DOI: 10.21451/1809-3000.RBRA2026.015

Anais da X Reunião Anual da Associação Brasileira de Andrologia Animal
Indaiatuba, SP, 4 a 6 de junho de 2026.

Next-Generation Fertility Assessment in Boars and Bulls Using Single-Cell Phenotyping and Artificial Intelligence

Avaliação de Fertilidade de Nova Geração em Varrões e Touros Utilizando Fenotipagem Unicelular e Inteligência Artificial

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Abstract

Conventional semen analysis based on motility and morphology remains the cornerstone of bull and boar fertility evaluation, yet morphologically normal, motile spermatozoa can still fail to produce a viable pregnancy or reduced litter sizes. Morphology is blind to unseen non-compensable defects, and population averages mask the subpopulation heterogeneity that determines fertilization. This review synthesizes single-cell sperm phenotyping advances that close this gap: capacitation-resolved biomarkers, multi-omic profiling, and AI-driven image analysis. The sperm zinc signature provides a tractable fluorescent readout of capacitation competence; capacitation-competent subpopulations correlate with in vitro embryo production in cattle and pigs; convolutional neural networks classify sperm morphology with F1 above 95%; label-free models predict acrosome and plasma-membrane status from brightfield images alone; and spectral flow cytometry resolves up to 14 biomarkers per sample. A five-tier framework integrating breeding soundness, CASA, AI morphology, functional and molecular biomarkers, and multi-parametric AI fusion is proposed as the path to fertility prediction that is objective, scalable, and biologically informed.

Keywords: *sperm capacitation; zinc signature; sire fertility; deep learning; flow cytometry.*

Resumo

A análise convencional do sêmen baseada em motilidade e morfologia continua sendo o principal método de avaliação da fertilidade de touros e varrões, embora espermatozoides morfologicamente normais e móveis ainda possam falhar em produzir uma gestação viável ou resultar em tamanhos de leitegada reduzidos. A morfologia não detecta defeitos não compensáveis ocultos, e médias populacionais mascaram a heterogeneidade entre subpopulações que determina a fertilização. Esta revisão sintetiza avanços em fenotipagem unicelular espermática que reduzem essa lacuna: biomarcadores resolvidos por capacitação, perfis multi-ômicos e análise de imagem orientada por inteligência artificial. A assinatura de zinco espermática fornece uma leitura fluorescente acessível da competência de capacitação; subpopulações competentes para capacitação correlacionam-se com a produção embrionária in vitro em bovinos e suínos; redes neurais convolucionais classificam morfologia espermática com F1 acima de 95%; modelos sem marcação predizem integridade acrossomal e de membrana plasmática a partir apenas de imagens em campo claro; e a citometria de fluxo espectral resolve até 14 biomarcadores por amostra. Propõe-se um arcabouço em cinco níveis integrando exame andrológico, CASA, morfologia por IA, biomarcadores funcionais e moleculares e fusão multi-paramétrica por IA, como caminho para predição de fertilidade objetiva, escalável e biologicamente informada.

Palavras-chave: capacitação espermática; assinatura de zinco; fertilidade do reprodutor; aprendizado profundo; citometria de fluxo.

Introduction

Bull and boar fertility evaluation has rested for more than half a century on a deceptively simple two-axis model: spermatozoa are considered acceptable if a sufficient fraction are progressively motile and morphologically normal. Yet a sire that meets every threshold of the breeding soundness examination (BSE) can still underperform in the field, and a sire that marginally fails one parameter can be a top producer. Motility and morphology measure necessary but not sufficient prerequisites for fertilization, and

Recebido: 18 de maio de 2026
Aceito: 20 de maio de 2026

conventional metrics report population averages that mask the subpopulation diversity that drives reproductive success [1, 2]. Population-level data from commercial pig herds show that pregnancy rate and number born alive vary more on the sire side than on the dam side, even after accounting for management, parity, and season [3]. A single subfertile boar inseminating hundreds of sows weekly, or a subfertile bull contributing to thousands of AI doses, can have large economical impacts.

