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Sperm-oviduct interaction and the binding test: insights into bull fertility and endocrine modulation

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Abstract

The interaction between spermatozoa and bovine oviductal epithelial cells (BOEC) plays a crucial role in fertilization, particularly in the formation of the sperm reservoir in the isthmus. This adhesion not only prolongs sperm viability and functionality but also regulates the timed release of spermatozoa towards the fertilization site. The process is mediated by specific molecular mechanisms, including sperm surface proteins (such as BSPs), annexins, and glycoproteins from the oviductal extracellular matrix. Moreover, the hormonal environment, especially circulating estradiol and progesterone concentrations, modulates the epithelial receptivity and the dynamics of sperm binding. Historically, the sperm-BOEC binding assay has been used to assess sperm viability and function. However, emerging studies have demonstrated its potential as a predictive biomarker of bull fertility. Notably, experiments using long-term co-incubation with oviductal explants have revealed significant correlations between sustained sperm binding capacity and field fertility outcomes. Bulls with higher pregnancy per artificial insemination tend to maintain sperm binding for longer periods, suggesting a link between binding longevity and reproductive competence. Additionally, despite widespread use, conventional semen analysis often fails to detect subfertile bulls, as many sperm traits do not consistently correlate with field fertility. The sperm-BOEC binding assay offers a more integrative approach by evaluating the ability of sperm to interact with the female reproductive tract under conditions that closely mimic the in vivo environment. This review highlights the physiological and molecular mechanisms involved in sperm-oviduct interaction and supports the binding assay as a potential tool for improving fertility assessment in bulls, with implications for AI programs and reproductive efficiency in cattle.

Keywords: *Oviduct; Artificial insemination; Sperm reservoir, Sperm longevity; Functional fertility markers*

Introduction

Artificial insemination (AI) plays a pivotal role in enhancing genetic progress and reproductive efficiency in both beef and dairy cattle. The development of hormonal protocols capable of synchronizing follicular development and ovulation has enabled the widespread use of timed-AI (TAI), allowing producers to concentrate breeding periods or strategically distribute calving across the year. Despite these advances, bull fertility remains a critical determinant of AI success, and there are currently no accurate tools to predict male fertility before field testing.

Reproductive efficiency is dependent on multifactorial factors, involving coordinated physiological and molecular events from sperm transport to fertilization and embryo development. While considerable research has focused on optimizing female reproductive responses, male fertility continues to contribute significantly to reproductive failures, often due to subfertility characterized by reduced semen quality or functionality (Butler et al., 2019). Various *in vitro* assessments, such as computer-assisted sperm analysis (CASA), flow cytometry, and genomic evaluations, have provided valuable insights into sperm characteristics and bull genetic potential, yet they still fall short in reliably predicting bull fertility outcomes under field conditions.

To ensure successful sperm fertility, a functional sperm reservoir is formed within the oviduct, particularly in the isthmic region, between 6 to 12 hours post-insemination (Wilmut and Hunter, 1984). Only spermatozoa, with intact membranes and non-hyperactivated motility, are capable of binding to bovine oviductal epithelial cells (BOEC), where they are maintained viable until ovulation (Lefebvre and Suarez, 1996). This binding not only preserves sperm metabolic energy and viability but also allows for regulated capacitation and release at the appropriate time, thus optimizing the chances of successful

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fertilization (Lefebvre and Suarez, 1996). Emerging evidence from our group suggests that the ability of spermatozoa to form and maintain a functional reservoir in the oviduct may vary according to the fertilizing potential of the semen. Differences have been observed between higher and lower fertility bulls (De Pauw et al., 2002; Silva, 2021), as well as in sperm populations with known reduced fertility, such as sex-sorted sperm (Carvalho et al., 2018). These findings indicate that sperm-oviduct interactions could serve as a potential marker of sperm quality. Building on this, our group has conducted studies evaluating the utility of sperm-oviduct binding assays to distinguish sperm samples with differing fertility potential (Carvalho et al., 2018; Silva, 2021), showing promising results, particularly for young bulls whose fertility cannot yet be reliably assessed under field conditions. However, the female reproductive environment, particularly its hormonal regulation, has been shown by Cortat (2024) to influence the sperm-oviduct binding process, a factor that needs to be explored in current binding tests.

Therefore, this review aims to explore the physiological and molecular mechanisms of sperm binding to BOEC, the development of a standardized *in vitro* sperm-oviduct binding test to assess bull fertility, and the influence of the endocrine *milieu* on the binding test. The ultimate goal is to establish a robust and predictive tool for bull fertility that reflects field performance and supports selection decisions in AI programs.

