, nutritional intake, and environmental exposure, and the two prespecified interaction terms: body condition score by integrated temperature-humidity index, and genomic estimated breeding value by forage dry matter intake. The random intercept is assumed normally distributed across strata, capturing unmeasured herd-, year-, and season-level management and climate factors shared by heifers within the same stratum. Continuous predictors are centered at their sample mean before interaction terms are formed, so that the main-effect coefficients remain interpretable as the association at the average value of the interacting variable rather than at an arbitrary zero point that may fall outside the observed data range. Coefficients are reported as odds ratios with profile confidence intervals, and the interaction coefficients are additionally reported as the predicted pregnancy probability across a grid of body-condition-score-by-temperature-humidity-index combinations, so that the practical, not only the statistical, magnitude of the interaction is visible to a reader without a background in logistic regression.

4.1 Sample size and statistical power

Because the primary analysis targets two prespecified interaction coefficients rather than two main effects, the sample size and power calculation must be anchored to the interaction, not to the main-effect detection that a simpler design would require. Detecting an interaction of a given standardized magnitude typically requires a sample several times larger than detecting a main effect of the same magnitude, and the clustered, herd-year-season structure of the data further inflates the effective variance through a design effect proportional to the intraclass correlation of pregnancy outcomes within stratum. The power calculation should therefore be performed at the level of the herd-year-season cluster, using an anticipated intraclass correlation informed by prior herd-fertility datasets where available, and should report the minimum detectable interaction effect at the planned number of herds and heifers per herd rather than reporting only an overall sample size. Where the anticipated cluster count cannot support adequate power for both prespecified interactions simultaneously, the protocol requires this limitation to be stated before data collection begins, with H1 (condition by heat) prioritized over H2 (genomic merit by nutrition) if a choice must be made, because the physiological case for H1 rests on a more direct and better-characterized mechanistic pathway.

4.2 Missing data and data governance

Herd records in practice arrive with structurally different missingness across the four predictor domains. Body condition score and pregnancy diagnosis are close to universally recorded on participating farms; genomic estimated breeding value is available only for genotyped heifers, typically a nonrandom and higher-value subset of the herd; and water-source contaminant testing is available only where a herd has independently commissioned it, which is neither random nor plausibly unrelated to herd management quality more broadly. Multiple imputation is appropriate for intermittently missing time-varying variables such as body condition score or forage dry matter intake, where the missingness mechanism is plausibly related to observed covariates. It is not appropriate for genomic estimated breeding value or contaminant-burden status, where missingness reflects a structural absence of testing rather than a value that exists but was not recorded; these variables are instead handled through the stratified genotyped and non-genotyped analysis described above, and through explicit reporting of which herds contributed water-source data rather than through imputed contaminant-burden values. All raw records enter an immutable raw data layer with source, timestamp, and unit recorded at ingestion; a versioned curated layer applies range and consistency checks; and a locked analysis dataset is frozen before the primary model is fit, with any subsequent correction documented as a new dataset version rather than as a silent edit. Farm-level records require data-sharing agreements specifying permitted use, retention period, and the herd owner's right to withdraw, negotiated as part of the readiness stage described in Section 7 and independent of the institutional animal-care approval required for any direct animal handling. Interpretability is treated as a reporting requirement rather than an afterthought for the tree-based architectures in Table 2. Where a random forest or gradient-boosted ensemble is used, the analysis plan requires reporting of permutation-based variable importance and partial-dependence profiles for each of the four predictor domains, so that a farm manager or attending veterinarian receiving a risk classification can be told which domain is driving it for a given heifer, rather than only the final probability. A model that outperforms the mixed-effects baseline on discrimination but cannot support this domain-level explanation is not preferred for the decision-support stage in Section 7 even if its raw accuracy is higher, because a recommendation a veterinarian cannot explain to a farm owner is unlikely to be acted upon. Sensitivity analyses vary the definition of pregnancy loss, the width of the temperature-humidity index integration window, the treatment of missing contaminant-burden data (available only for herds with water testing on record), and the choice of primary versus first-service-only outcome. Where the two candidate interaction terms fail to reach the prespecified minimum meaningful effect, the framework requires that the failure be reported and the proposed mechanism reconsidered, rather than that the interaction term be quietly dropped and the model re-specified as though it had never been proposed.