A century-old dogma is now being challenged by the convergence of three technical revolutions. Image-based flow cytometry enables simultaneous integration of sperm morphology with approximately 5-6 functional biomarkers at the single-cell level, whereas spectral flow cytometry expands multiplexing capacity to approximately 7-14 biomarkers on tens of thousands of spermatozoa per ejaculate. Multi-omic platforms have brought the proteome, metabolome, and lipidome of the semen within reach [4, 5, 6]. Deep learning has eliminated the inter-observer variability of visual morphology assessment and is beginning to extract functional information from images that the human eye does not register [7]. Together these technologies recast sire fertility evaluation as a single-cell phenotyping problem (Figure 1) [8, 9]. This review organizes the current evidence around that reframing, with parallel attention to the boar and the bull, and articulates where the field is moving over the next two to five years.

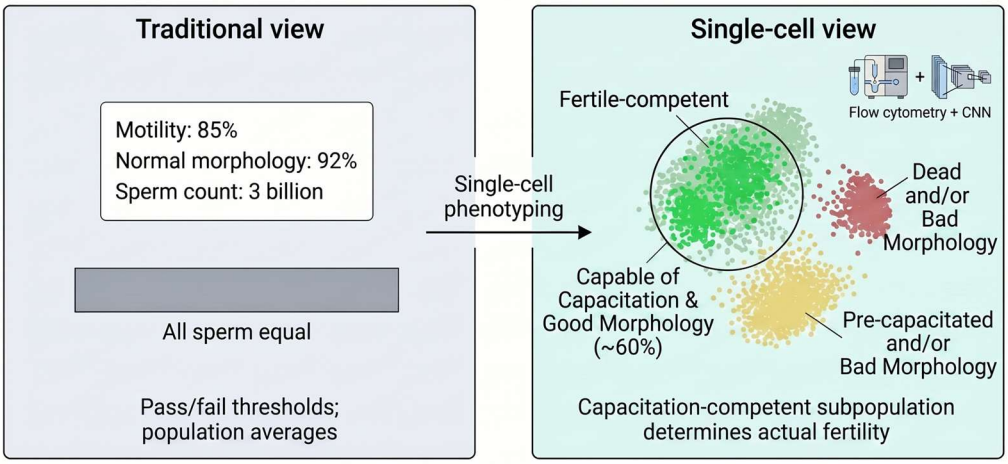

Figure 1. From population averages to single-cell fertility distributions. The traditional view (left) reports flat ejaculate-level percentages of motility, morphology, and concentration, treating all spermatozoa as functionally equivalent. The single-cell view (right), accessed through image-based flow cytometry and AI-driven classification, resolves distinct subpopulations: fertile-competent (capable of capacitation & good morphology), pre-capacitated and/or morphologically abnormal, and dead and/or morphologically abnormal, and reveals the capacitation-competent subpopulation that actually determines fertilization success. *Created in BioRender. Kerns, K. (2026) https://BioRender.com/5471whf.*

Compensable and non-compensable defects in the fertilization cascade

A spermatozoon traverses a sequential series of selection barriers between deposition and syngamy. Of the millions (bull) or billion (boar) deposited, a relative few reach the site of fertilization in the oviduct [10]. At each step the female tract selects for functional competence rather than structural normality: cervical mucus filtration, uterotubal-junction transit, oviductal reservoir binding, cumulus penetration, and zona pellucida recognition & penetration [10, 11]. The biochemical pivot in this cascade is sperm capacitation, defined by Austin and Chang in 1951 as the acquisition of fertilizing capacity. Capacitation involves bicarbonate-driven activation of sperm-soluble adenylyl cyclase, cAMP-dependent phosphorylation, membrane lipid remodeling, hyperactivation, ubiquitin–proteasome activity, and zinc efflux [12, 13, 14]. Once fully capacitated, the capacitated state is brief and underappreciated. From a biomarker perspective under the microscope, it's likely tens of seconds to a few minutes. After fertilization the spermatozoon must additionally activate the oocyte through release of phospholipase C zeta and related factors that trigger Ca^{2+} oscillations, cortical granule exocytosis, zona hardening, and the cell-cycle restart [15].

Saacke [1, 16] formalized the framework that explains why some subfertile ejaculates can be rescued by increasing AI dose while others cannot. Compensable defects prevent the spermatozoon from reaching or penetrating the oocyte and can be offset by depositing more sperm. Non-compensable defects

allow fertilization to proceed but block embryogenesis: DNA fragmentation, aberrant chromatin remodeling, oxidative damage, and the silent functional defects that morphology cannot see. Conventional BSE captures compensable defects well and is largely blind to non-compensable defects. Closing this gap is the central motivation for biomarker-driven sperm assessment [9].