Anatomy and physiology of the oviduct

The oviduct is a complex and dynamic segment of the female reproductive tract, playing a central role in mammalian fertility. Anatomically, it connects the ovary to the uterus and is divided into three distinct regions: infundibulum, ampulla, and isthmus, each one exhibiting unique morphological, cellular, and functional characteristics. These segments are lined by a simple columnar epithelium composed of ciliated and secretory cells, together with a finely tuned muscular layer and intricate mucosal folds (Yániz et al., 2000). Steroid hormones, particularly estradiol (E2) and progesterone (P4), play a pivotal role in regulating oviductal function by modulating epithelial cell activity, luminal fluid composition, and muscular contractility, thereby creating a dynamic microenvironment essential to several important events related to the success of fertility (Binelli et al., 2018). These features enable the oviduct to orchestrate critical reproductive events, including sperm reservoir formation, transport of gametes and embryo, fertilization, and early embryo development (Kölle et al., 2015). Moreover, the oviduct also serves as the site of the first maternal-embryo crosstalk, where the early embryo undergoes its initial mitotic divisions and embryonic genome activation (Ferraz et al., 2018).

Initiating the cascade of oviductal functions that support fertilization, the formation of sperm reservoirs represents a pivotal event in orchestrating the sequential processes required for successful fertilization. The sperm reservoir constitutes a dynamic molecular interface formed through specific and reversible interactions between spermatozoa and the cilia of bovine oviduct epithelium cells (BOEC) (Lefebvre and Suarez, 1996). This reservoir arises following the migration of sperm through the uterus and their selective passage across the utero-tubal junction, a physiological barrier that permits only sperm with functional competence to proceed into the oviduct. This natural sperm selection ensures the establishment of stable interactions with the BOEC, which prolong sperm viability, regulate the timing of capacitation, and synchronize gamete arrival for fertilization (Ignotz et al., 2007). These interactions are mediated by a complex interplay of carbohydrate-lectin recognition, protein-protein binding, and dynamic modifications of membrane molecules on sperm (Ignotz et al., 2007). It maintains sperm viability not only by delaying capacitation but also by providing a supportive microenvironment enriched with oviductal secretions, such as proteins, ions, and extracellular vesicles, that help to modulate sperm metabolism, motility, and function (Ferraz et al., 2019). Moreover, the reservoir enables the temporally controlled release of spermatozoa in response to ovulatory cues, ensuring their arrival at the fertilization site in an optimal physiological state.

A key mechanism underlying this binding involves carbohydrate-protein interactions, in which specific glycoconjugates on the apical membrane of oviduct epithelial cells serve as ligands for lectin-like receptors on the sperm surface (Kadirvel et al., 2012). In several mammalian species, including bovine, fucosylated and sialylated oligosaccharides, such as Lewis-a (Le^a) and sialyl-Lewis^x (sLe^x) motifs, are highly expressed on the oviduct epithelial cells' surface and are recognized by sperm surface receptors. However, while the interaction between specific glycans on the oviduct cells' surface and sperm receptors is a conserved mechanism among mammals, the exact oligosaccharide structures involved may vary between species (Kadirvel et al., 2012). In bovines, binder of sperm protein 1 (BSP1) and BSP3, secreted by the seminal vesicles and incorporated onto the sperm plasma membrane during ejaculation, have been associated with mediating adhesion to the oviduct via binding to fucosylated glycoproteins on BOEC

(Figure 1). These BSP proteins interact with epithelial annexins and sulfated glycoproteins, which further facilitate the sperm-oviduct binding process (Ignotz et al., 2007).

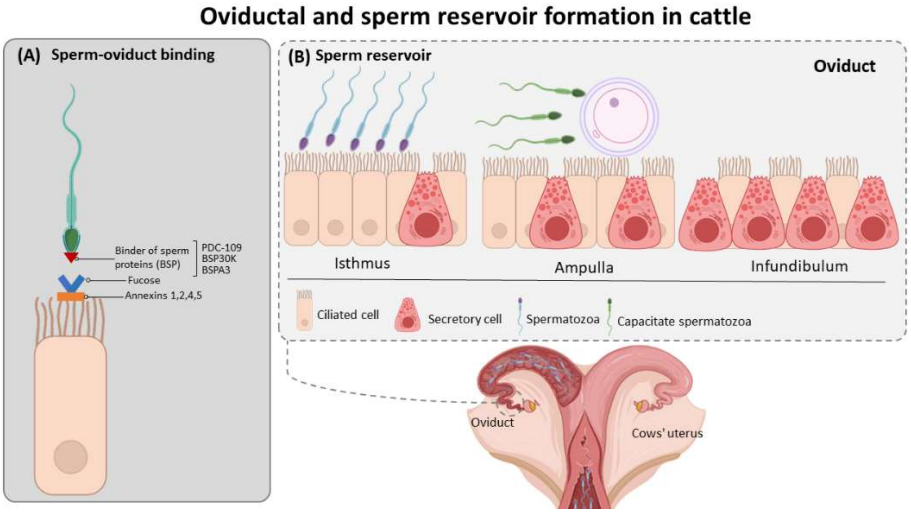

Figure 1. The bovine oviduct, particularly the isthmus region, plays a key role in forming the sperm reservoir, where spermatozoa temporarily bind to the bovine oviductal epithelial cells (BOEC). This interaction occurs between apical region of the sperm head and cilia of ciliated cells. It is mediated by a series of molecular interactions involving binder of sperm proteins (BSPs) such as PDC-109, BSP30K, and BSPA3, which are present on the sperm membrane, and receptors on the epithelial surface including fucose residues and annexins 1, 2, 4, and 5 (A). The middle panel (B) illustrates the oviductal epithelium across its segments - isthmus, ampulla, and infundibulum - highlighting the distribution of secretory and ciliated cells, as well as the transition from bound to capacitated spermatozoa as they progress toward the isthmus to ampulla, where fertilization occurs.