5 Predictor specification and measurement

Table 3 specifies the operational definition, measurement window, primary data source, and expected direction of association with pregnancy success for the core variables within each of the four domains. Expected direction is stated as a hypothesis to be tested, not as an assumed result; several variables carry a conditional direction because their effect is hypothesized to depend on the value of another domain, consistent with the interaction structure specified in Section 3.

Table 3. Operational definitions and measurement specification of core predictor variables.

Variable	Definition and unit	Measurement window	Primary source	Expected direction
Breed composition	Proportion Bos indicus ancestry, 0 to 1, from pedigree or genomic estimate	Fixed at birth	Herd genetic records / genomic report	Higher indicus proportion favors pregnancy success under high heat load; direction conditional on THI
Genomic EBV, fertility	Genomic estimated breeding value for heifer conception rate, standardized units	Fixed at genotyping	Genomic evaluation provider	Positive; magnitude conditional on nutritional intake
Body condition score	1 to 5 scale (quarter-point), visual or image-based	Repeated: minus 90 d, at service, plus 30 d	On-farm scoring / automated imaging	Positive up to a herd-specific optimum, then flat
Average daily gain	kg per day, rolling 60-day window	Continuous, pre-breeding	Scale records	Positive up to attainment of target breeding weight
Forage dry matter intake	kg DM per head per day, pen-level estimate	Continuous, breeding window	Ration formulation / intake monitoring	Positive, subject to diminishing return above formulated requirement
Dietary energy density	Mcal net energy for maintenance per kg DM	Continuous, breeding window	Ration formulation records	Positive; interacts with forage dry matter intake
Water access	Linear trough space per head, cm; flow rate, L per min	Static, checked seasonally	Facility audit	Positive; compounds with THI when restricted
Temperature-humidity index (integrated)	THI, area under curve above 68 threshold across breeding window	Continuous, breeding and early gestation window	On-farm or nearest-station weather data	Negative; interacts with body condition score
Night cooling recovery	Hours per night with THI below 68	Continuous, breeding window	Weather data	Positive
Respiration rate	Breaths per minute, individual animal	Spot-checked on high-THI days	Direct observation	Negative; physiological readout of realized heat load
Water-source	Categorical:	Annual or as	Water-quality	Negative where

contaminant burden	tested/untested; if tested, PFAS above or below regional reference level	tested	testing records	above reference level; hypothesis-generating, not confirmed in cattle
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Each variable in Table 3 requires, at minimum, a recorded source, timestamp, permissible range, and missing-value code before it enters the analysis dataset, and repeated variables require a defined measurement window rather than a single value carried forward. Breed composition and genomic estimated breeding value are the two variables least likely to change within a heifer's breeding season and are therefore recorded once and applied as time-invariant effect modifiers; body condition score, growth rate, forage dry matter intake, and thermal exposure are recorded repeatedly because their trajectories, not only their values at a single timepoint, carry predictive information. Where genomic data are unavailable for a given heifer, breed composition from pedigree is used as a lower-resolution substitute rather than as a missing-data placeholder, and the model architecture comparison in Section 4 is repeated separately for the genotyped and non-genotyped subpopulations to test whether genomic resolution changes which architecture performs best.