The sperm zinc signature as a single-cell capacitation biomarker

Zinc is the most abundant transition metal in semen, and its dynamic redistribution on the spermatozoon over the course of capacitation provides a tractable, fluorescent readout of capacitation competence [12, 13]. Using image-based flow cytometry with the zinc-specific probe FluoZin-3 AM, four distinct sperm zinc signatures (S1 through S4) were resolved in both bovine and porcine ejaculates. S1 represents the least capacitated spermatozoan with zinc localized across the entire sperm head and tail; S2 represents early stages of capacitation with zinc on the sperm and midpiece but not the principal piece; S3 represents mid-late stages of sperm capacitation with midpiece zinc localization alone, coincident with acrosomal remodeling; and S4 represents late capacitation and often at this point cell death. In vitro capacitation shifts the population distribution from S1-dominant to S2- and S3-enriched in a sire-specific and time-dependent manner [12].

The biological significance of the zinc redistribution maps to known capacitation events. Zinc stabilizes chromatin through zinc-finger proteins, gates the sperm-soluble adenylyl cyclase pathway, modulates ubiquitin-proteasome activity required for zona penetration, and triggers downstream Ca^{2+} flux and oviductal reservoir release [13, 17]. The S2-to-S3 transition is accompanied by a posterior-to-anterior plasma-membrane permeability wave requiring ≈2 seconds in fresh boar spermatozoa and 2–5 minutes in frozen-thawed bull spermatozoa, illustrating either species-specific kinetics or cryopreservation-induced deacceleration of capacitation [14]. Functionally, only S1 and S2 spermatozoa bind oviductal-like glycan beads in vitro, and zinc efflux is required for release [14]. This recapitulates the in vivo wave-like release from the oviductal reservoir [11, 18] and underscores that capacitation is a timing-sensitive trajectory rather than a switch: spermatozoa that capacitate too early die before reaching the oocyte, those that capacitate too slowly miss the fertilization window, and only the cell that is timed correctly fertilizes (Figure 2).

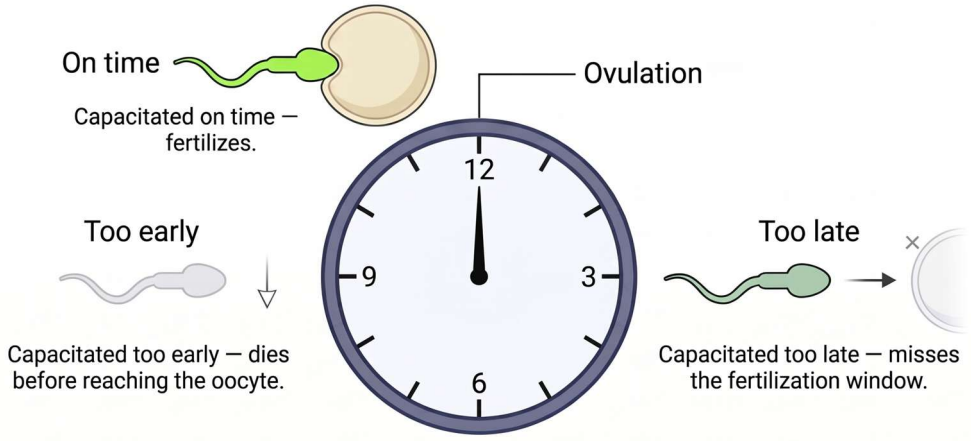

Figure 2. The capacitation timing window. Capacitation is a trajectory along the zinc signature (S1→S2→S3→S4) continuum rather than a discrete switch, and only spermatozoa that complete the capacitation in synchrony with the arrival of the oocyte are capable of fertilizing. Cells that capacitate too early exhaust their fertile lifespan before reaching the oocyte; cells that capacitate too slowly remain quiescent past the fertilization window. The cell that fertilizes is the cell timed correctly. Created in BioRender. Kerns, K. (2026) https://BioRender.com/1pyombp.

From biomarker to fertility predictor

Multivariate models that combine CASA-derived motility parameters with biomarker measurements at 0 and 4 hours of in vitro capacitation explain substantially more of the variance in porcine in vitro fertilization than either category alone. Motility alone is essentially uninformative after standard quality metrics are met; biomarker-only models outperform motility-only models by an order of magnitude; and the combined motility + biomarker model with paired pre- and post-capacitation timepoints is the best predictor [19]. This underscores that sperm in vitro capacitation can be utilized as a functional assay of sperm potential fertility; the informative quantity is the rate of progression through the zinc signature states,

not the proportion at any one time [20].