Importantly, the oviductal epithelium is not a passive surface; it responds dynamically to the presence of sperm, constituting an active biological interaction that significantly modulates the local oviductal environment. *In vitro* and *in vivo* studies have shown that sperm-oviduct interactions can induce changes in gene expression and in protein secretion, modulating genes involved in immune responses, cellular signaling, and molecular transport (Fazeli et al., 2004; López-Úbeda at., 2015; Reshi et al., 2020). Therefore, chemical characteristics of oviductal fluids are very dynamic and change continuously depending on the stage of the estrous cycle (Binelli et al., 2018) and on the presence of sperm and oocyte (Georgiou et al., 2005, 2007). Indeed, most of the proteins identified after exposing the oviduct to gametes are involved in gamete maturation, viability, and function (Georgiou et al., 2007). This regulation, initiated by sperm binding, appears to create a favorable microenvironment for the gametes while simultaneously inducing changes that prepare the oviductal milieu for successful fertilization and optimal early embryo development.

In this regard, our group recently published a study (Ribeiro et al., 2025) evaluating the effects of sex-sorted and non-sorted sperm on the proteome of BOEC. It was demonstrated that exposure of BOEC after insemination with sex-sorted sperm induces more pronounced proteomic changes compared to non-sorted sperm or a control group formed of BOEC from females "inseminated" with saline. Proteomic analysis identified 3,311 proteins, of which 601 had significant differences in abundance ($P \leq 0.05$). The presence of sex-sorted sperm led to an overall increase in protein abundance, particularly involving pathways related to metabolism and immune responses. The comparison between BOEC proteome exposed to sex-sorted or non-sorted sperm revealed 411 differentially abundant proteins, with 347 upregulated in the sex-sorted sperm group, including those associated with immune activation and oxidative stress response. Conversely, proteins related to cell adhesion, such as tight junction and talin proteins, were downregulated in the presence of sex-sorted sperm, suggesting a potential impact on epithelial functionality. These findings indicate that the type of sperm influences the oviductal environment, potentially affecting fertilization processes and early embryonic development. It is possible that the altered proteomic response of the oviductal epithelium exposed to sex-sorted sperm may be due to incorrect maintenance of the sperm reservoir, as shown by Carvalho et al. (2018). Considering that sex-sorted sperm is associated with reduced fertility, it is plausible that these molecular changes impair the proper formation

or stability of the sperm reservoir, which is essential for sperm survival and timely release for fertilization. A similar mechanism may underlie the reduced fertility observed in lower-fertility bulls, where inadequate sperm-oviduct interactions could result in suboptimal reservoir formation and, consequently, compromised fertilization efficiency compared to high-fertility sires.

Due to the limited access to oviducts in live animals, conducting *in vivo* studies on sperm-oviduct interactions remains a significant challenge. This limitation has prompted the development of *in vitro* oviduct cell culture models, designed to closely replicate the physiological conditions of the oviduct. Among these models, the use of oviductal explants has gained prominence for their application in sperm-oviduct binding assays.

The sperm-oviduct epithelial cell binding assay is an *in vitro* functional test designed to evaluate the capacity of spermatozoa to bind and maintain the interaction with the BOEC, mimicking a key physiological event of fertilization. This assay serves as a valuable tool to investigate sperm reservoir formation, including mechanisms of storage, release, and modulation of the oviductal environment, key factors in improving bovine fertility, and assisted reproductive outcomes.

This approach can be used to study the molecular and cellular mechanisms underlying sperm receptor formation, release dynamics, and oviductal environmental modulation. Over time, the assay has gained attention as a potential predictor of bull fertility, especially in identifying subtle functional deficiencies in sperm from subfertility bulls that may not be detectable by routine semen analysis. In addition, more physiological models able to mimic the oviduct microenvironment throughout the estrous cycle are being developed (Cortat et al., 2024) and will be helpful to proceed further on those unsolved questions.

Sperm-oviduct binding assay: mechanisms and fertility implications

Historically, the oviductal epithelial cell binding assay has been used primarily to assess sperm viability and functional integrity over short incubation periods (typically 13 to 30 minutes), focusing on the initial ability of spermatozoa to adhere to the female reproductive tract (Suarez, 1998; Suarez et al., 2008; Sostaric et al. 2008; Table 1). However, a pivotal study by De Pauw et al. (2002) marked a turning point in this field. For the first time, researchers evaluated the association between sperm binding ability and actual field fertility. Using bovine oviduct explants co-incubated with sperm for 24 hours, they observed a positive correlation between the number of sperm bound per mm of explant and the non-return rate in cattle. This was the first indication that sustained sperm binding capacity could serve as a functional marker of fertility.

