



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/237380/>

Version: Published Version

---

**Article:**

Mason, C., Garlovsky, M., Vedder, O. et al. (2026) Experimental evidence that prenatal and postnatal developmental stress affects the adult seminal fluid proteome in a precocial bird. *Molecular Ecology*, 35 (2). e70257. ISSN: 0962-1083

<https://doi.org/10.1111/mec.70257>

---

**Reuse**

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

## ORIGINAL ARTICLE OPEN ACCESS

# Experimental Evidence That Prenatal and Postnatal Developmental Stress Affects the Adult Seminal Fluid Proteome in a Precocial Bird

Chloe Mason<sup>1</sup>  | Martin Garlovsky<sup>2</sup>  | Oscar Vedder<sup>3</sup> | Trong Khoa Pham<sup>4</sup> | Rachel George<sup>4</sup> | Barbara Tschirren<sup>5</sup>  | Nicola Hemmings<sup>1</sup> 

<sup>1</sup>School of Biosciences, University of Sheffield, Sheffield, UK | <sup>2</sup>Applied Zoology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany | <sup>3</sup>Institute of Avian Research, Wilhelmshaven, Germany | <sup>4</sup>biOMICS Mass Spectrometry Facility, University of Sheffield, Sheffield, UK | <sup>5</sup>Centre for Ecology and Conservation, University of Exeter, Penryn, UK

**Correspondence:** Chloe Mason ([chloemason178@gmail.com](mailto:chloemason178@gmail.com))

**Received:** 7 September 2025 | **Revised:** 18 December 2025 | **Accepted:** 10 January 2026

**Keywords:** fertility | liquid chromatography–tandem mass spectrometry | maternal investment | proteomics | seminal fluid protein composition | silver spoon effect

## ABSTRACT

Seminal fluid proteins are important modulators of male fertility and reproductive success, yet little is known about how their abundance responds to early-life developmental stress. Japanese quail *Coturnix japonica* males produce a unique seminal foam that enhances fertilisation success. We characterised the proteome of the seminal foam for the first time and assessed how its composition is influenced by prenatal and postnatal developmental stress. Proteomic identification using liquid chromatography–tandem mass spectrometry and subsequent gene ontology (GO) analysis of chicken (*Gallus gallus domesticus*) orthologs suggested roles for the foam proteome in sperm maturation and DNA protection, semen liquefaction, sperm plasma membrane homeostasis and energy production for sperm motility. Males that experienced prenatal stress exhibited increased abundance of proteins involved in lipid metabolic processes, inflammation and oxidative stress, including proteolytic enzymes, interleukin receptors and avidin-like proteins. Similarly, males that exhibited postnatal stress exhibited increased abundance of proteins involved in chromatin organisation, carbon metabolism and oxidative stress. Nine proteins involved in metabolic processes and antioxidant processes were consistently more abundant across developmentally stressed males from both experiments, suggesting convergent responses to early-life stress. These results demonstrate that early development environments can alter the seminal foam proteome of adult males, with potential implications for ejaculate quality and fertilisation ability.

## 1 | Introduction

Identifying the causes of variation in male reproductive success is essential to understanding the process of sexual selection (Andersson 1994; Birkhead 2010). Variation in sperm and testes traits influences male fertilisation success under sperm competition (Lüpold et al. 2020). However, research into the role of non-sperm ejaculate components in post-copulatory sexual selection has only gained momentum in recent years. In internally fertilising species, males transfer sperm along with seminal

fluid comprised of somatic cells (e.g., immune cells), macromolecules (carbohydrates, fats, vitamins and minerals), hormones and proteins (Hopkins et al. 2017; Poiani 2006).

Seminal fluid proteins (SFPs) are particularly important in male and female reproduction and include antioxidants, lipases, lectins, proteases and protease inhibitors with a diverse range of functions (Chapman 2001; Avila et al. 2011; Perry et al. 2013; Ramm 2020; Santiago-Moreno and Blesbois 2020). The function of SFPs has mainly been studied in mammals,

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Molecular Ecology* published by John Wiley & Sons Ltd.

insects and to some extent, birds, where they can promote sperm competition success through mediating sperm function (den Boer et al. 2008; Labas et al. 2015; Jodar et al. 2017; Th  lie et al. 2019; Santiago-Moreno and Blesbois 2020), storage (den Boer et al. 2009; King et al. 2011) and maturation (Manjunath and Th  rien 2002; Douard et al. 2004), as well as providing immune protection (Dorus et al. 2012; Atikuzzaman et al. 2017). SFPs can also act beyond sperm traits by modifying female reproductive behaviour (Chapman et al. 2003; Liu and Kubli 2003; Bath et al. 2017), mating plug formation (Ram and Wolfner 2009; Stockley et al. 2020), promoting oviposition (Chapman et al. 2003; Liu and Kubli 2003; Goenaga et al. 2015), and modulating female immune responses (Short and Lazzaro 2010; Schjenken and Robertson 2014) and physiology (Sasanami et al. 2015; Schjenken and Robertson 2020). Furthermore, SFPs exhibit high rates of evolutionary change, resulting in between-species divergence and within-species variation (Chapman 2001; Ramm et al. 2009; Goenaga et al. 2015; Garlovsky et al. 2020).

Across species, there is evidence that seminal fluid production is affected by the environment. A meta-analysis found investment in SFP production is highly sensitive to nutrient availability, particularly dietary protein intake, whilst sperm traits were only moderately affected (Macartney et al. 2019). However, how environmental factors influence the protein composition of seminal fluid, as opposed to just the amount produced, is less well understood across taxa. In *Drosophila*, the main genus in which this work has been carried out, SFP abundances have been shown to be affected by diet (Zendeer et al. 2023), male-male competition (Hopkins et al. 2019; Ramm 2020), female-mating status (Sirot et al. 2011) and male age (Sepil et al. 2020), but whether these effects are consistent across other taxa remains unclear.

Developmental conditions are likely to have long-term effects on male reproductive investment, with nutritional conditions during postnatal development emerging as a particularly important factor (Edwards et al. 2019; Garc  a-Vargas et al. 2019). In *Drosophila* for example, males reared on a nutrient-restricted diet were typically smaller and partially compensated for lower mating success by investing in increased sperm numbers per ejaculate under competitive mating situations (De Nardo et al. 2021). In non-competitive matings, however, males reared on poor-quality diets transferred fewer sperm across successive matings compared to high-quality diet males (Macartney et al. 2021), suggesting resource-limited males are constrained in their long-term investment in sperm traits. However, less is known about how dietary stress influences other components of the ejaculate, such as SFPs (Macartney et al. 2019). Investment in seminal fluid production is a large energetic expense (Friesen et al. 2015) and seminal fluid may deplete faster than sperm (Linklater et al. 2007; Reinhardt et al. 2011). *Drosophila* males reared at a higher population density with limited food were smaller in body size and transferred a greater proportion of SFPs during mating, possibly reflecting a response to increased risk of sperm competition (Wigby et al. 2016). Further proteomic analysis revealed that although larger males produced higher abundance of SFPs in their accessory glands, smaller males transferred greater quantities of SFPs during mating, suggesting that males exposed to postnatal developmental stress may invest more per mating (von Hellfeld et al. 2025).

Although resource demand is highest during postnatal development, the prenatal development environment provided by the mother can also have significant and long-term effects on offspring phenotype and fitness (Mousseau and Fox 1998; Rhind et al. 2001). Prenatal developmental stress, such as malnutrition, can influence embryo growth, organ development, immune function and adult reproductive behaviour (Clark and Galef 1995; Desai and Hales 1997; Gorman and Nager 2004; Giordano et al. 2014). In sheep (*Ovis aries*), maternal undernutrition reduces the lifetime reproductive capacity of female offspring, while in males, it alters fetal plasma testosterone concentrations despite having no detectable effect on testis structure, potentially affecting later reproductive development (Rae et al. 2002).

Birds provide an ideal system for investigating the effects of prenatal and postnatal conditions on adult reproductive traits, as embryonic development occurs externally, allowing direct measurement of prenatal factors (Groothuis et al. 2005). Moreover, egg size and its composition (i.e., the proportion of nutrients, hormones and immunoglobulins) are known to alter offspring phenotype (Williams 1994). In zebra finches (*Taeniopygia guttata*), offspring of females with a poor diet prior to breeding have lower fecundity (Gorman and Nager 2004), but the effect of prenatal conditions on adult male reproductive investment, particularly the composition of SFPs, remains unexplored.

In this study, we experimentally investigate if pre- and postnatal developmental stress influences the proteomic composition of a unique seminal foam produced by male Japanese quail (*Coturnix japonica*). Species of the *Coturnix* (quail) genus produce a foam-like substance upon ejaculation, secreted from a specialised gland known as the 'proctodeal gland' or 'cloacal gland' (Klemm et al. 1973). The foam is a viscous glycomuco-protein aerated by male cloacal muscle contractions (Seiwert and Adkins-Regan 1998), as well as interactions with carbon dioxide and hydrogen produced by cloacal bacteria (Mohan et al. 2004). In Japanese quail, seminal foam constitutes a novel component of the quail ejaculate that is stored separately from sperm and is not mixed with semen until inside the female reproductive tract (Fujihara 1992; Klemm et al. 1973). SFPs may also be found in semen produced in the testes, epididymis and ductus deferens, as in other Galliformes (chicken *Gallus gallus domesticus* and turkey *Meleagris gallopavo*) (Fujihara 1992), but these are not considered here. Male proctodeal gland size is a predictor of fertilisation success, and natural copulations with foam have higher fertilisation success compared to copulations without foam or with artificially placed foam (Ogawa et al. 1974; Cheng et al. 1989; Abuoghaba et al. 2024). Foam may extend the female's fertile period, increasing the likelihood of successful fertilisation (Singh et al. 2012; Abuoghaba et al. 2024), or improve sperm motility. Foam significantly prolongs sperm motility and increases sperm velocity in vitro, suggesting its components supply energy to sperm (Singh et al. 2011; Farooq et al. 2015). Lactate dehydrogenase is a protein found at high levels in quail seminal plasma (Buxton and Orcutt 1975), and lactate in foam may act as an energy source for sperm transport (Singh et al. 2011) as it does in the seminal fluid of mammals (Odet et al. 2011; Saeed et al. 2021). Foam also disaggregates sperm clumps, leading to more vigorous motility, possibly due to non-protein components (Singh et al. 2011). Most studies

investigating the effect of foam on male fertilisation success employ a foam removal technique which requires invasive surgery and is likely to interfere with copulation. However, Finseth et al. (2013) showed that non-invasive foam removal in natural mating scenarios reduces male fertilisation success under sperm competition. The function of foam during sperm competition, possibly mediated through positive effects on the male's own sperm at a cost to a rival's fertility, suggests it evolved under sexual selection (Finseth et al. 2013). Although Japanese quail mating behaviour is difficult to observe in the wild, due to their cryptic nature, the species is most likely polyandrous, with high levels of sperm competition, evidenced by their frequent mate switching and forced copulations under semi-natural conditions (Nichols 1991) and laboratory settings (Adkins-Regan 1995), prolonged female sperm storage (Birkhead and Fletcher 1994; Beccardi, Tschirren, and Vedder 2025), and large testes relative to body size (Clulow and Jones 1982; Møller 1991).

The broader protein composition and function of quail foam have not yet been studied but could offer an interesting comparison to SFPs that are differentially produced but play a similar role in other Galliformes (Labas et al. 2015; Borziak et al. 2016; Słowińska et al. 2017). In this study, we used high-throughput proteomics using liquid chromatography–tandem mass spectrometry (LC–MS/MS) to characterise the protein composition of the unique seminal foam of the Japanese quail for the first time, offering new insights as to its evolution and function. We then assess whether developmental stress affects the proteomic composition of seminal foam that males produce as adults, using males from two experiments that were aimed at manipulating resources available during pre- vs. postnatal development. We hypothesised that male investment in seminal foam would be more constrained if they developed in an environment with limited resources, and this would impact adult SFP production, with developmentally stressed males exhibiting lower abundances of SFPs, particularly those that modulate fertilisation success, than those that were not resource-limited during development. Together, these experiments provide the first assessment of how early developmental stress affects the seminal fluid proteome in any vertebrate species, advancing our understanding of the function of this unique reproductive fluid and early-life effects on ejaculate investment more broadly.