The molecular phenotype of semen is also accessible through multi-omics. Whole-cell proteomic profiling cleanly separates capacitated from non-capacitated samples on principal-components analysis, and more than 100 of the proteins that change with capacitation are zinc-interacting [17]. Capacitation-induced zinc flux drives a measurable metabolic shift involving 79 metabolites with glycolytic rerouting as a dominant feature [4], and lipidomic profiling of boar sperm membranes during capacitation identifies discrete lipid classes whose remodeling tracks capacitation progression [5]. These layers are complementary: the protein layer reports cellular machinery, the metabolite layer reports its current metabolic state, and the lipid layer reports the structural matrix in which both occur.

AI-driven morphology and label-free functional assessment

Morphology assessment as practiced has two persistent failure modes. First, experienced evaluators disagree on classification in 10% to 20%, and at times up to 30%, of cells. Second, evaluator accuracy declines after 15–30 minutes of continuous microscope work due to visual fatigue. eLearning interventions improve inter-observer agreement by only 3% to 5%, with the effect decaying within 2–4 months. Convolutional neural networks (CNNs) trained on image-based flow cytometry datasets eliminate this variability [7]. At 60× magnification, CNN models trained on 2,000 sperm images/class across five boars classify normal, proximal cytoplasmic droplet (PCD), distal cytoplasmic droplet (DCD), and distal midpiece reflux (DMR) with precision, recall, and F1 above 95% for every class, with a weighted F1 of 97.5% and 100% recall for DMR [7]. The clinical relevance of these categories is well established: retained cytoplasmic droplets reduce the response of boar spermatozoa to bicarbonate-induced capacitation, shorten the shelf life of fresh boar semen, and correlate negatively with both pregnancy rate and litter size [21].

A more recent development is the use of CNNs to predict functional biomarker status from brightfield imagery alone. Label-free models trained on paired stained and unstained image-based flow cytometry data predict peanut agglutinin-positive (PNA+) acrosomal disruption directly from brightfield sperm images, demonstrating that deep-learning approaches can extract functional information associated with acrosomal status without fluorescent labeling [7].

Spectral flow cytometry for high-parameter single-cell phenotypes

Traditional flow cytometry is constrained by spectral overlap between fluorophores to roughly one to five colors per panel. Spectral flow cytometry, which unmixes the full emission spectrum across multiple lasers, resolves >10-14 or more fluorophores from a single tube. For spermatozoa this enables simultaneous measurement of viability, acrosome status, mitochondrial membrane potential, intracellular zinc, capacitation markers, intracellular pH, reactive oxygen species, glutathione, DNA fragmentation, membrane fluidity, and additional functional endpoints on the same cells as long as live/viable cell and fixed cell assays are maintained within their respective treatments. Multiplexed panels have already proven decisive in detecting subtler degradation modes during liquid semen storage, including ferroptotic cell death that is invisible to single-color assays [22]. The biological value of multiplexing is the removal of cell-to-cell uncertainty inherent to one-color-at-a-time or few-colors-at-a-time assays: a spermatozoon that is PI-negative, JC-1-high, and FluoZin-3-S2 is a different cell from what the population mean of any single channel would suggest.

A five-tier integrated framework for sire fertility

The foregoing evidence supports a five-tier integrated framework. Tier 1 is the field BSE and Tier 2 is CASA-derived kinematics with subpopulation analysis; both are routinely deployed or capable of being deployed easily. Tier 3 is AI morphology, which is near deployment-ready with image-based flow cytometry and eliminates inter-observer variability [7]. Tier 4A introduces functional biomarkers (such as plasma-membrane integrity, acrosome status, mitochondrial potential, zinc signature) and Tier 4B introduces molecular biomarkers (such as DNA fragmentation, ubiquitin, oxidative stress, sperm proteomics, lipidomics). Tier 5 integrates all preceding tiers through multi-parametric AI fusion into a single, sire-level fertility score. A pragmatic, near-term implementation rule emerges from combining Tier 3 and Tier 4A: a sample with 40% morphologically unacceptable spermatozoa and 40% biomarker-unacceptable spermatozoa has been assumed to be 40% unacceptable; however, it is quite normal for a negative biomarker to be present on a normal morphology sperm and an abnormal sperm is capable of having normal biomarker status, thus such a sample could contain ≈70% functionally unacceptable cells,

because the two failure modes are only partially overlapping. Combining assays therefore identifies a substantially larger pool of compromised cells than either alone.