Building on this foundational work, subsequent studies have further explored the predictive potential of the assay. For example, Saraf et al. (2018) extended this concept to buffalo, finding that bulls with higher field fertility had greater sperm binding capacity after 1 hour of co-incubation, which was moderately correlated with reproductive performance ($R^2 = 0.47$). Similarly, Carvalho et al. (2018) utilized the sperm binding assay with BOEC to evaluate the longevity of sex-sorted vs. non-sorted spermatozoa by assessing their binding capacity at 0.5 and 24 hours of co-incubation. While no significant difference was observed in the number of bound sperm per mm at the 0.5-hour timepoint, a marked reduction was evident at 24 hours for sex-sorted sperm compared to non-sorted sperm (6.7 vs. 23.6 sperm/mm; $P<0.05$). These results suggest that the lower pregnancies per AI (P/AI) commonly associated with sexed semen may, at least in part, be due to reduced sperm longevity in the female reproductive tract. This evidence further reinforces the association between sperm binding to BOEC and bull fertility.

In cattle production systems that rely heavily on AI, the use of subfertile bulls can result in significant reproductive losses (Northrop et al., 2019; Reese et al., 2020). Although semen analysis is widely used to evaluate breeding performance, bulls with similar semen profiles often have significantly different P/AI under field conditions (Oliveira et al., 2014). This discrepancy highlights the need for functional tests that are more reflective of the fertilization potential of the sperm.

Despite its widespread use, conventional semen analysis may not fully reflect the fertilization potential of spermatozoa. Parameters such as motility, mitochondrial membrane potential, and membrane integrity-assessed by CASA, fluorescence microscopy, or flow cytometry, are informative, yet often fail to predict field fertility accurately (Vicent et al., 2008; Harstine et al., 2018; Raina et al., 2020). These laboratory metrics do not consistently correlate with P/AI in commercial herds (Oliveira et al., 2014). Thus, while semen quality is critical, successful fertilization also depends on the sperm's ability to interact with the female reproductive tract, particularly in processes such as capacitation, oviductal reservoir formation, and sustained viability within the oviduct (Saacke et al., 2008; Saint-Dizier et al., 2020).

Table 1. Overview of experimental designs and main findings from bovine sperm-oviduct binding assays

Reference	Experimental groups	Tissue origin (species, region, estrous cycle phase)	Sperm type	Co-incubation time (hour)	Culture methodology	Key findings
Lefebvre et al. (1997)	Epithelium pretreated with various carbohydrates/glycoproteins or fucosidase	Bovine isthmus and ampulla, preovulatory heifers (surgically removed oviducts)	Frozen-thawed (swim-up selected)	1/4	Explant	Fucose and fucoidan inhibited sperm binding; fucosidase treatment reduced adhesion, suggesting fucose mediates specific sperm-oviduct interaction
Gualtieri and Talevi (2000)	Ampullary vs. isthmic epithelial monolayers	Bovine oviduct (ampulla and isthmus), selection based on ciliary activity; estrous cycle phase not specified	Frozen-thawed	1	Monolayers	Only acrosome-intact sperm bind; binding preserves acrosomes; release likely due to sperm surface changes during capacitation
De Pauw et al. (2003)	Bulls with varying non-return rates (NRR)	Bovine oviductal explants (region not specified), slaughterhouse	Frozen- thawed, and fresh	24	Explant	Sperm binding was positively associated with NRR when membrane integrity was >60%; the method showed potential as an *in vitro* fertility predictor
Sostaric et al. (2008)	Inseminated and non-inseminated cows; ipsilateral vs. contralateral oviducts; pre- and post-ovulation; explants and monolayers	Bovine isthmus and ampulla, synchronized cows (6 hours before to 5 hours after ovulation)	Frozen-thawed	1/4 (explants), variable in vivo and in vitro	Explant and monolayer; *in vivo*	Ovulation reduced sperm binding capacity, especially in the isthmus; binding was fucose-dependent; monolayers bound fewer sperm than explants; no differences between ipsi and contralateral oviducts
Carvalho et al. (2018)	Non-sexed (NS) vs. sex-sorted (XY) sperm; same ejaculate	Bovine isthmus, slaughterhouse oviducts	Frozen-thawed	0.5 and 24	Explant	Sex-sorted sperm (XY) had reduced longevity and lower binding to BOEC after 24 hours; no binding difference at 30 minutes despite poorer semen quality
Silva (2021)	Bulls classified as of higher or lower fertility based on large-scale TAI data (P/AI)	Bovine isthmus, slaughterhouse oviducts	Frozen-thawed	0,5, 12, 24, and 36	Explant	Higher sperm binding in higher-fertility bulls; strong correlation with field fertility at 36 hours (r = 0.89); no differences in conventional semen traits
Donnellan et al. (2022)	Bulls classified as of higher or lower fertility based on P/AI data	Reproductive tracts were collected from non-pregnant nulliparous heifers at slaughterhouse, follicular phase	Frozen-thawed	1/2	Explant	A higher number of spermatozoa from the higher fertility group compared to the lower fertility group bound to oviductal explants
Cortat et al. (2024)	Groups based on ovarian structure (CL or DF), ipsilateral or contralateral to the oviduct. *	Bovine isthmus, synchronized cows (preovulatory/diestrus), slaughterhouse	Frozen-thawed	0,5, 12, and 24	Explant	Fewer sperm bound to BOEC from oviducts ipsilateral to CL; binding modulated by local hormonal environment