## 2 | Materials and Methods

### 2.1 | Study Population and Experimental Manipulation of Early Life Stress

#### 2.1.1 | Experiment 1—Prenatal Developmental Stress

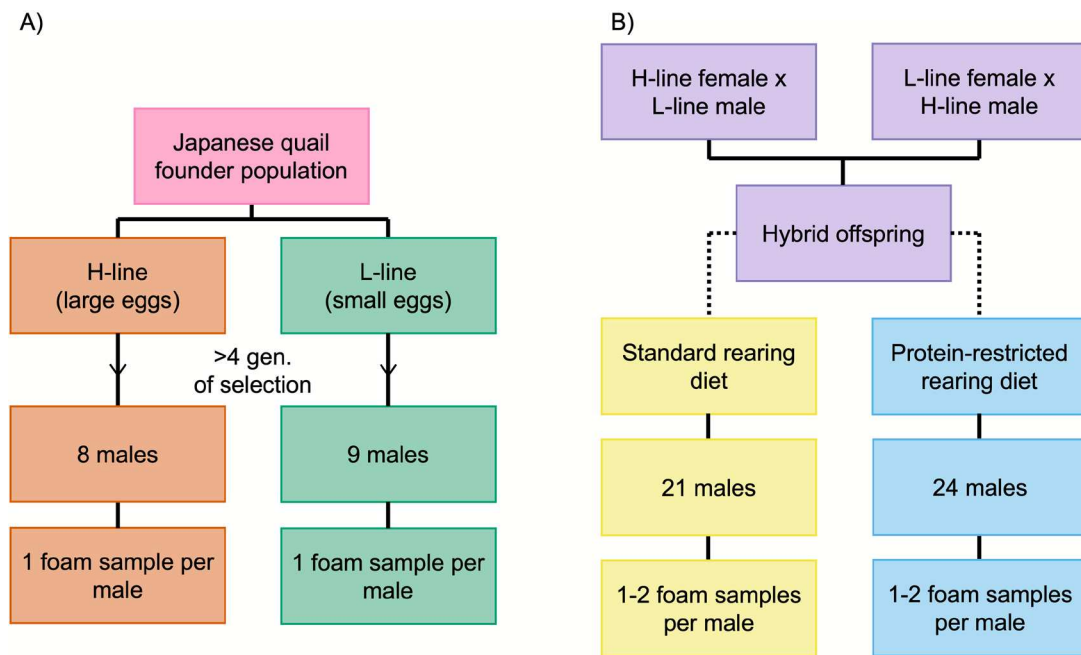
We used artificial selection for increased and reduced maternal egg investment to manipulate prenatal developmental stress (Pick, Hutter, and Tschirren 2016). Briefly, from a founder population of Japanese quail housed in a breeding facility at the University of Zurich, Switzerland, the 10 females producing the largest and smallest eggs (relative to their body size) were assigned to the high and low maternal reproductive investment lines, respectively, in two independent biological replicates. Two sons and 2 daughters of each of the 10 females producing the largest eggs (20 sons and 20 daughters total) and 10 females

producing the smallest eggs (20 sons and 20 daughters total) within their respective lines and replicates were selected for the next generation of breeding (20 breeding pairs per line). Breeding pairs consisted of unrelated males and females (not sharing any grandparents) and individuals were paired once only. See Pick, Hutter, and Tschirren (2016) for a full description of the breeding conditions. By generation four, there was a strong divergence in egg size and dried egg components (i.e., resource availability for the developing embryo) between lines (mean  $\pm$  SDs: high maternal investment line =  $12.46 \pm 0.94$  g, low maternal investment line =  $11.12 \pm 0.91$  g; difference in absolute egg size = 1.06 standard deviations), but no difference in laying rate (Pick, Hutter, and Tschirren 2016). In 2017, individuals were transferred to the Institute of Avian Research, Wilhelmshaven, Germany, and in 2018, we sampled foam from 1-year-old non-sib male offspring from the 5th and 6th generations of the selection experiment (8 males from the high maternal investment lines and 9 males from the low maternal investment lines; Figure 1A). We collected foam via cloacal and proctodeal gland massage, removing it before semen was ejaculated to avoid contamination, and stored aliquots of pure foam at  $-80^{\circ}\text{C}$  until analysis.

#### 2.1.2 | Experiment 2—Postnatal Developmental Stress

We used a post-hatching food quality experiment to manipulate postnatal developmental stress (Vedder et al. 2023). In 2019, 1-year-old birds from the 6th and 7th generation of the selection experiment (see above) were mated with a partner from the opposite maternal investment selection line within each replicate to produce hybrid offspring. See Vedder et al. (2023) for a full description of the breeding conditions. Hatchlings were randomly distributed over rearing cages with one of two rearing diets that differed in protein content: a standard protein diet (21.0% protein, 4.0% fat and 1.1% calcium; calorific value 11.4 MJ/kg) or a protein-restricted diet (14.5% protein, 4.0% fat and 1.0% calcium; calorific value 11.4 MJ/kg), both of which are commercially available (GoldDott, DERBY Spezialfutter GmbH, Münster, Germany). There was no difference in the size of the eggs that the hatchlings originated from between treatment groups (overall mean  $\pm$  SE:  $12.04 \text{ g} \pm 0.19$ ; standard-protein diet group:  $12.18 \text{ g} \pm 0.27$ ; protein-restricted diet group:  $11.92 \text{ g} \pm 0.27$ ). The protein-restricted diet substantially reduced growth rate without compromising chick survival, whilst the standard diet increased growth rate without leading to impairments associated with too rapid growth (Vedder et al. 2023; Vedder and Beccardi 2025). From 5 weeks onwards, birds were individually checked for cloacal foam production every 2–3 days, indicating sexual maturity (Sachs 1969). The average age for onset of sexual maturity for males was 45 days with the standard diet, and 69 days with the protein-restricted diet. After sexual maturity, all males received a standard adult diet (19.0% protein, 4.6% fat and 4.8% calcium; calorific value 9.8 MJ/kg) (GoldDott, DERBY Spezialfutter GmbH, Münster, Germany). In 2019, we collected 1–2 foam samples per male from 21 standard rearing diet and 24 protein-restricted rearing diet non-sib males (Table S1; Figure 1B) while they were housed in individual cages, with the standard adult diet. The average age of males when foam was collected was  $117 \text{ days} \pm 10$ . Foam samples were collected and stored as described above. In both experiments, birds were maintained on a 16:8 light: dark cycle at  $\sim 20^{\circ}\text{C}$  and had constant access to water,





**FIGURE 1** | Experimental design and sample collection: (A) prenatal developmental stress (Experiment 1) and (B) postnatal developmental stress (Experiment 2).

grit and food. All animals were kept under licences provided by the Veterinäramt JadeWeser (permit number 42508\_03122020).

## 2.2 | Sample Preparation

We defrosted foam samples at room temperature for 12 h, then pooled smaller samples from the same selection lines/diet treatments within each replicate to ensure sufficient material for mass spectrometry (MS) analysis. Overall, this gave us 4 pooled replicate samples per selection line (high maternal investment line and low maternal investment line) from Experiment 1, and 12 pooled replicate samples per dietary treatment (standard-diet and protein-restricted diet) from Experiment 2. In Experiment 1, 4 high line samples and 3 low line samples contained foam from 2 males (1 foam sample per male), and 1 low line sample contained foam from 3 males (1 foam sample per male). In Experiment 2, we had 6 replicates from each hybrid cross type (high line mother × low line father, and low line mother × high line father). Twelve standard-diet samples contained foam from 3 males (1 foam sample per male), and 12 protein-restricted samples contained foam from 4 males (1 foam sample per male).

We added four times the sample volume of ice-cold acetone ( $-20^{\circ}\text{C}$ ) to each sample, then vortexed and incubated them for 4 h at  $20^{\circ}\text{C}$  before centrifuging for 10 min at 13,000 rpm at  $4^{\circ}\text{C}$  to isolate foam proteins. The remaining supernatant was discarded, and we left the protein pellet at room temperature until the acetone evaporated completely.

We resuspended the protein pellet in 25  $\mu\text{L}$  of protein lysis buffer (5% SDS (Sigma–Aldrich) and 100 mM TEAB (ThermoFisher), pH 8.5). We quantified the total protein concentration using a BCA assay (ThermoFisher) and normalised to 25  $\mu\text{g}$  of protein per replicate for proteomic analysis. We reduced the protein

by 20 mM DTT (ThermoFisher) using a thermoshaker at  $95^{\circ}\text{C}$ , 800 rpm for 10 min, and after cooling for 5 min at room temperature, we alkylated proteins using 40 mM 2-iodoacetamide (Sigma–Aldrich) using a thermoshaker at room temperature, 800 rpm for 30 min in the dark. We then digested proteins using a suspension trapping technique (S-Trap) according to the manufacturer's protocol (ProtiFi). Briefly, alkylated proteins were acidified by adding 2.5  $\mu\text{L}$  12% phosphoric acid, followed by 365  $\mu\text{L}$  S-trap binding buffer (90% aqueous methanol, 0.1 M TEAB, pH 7.1). We transferred samples to S-Trap columns gently and centrifuged for 60 s at  $4000\times g$  to trap the denatured proteins.

We washed trapped proteins 5 times with 150  $\mu\text{L}$  binding buffer, centrifuging between each addition, before transferring proteins to clean 1.5 mL Eppendorf tubes for protein digestion. We added 25  $\mu\text{L}$  of MS grade Trypsin (ThermoFisher) in 50 mM TEAB buffer (concentration 0.1  $\mu\text{g}/\mu\text{L}$ ) to each S-Trap and incubated at  $47^{\circ}\text{C}$  for 1.5 h without shaking to digest the proteins into peptides. Peptides were then eluted using a series of solvents: 40  $\mu\text{L}$  50 mM TEAB, 40  $\mu\text{L}$  0.2% aqueous formic acid (ThermoFisher), 40  $\mu\text{L}$  50% ACN in 0.2% formic acid and 40  $\mu\text{L}$  80% ACN in 0.2% formic acid. Samples were centrifuged at  $4000\times g$  for 60 s between solvent additions. We collected eluted peptides and dried them in a vacuum concentrator (Eppendorf) for 2 h before re-constituting them in 60  $\mu\text{L}$  0.5% formic acid and withdrawing 4  $\mu\text{L}$  for MS analysis.

## 2.3 | Liquid Chromatography-Tandem MS Analysis

We performed sample processing separately for each experiment. All MS proteomics analyses were performed at the bioMICS Mass Spectrometry Facility, University of Sheffield (<https://www.sheffield.ac.uk/mass-spectrometry>) on an Orbitrap

Exploris E480 mass spectrometer (ThermoFisher) equipped with a nanospray source, coupled to a Vanquish HPLC System (ThermoFisher). Peptides were desalted online using a Nano-Trap Column (75  $\mu$ m I.D.  $\times$  20 mm; ThermoFisher) and then separated using an EASY-Spray column (50 cm  $\times$  50  $\mu$ m I.D., PepMap C18, 2  $\mu$ m particles, 10  $\text{\AA}$  pore size; ThermoFisher). We used a 100-min gradient, starting from 3% to 20% buffer B (0.5% formic acid in 80% ACN) for 68 min, followed by a ramp-up to 35% buffer B for 23 min, then to 99% buffer B for 1 min, and maintained at 99% buffer B for 9 min. MS was operated in positive mode with a cycle of 1 MS acquired at a resolution of 120,000, at  $m/z$  of 400. We subjected the top 20 most abundant multiply charged ( $2^+$  and higher) ions in a given chromatographic window to MS/MS fragmentation in the linear ion trap, with a scan range ( $m/z$ ) of 375–1200, normalised AGC target of 300%, microscan 1, an FTMS target value of  $1e4$  and a resolution of 15,000.