The clinical consequence of these multi-tier readouts is that two sires with identical conventional metrics can carry vastly different fertility ceilings, especially when including the capacitation-competent fraction of each ejaculate (**Figure 3**). No amount of additional motility or morphology pushes a sire past his capacitation ceiling, and only single-cell biomarker readout reveals that ceiling.

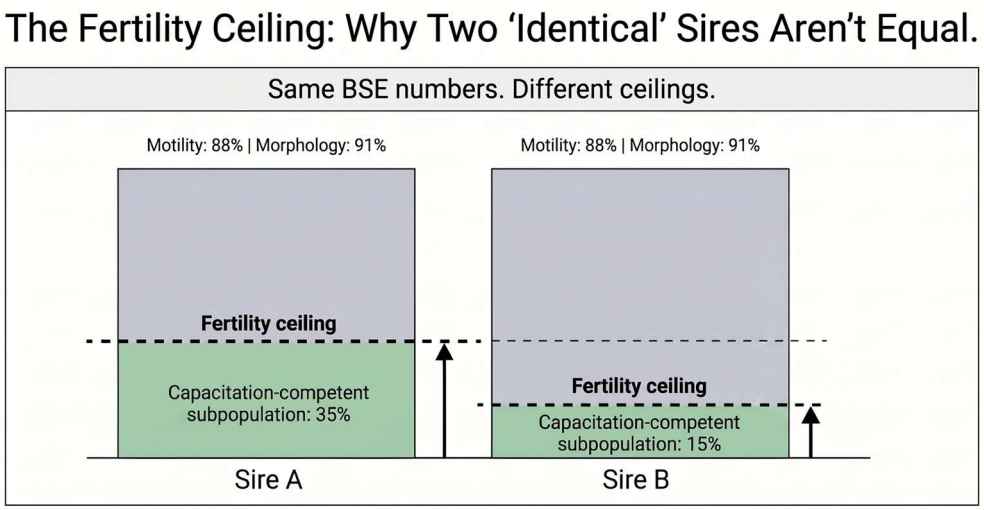

Figure 3. The fertility ceiling. Two sires with identical conventional BSE metrics (motility 88%, morphology 91%) can carry markedly different capacitation-competent subpopulations: 35% in Sire A and 15% in Sire B in this illustration, each of which sets a hard ceiling on attainable fertility regardless of further motility or morphology gains. Single-cell biomarker readout reveals the true fertility ceiling that conventional metrics mask and motivates the multi-tier integrated framework proposed in this review. *Created in BioRender. Kerns, K. (2026) https://BioRender.com/zdxgtfs.*

Final considerations

Motility and morphology are necessary prerequisites but not sufficient diagnostics of sire fertility. They detect visible compensable and non-compensable defects well, miss invisible non-compensable defects almost entirely, and report population averages that obscure the subpopulation heterogeneity in which fertility actually resides. Single-cell phenotyping with capacitation-resolved biomarkers, multi-omic profiling, AI-driven morphology, label-free deep-learning functional assessment, and spectral multi-parameter flow cytometry provide complementary windows into the molecular and functional readiness of the spermatozoon. The sperm zinc signature provides a tractable single-cell readout of capacitation that correlates with in vitro embryo production in both cattle and pigs [19]. Combined assays, framed within a five-tier integrated framework, will reshape sire selection and quality control in both boar and bull AI programs over the coming five years. The challenge ahead is not whether to integrate these tools but how rapidly the field can do so without losing the standardization that current practice provides. The opportunity is a precision-medicine analog for reproductive livestock science, in which each ejaculate is characterized by its single-cell fertility distribution and each sire is managed by what his spermatozoa can actually do rather than by what they appear to be.

Acknowledgments: The author thanks current and past trainees in the Kerns laboratory along with collaborators. This project was supported by Agriculture and Food Research Initiative (AFRI) Competitive Grant no. 2022-67015-36298 (K.K.) from the USDA National Institute of Food and Agriculture (NIFA), the Iowa Pork Producers Association Award no. 22015 (K.K.), and the National Pork Board.