*CL: corpus luteum; DF: dominant follicle

In a recent study, Silva et al. (2021) investigated *Bos indicus* bulls classified as of higher or lower fertility based on extensive TAI data collected under field conditions. Frozen-thawed semen samples were collected from six Nelore sires with known field fertility. These samples were provided by Alta Genetics (Uberaba, Brazil). Fertility classification was based on retrospective data from the Concept Plus Beef program, which compiles results from more than six million inseminations in commercial TAI programs. For this study, three bulls were classified as of higher fertility (average P/AI = 55%) and three bulls were classified as of lower fertility (average P/AI = 45%). Each bull was represented by two to three commercially approved ejaculates used for field AI. Frozen-thawed semen samples from these bulls were evaluated for sperm motility and kinetics (CASA), morphology, and membrane integrity by fluorescence microscopy and flow cytometry. Interestingly, no significant differences were observed between the fertility groups for most of these conventional semen parameters, confirming that standard analyses are often insufficient to explain differences in fertility. However, when the ability of spermatozoa to bind to BOEC was evaluated, a clear distinction between the groups was observed. To assess the ability of spermatozoa to interact with the female reproductive tract, a binding assay was performed using oviductal epithelial explants derived from the isthmus region of bovine oviducts. Reproductive tracts were collected from slaughterhouse animals, and the isthmic segments were dissected and cultured for 24 hours to form explants. Explants were then co-incubated with spermatozoa for 0.5, 12, 24, and 36 hours from frozen-thawed semen samples adjusted to a final concentration of 1×10^5 motile spermatozoa/mL. The results showed that higher fertility bulls had a greater number of bound spermatozoa at 12, 24, and 36 hours compared to lower fertility bulls ($P<0.05$). Although time-dependent declines in sperm binding were observed in both groups, reflecting progressive loss of sperm function, the superior ability of the higher fertile bulls to bind remained at 36 hours.

In a similar study conducted with *Bos taurus* (Holstein) bulls, Silva et al. (2024) evaluated the relationship between sperm binding and field fertility in dairy cattle with documented reproductive performance. Fertility data from 1,833 inseminations over 2 years in a commercial dairy herd (Tainá Farm, São Pedro, SP, Brazil) were retrospectively collected from seven Holstein sires. Based on P/AI data, bulls were classified as of higher fertility (n = 3; average P/AI = 35.0%) or lower fertility (n = 4; average P/AI = 21.4%). Cryopreserved semen batches (0.25 mL straws containing ~5.9×10^6 motile sperm/straw) were provided by CRV Lagoa (Ribeirão Preto Brazil), with each sire contributing with two to four batches to ensure balanced group representation.

As in *Bos indicus*, no differences between higher and lower fertility bulls were observed in conventional semen evaluations, including kinetic parameters by CASA, morphology, and membrane/acrosomal integrity (fluorescence microscopy and flow cytometry). However, when the ability of sperm to bind to BOEC was evaluated, differences were found. The sperm binding test was performed using the same methodology as previously described, and from 12 hours on, more sperm from higher fertility bulls were bound to BOEC than those from lower fertility bulls ($P < 0.05$). Notably, at the 36-hour timepoint, this binding capacity had a strong correlation with field fertility ($r = 0.89$), highlighting the potential of this assay as a reliable and predictive functional biomarker of bull fertility under commercial production conditions.

The results from both *Bos indicus* and *Bos taurus* bulls show that conventional semen analysis is not sufficient to distinguish differences in fertility under field conditions. Conversely, the oviductal epithelial cell sperm binding assay was able to discriminate between higher and lower fertility sires as early as at 12 hours of co-incubation, with the strongest correlation observed at 36 hours (Silva et al., 2021). These results highlight the value of the assay as a potential functional biomarker of fertility, particularly for the detection of subclinical deficiencies. Incorporating this methodology into routine fertility assessments may improve selection of more fertile sires and contribute to greater reproductive efficiency in AI programs.