5.1 Reliability and quality control

A predictor is only as useful as its reliability allows. Body condition score assigned by visual appraisal is subject to inter-observer disagreement that widens with the number of scorers involved across a multiherd study; the protocol therefore requires either a single trained scorer per herd-visit, periodic inter-observer calibration sessions with a reported agreement statistic, or adoption of the image-based automated scoring methods documented in Section 2, which remove between-observer variability at the cost of requiring calibration against visual scores at least once per herd. Temperature-humidity index derived from a single fixed weather station may not represent the microclimate a given heifer actually experiences if shade, airflow, or stocking density vary within a pen; on-animal or in-pen sensors, where feasible, should be prioritized over station-level estimates for the subset of herds able to support them, with the resulting discrepancy between station and in-pen measurement reported as a methods finding in its own right rather than discarded. Genomic estimated breeding values carry their own reliability statistic, reported by the genomic evaluation provider, that should be retained alongside the point estimate and used to downweight low-reliability genomic values in the mixed-effects model rather than treating all genomic estimated breeding values as equally precise regardless of the reference population size behind them. Laboratory or diagnostic assays used for pregnancy confirmation should report their own sensitivity and specificity at the gestational age of testing, since early transrectal palpation and early ultrasonography carry materially different false-negative rates, and a difference in diagnostic method across herds that is not recorded as a covariate could otherwise masquerade as a difference in true pregnancy rate. Reliability reporting also extends to the contaminant-burden indicator in the environmental exposure domain. Because water-source testing is neither universal nor performed on a fixed schedule, the study should record the test date, analyte panel, and detection limit for every herd that contributes this variable, and should treat a herd with an old or partial test differently from one with a recent, full-panel test rather than collapsing both into a single binary tested-or-not indicator. A stale or partial test can understate current exposure in a way that biases hypothesis H6 toward the null, which is a further reason H6 is designated exploratory rather than confirmatory in the sequencing set out below.

Laboratory batch and technician effects, well documented as sources of variance in bovine in vitro production work, apply equally to on-farm diagnostic and scoring procedures and should be captured as random effects in the primary model wherever more than one technician or diagnostic team contributes records within a herd. Where a single technician effect is confounded with a single herd, as is common on smaller operations, this should be reported as a limitation on the interpretability of any residual herd-level variance component rather than silently absorbed into the herd random intercept.

6 Testable hypotheses

Six hypotheses follow directly from the physiological foundations in Section 2 and the framework in Section 3. Each is stated with a direction, the comparison that would support or refute it, and the minimum evidence a prospective study should require before treating the hypothesis as supported.

H1 (condition by heat interaction). The association between integrated temperature-humidity index and pregnancy success is more strongly negative among heifers in the lowest body-condition-score tertile than among heifers in the highest tertile. Support requires a statistically and practically meaningful interaction coefficient in the mixed-effects model that survives herd-held-out validation, not merely a difference in raw pregnancy rate across unadjusted subgroups.

H2 (genomic merit by nutrition interaction). Genomic estimated breeding value for fertility predicts pregnancy success more strongly among heifers above herd-median forage dry matter intake than among heifers below it. Support requires the interaction term, not only the genomic EBV main effect, to add predictive value in held-out herds.

H3 (breed composition by thermal load). Proportion *Bos indicus* ancestry is associated with higher pregnancy success specifically in herd-year-season strata with integrated temperature-humidity index above the study median, with no meaningful association, or a reversed association favoring conventional genetics, below that median. This hypothesis treats breed composition as conditionally rather than universally advantageous.

H4 (measurement resolution). A model incorporating genomic estimated breeding value and a genomic relationship matrix achieves better calibration in an unseen herd than an otherwise identical model using pedigree-based breed composition alone, among genotyped heifers.

H5 (architecture transfer gap). The performance gap between development-herd and held-out-herd evaluation is larger for gradient-boosted trees than for the mixed-effects baseline, reflecting greater susceptibility of flexible learners to herd-specific management signal that does not transfer.