## 2.4 | Protein Identification and Bioinformatic Analysis

We compared protein abundances between the selection lines and rearing diet groups described above, as well as producing an overall characterisation of the Japanese quail foam proteome using data from all samples combined. We analysed all MS data with MaxQuant (v.1.6.10.43) and searched data against the *Coturnix japonica* protein database consisting of 27,875 proteins (Uniprot proteome ID: UP000694412) with the following search parameters: trypsin/P (2 missed cleavages) as the enzyme, methionine oxidation and N-terminal protein acetylation as variable modifications and cysteine carbamidomethylation as a fixed modification. We set FDR for both peptides and proteins to 1% using target-decoy approaches. Since using a two unique peptide requirement can reduce proteome coverage without substantially improving identification confidence when a robust FDR control is used, we used a  $\geq 1$  unique peptide cutoff.

We loaded the MaxQuant output into Perseus (v1.5.6.0) for downstream data analysis, including filtering, normalisation and statistics (Cox and Mann 2008; Cox et al. 2014). We set all label-free quantification (LFQ) intensities as main columns, filtered the matrix to remove potential contaminant proteins and reverse sequences, then transformed LFQ intensities using the  $\log_2(x)$  function. For the selection treatment, we categorically annotated rows with either H (high maternal investment) or L (low maternal investment), and filtered proteins to identify those present in at least 3 replicates in at least one of the selection lines. For the diet treatment, we categorically annotated rows with either SD (standard diet) or PR (protein-restricted diet), and filtered proteins to identify those present in at least 3 replicates in at least one of the diet groups. We examined the quality of replicates using Pearson's correlations and performed Principal Component Analysis (PCA) on the  $\log_2$ -transformed LFQ intensities to assess sample clustering.

We normalised each protein's ( $\log_2$ ) intensity in each sample to its median value and missing values were imputed from a normal distribution (Experiment 1: 472 values imputed, 30% and Experiment 2: 1521 values imputed, 56%). We calculated  $\log_2$  fold-changes to describe differences between maternal investment selection lines or diet groups within each experiment.

We then evaluated differences in protein abundance between selection lines or treatment groups for statistical significance ( $p < 0.05$ ) using unpaired two-sided Student's  $t$ -tests with Benjamini-Hochberg correction for multiple testing (FDR) on normally distributed data.

## 2.5 | Gene Ontology (GO) Analysis

As the *C. japonica* genome has not been fully annotated, we identified chicken (*Gallus gallus domesticus*) orthologs using OrthoFinder v.3.0 (Emms and Kelly 2019) and the chicken reference proteome (UniProt Proteome ID: UP000000539). We assessed the overlap between the quail foam proteome, the seminal fluid proteome of the red junglefowl (*Gallus gallus*; Borziak et al. 2016) and domestic chicken (Labas et al. 2015), and the chicken spermatozoa proteome (Labas et al. 2015). We used the list of orthologs that were differentially abundant in a selection line or diet group for GO analysis to identify functional differences in the foam's proteome between treatments.

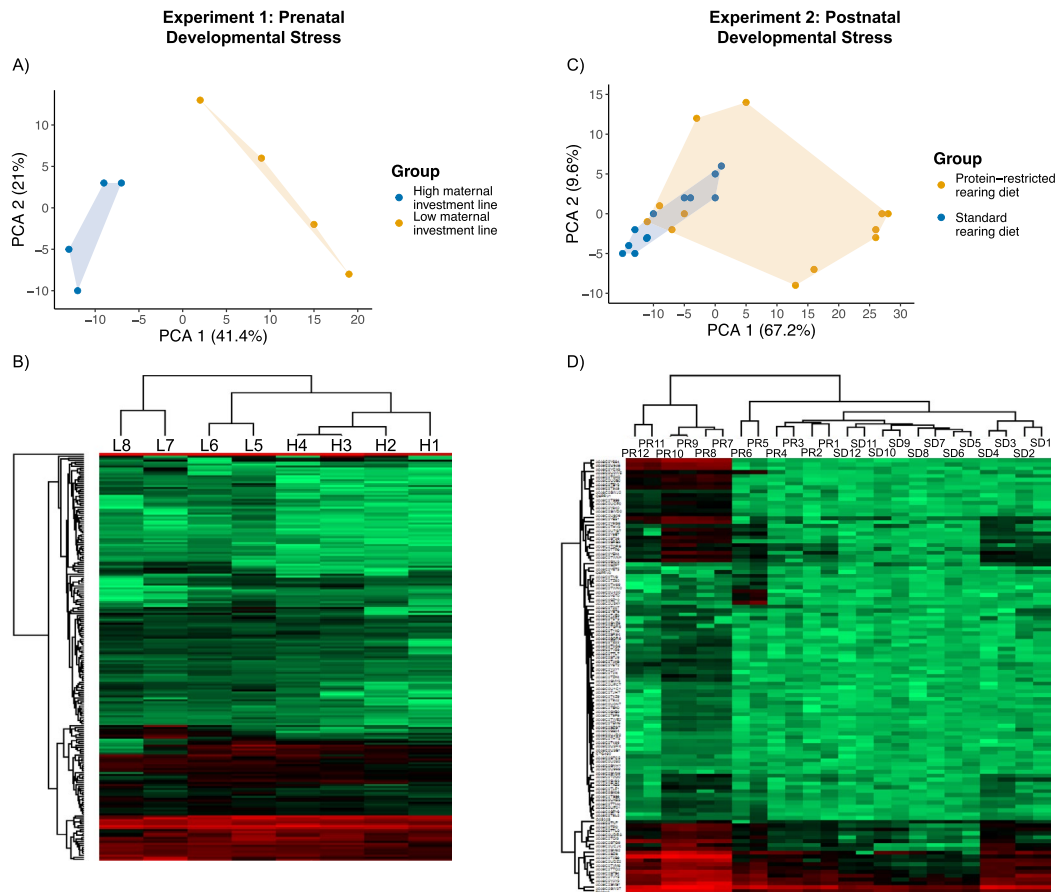
We performed GO enrichment analyses using the website version of DAVID v.2023q4 (Huang et al. 2009; Sherman et al. 2022). We uploaded the list of proteins that were differentially abundant within a selection line or diet group to DAVID (<https://david.ncifcrf.gov/tools.jsp>) and used the total seminal foam proteome as the background. In instances where a quail protein had multiple orthologs in chicken, we selected one representative ortholog for GO analysis. We downloaded the outputs for all three GO categories (biological processes, cellular components and molecular functions) including their associated statistical values, as well as the output table from the Functional Annotation Clustering Tool that clusters redundant annotation terms to identify biological themes associated with proteomes. Figures were created in R v.4.4.2 (R Core Team 2024).

## 3 | Results

### 3.1 | Effect of Prenatal Developmental Stress on the Seminal Foam Proteome

In the prenatal stress experiment, we identified 425 proteins across 17 Japanese quail foam samples (pooled into 4 replicates per treatment; Table S2). Of these, 196 (46.2%) were identified in at least 3 replicates within a selection line and included in the final dataset (Table S3). The relative protein abundances were strongly correlated between replicates (mean Pearson's correlation coefficient  $r = 0.86$ , range = 0.69–0.96). A principal component analysis showed PC1 explained 41.1% and PC2 explained 21% of the variation in the data and revealed a clear separation between males from the high and low maternal investment lines (Figure 2A,B).

We found 48 proteins that were significantly more abundant in foam of males from the low maternal investment lines compared to males from the high maternal investment lines (for each protein  $\log_2$  fold change  $> 1.5$ ,  $p < 0.05$ ; Table S3; Figure 3A) and 148 proteins that were present in similar quantities in both the high and low maternal investment lines ( $p > 0.05$ ). No proteins were significantly more abundant in the high maternal investment



**FIGURE 2** | Proteomic profiles of foam samples from both experiments. Experiment 1: (A) principal component analysis (PCA) plot of protein abundance of samples from high (blue) and low (orange) maternal investment lines along PC1 (41.4%) and PC2 (21%), and (B) heat map showing the abundance of 196 detected seminal foam proteins. Rows represent individual proteins and columns represent replicate samples from high (H) and low (L) maternal investment lines. Experiment 2: (C) principal component analysis (PCA) plot of protein abundance of samples from standard (blue) and protein-restricted (orange) rearing diet groups along PC1 (67.2%) and PC2 (9.6%) and (D) heat map showing the abundance of 103 detected seminal foam proteins. Rows represent individual proteins and columns represent replicate samples from standard rearing diet (SD) and protein-restricted rearing diet (PRD) groups.

line compared to the low maternal investment line ( $\log_2$  fold change  $< 1.5$ ,  $p < 0.05$ ). See Table S3 for results of the two-sided Student's *t*-tests.

The 48 proteins that were more abundant in foam samples from the low maternal investment line 47 had chicken orthologs, and 17 had more than one (106 orthologs in total; see Table S3 for a complete list). Of these proteins, 46 (96%) had GO annotations. Our GO analysis showed that proteins that were more abundant in the low maternal investment lines were associated with lipid metabolic processes (see Table S4 for a complete list of GO terms associated with proteins that were more abundant in the low maternal investment line and associated *p*-values). No annotation clusters were enriched in proteins that were more abundant in the low maternal investment line. See Table S5 for all annotation clusters, associated terms and *p*-values.

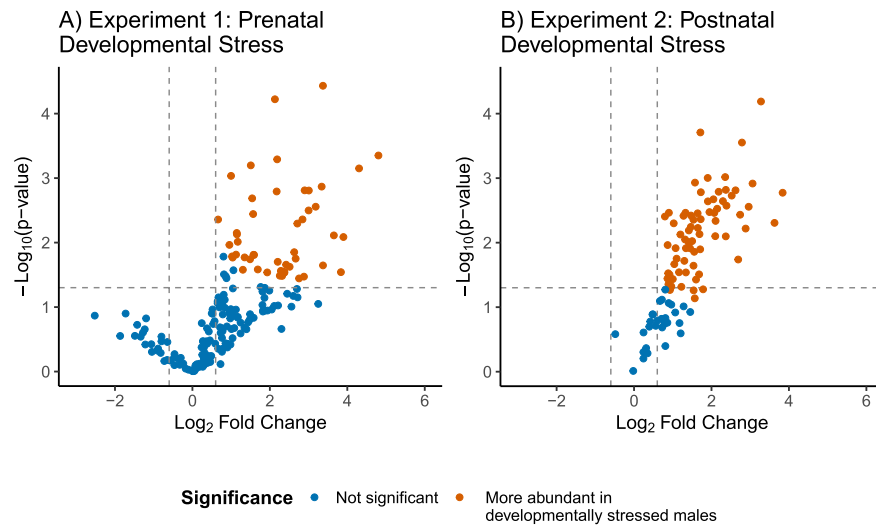
### 3.2 | Effect of Postnatal Developmental Stress on the Seminal Foam Proteome

In the postnatal stress experiment, we identified 401 proteins across 79 Japanese quail foam samples (pooled into 12 replicates

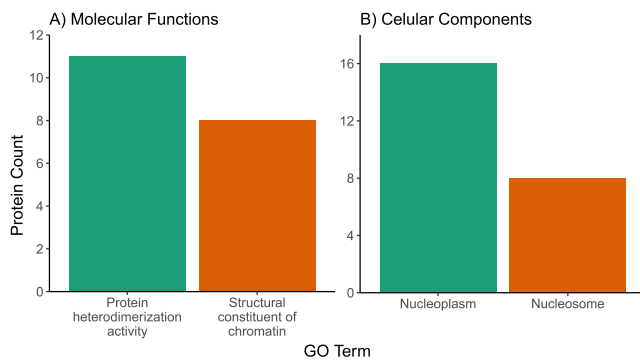
per treatment; Table S2). Of these, 103 (25.7%) were identified in at least 3 replicates within a treatment group and included in the final dataset (Table S6). The relative protein abundances were strongly correlated between replicates (mean Pearson's correlation coefficient = 0.83, range = 0.55–0.99). A principal component analysis showed PC1 explained 67.2% and PC2 explained 9.6% of the variation in the data (Figure 2C,D).