Similarly, Donnellan et al. (2022) used an *ex vivo* approach with isthmic BOEC explants to investigate sperm binding ability in Holstein bulls with higher or lower field fertility. The explants were cultured for 24 hours before co-incubation with spermatozoa from frozen-thawed semen samples. After a 30-minute incubation period and subsequent washing, the number of spermatozoa bound to the epithelium was quantified. Higher-fertility bulls had a greater number of bound spermatozoa compared to lower-fertility bulls (15.1 ± 0.98 vs. 12.5 ± 0.76, respectively; $P < 0.05$). Although most conventional sperm parameters, such as motility and membrane integrity, did not differ between groups, the BOEC explant assay revealed functional differences aligned with field fertility, reinforcing its potential as a complementary tool in fertility assessment.

Influence of the endocrine milieu on sperm-oviduct binding assay

Numerous *in vitro* studies have evaluated the ability of sperm to bind to BOEC explants as a potential indicator of male fertility. However, when BOEC from females at various stages of the estrous cycle are placed in an *in vitro culture* system, thereby removing the *in vivo* endocrine stimulus from these cells, it is unknown whether they will retain their characteristics. This intra-assay variability is a significant challenge for assay standardization, especially with regard to future large-scale applications in fertility diagnostics. A likely source of this variability is the endocrine status of the donor animal at the time of oviduct collection, which may influence the receptivity of epithelial cells to sperm binding. Therefore, elucidating how the hormonal milieu affects the sperm-oviduct interaction is crucial for optimizing the test, enhancing its reproducibility, and increasing its predictive power in assessing bull fertility.

It is well known that oviductal epithelial cells undergo dynamic morphological and functional changes during the estrous cycle (Binelli et al., 2018). These changes are largely in response to fluctuations in circulating concentrations of E2 and P4 (Gonella-Diaza et al., 2017; Binelli et al., 2018). These hormonal variations modulate the oviductal microenvironment, affecting not only the structural composition of the epithelial layer but also its secretory activity, immune modulation, and ciliary beating patterns, all of which are critical for successful fertilization and early embryo development (Binelli et al., 2018).

Regulation of E2 and P4 within the oviduct is finely controlled by their interaction with specific nuclear receptors expressed in oviductal epithelial cells, E2 receptors (ERα and ERβ), and P4 receptors (PR; Binelli et al., 2018). These receptors mediate the genomic effects of their respective hormones and are essential for orchestrating the structural and functional changes of the oviduct throughout the estrous cycle. The E2, for example, not only enhances the expression of its receptors (ERα and ERβ) but also induces morphological adaptations such as increased epithelial cell height, ciliation, and secretory activity (Binelli et al., 2018). These features are associated with improved sperm transport and survival. Conversely, P4 induces the expression of PR and contributes to the modulation of the epithelial phenotype to create a more receptive environment for fertilization. At the same time, P4 exerts a suppressive effect on oviductal secretion and motility. Recent studies have confirmed the presence of ESR1, ESR2, and PGR transcripts and proteins in BOEC, with their expression levels fluctuating in response to the hormonal status of the female. This dynamic interplay between E2 and P4 not only fine-tunes oviductal physiology but also influences sperm-epithelium interactions, highlighting the importance of the hormonal *milieu* in optimizing fertilization conditions (Binelli et al., 2018).

Some studies suggest that sperm transport involves a cycle of detachment and attachment in the oviduct before capacitation, but once capacitated, sperm lose their affinity for the oviductal epithelium (Lefebvre et al., 1995; Suarez, 1998; Gualtieri and Talevi, 2000). Progesterone may highlight two key factors that may help explain this effect. First, the presence of P4 during diestrus may be associated with epithelial self-renewal (Binelli et al., 2018), potentially affecting the quality and viability of cells for culture. Second, P4 can activate a biphasic response involving repeated intracellular Ca2+ oscillations (Harper et al., 2004). In particular, P4 is a key regulator of the timing and mechanism of sperm release from the oviductal reservoir (Kirkman-Brown et al., 2004; Mirihagalle et al., 2022). One of the main pathways by which P4 exerts this effect is by activating CatSper (sperm cation channel), a sperm-specific calcium ion channel located in the main part of the flagellum. Upon exposure to P4, CatSper channels open, resulting in a biphasic intracellular Ca^{2+} response, initial rapid influx followed by a sustained phase, that is thought to result from mobilization of internal Ca^{2+} stores (Harper et al., 2004; Kirkman-Brown et al., 2004; Mirihagalle et al., 2022). This calcium surge is critical for triggering hyperactivated motility, a vigorous and asymmetric flagellar beating pattern that allows sperm to navigate the viscous fallopian fluid and overcome barriers such as the zona pellucida. The increased propulsive force generated by hyperactivation is also thought to directly contribute to sperm detachment from BOEC, allowing spermatozoa to resume their progression towards the oocyte (Leclerc et al., 1996; Calogero et al., 2000; Naz and Rajesh, 2004).