H6 (contaminant burden). Among herds with water-source testing on record, categorical contaminant-burden status is associated with pregnancy success after adjustment for temperature-humidity index and body condition score. This hypothesis is explicitly exploratory given the absence of bovine-specific dose-response data and is retained to generate, not confirm, an effect estimate. For each hypothesis, a null or reversed result is treated as informative. A failed interaction term indicates either that the proposed physiological mechanism does not operate at the measured resolution, that the measurement itself is too coarse to detect it, or that a confound absorbed the effect; distinguishing among these explanations requires the process- and implementation-level evidence specified in Sections 4 and 5, not a redefinition of pregnancy success after the fact.

6.1 Sequencing of hypothesis tests

The six hypotheses are not of equal priority and should not be tested as an undifferentiated set. H1 carries the strongest a priori physiological support from the neuroendocrine convergence described in Section 2 and should be treated as the primary confirmatory test, with a single preregistered significance threshold and no adjustment for the other five hypotheses. H2 and H3 are secondary confirmatory tests, each with a preregistered direction but tested at a threshold adjusted for the two comparisons; both depend on genotyped or breed-composition data of variable availability across herds and should be reported with the achieved sample size for the relevant subpopulation stated explicitly rather than implied by the overall study sample size. H4 and H5 are methodological rather than biological hypotheses and are appropriately evaluated descriptively, by direct comparison of calibration and transfer performance across architectures, without a formal significance threshold. H6 is exploratory by design, given the absence of bovine-specific dose-response evidence for contaminant exposure, and any effect estimate it produces should be reported with explicit language identifying it as hypothesis-generating, not confirmatory, regardless of the p-value obtained. This sequencing exists so that a null result on an exploratory hypothesis does not retrospectively discredit the confirmatory tests, and so that a positive result on H1 is not diluted by simultaneous testing of five other comparisons of lower prior probability.

7 From model to on-farm decision support

A validated prediction model changes practice only if its output reaches a decision at the right time. The proposed pathway moves in four stages. Definition and readiness establish agreed variable definitions, data sources, and the ethical and data-governance approvals required before any heifer-level record leaves the farm management system. A pilot phase tests data capture and model scoring on a small number of herds without acting on the output, so that data quality problems are surfaced before they affect management decisions. Protected evaluation applies the held-out validation design in Section 4 on a larger multiherd sample, with the model used only in an advisory capacity alongside standard practice. Only after calibration and transfer performance meet prespecified thresholds does the model move to decision support, where its output informs, but does not replace, breeding and culling decisions made by farm management and attending veterinarians. Throughout this pathway, a heifer flagged as low-probability for pregnancy success should trigger a defined management response, such as targeted nutritional supplementation, delayed rather than accelerated culling pending a repeat assessment, or, where thermal load is the dominant driver, provision of shade or cooling rather than a genetic or nutritional intervention that would not address the operative mechanism. A model that reports risk without a corresponding action pathway converts a measurement exercise into an administrative burden rather than a management tool. The decision value of the prediction model depends on the asymmetry between the two error types it can make. A false-negative heifer, incorrectly predicted low-probability and consequently culled or withheld from further breeding investment, forfeits her full remaining productive value. A false-positive heifer, incorrectly predicted high-probability and consequently held in the breeding program without the corrective management a true low-probability animal would have received, extends the herd's non-productive interval and delays realization of the same information at the next diagnosis. These costs are not symmetric and are not constant across herds, since replacement heifer cost, feed cost, and the opportunity cost of a delayed diagnosis vary by operation and by season. Rather than reporting a single accuracy figure, the protected-evaluation and decision-support stages

should report net benefit across a plausible range of decision thresholds, so that a given herd can select the threshold appropriate to its own cost structure instead of inheriting a default threshold optimized for the average development-herd cost ratio.

None of the four stages is meaningful without capacity building among the staff who record the underlying data. Body condition scoring, respiration-rate spot checks, and water-trough audits are only as reliable as the training of the person performing them, and a multiherd study that trains scorers once at enrollment without periodic refresher calibration should expect measurement drift over the course of a multiyear study. The readiness stage should therefore include a documented training protocol for each manually recorded variable, with a scheduled recalibration interval, rather than treating training as a one-time event that precedes, rather than continues alongside, data collection.