We found 72 proteins that were significantly more abundant in foam samples from males raised on a protein-restricted diet compared to a standard diet (for each protein  $\log_2$  fold change  $> 1.5$ ,  $p < 0.05$ ; Table S6; Figure 3B) and 31 proteins that were present in similar quantities in foam from the standard and protein-restricted diet males ( $p > 0.05$ ). No proteins were significantly more abundant in foam from males fed the standard diet compared to the protein-restricted diet ( $\log_2$  fold change  $< 1.5$ ,  $p < 0.05$ ). See Table S6 for results of the two-sided Student's *t*-tests.

Of the 72 proteins that were more abundant in foam samples from males fed the protein-restricted diet, 71 had chicken orthologs and 11 had more than one (128 orthologs in total; see Table S6 for a complete list). Of these proteins, 69 (96%)



**FIGURE 3** | Effect of developmental stress on the quail seminal foam proteome: (A) Volcano plot of protein abundance comparing low vs. high maternal investment lines (two-sample *t*-test,  $n = 4$  biological replicates per selection line). Orange indicates 48 of 196 proteins were more abundant in the low maternal investment lines. No proteins were more abundant in the high maternal investment lines. Blue proteins had no significant change in abundance between the high and low maternal investment lines. (B) Volcano plot of protein abundance comparing protein-restricted vs. standard rearing diet groups (two-sample *t*-test,  $n = 12$  biological replicates per diet treatment). Orange indicates 72 of 103 proteins were more abundant in the protein-restricted diet group. No proteins were more abundant in the standard diet group. Blue proteins had no significant change in abundance between the standard diet and protein-restricted diet group.



**FIGURE 4** | GO analysis of the proteins with increased abundance in the protein-restricted diet group: (A) 2 molecular functions, and (B) 2 cellular components all enriched in proteins that were significantly more abundant in Japanese quail seminal foam of males fed a protein-restricted diet compared to a standard diet. Only functional enrichment groups with  $p$ -values  $< 0.05$  are shown.

had GO annotations. Our GO analysis found that proteins that were more abundant in the protein-restricted diet group were associated with nucleosome assembly, the molecular functions: protein heterodimerisation activity and structural constituent of chromatin (Figure 4A); and the cellular components: nucleoplasm and nucleosome (Figure 4B; see Table S7 for a complete list of GO terms associated with proteins that were more abundant in the protein-restricted diet group and associated  $p$ -values).

Using the Functional Annotation Clustering Tool in DAVID (Huang et al. 2009; Sherman et al. 2022), we identified that the annotation cluster histone modification and chromatin structure was enriched in proteins that were more abundant in the protein-restricted diet group. See Table S8 for all annotation clusters, associated terms and  $p$ -values.

### 3.3 | Overall Characterisation of the Japanese Quail Seminal Foam Proteome

Finally, we combined data across both experiments to provide an overall characterisation of the seminal foam proteome. We identified 608 foam proteins across the two cohorts, of which 224 were identified in at least 3 replicates in a single cohort (36.8%) (Table S2). Relative protein abundances were strongly correlated between replicates (mean Pearson's correlation coefficient = 0.85, range = 0.55–0.99). See Table S9 for the 20 most abundant proteins in the foam.

Genes encoding foam proteins were highly conserved, with 96.4% of proteins (216/224) having chicken orthologs (Table S2) compared to 82.0% of all Japanese quail proteins (22,858/27,875;  $\chi^2 = 31.17$ ,  $df = 1$ ,  $p < 0.001$ ). We found more than one chicken ortholog for 27.7% (62/224) of quail proteins (Figure S1), and 449 chicken protein orthologs in total, which are encoded by 266 distinct chicken genes. See Table S10 for full ortholog protein list.

Eight foam proteins (4%) did not have chicken orthologs (Table S11) and we inferred their functions in UniProt (The UniProt Consortium 2025). Four were predicted to be isoforms of neuroblast differentiation-associated protein AHNAK-like and associated with the nucleus and regulation of RNA splicing. Two predicted Ig-like domain-containing proteins may be associated with an immunoglobulin-mediated immune response. We found aldo-keto reductase family 1 member B1 (aldose reductase) in foam, inferred from homology, and finally, we found a predicted secreted protein associated with the transmembrane helix with no known function.

Comparing proteomes, we found that 7.6% (34/449) and 14.7% (66/449) of chicken orthologs identified in quail foam were also

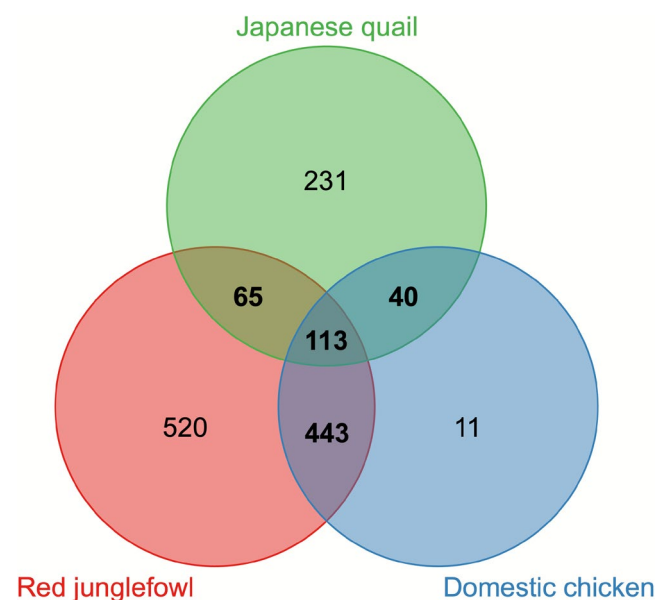


in domestic chicken and red junglefowl seminal fluid, respectively, and 34.1% (153/449) and 39.6% (178/449) of foam proteins had isoforms found in chicken and red junglefowl seminal fluid, respectively (Figure 5; Table S10). Comparing genomes, we found 31.6% (84/266) and 40.2% (107/266) of genes encoding quail foam proteins encoded SFPs in chicken and red junglefowl seminal fluid, respectively (Table S10). There was evidence that proteins found in chicken sperm were also present in quail foam, with 29.2% (131/449) of foam protein having isoforms identified in chicken spermatozoa (Table S10). Of these, 48 were not found in chicken seminal fluid. The remaining 231 quail foam proteins were not found in either chicken or red junglefowl seminal fluid. GO analysis of proteins unique to quail foam revealed a significant association with proteolysis (Benjamini-Hochberg corrected  $p = 4.5E-6$ , FDR = 4.5E-6).

GO analysis of chicken orthologs using the *G. gallus* gene list as the background identified 3 biological process terms (Figure 6A), 16 molecular functions (Figure 6B) and 16 cellular components (Figure 6C) significantly enriched in quail foam (see Table S12 for all GO terms and associated  $p$ -values), together suggesting the foam plays a role in sperm maturation and motility, proteolysis, protein binding and immune regulation. The Functional Annotation Clustering tool in DAVID (Huang et al. 2009; Sherman et al. 2022) identified 35 clusters (Table S13), 17 of which were significantly enriched in the foam proteome (Table S14). As a relatively high number of proteins were associated with proteolysis, these proteins were identified individually and assigned to their appropriate protease class (Table S15).

### 3.4 | Overlaps in Proteomes

Nine proteins that were more abundant in foam samples from the low maternal investment line in Experiment 1 were also



**FIGURE 5** | Venn diagram indicating the size and overlap between the seminal foam proteomes of the Japanese quail (*Coturnix japonica*), red junglefowl (*Gallus gallus*) and domestic chicken (*Gallus gallus domesticus*).

more abundant in foam of the protein-restricted diet group in Experiment 2 (Figure 7). Of these, 6 were associated with metabolic pathways (Table 1). Their functions were inferred using the UniProt database: alpha-amylase is an enzyme involved in glucose production (Zakowski and Bruns 1985). Transketolase catalyses the reversible conversion of sugar phosphates into glycolytic intermediates, providing a link between the non-oxidative pentose phosphate shunt and glycolysis (Zhao and Zhong 2009). Malate dehydrogenase plays a central role in aerobic cellular respiration, facilitating ATP generation from glucose (Wolyniak et al. 2024), whilst ATP synthase drives ATP production from ADP during oxidative phosphorylation (Neupane et al. 2019). Aldo-keto reductase family 1 member B10 detoxifies reactive carbonyl compounds in cells, and aldehyde dehydrogenase 1 family member A1 detoxifies aldehydes, both protecting cells from oxidative stress and cellular death (Shortall et al. 2021; Wang et al. 2009). Although many of these proteins are involved in metabolic pathways, no biological process, molecular function or cellular component GO terms were significantly enriched after multiple testing correction, as expected with a small number of proteins.