The importance of CatSper-mediated Ca^{2+} influx has been demonstrated in bovine sperm (Johnson et al., 2017; Romero-Aguirregomezcorta et al., 2019; Mirihagalle et al., 2022), and the interaction of P4 with this channel occurs via a non-genomic mechanism. This interaction indirectly activates protein kinase A (PKA), which then triggers tyrosine phosphorylation of sperm proteins, further enhancing motility patterns associated with detachment and capacitation. Supporting the hypothesis that hyperactivated motility is a key factor in BOEC detachment, a study in cattle confirmed the beneficial role of P4 in inducing sperm hyperactivation (Romero-Aguirregomezcorta et al., 2019)

Therefore, the local hormonal *milieu*, in particular the concentration of P4, not only affects the receptivity and phenotype of epithelial cells but also modulates sperm behavior at the molecular level via

CatSper activation. Understanding these interactions is crucial for improving the interpretation and standardization of *in vitro* sperm-oviduct binding assays, especially in the context of fertility prediction and assisted reproduction.

To investigate the influence of the endocrine milieu on the sperm-oviduct interaction, Cortat et al. (2024) performed two experiments: one with reproductive tracts collected from slaughter cows and the other from synchronized cows with known cyclic status. In both cases, oviducts were classified according to the stage of the estrous cycle based on ovarian morphology. In Experiment 1, reproductive tracts from 12 cows were collected at slaughter and classified based on ovarian structures: either a functional corpus luteum (CL) or a dominant follicle (DF). In Experiment 2, seven *Bos indicus* cows were hormonally synchronized to present at slaughter either a 14-day CL (n = 3) or a DF in the absence of a CL (n = 4). In both experiments, oviductal isthmus tissue was further categorized according to the laterality of the ovarian structure, ipsilateral or contralateral to the ovary containing the CL or DF. Based on this classification, oviducts were cultured individually according to ovarian structure and laterality. Isthmic epithelial oviducts were then cultured for 24 hours to form explants and then co-cultured with frozen-thawed spermatozoa (1×10^5 motile sperm/mL) for 0.5, 12, and 24 hours.

To complement the estrous stage classification, Cortat et al. (2024) also analyzed the hormonal profile of the animals. In both experiments, follicular fluid was aspirated from all follicles > 4 mm, and the concentrations of E2 and P4 were measured. Blood samples were also collected to measure plasma P4. In Experiment 1, P4 concentrations in follicular fluid (FF) were significantly higher in the CL-Ipsi group (193.0 ± 26.2^{a} ng/mL) compared to the DF-Contra (39.5 ± 13.7^{b} ng/mL), DF-Ipsi (62.7 ± 19.9^{b} ng/mL), and CL-Contra ($<20.0^{b}$ ng/mL) groups. Estradiol concentrations in the FF did not differ among groups in this experiment. Otherwise, in Experiment 2, involving synchronized cows, P4 concentrations in the FF were also higher in the CL-Ipsi group (112.7 ± 32.9^{a} ng/mL) than in DF-Contra (24.5 ± 2.6^{b} ng/mL), while DF-Ipsi (81.2 ± 21.9^{ab} ng/mL) and CL-Contra (34.4 ± 8.6^{ab} ng/mL) had intermediate values. Regarding E2 concentrations, DF-Ipsi had the highest concentrations ($>200.0^{a}$ ng/mL), differing from CL-Contra (75.5 ± 62.4^{b} ng/mL), CL-Ipsi (19.3 ± 8.0^{b} ng/mL), and DF-Contra (13.7 ± 1.3^{b} ng/mL). Cows in the luteal phase (14-d old CL) presented higher plasma P4 concentrations (5.4 ± 1.1 vs. 2.0 ± 0.3 ng/mL; $P < 0.05$), confirming their endocrine status and supporting the morphological classification based on ovarian structure.

Regarding sperm binding, there was a significant effect of the endocrine status on sperm binding capacity. Photographs of sperm attachment to oviduct explants are shown in Figure 2. The BOEC derived from oviducts ipsilateral to a functional CL had reduced sperm binding per mm of explant ($P<0.05$) in all points of evaluation. These findings suggest that the hormonal *milieu* typical of the follicular phase, characterized by elevated E2 concentrations, creates a more favorable environment for sperm adhesion. In contrast, tissues collected during the luteal phase, which have lower or absent estrogenic activity, had reduced binding capacity. These results highlight the importance of considering the endocrine context during oviduct collection to reduce variability and increase the reliability of the sperm-oviduct binding assay.

In another study, Lefebvre et al. (1995) evaluated sperm binding to oviduct explants obtained from synchronized cows at different stages of the estrous cycle, specifically, preovulatory, early postovulatory (approximately 12 hours), and mid-diestrus (day 10), and from different anatomical regions of the oviduct (isthmus and ampulla). Contrary to the reported study, the authors found no significant effect of estrous phase or oviduct region on the number of sperm bound to the explants. It should be noted, however, that the co-incubation period in this study was limited to only 15 minutes, which may not have been sufficient to fully capture the dynamics of the sperm-epithelium interaction and may have underestimated subtle effects of the endocrine *milieu*.