Governance of the decision-support stage should specify who is authorized to act on a model output, how a heifer's record can be corrected if a variable was misrecorded, and how the model's classification is documented alongside the eventual outcome so that miscalibration can be detected in ongoing use rather than only at the next formal validation cycle. Version control should tie each classification to the specific model version and the predictor values active at the time of scoring, so that a later audit can reconstruct why a given heifer received a given risk classification.

8 Discussion

The physiological literature reviewed in Section 2 converges on a single structural point: nutritional status and thermal load act on the same upstream endocrine pathway, so a prediction model that enters them as additive covariates is not a simplification of the biology but a misspecification of it. The practical implication is that herds correcting only one limiting factor, for example investing in shade structures while allowing body condition to drift below the herd-specific optimum, should not expect the full pregnancy-rate benefit that either intervention would produce in isolation, because the two factors are expected to interact rather than to sum.

The genetic literature carries a second, less intuitive implication. Genomic selection and breed-composition choices that raise average fertility merit or average thermotolerance do not remove the value of nutritional and thermal management; the comparative embryo-transfer literature indicates that the pregnancy-rate advantage associated with heat-adapted genetics is itself conditional on the thermal environment in which it is measured (Eberhardt et al., 2009), which is exactly the conditional structure proposed for hypothesis H3. A herd that adopts heat-tolerant genetics without also addressing body condition and forage quality should not expect the genetic gain to be fully expressed. Methodologically, the comparison across mixed-effects, tree-based, and genomic-enhanced architectures in Section 4 is included because no single architecture is expected to dominate across all use cases. A mixed-effects model with prespecified interactions is the more defensible choice where interpretability and physiological grounding matter, such as when the output must be explained to a farm manager or attending veterinarian, while a tree-based ensemble may recover thresholds, such as a temperature-humidity index breakpoint, that the linear model would need to be told to look for. The herd-held-out and season-held-out validation design exists specifically to prevent either architecture from being credited with accuracy that reflects development-herd management idiosyncrasy rather than a transferable predictor-outcome relationship. The inclusion of water-source contaminant burden as an exploratory covariate is deliberately conservative. The supporting evidence is drawn from human and environmental-health literatures rather than cattle-specific trials (Oyelade & Ohanebo, 2024;

Oyelade & Ohanebo, 2025), and hypothesis H6 is framed accordingly as hypothesis-generating. Its inclusion nonetheless follows directly from the definition of environmental exposure used throughout this paper: an exposure that is present in a herd's water supply and independently linked to endocrine-relevant developmental outcomes in other species should not be excluded from a livestock reproduction model on the grounds that a bovine dose-response study does not yet exist. Absence of species-specific evidence is a reason to test the association, not a reason to omit the variable. The predictor taxonomy in Table 1 is also written to anticipate a shift already underway in commercial dairy management, in which continuous sensor streams, including rumination and activity collars, in-line milk and rumen sensors, and automated imaging for body condition, are replacing the periodic manual observations on which most existing fertility prediction work has relied. A framework specified only in terms of periodic manual measurement risks becoming obsolete as soon as continuous data become available, while a framework specified in terms of the underlying physiological construct, such as energy balance or integrated thermal load, remains valid regardless of whether the construct is measured by a monthly visual body condition score or a continuous automated proxy. The variable-level measurement matrix in Table 3 is deliberately written at the level of the construct and its currently practical measurement method rather than assuming any particular sensor technology, so that a herd with access to continuous monitoring and a herd relying on periodic manual scoring can both contribute data to the same underlying model, with measurement method itself retained as a covariate rather than treated as an unimportant implementation detail.