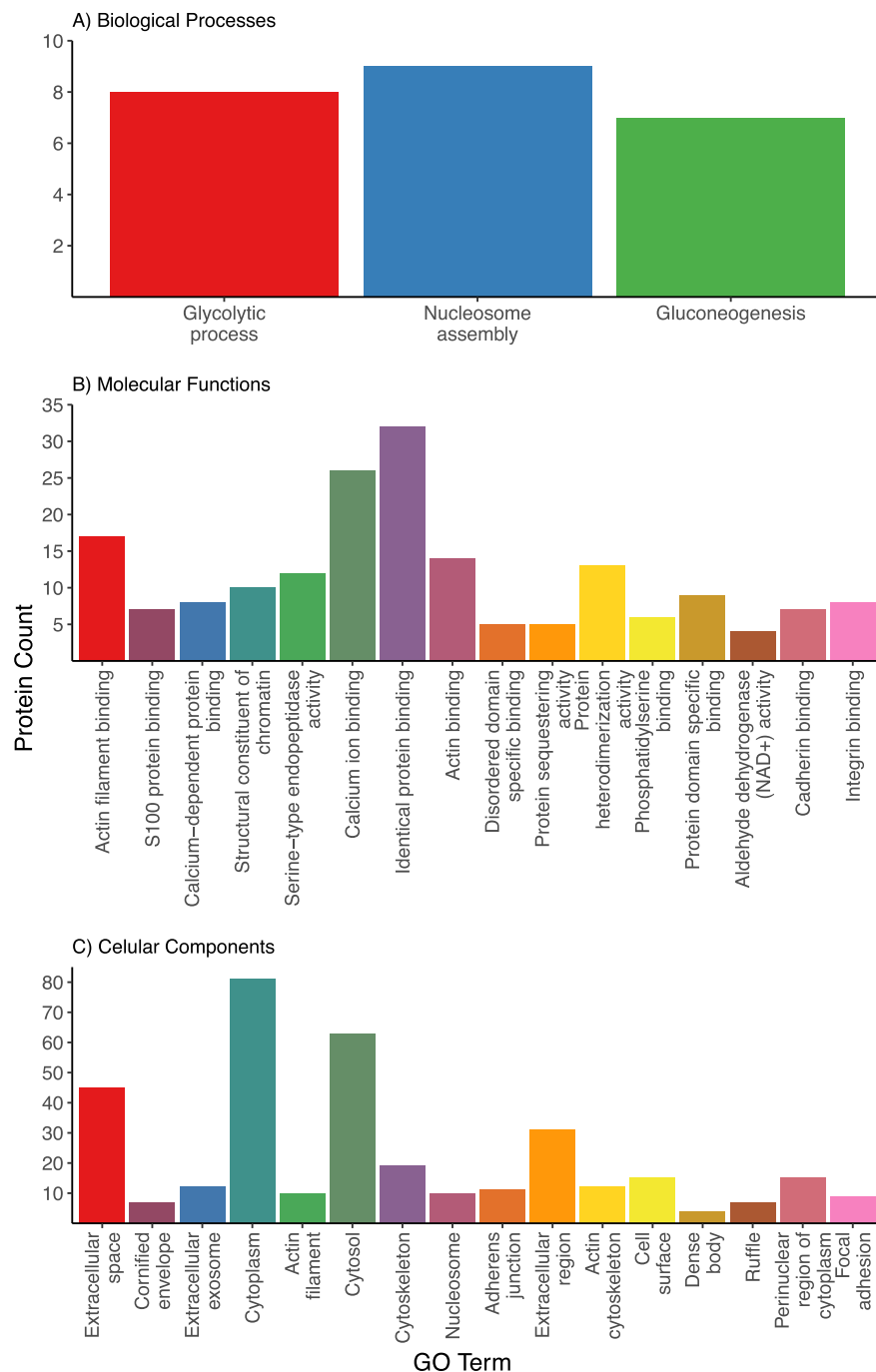
## 4 | Discussion

Here, we characterised the proteome of a unique reproductive foam produced by male Japanese quail and tested how it is influenced by developmental stress. We identified a total of 224 proteins in the foam, of which over 96% have orthologs in the closely related chicken, and at least 48% have been identified in chicken seminal fluid. Our interrogation of the foam proteome suggests roles in sperm maturation and DNA protection, semen liquefaction, sperm plasma membrane homeostasis and energy production for sperm motility.

We found that both pre- and postnatal developmental stress can affect reproductive traits in adult male Japanese quail by altering the protein composition of seminal foam. First, using lines selected for divergent maternal investment (measured as egg size relative to female body size), we found that males from the low maternal investment lines, which developed in relatively small, less-provisioned eggs, exhibited increased abundance of 48 proteins in their seminal foam. These proteins were primarily associated with lipid metabolic processes. Second, to measure an alternative axis of early developmental stress, we compared males that received different quality diets post-hatching and found that males that received a protein-restricted rearing diet exhibited increased abundance of 72 proteins. These proteins were primarily associated with nucleosome assembly and chromatin structure. Notably, nine proteins were consistently more abundant in foam samples from both the low maternal investment line and the protein-restricted males. These proteins were primarily associated with metabolic pathways, suggesting a convergent molecular response to early developmental stress across pre- and post-hatching stages.

### 4.1 | Prenatal Developmental Stress and Impaired Reproductive Function

Several proteins that were more abundant in foam of males from the low maternal investment lines (Experiment 1) suggest physiological dysregulation associated with oxidative stress,



**FIGURE 6** | GO analysis of the Japanese quail (*Coturnix japonica*) seminal foam proteome: (A) 7 biological processes, (B) 18 molecular functions and (C) 15 cellular components significantly enriched in the Japanese quail foam proteome, as identified by DAVID. Only functional enrichment groups with Benjamini-Hochberg corrected  $p$ -values  $< 0.01$  and passing a 1% false discovery rate threshold are shown.

inflammation and impaired reproductive function. The low maternal investment lines used in this study have previously been shown to have lower fertilisation success compared to males of the high maternal investment lines (Pick et al. 2017), however, few differences in sperm form or function have been detected between the lines (Mason et al. 2024). Differences in foam composition may therefore go some way to explaining their different fertilising potential between investment lines.

Several proteins identified in the foam were associated with lipid metabolic processes, indicating that lipid regulation may be

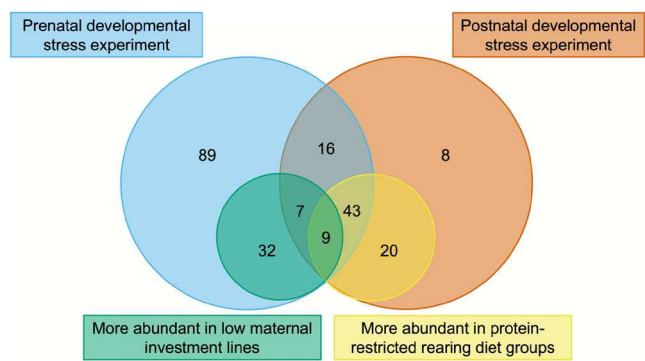
an important component of seminal fluid composition in males with reduced fertilisation success. Lipid-modifying enzymes and lipid binding proteins, including pancreatic lipase-related proteins, can influence membrane stability, energy availability and the biochemical conditions encountered by sperm (Sias et al. 2005; Furse et al. 2022; Tao et al. 2023). Differences in the lipid metabolic activity between males from high and low maternal investment lines may reflect altered physiological pathways associated with reduced fertilisation success. Abnormal lipid homeostasis can cause spermatogenic dysfunction and consequential infertility (Davis 1980; Lu et al. 2016; Thankamoni

et al. 2024), with elevated lipid levels further promoting reactive oxygen species production (Agarwal et al. 2014). In low maternal investment line males, the increased abundance of lipid metabolic enzymes may impair sperm function during fertilisation. These differences in lipid metabolic activity provide useful context for interpreting other molecular differences between selection lines, including those linked to oxidative stress.

Several proteins that were more abundant in foam from low maternal investment line males are biomarkers of oxidative stress. For example, fructose-bisphosphate aldolase is an enzyme that catalyses a key step in glycolysis (Bhagavan and Ha 2015) and is overexpressed in human seminal ejaculates with high levels of oxidative stress (Sharma et al. 2013). Additionally, alpha-enolase is used as a biomarker of reduced semen quality in several species including humans (Force et al. 2002; He et al. 2014) and mallards (*Anas platyrhynchos*) (Tang et al. 2022), and increased expression may over-activate the apoptosis signal pathway, resulting

in disturbances during spermatogenesis (Xiong et al. 2022). These enzymes, when present in high concentrations, could signal increased oxidative stress and impaired sperm maturation in adult males that experienced prenatal developmental stress, with potential consequences for fertilisation. While oxidative stress in females does not differ between the lines (measured as reactive oxygen species production in blood plasma) (Pick, Hutter, Ebner, et al. 2016), females from the low maternal investment lines (but not high maternal investment lines) exhibited a marked increase in oxidative stress under breeding conditions (Pick, Hutter, Ebner, et al. 2016). It is therefore possible that both males and females from the low maternal investment lines are susceptible to oxidative stress, and this may be a consequence of prenatal developmental stress or a correlated response to relaxed selection on maternal investment.

Increased abundance of avidin and interleukin receptors in the foam from low maternal investment line males provides evidence of inflammation in reproductive tissue. Interleukins are cytokines involved in immune cell regulation and inflammation modulation (Verkhatskii 2023). White blood cells, which produce interleukins, are often in higher abundance in semen of humans with genital tract inflammation and fertility issues (Barratt et al. 1990; Sharma et al. 2013). Interleukins are activated by binding to interleukin receptors (Martin and Falk 1997) and interleukin receptor accessory proteins (Martin and Falk 1997; Casadio et al. 2001). Specifically, interleukin 1 receptor accessory protein (IL1RAP) induces multiple physiological responses to inflammation, infection and tissue damage (Dinarello 1996). Additionally, avidin-related proteins are known to increase in response to infection, tissue injury and inflammatory stress (Board and Fuller 1974; Kunas et al. 1993) and bind to biotin in bacteria to prevent bacterial growth as part of the immune defence (Dillon 2014). Avidin is linked to promoting sperm activation and longevity in the female reproductive tract of turkeys (*Meleagris gallopavo domesticus*) (Foye-Jackson et al. 2011) as well as protecting developing embryos



**FIGURE 7** | The number of proteins differentially expressed between the prenatal and postnatal developmental stress experiments, and proteins that were significantly more abundant within treatments in both experiments. The number of the same overlapping proteins between experiments and treatments are shown.

**TABLE 1** | Quail proteins and their chicken orthologs that were significantly more abundant in males exposed to stressful pre- and postnatal developmental conditions, along with their annotations from the Functional Annotation Chart in DAVID (Huang et al. 2009; Sherman et al. 2022).

Quail protein Uniprot AC	Chicken ortholog Uniprot AC	Chicken ortholog protein name	Chicken ortholog gene symbol	Functional annotation
A0A8C2T207	A0A8V0Z6F8	Mesothelin-like protein	MSLNL	
A0A8C2U7G7	A0A8V0YKA1	Aldo-keto reductase family 1 member B10	AKR1B10	Metabolic pathway, cytosol
A0A8C2SWD0	A0A1D5PFB8	Dynactin subunit 2	DCTN2	Cytosol
A0A8C2UFC7	Q98942	Alpha-amylase	amy	Metabolic pathway
A0A8C2TLF1	F1P1A5	Transketolase	TKTL1	Metabolic pathway, cytosol
A0A8C2TH69	E1BVT3	Malate dehydrogenase	MDH2	Metabolic pathway
A0A8C2TV59	A0A1D5PE96	Plastin-3	PLS3	Cytosol
A0A8C2T9A2	F1NJC7	Aldehyde dehydrogenase 1 family member A1	ALDH1A1	Metabolic pathway, cytosol
A0A8C2SZ10	A0A1D5PN54	ATP synthase subunit alpha	ATP5F1AZ	Metabolic pathway

against microbial infections in the female oviduct (Korenman and O'Malley 1968; Board and Fuller 1974). The increased abundance of the immune proteins suggests that males that undergo prenatal developmental stress may face inflammation as adults.

Males from the low maternal investment line exhibited increased abundance of proteases, including higher levels of trypsin and pancreatic elastase II, without corresponding increased abundance of protease inhibitors. Numerous proteases found in seminal fluid across taxa regulate multiple downstream activities involved in immunity, cell cycle regulation and tissue morphogenesis via the hydrolysis of peptide bonds (Ram and Wolfner 2009; Laflamme et al. 2014). However, these proteases require tight control by protease inhibitors to prevent premature activation of pathways or tissue damage (Laflamme and Wolfner 2013). In human seminal fluid, pancreatic elastase II plays a role in elastin hydrolysis, and increased elastase levels are associated with increased white blood cells, bacterial infection and inflammation in infertile males (Zorn et al. 2003). Whilst increased elastase levels are negatively correlated with semen volume, they have limited effects on sperm characteristics. Instead, elastase may damage the female reproductive tract, preventing fertilisation (Zorn et al. 2000). Furthermore, trypsin-like proteases have diverse roles, including induction of egg-laying in insects (Marshall et al. 2009) and mediation of sperm function and fertility across taxa (Green and Summers 1980; Inaba et al. 1993; Friedländer et al. 2001; Kodama et al. 2002; Miyata et al. 2012; Stephens et al. 2018). In mammals, increased trypsin concentrations lead to more rapid liquefaction of viscous semen and increased motility, which is a possible function of the quail foam (Singh et al. 2011), but at too high a concentration can lead to sperm fragmentation and compromised sperm function (Marson et al. 1988; Flores-Herrera et al. 2012). The elevated abundance of these proteases, without corresponding regulatory proteins, supports the hypothesis that low maternal investment line males are susceptible to inflammation and impaired reproductive function, either through sperm fragmentation or by altering the environment of the female reproductive tract.