Overall, studies investigating the influence of the endocrine *milieu* on sperm-oviduct binding collectively suggest that fluctuations in steroid hormone concentrations throughout the estrous cycle significantly affect both sperm behavior and oviductal epithelial cell function. In particular, P4 has been shown to modulate sperm detachment through mechanisms involving calcium influx and hyperactivation, while also affecting epithelial receptivity and cell turnover. Estradiol, on the other hand, appears to increase epithelial ciliation and secretory activity, creating a more favorable environment for sperm binding. These hormonal dynamics underscore the complexity of the sperm reservoir and highlight the importance of considering the hormonal stage when interpreting results from *in vitro* binding assays. Ongoing investigation into endocrine-cell interactions is essential for advancing our understanding of fertilization biology and for refining tools to assess male fertility.

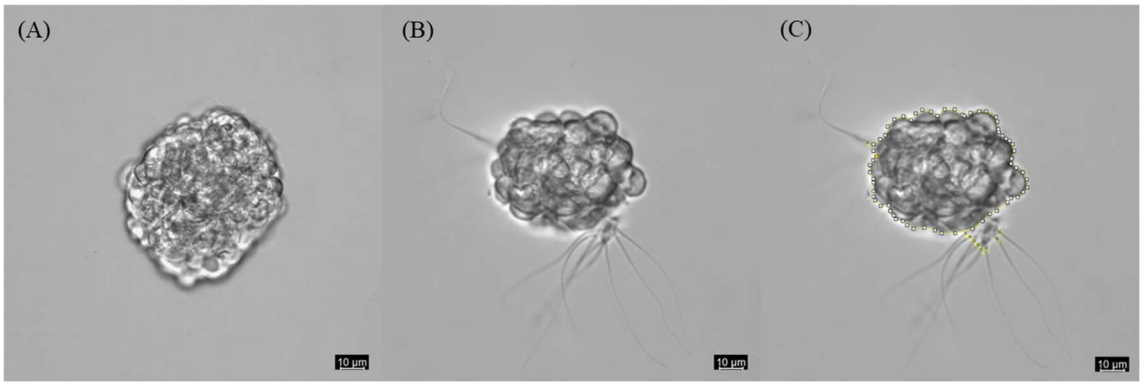

Figure 2. Phase contrast images showing *in vitro* sperm binding assay using bovine oviductal epithelial cell (BOEC) explants. Image (A) shows a BOEC explant formed after 24 hours of culture, preserving the three-dimensional structure of the epithelial cells. In (B), the explant was co-incubated with motile spermatozoa, allowing for the visualization of sperm binding to the epithelial surface 24 h of co-incubation. Image (C) shows the same explant with its perimeter delineated for analysis, and sperm bound to the outer edge were counted individually. The explants were photographed are assessed under an optical microscope for sperm binding quantification and measurement of the aggregate perimeter (in millimeters), allowing characterization of the sperm-oviduct epithelial cell interaction. All images were captured using a Thunder Imager 3D Assay inverted biological microscope (Leica Microsystems, Wetzlar, Germany) at 40 × magnification. Scale bars: 10 µm.

Future perspectives

The interaction between sperm and BOEC is thought to play an important role in sperm survival, capacitation, and fertilization. Recent studies suggest that the ability of spermatozoa to bind and remain attached to oviductal cells may be closely associated with bull fertility. These findings support the potential of sperm-oviduct binding assay as a functional tool to complement conventional semen evaluations and to aid in the identification of bulls with suboptimal reproductive performance.

Looking ahead, future studies should focus on elucidating the molecular mechanisms involved in sperm binding, particularly the role of specific adhesion molecules and how this process is modulated by the hormonal environment of the oviduct.

In this context, considering that BOEC lose exposure to the physiological endocrine environment once placed in culture, it becomes essential to investigate whether the supplementation of steroid hormones in the in vitro culture medium can preserve or enhance their sperm-binding capacity. Recreating the hormonal conditions typical of the in vivo oviduct may help maintain epithelial functionality and improve the consistency and predictive value of sperm-oviduct binding assays.

To fully realize the potential of this assay as a practical tool for fertility assessment, biological improvements must be accompanied by methodological advances, including the standardization of assay protocols and validation of their predictive value in large-scale studies under commercial conditions to enable widespread application in AI programs. One major limitation to the large-scale application of the sperm-oviduct binding assay is the need to collect oviducts from slaughterhouses, preferably from animals in the follicular phase of the estrous cycle, when elevated E2 levels create a more favorable environment for sperm binding. To overcome this, it is essential to evaluate whether BOEC explants can be cryopreserved without losing their structural and functional characteristics after thawing.

In conclusion, the sperm-oviduct binding assay offers a promising approach to improve fertility assessment by considering physiological interactions between spermatozoa and the female reproductive tract. Its application could contribute to more informed sire selection and improved reproductive efficiency in cattle.

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