References

Saacke RG, Dalton JC, Nadir S, Nebel RL, Bame JH. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. *Animal Reproduction Science.* 2000;60-61:663-77.
Sutovsky P, Hamilton LE, Zigo M, Ortiz D'Avila Assumpção ME, Jones A, Tirpak F, et al. Biomarker-

based human and animal sperm phenotyping: the good, the bad and the ugly. *Biology of Reproduction*. 2024;110:1135-56.
Flowers WL. Triennial Reproduction Symposium: sperm characteristics that limit success of fertilization. *Journal of Animal Science*. 2013; 91:3022-9.
Weide T, Mills K, Shofner I, Breitzman MW, Kerns K. Metabolic shift in porcine spermatozoa during sperm capacitation-induced zinc flux. *International Journal of Molecular Sciences*. 2024; 25:7919.
Shofner IJ, Mills K, Weide T, Breitzman MW, Kerns K. Zinc regulation of lipidome remodeling during boar sperm capacitation. *Journal of Animal Science*. 2026;104:skag009.
Zigo M, Kerns K, Sen S, Essien C, Oko R, Xu D, et al. Zinc is a master-regulator of sperm function associated with binding, motility, and metabolic modulation during porcine sperm capacitation. *Communications Biology*. 2022; 5:538.
Keller A, Maus MK, Keller E, Kerns K. Deep learning classification method for boar sperm morphology analysis. *Andrology*. 2024; 13:1615-25.
Zuidema D, Kerns K, Sutovsky P. An exploration of current and perspective semen analysis and sperm selection for livestock artificial insemination. Animals. 2021; 11:3563.
Keller A, Kerns K. Sperm capacitation as a predictor of boar fertility. *Molecular Reproduction and Development*. 2023; 90:594-600.
Suarez SS, Pacey AA. Sperm transport in the female reproductive tract. *Human Reproduction Update*. 2006; 12:23-37.
Hung PH, Suarez SS. Alterations to the bull sperm surface proteins that bind sperm to oviductal epithelium. *Biology of Reproduction*. 2012; 87:88.
Kerns K, Zigo M, Drobnis EZ, Sutovsky M, Sutovsky P. Zinc ion flux during mammalian sperm capacitation. *Nature Communications*. 2018; 9:2061.
Kerns K, Zigo M, Sutovsky P. Zinc: a necessary ion for mammalian sperm fertilization competency. *International Journal of Molecular Sciences*. 2018; 19:4097.
Kerns K, Sharif M, Zigo M, Xu W, Hamilton LE, Sutovsky M, et al. Sperm cohort-specific zinc signature acquisition and capacitation-induced zinc flux regulate sperm-oviduct and sperm-zona pellucida interactions. *International Journal of Molecular Sciences*. 2020; 21:2121.
Yeste M, Jones C, Amdani SN, Patel S, Coward K. Oocyte activation deficiency: a role for an oocyte contribution? *Human Reproduction Update*. 2016; 22:23-47.
Saacke RG. Sperm morphology: its relevance to compensable and uncompensable traits in semen. *Theriogenology*. 2008; 70:473-8.
Zigo M, Maňásková-Postlerová P, Zuidema D, Kerns K, Jonáková V, Tůmová L, et al. Porcine model for the study of sperm capacitation, fertilization and male fertility. *Cell and Tissue Research*. 2020; 380:237-62.
Wallgren M, Saravia F, Rodriguez-Martinez H. The vanguard sperm cohort of the boar ejaculate is overrepresented in the tubal sperm reservoir in vivo. *Journal of Reproduction and Development*. 2009; 56:68-72.
Rodriguez I, Keller A, Jennett L, Johnson M, Shofner I, Mahmood M, et al. Capacitation-induced zinc ion flux and sperm plasma membrane remodeling predict porcine in vitro fertilization cleavage success. *Molecular Reproduction and Development*. 2026;93:e70085.
Visconti PE, Krapf D, de la Vega-Beltrán JL, Acevedo JJ, Darszon A. Ion channels, phosphorylation and mammalian sperm capacitation. *Asian Journal of Andrology*. 2011;13:395-405.
Henning H, Luther AM, Waberski D. A high incidence of sperm with cytoplasmic droplets affects the response to bicarbonate in preserved boar semen. *Animals*. 2021;11:2570.
Mahmood M, Weide T, Kerns K. Ferroptosis contributes to boar sperm deterioration during liquid semen storage. *Frontiers in Veterinary Science*. 2026; 13:1816003.