Access to genomic testing is not uniform across the herds to which this framework would ideally apply. The genomic-enhanced architecture in Table 2 and hypothesis H4 both depend on genotyping, which remains cost-prohibitive for many smallholder and pastoral operations, including systems that rely most heavily on *Bos indicus*-influenced genetics for thermotolerance and that therefore stand to benefit most from a validated breed-composition-by-heat-load interaction. A framework that reports genomic-enhanced performance without also validating the pedigree-only architecture on the same held-out herds would produce a tool usable only by the subset of operations that can already afford genotyping, reproducing an access gradient rather than narrowing it. The stratified genotyped and non-genotyped validation specified in Section 4 exists specifically to prevent this outcome and to report honestly where the pedigree-only model falls short. The proposed protocol also differs from common current practice in a way worth stating plainly. Many on-farm decisions aids compute a single composite fertility score from a weighted sum of available variables, chosen for computational convenience rather than derived from the interaction structure argued for here. Such a composite is not wrong so much as underspecified: it can achieve reasonable average performance while systematically misclassifying the heifers at the tails of the condition, heat-load, or genomic-merit distributions, precisely because a weighted sum cannot represent a multiplicative interaction unless the interaction term is entered explicitly. The comparative architecture design in Section 4 is constructed to make this difference visible rather than assumed, by testing whether the mixed-effects model with explicit interaction terms outperforms both a simpler additive baseline and the more flexible tree-based learners on calibration in held-out herds, not only on raw discrimination in the development sample. The framework's limitations follow from its status as a design rather than a completed study. No accuracy, calibration, or effect-size estimate is reported here because none has been produced under this protocol; every quantitative claim in Sections 3 through 6 is stated as a hypothesis with a specified test, not as a result. The value of the paper lies in specifying that test precisely enough that a future study, using authorized herd data and

appropriate institutional animal-care approval, can be judged against a preregistered plan rather than against outcomes chosen after the data were seen.

9 Conclusion

Predicting pregnancy success in dairy heifers from body condition, forage dry matter intake, environmental exposure, and genetic background requires treating these four domains as a single, interacting predictor architecture rather than as four independent covariates entered additively into a regression. This paper has specified that architecture: a physiological rationale for two a priori interaction terms, a herd-clustered and temporally held-out validation design compared across four candidate modeling architectures, a variable-level measurement matrix with defined sources and timing, and six falsifiable hypotheses. The central claim is that no single domain, whether nutritional management, cooling infrastructure, or genomic selection, is sufficient on its own to maximize heifer pregnancy rates once any of the other three domains is limiting. Future empirical work should apply this protocol to authorized, multiherd data under appropriate institutional animal-care oversight, report calibration and transfer performance alongside discrimination, and treat a failed interaction hypothesis as informative rather than as a reason to abandon the interaction-based framing. If H1 and H2 are confirmed under held-out validation, the practical consequence for herd management is that nutritional, cooling, and genetic investments should be sequenced and co-targeted at the individual heifer level rather than applied as uniform herd-wide interventions, since the framework predicts that the marginal value of any one investment depends on the heifer's standing in the other domains. If they are not confirmed, the framework's more conservative but still useful contribution is the variable-level measurement matrix and validation design itself, which would allow the field to move toward a properly specified additive model with the same rigor this paper has applied to the interaction hypothesis, rather than defaulting back to an unvalidated composite score.

Declarations

Ethics approval and consent to participate: This manuscript is a predictive-modeling framework and protocol paper; no animal observations were collected for it. A future empirical study implementing this protocol must obtain institutional animal care and use committee approval for all animal handling, sampling, and management procedures, and must obtain the consent of participating herd owners for use of farm records, before any data are collected.

Consent for publication: Not applicable.

Availability of data and materials: No new animal, herd, or laboratory dataset was generated or analyzed for this paper. The predictor taxonomy, modeling comparison protocol, and measurement specifications required to reproduce the proposed study are reported in full within the manuscript.

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