Given the limited evidence for reproductive costs of oxidative stress in birds (Speakman and Garratt 2014; Beccardi, Salmón, and Vedder 2025), defining reliable markers of oxidative stress and inflammation is essential for clarifying how early developmental stress influences adult male ejaculate quality. Individuals that experience prenatal developmental stress may exhibit hidden costs in their ejaculate composition, with metabolic and immune protein expression reflecting stress responses rather than enhanced functional investment. Increased SFP abundance may indicate constrained or dysregulated reproductive physiology, consistent with the reduced fertilisation success reported for the low maternal investment selection lines in this system (Pick et al. 2017). Previous work demonstrated that high investment line males caused greater oxidative damage in females following mating (Romero-Haro et al. 2023), highlighting that male oxidative condition and female post mating oxidative costs are decoupled.

## 4.2 | Postnatal Dietary Protein Restriction and Its Impact on Seminal Fluid Function

Similar to males that experienced pre-natal developmental stress, our proteomic analyses identified several proteins that

were more abundant in males raised on a post-hatching protein-restricted diet (Experiment 2), which may indicate oxidative stress and reduced semen quality. For example, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) abundance increases in response to heat shock and oxidative stress in human sperm (Sharma et al. 2013) and is higher in abundance in chicken (*G. gallus domesticus*) semen with reduced sperm motility (Li et al. 2020). Additionally, triosephosphate isomerase is involved in the sperm acrosome reaction and binding of sperm to the egg's zona-pellucida (Auer et al. 2004), and its increased abundance indicates reduced sperm quality in boar (*Sus domesticus*) and human seminal fluid (Siva et al. 2010; Vilagran et al. 2016), and reduced fertility in bulls (*Bos taurus*) (Soggiu et al. 2013).

Males that were protein-restricted during development showed an increased abundance of histones involved in protein heterodimerisation activity and chromatin organisation in the nucleus, potentially indicating inflammation (Singh et al. 2025). Elevated extracellular histone levels in biofluids are also associated with inflammation, infection and tissue damage (Singh et al. 2022, 2025). Specifically, histone H4 can affect sperm morphology and motility (Schon et al. 2019) and impaired histone-protamine exchange during spermatogenesis can reduce fertilisation (Carrell et al. 2007). The function of extra-nuclear histones, particularly in seminal fluids, and their effect on reproduction is an ongoing area of research. Additionally, immunoglobulin lambda-like polypeptide 1 (IGLL1) is a type of immunoglobulin free light chain which has been associated with inflammation and reduced sperm quality in humans (Basile et al. 2022; Bruno et al. 2022). In other biofluids, lambda light chains are responsible for activating inflammatory responses in autoimmune disorders (Napodano et al. 2019). Our data provide the first evidence that IGLL1 originates from a reproductive accessory gland and is modulated by diet early in life. Overall, this provides evidence that post-natal developmental stress can have long term effects on reproductive accessory gland inflammation status in adults, with potential negative consequences for sperm quality.

The increased abundance of glycolytic enzymes (such as pyruvate kinase and malate dehydrogenase), S100 calcium ion binding proteins and cytoskeletal proteins (such as tropomyosin, myosin, actin, spectrin and desmoplakin) in the foam of males raised on a protein-restricted diet may reflect compensatory mechanisms to preserve reproductive function (Donato et al. 2013). In birds, glycolysis is an important energy-generating pathway for sperm motility (Froman and Kirby 2005). Protein-restricted males may rely more greatly on glycolysis to compensate for impaired sperm mitochondrial function (Ford 2006). Furthermore, S100 proteins are known to regulate calcium-dependent cellular processes including energy metabolism, cytoskeletal organisation and cell survival, and may serve a protective role by supporting sperm function in the female reproductive tract (Donato 2001; Sakaguchi et al. 2008; Leśniak et al. 2009; Donato et al. 2009). S100 proteins may also promote immune tolerance in the female reproductive tract, preventing the female's immune system attacking foreign sperm cells (Sorci et al. 2011; Schjenken and Robertson 2020), but see Słomnicki et al. (2009). These findings suggest developmentally stressed males invest in the maintenance and survival of sperm, and in the literature, quail show preservation of sperm quality under dietary-protein restriction (Arcsott and Parker 1963; Retes et al. 2019; Tyler et al. 2021).



However, developmental stress may elicit physiological trade-offs, balancing immediate reproductive performance against potential long-term fitness costs. For example, in *Drosophila*, males have been shown to buffer ejaculates against adverse conditions by transferring higher abundances of SFPs and investing more in each mating opportunity, potentially as a response to fewer mating opportunities overall (von Hellfeld et al. 2025).

Dietary-protein availability is a major determinant of oxidative balance, as amino acids underpin the synthesis of antioxidants (Egbujor et al. 2024). In birds specifically, protein restriction during development can increase oxidative stress by disrupting the production and function of antioxidants, leading to an increase in reactive oxygen species (Faraguna et al. 2025). Whilst some reactive oxygen species are required for reproduction, an imbalance between reactive oxygen species and the capacity of antioxidant mechanisms results in oxidative stress (Costantini 2008). Oxidative stress may lead to alterations in protein expression in sperm and seminal fluid, accelerating functional decline by promoting lipid membrane peroxidation, alkylation of proteins associated with mitochondrial function and flagellar movement, and loss of motility and membrane integrity (Aitken et al. 2012; Moazamian et al. 2015). Damage to sperm morphology and DNA integrity impairs fertilisation (Azenabor et al. 2015; Dada 2017; Agarwal et al. 2018; Barati et al. 2020; Candela et al. 2021). Rather than reflecting increased reproductive investment, an increased abundance of metabolic enzymes and oxidative stress biomarkers in protein-restricted males may represent compensatory mechanisms that sustain sperm performance under oxidative stress. We show that protein intake during early-life development can have long-term effects on the proteome with potential consequences for oxidative balance.

### 4.3 | Metabolic Adaptations to Early Developmental Stress

Adult males exposed to early developmental stress, either pre- or postnatally, exhibited increased abundance of seminal foam proteins associated with metabolic pathways, particularly glycolysis, suggesting a conserved response. Malate dehydrogenase and alpha-amylase, both involved in glycolysis, may support energy production and thereby sperm function. In mammalian seminal fluid, alpha-amylase reduces sperm viscosity (Bunge and Sherman 1954; Mendeluk et al. 2000). In red junglefowl (*G. gallus*) seminal fluid, alpha-amylase is present at low abundance and does not contribute to sperm performance (Borziak et al. 2016), but it may play a more important role in quail semen (Buxton and Orcutt 1975). ATP synthase may also contribute to energy homeostasis by producing extracellular ATP (Guo et al. 2019). Since both treatment groups also exhibited biomarkers of oxidative stress, it is possible developmental stress can have generalised long-term physiological consequences on male fertility, even when no longer exposed to stress as an adult (Breitbart et al. 2005; Xiao and Yang 2007), and the increased abundance of glycolytic enzymes may be an adaptive response to maintain sperm function and fertility. Alternatively, increased metabolic activity could contribute to oxidative stress, suggesting a trade-off between compensatory metabolism and redox imbalance (Speakman and Garratt 2014).

Several proteins that were more abundant in developmentally stressed males are involved in oxidative stress responses and may mitigate sperm against oxidative damage. Transketolase contributes to the production of NADPH which balances reactive oxygen species and maintains mitochondrial membrane potential (Perl et al. 2006) and is critical for ATP synthesis and sperm cell survival. Impaired function of transketolase may lead to oxidative damage and male infertility (Li et al. 2013; Perl 2007). Aldo-keto reductase B10 (AKR1B10) and aldehyde dehydrogenase A1 detoxify reactive carbonyl and aldehyde compounds that are produced under oxidative stress (Wang et al. 2009; Shortall et al. 2021). Whilst AKR enzymes are found in male mammalian reproductive tissue (Kobayashi et al. 2002; Iuchi et al. 2004), the physiological function of other AKR enzymes other than AKR1B1 remains poorly characterised. Detoxification of reactive oxygen species may help preserve fertility under developmental stress (Gibb et al. 2016), consistent with adaptive increases in antioxidant activity seen across taxa during oxidative stress (Faraguna et al. 2025). Future research into trade-offs between reproductive investment and other physiological functions, such as immune regulation (Knowles et al. 2009) and brain size (Kotrschal et al. 2013), in developmentally stressed males will be a fruitful area for further investigation.

Differences in the number of proteins identified between Experiment 1 and Experiment 2, as well as the relatively low overlap in proteins detected (33%), may be due to sampling limitations. Pooled samples were used to ensure sufficient material for MS analysis; however, pooling limits the ability to detect proteins that vary among individuals. In Experiment 2, some pooled replicates contained more males, increasing the risk that low-abundance peptides were diluted below the MS detection threshold. We reduced potential individual-level bias by using only 1 sample per male per replicate, ensuring no individuals disproportionately influenced the proteomic profile. This limits treatment comparisons to the level of the pooled sample rather than the individual, and observed differences in proteomic profiles should be interpreted as population-level trends rather than individual-level effects. In addition, males differed in age between the two experiments, which may also contribute to the small proportion of overlapping proteins. Older males may produce different relative abundances of specific proteins. Such biological variation as well as the constraints of pooled sampling likely reduced the consistency of protein detection across experiments.

## 5 | Conclusion

In conclusion, we provide the first experimental evidence that pre- and postnatal developmental stress alters the proteome of a unique seminal foam produced by male Japanese quail, using an approach that is portable for work on seminal fluids across vertebrates. Our findings show that different types of early developmental stress exert a similar influence on the molecular composition of this seminal fluid, with potential long-term impacts on ejaculate function and fertility. Contrary to our original hypothesis, developmentally stressed males do not reduce investment in SFP production, but instead we find evidence of oxidative stress and inflammation in their reproductive tissue

with potential long-term consequences for sperm function, future matings and other physiological functions. The elevated abundance of metabolic proteins that support sperm function in developmentally stressed males may reflect adaptive plasticity to safeguard fertilisation despite physiological constraints imposed by early-life stress, potentially at the expense of future reproductive performance. Our findings provide new insights into mechanisms by which early-life environmental conditions can influence reproductive fluids and the consequences of this for fertility in adulthood. Furthermore, they highlight the importance of developmental plasticity in modulating reproductive investment and function, potentially as an adaptive response to mitigate oxidative and inflammatory damage.

## Author Contributions

C.M. conceptualised the study, designed the methods, collected data, analysed the data, created the figures and wrote the manuscript; M.G. designed the methods and reviewed the manuscript; O.V. designed the methods, collected samples/data, and reviewed the manuscript; T.K.P. designed the methods, collected the data, analysed the data and reviewed the manuscript; R.G. contributed to the collection of data and reviewed the manuscript; B.T. designed the methods, collected samples and reviewed the manuscript; N.H. conceptualised the study, designed the methods and reviewed the manuscript.

## Acknowledgements

C.M. was funded by a NERC ACCE studentship (NE/S00713X/I), O.V. was funded by the German Research Foundation (DFG, Deutsche Forschungsgemeinschaft, 428800869), B.T. was funded by the Swiss National Science Foundation (PP00P3 128386 and PP00P3 157455) and N.H. was funded by a Royal Society Dorothy Hodgkin Fellowship (DHF160200). Mass spectrometry analyses were performed by the biOMICS Facility at the University of Sheffield. We thank two anonymous reviewers for their constructive comments on the manuscript.

## Funding

This work was supported by the Natural Environment Research Council (NE/S00713X/I), Deutsche Forschungsgemeinschaft (428800869), Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung (PP00P3 128386, PP00P3 157455), and Royal Society (DHF160200).

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al. 2025) partner repository with the dataset identifier PXD063034.

## References

Abuoghaba, A. A. K., M. G. Abdelfattah, M. Abdelhamid Mohamed Sayed, and M. Hosny. 2024. "The Relationship of the Male's Proctodeal Gland Size to Sperm-Egg Interaction and the Duration of Fertility in Japanese Quail." *Poultry Science* 103, no. 8: 103809. <https://doi.org/10.1016/j.psj.2024.103809>.

Adkins-Regan, E. 1995. "Predictors of Fertilization in the Japanese Quail, *Coturnix Japonica*." *Animal Behaviour* 50, no. 5: 1405–1415. [https://doi.org/10.1016/0003-3472\(95\)80055-7](https://doi.org/10.1016/0003-3472(95)80055-7).

Agarwal, A., M. Rana, E. Qiu, H. Alburni, A. D. Bui, and R. Henkel. 2018. "Role of Oxidative Stress, Infection and Inflammation in Male Infertility." *Andrologia* 50, no. 11: e13126. <https://doi.org/10.1111/and.13126>.

Agarwal, A., G. Virk, C. Ong, and S. S. du Plessis. 2014. "Effect of Oxidative Stress on Male Reproduction." *World Journal of Men's Health* 32, no. 1: 1–17. <https://doi.org/10.5534/wjmh.2014.32.1.1>.

Aitken, R. J., S. Whiting, G. N. De Iuliis, S. McClymont, L. A. Mitchell, and M. A. Baker. 2012. "Electrophilic Aldehydes Generated by Sperm Metabolism Activate Mitochondrial Reactive Oxygen Species Generation and Apoptosis by Targeting Succinate Dehydrogenase." *Journal of Biological Chemistry* 287, no. 39: 33048–33060. <https://doi.org/10.1074/jbc.M112.366690>.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press.

Arscott, G. H., and J. E. Parker. 1963. "Dietary Protein and Fertility of Male Chickens." *Journal of Nutrition* 80, no. 3: 311–314. <https://doi.org/10.1093/jn/80.3.311>.

Atikuzzaman, M., L. Sanz, D. Pla, et al. 2017. "Selection for Higher Fertility Reflects in the Seminal Fluid Proteome of Modern Domestic Chicken." *Comparative Biochemistry and Physiology. Part D, Genomics & Proteomics* 21: 27–40. <https://doi.org/10.1016/j.cbd.2016.10.006>.

Auer, J., L. Camoin, A. M. Courtot, F. Hotellier, and M. De Almeida. 2004. "Evidence That P36, a Human Sperm Acrosomal Antigen Involved in the Fertilization Process Is Triosephosphate Isomerase." *Molecular Reproduction and Development* 68, no. 4: 515–523. <https://doi.org/10.1002/mrd.20107>.

Avila, F. W., L. K. Sirot, B. A. Laflamme, C. D. Rubinstein, and M. F. Wolfner. 2011. "Insect Seminal Fluid Proteins: Identification and Function." *Annual Review of Entomology* 56, no. 1: 21–40. <https://doi.org/10.1146/annurev-ento-120709-144823>.

Azenabor, A., A. O. Ekun, and O. Akinloye. 2015. "Impact of Inflammation on Male Reproductive Tract." *Journal of Reproduction & Infertility* 16, no. 3: 123–129.

Barati, E., H. Nikzad, and M. Karimian. 2020. "Oxidative Stress and Male Infertility: Current Knowledge of Pathophysiology and Role of Antioxidant Therapy in Disease Management." *Cellular and Molecular Life Sciences* 77, no. 1: 93–113. <https://doi.org/10.1007/s00018-019-03253-8>.

Barratt, C. L. R., A. E. Bolton, and I. D. Cooke. 1990. "Functional Significance of White Blood Cells in the Male and Female Reproductive Tract." *Human Reproduction* 5, no. 6: 639–648. <https://doi.org/10.1093/oxfordjournals.humrep.a137162>.

Basile, U., E. Vergani, C. Napodano, et al. 2022. "Evaluation of Free Light Chains of Immunoglobulins in Normal and Pathological Seminal Fluids: Preliminary Data." *Andrologia* 54, no. 2: e14317. <https://doi.org/10.1111/and.14317>.

Bath, E., S. Bowden, C. Peters, et al. 2017. "Sperm and Sex Peptide Stimulate Aggression in Female *Drosophila*." *Nature Ecology & Evolution* 1, no. 6: 0154. <https://doi.org/10.1038/s41559-017-0154>.

Beccardi, M., P. Salmón, and O. Vedder. 2025. "Experimental Evidence for Reproduction to Increase Mitochondrial Respiration, but Decrease Mitochondrial Efficiency, in Females of a Short-Lived Bird." *bioRxiv*. <https://doi.org/10.1101/2025.05.12.653466>.

Beccardi, M., B. Tschirren, and O. Vedder. 2025. "Male Inbreeding Reduces Fertility, but Does Not Accelerate In Vivo Sperm Senescence: An Experimental Approach in a Polyandrous Bird." *Biology Letters* 21, no. 4: 20250083. <https://doi.org/10.1098/rsbl.2025.0083>.

Bhagavan, N. V., and C. E. Ha. 2015. "Carbohydrate Metabolism I." In *Essentials of Medical Biochemistry*, 165–185. Academic Press. <https://doi.org/10.1016/B978-0-12-416687-5.00012-9>.

Birkhead, T. R. 2010. "How Stupid Not to Have Thought of That: Post-Copulatory Sexual Selection." *Journal of Zoology* 281, no. 2: 78–93. <https://doi.org/10.1111/j.1469-7998.2010.00701.x>.

- Birkhead, T. R., and F. Fletcher. 1994. "Sperm Storage and the Release of Sperm From the Sperm Storage Tubules in Japanese Quail *Coturnix Japonica*." *Ibis* 136, no. 1: 101–105. <https://doi.org/10.1111/j.1474-919X.1994.tb08137.x>.
- Board, R. G., and R. Fuller. 1974. "Non-Specific Antimicrobial Defences of the Avian Egg, Embryo and Neonate." *Biological Reviews* 49, no. 1: 15–49. <https://doi.org/10.5555/19742281359>.
- Borziak, K., A. Álvarez-Fernández, T. L. Karr, T. Pizzari, and S. Dorus. 2016. "The Seminal Fluid Proteome of the Polyandrous Red Junglefowl Offers Insights Into the Molecular Basis of Fertility, Reproductive Ageing and Domestication." *Scientific Reports* 6, no. 1: 35864. <https://doi.org/10.1038/srep35864>.
- Breitbart, H., G. Cohen, and S. Rubinstein. 2005. "Role of Actin Cytoskeleton in Mammalian Sperm Capacitation and the Acrosome Reaction." *Reproduction* 129, no. 3: 263–268. <https://doi.org/10.1530/rep.1.00269>.
- Bruno, C., U. Basile, E. Vergani, et al. 2022. "Inflammation and Oxidative Stress in Seminal Plasma: Search for Biomarkers in Diagnostic Approach to Male Infertility." *Journal of Personalized Medicine* 12, no. 6: 857. <https://doi.org/10.3390/jpm12060857>.
- Bunge, R. G., and J. K. Sherman. 1954. "Liquefaction of Human Semen by Alpha-Amylase." *Fertility and Sterility* 5, no. 4: 353–356. [https://doi.org/10.1016/S0015-0282\(16\)31684-3](https://doi.org/10.1016/S0015-0282(16)31684-3).
- Buxton, J. R., and F. S. Orcutt. 1975. "Enzymes and Electrolytes in the Semen of Japanese Quail." *Poultry Science* 54, no. 5: 1556–1566. <https://doi.org/10.3382/ps.0541556>.
- Candela, L., L. Boeri, P. Capogrosso, et al. 2021. "Correlation Among Isolated Teratozoospermia, Sperm DNA Fragmentation and Markers of Systemic Inflammation in Primary Infertile Men." *PLoS One* 16, no. 6: e0251608. <https://doi.org/10.1371/journal.pone.0251608>.
- Carrell, D. T., B. R. Emery, and S. Hammoud. 2007. "Altered Protamine Expression and Diminished Spermatogenesis: What Is the Link?" *Human Reproduction Update* 13, no. 3: 313–327. <https://doi.org/10.1093/humupd/dml057>.
- Casadio, R., E. Frigimelica, P. Bossù, et al. 2001. "Model of Interaction of the IL-1 Receptor Accessory Protein IL-1RAcP With the IL-1 $\beta$ /IL-1R(I) Complex." *FEBS Letters* 499, no. 1–2: 65–68. [https://doi.org/10.1016/S0014-5793\(01\)02515-7](https://doi.org/10.1016/S0014-5793(01)02515-7).
- Chapman, T. 2001. "Seminal Fluid-Mediated Fitness Traits in *Drosophila*." *Heredity* 87, no. 5: 511–521. <https://doi.org/10.1046/j.1365-2540.2001.00961.x>.
- Chapman, T., J. Bangham, G. Vinti, et al. 2003. "The Sex Peptide of *Drosophila melanogaster*: Female Post-Mating Responses Analyzed by Using RNA Interference." *Proceedings of the National Academy of Sciences* 100, no. 17: 9923–9928. <https://doi.org/10.1073/pnas.1631635100>.
- Cheng, K. M., A. R. Hickman, and C. R. Nichols. 1989. "Role of the Proctodeal Gland Foam of Male Japanese Quail in Natural Copulations." *Auk* 106, no. 2: 279–285. <https://doi.org/10.1093/auk/106.2.279>.
- Clark, M. M., and B. G. Galef. 1995. "Prenatal Influences on Reproductive Life History Strategies." *Trends in Ecology & Evolution* 10, no. 4: 151–153. [https://doi.org/10.1016/S0169-5347\(00\)89025-4](https://doi.org/10.1016/S0169-5347(00)89025-4).
- Clulow, J., and R. C. Jones. 1982. "Production, Transport, Maturation, Storage and Survival of Spermatozoa in the Male Japanese Quail, *Coturnix coturnix*." *Reproduction* 64, no. 2: 259–266. <https://doi.org/10.1530/jrf.0.0640259>.
- Costantini, D. 2008. "Oxidative Stress in Ecology and Evolution: Lessons From Avian Studies." *Ecology Letters* 11, no. 11: 1238–1251. <https://doi.org/10.1111/j.1461-0248.2008.01246.x>.
- Cox, J., M. Y. Hein, C. A. Lubner, I. Paron, N. Nagaraj, and M. Mann. 2014. "Accurate Proteome-Wide Label-Free Quantification by Delayed Normalization and Maximal Peptide Ratio Extraction, Termed MaxLFQ." *Molecular & Cellular Proteomics* 13: 2513–2526.
- Cox, J., and M. Mann. 2008. "MaxQuant Enables High Peptide Identification Rates, Individualized p.p.b.-Range Mass Accuracies and Proteome-Wide Protein Quantification." *Nature Biotechnology* 26: 1367–1372. <https://doi.org/10.1038/nbt.1511>.
- Dada, R. 2017. "Oxidative Stress Major Executioner in Disease Pathology Role in Sperm DNA Damage and Preventive Strategies." *Frontiers in Bioscience* 9, no. 3: 420–447. <https://doi.org/10.2741/s495>.
- Davis, B. K. 1980. "Interaction of Lipids With the Plasma Membrane of Sperm Cells. I. The Antifertilization Action of Cholesterol." *Archives of Andrology* 5, no. 3: 249–254. <https://doi.org/10.3109/01485018008986993>.
- De Nardo, A. N., J. Roy, S. H. Sbilordo, and S. Lüpold. 2021. "Condition-Dependent Interaction Between Mating Success and Competitive Fertilization Success in *Drosophila melanogaster*." *Evolution* 75, no. 8: 2014–2026. <https://doi.org/10.1111/evo.14228>.
- den Boer, S. P. A., J. J. Boomsma, and B. Baer. 2009. "Honey Bee Males and Queens Use Glandular Secretions to Enhance Sperm Viability Before and After Storage." *Journal of Insect Physiology* 55, no. 6: 538–543. <https://doi.org/10.1016/j.jinsphys.2009.01.012>.
- den Boer, S. P. A., J. J. Boomsma, B. Baer, S. P. A. den Boer, J. J. Boomsma, and B. Baer. 2008. "Seminal Fluid Enhances Sperm Viability in the Leafcutter Ant *Atta Colombica*." *Behavioral Ecology and Sociobiology* 62, no. 12: 1843–1849. <https://doi.org/10.1007/s00265-008-0613-5>.
- Desai, M., and C. N. Hales. 1997. "Role of Fetal and Infant Growth in Programming Metabolism in Later Life." *Biological Reviews of the Cambridge Philosophical Society* 72, no. 2: 329–348. <https://doi.org/10.1017/S0006323196005026>.
- Dillon, V. M. 2014. "Natural Anti-Microbial Systems. Preservative Effects During Storage." In *Encyclopedia of Food Microbiology*, edited by C. A. Batt and M. L. Tortorello, Second ed., 941–947. Academic Press. <https://doi.org/10.1016/B978-0-12-384730-0.00238-X>.
- Dinarello, C. 1996. "Biologic Basis for Interleukin-1 in Disease." *Blood* 87, no. 6: 2095–2147. <https://doi.org/10.1182/blood.v87.6.2095.bloodjournal8762095>.
- Donato, R. 2001. "S100: A Multigenic Family of Calcium-Modulated Proteins of the EF-Hand Type With Intracellular and Extracellular Functional Roles." *International Journal of Biochemistry & Cell Biology* 33, no. 7: 637–668. [https://doi.org/10.1016/S1357-2725\(01\)00046-2](https://doi.org/10.1016/S1357-2725(01)00046-2).
- Donato, R., B. R. Cannon, G. Sorci, et al. 2013. "Functions of S100 Proteins." *Current Molecular Medicine* 13, no. 1: 24–57. <https://doi.org/10.2174/156652413804486214>.
- Donato, R., G. Sorci, F. Riuizi, et al. 2009. "S100B's Double Life: Intracellular Regulator and Extracellular Signal." *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research* 1793, no. 6: 1008–1022. <https://doi.org/10.1016/j.bbamcr.2008.11.009>.
- Dorus, S., S. Skerget, and T. L. Karr. 2012. "Proteomic Discovery of Diverse Immunity Molecules in Mammalian Spermatozoa." *Systems Biology in Reproductive Medicine* 58, no. 4: 218–228. <https://doi.org/10.3109/19396368.2012.700442>.
- Douard, V., A. Gassama-Diagne, D. Hermier, and E. Blesbois. 2004. "Activity of Phospholipases A and Lysophospholipase in Turkey Semen and Oviducal Fluid." *Poultry Science* 83, no. 8: 1385–1393. <https://doi.org/10.1093/ps/83.8.1385>.
- Edwards, K. L., A. N. Edes, and J. L. Brown. 2019. "Stress, Well-Being and Reproductive Success." *Advances in Experimental Medicine and Biology* 1200: 91–162.
- Egbujor, M. C., O. T. Olaniyan, C. N. Emeruwa, S. Saha, L. Saso, and P. Tucci. 2024. "An Insight Into Role of Amino Acids as Antioxidants via NRF2 Activation." *Amino Acids* 56, no. 1: 23. <https://doi.org/10.1007/s00726-024-03384-8>.



- Emms, D. M., and S. Kelly. 2019. "OrthoFinder: Phylogenetic Orthology Inference for Comparative Genomics." *Genome Biology* 20, no. 1: 238. <https://doi.org/10.1186/s13059-019-1832-y>.
- Faraguna, S., S. Milinković Tur, S. Sobočanec, M. Pinterić, and M. Belić. 2025. "Assessment of Oxidative Stress and Associated Biomarkers in Wild Avian Species." *Animals* 15, no. 9: 1203. <https://doi.org/10.3390/ani15091203>.
- Farooq, U., S. Cho, P. K. Rybnik-Trzaskowska, R. P. Singh, and I. A. Malecki. 2015. "Effect of Proctodeal Gland Foam on Sperm Kinetics in Japanese Quail (*Coturnix japonica*)."
- Theriogenology* 83, no. 2: 162–167. <https://doi.org/10.1016/j.theriogenology.2014.09.006>.
- Finseth, F. R., S. R. Iacovelli, R. G. Harrison, and E. K. Adkins-Regan. 2013. "A Nonsemen Copulatory Fluid Influences the Outcome of Sperm Competition in Japanese Quail." *Journal of Evolutionary Biology* 26, no. 9: 1875–1889. <https://doi.org/10.1111/jeb.12189>.
- Flores-Herrera, H., D. G. Acuña-Hernández, J. A. Rivera-Rebolledo, M. A. González-Jiménez, A. Z. Rodas-Martínez, and W. F. Swanson. 2012. "Effect of Increasing Trypsin Concentrations on Seminal Coagulum Dissolution and Sperm Parameters in Spider Monkeys (*Ateles geoffroyi*)."
- Theriogenology* 78, no. 3: 612–619. <https://doi.org/10.1016/j.theriogenology.2012.03.007>.
- Force, A., J. L. Viillard, G. Grizard, and D. Boucher. 2002. "Enolase Isoforms Activities in Spermatozoa From Men With Normospermia and Abnormospermia." *Journal of Andrology* 23, no. 2: 202–210. <https://doi.org/10.1002/j.1939-4640.2002.tb02616.x>.
- Ford, W. C. L. 2006. "Glycolysis and Sperm Motility: Does a Spoonful of Sugar Help the Flagellum Go Round?" *Human Reproduction Update* 12, no. 3: 269–274. <https://doi.org/10.1093/humupd/dmi053>.
- Foye-Jackson, O. T., J. A. Long, M. R. Bakst, et al. 2011. "Oviductal Expression of Avidin, Avidin-Related Protein-2, and Progesterone Receptor in Turkey Hens in Relation to Sperm Storage: Effects of Oviduct Tissue Type, Sperm Presence, and Turkey Line." *Poultry Science* 90, no. 7: 1539–1547. <https://doi.org/10.3382/ps.2010-01159>.
- Friedländer, M., A. Jeshtadi, and S. E. Reynolds. 2001. "The Structural Mechanism of Trypsin-Induced Intrinsic Motility in *Manduca sexta* Spermatozoa In Vitro." *Journal of Insect Physiology* 47, no. 3: 245–255. [https://doi.org/10.1016/S0022-1910\(00\)00109-8](https://doi.org/10.1016/S0022-1910(00)00109-8).
- Friesen, C. R., D. R. Powers, P. E. Copenhaver, and R. T. Mason. 2015. "Size Dependence in Non-Sperm Ejaculate Production Is Reflected in Daily Energy Expenditure and Resting Metabolic Rate." *Journal of Experimental Biology* 218, no. 9: 1410–1418. <https://doi.org/10.1242/jeb.120402>.
- Froman, D. P., and J. D. Kirby. 2005. "Sperm Mobility: Phenotype in Roosters (*Gallus domesticus*) Determined by Mitochondrial Function." *Biology of Reproduction* 72, no. 3: 562–567. <https://doi.org/10.1095/biolreprod.104.035113>.
- Fujihara, N. 1992. "Accessory Reproductive Fluids and Organs in Male Domestic Birds." *World's Poultry Science Journal* 48, no. 1: 39–56. <https://doi.org/10.1079/wps19920005>.
- Furse, S., L. C. Kusinski, A. Ray, et al. 2022. "Relative Abundance of Lipid Metabolites in Spermatozoa Across Three Compartments." *International Journal of Molecular Sciences* 23, no. 19: 11655. <https://doi.org/10.3390/ijms231911655>.
- García-Vargas, D., L. Juárez-Rojas, S. Rojas Maya, and S. Retana-Márquez. 2019. "Prenatal Stress Decreases Sperm Quality, Mature Follicles and Fertility in Rats." *Systems Biology in Reproductive Medicine* 65, no. 3: 223–235. <https://doi.org/10.1080/19396368.2019.1567870>.
- Garlovsky, M. D., C. Evans, M. A. Rosenow, T. L. Karr, and R. R. Snook. 2020. "Seminal Fluid Protein Divergence Among Populations Exhibiting Postmating Prezygotic Reproductive Isolation." *Molecular Ecology* 29, no. 22: 4428–4441. <https://doi.org/10.1111/mec.15636>.
- Gibb, Z., S. R. Lambourne, B. J. Curry, S. E. Hall, and R. J. Aitken. 2016. "Aldehyde Dehydrogenase Plays a Pivotal Role in the Maintenance of Stallion Sperm Motility." *Biology of Reproduction* 94, no. 6: 133. <https://doi.org/10.1095/biolreprod.116.140509>.
- Giordano, M., T. G. G. Groothuis, and B. Tschirren. 2014. "Interactions Between Prenatal Maternal Effects and Posthatching Conditions in a Wild Bird Population." *Behavioral Ecology* 25, no. 6: 1459–1466. <https://doi.org/10.1093/beheco/arv149>.
- Goenaga, J., T. Yamane, J. Rönn, and G. Arnqvist. 2015. "Within-Species Divergence in the Seminal Fluid Proteome and Its Effect on Male and Female Reproduction in a Beetle." *BMC Evolutionary Biology* 15, no. 1: 266. <https://doi.org/10.1186/s12862-015-0547-2>.
- Gorman, H. E., and R. G. Nager. 2004. "Prenatal Developmental Conditions Have Long-Term Effects on Offspring Fecundity." *Proceedings of the Royal Society B: Biological Sciences* 271, no. 1551: 1923–1928. <https://doi.org/10.1098/rspb.2004.2799>.
- Green, J. D., and R. G. Summers. 1980. "Ultrastructural Demonstration of Trypsin-Like Protease in Acrosomes of Sea Urchin Sperm." *Science* 209, no. 4454: 398–400. <https://doi.org/10.1126/science.6992277>.
- Groothuis, T. G. G., W. Müller, N. von Engelhardt, C. Carere, and C. Eising. 2005. "Maternal hormones as a tool to adjust offspring phenotype in avian species." *Neuroscience & Biobehavioral Reviews* 29, no. 2: 329–352. <https://doi.org/10.1016/j.neubiorev.2004.12.002>.
- Guo, H., Z. Chang, Z. Zhang, et al. 2019. "Extracellular ATPs Produced in Seminal Plasma Exosomes Regulate Boar Sperm Motility and Mitochondrial Metabolism." *Theriogenology* 139: 113–120. <https://doi.org/10.1016/j.theriogenology.2019.08.003>.
- He, Y., H. Li, J. He, and X. Zhao. 2014. "Heavy Ion Radiation Can Promote Greater Motility and Enolase Protein Expression in Ram Sperm in In Vitro Liquid Storage." *Animal Reproduction Science* 148, no. 3–4: 260–266. <https://doi.org/10.1016/j.anireprosci.2014.06.019>.
- Hopkins, B. R., I. Sepil, M.-L. Thézénas, et al. 2019. "Divergent Allocation of Sperm and the Seminal Proteome Along a Competition Gradient in *Drosophila melanogaster*." *Proceedings of the National Academy of Sciences* 116, no. 36: 17925–17933. <https://doi.org/10.1073/pnas.1906149116>.
- Hopkins, B. R., I. Sepil, and S. Wigby. 2017. "Seminal Fluid." *Current Biology* 27, no. 11: R404–R405. <https://doi.org/10.1016/j.cub.2017.03.063>.
- Huang, D. W., B. T. Sherman, and R. A. Lempicki. 2009. "Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources." *Nature Protocols* 4, no. 1: 44–57. <https://doi.org/10.1038/nprot.2008.211>.
- Inaba, K., Y. Akazome, and M. Morisawa. 1993. "Purification of Proteasomes From Salmonid Fish Sperm and Their Localization Along Sperm Flagella." *Journal of Cell Science* 104, no. 3: 907–915. <https://doi.org/10.1242/jcs.104.3.907>.
- Iuchi, Y., T. Kaneko, S. Matsuki, et al. 2004. "Carbonyl Stress and Detoxification Ability in the Male Genital Tract and Testis of Rats." *Histochemistry and Cell Biology* 121, no. 2: 123–130. <https://doi.org/10.1007/s00418-003-0607-3>.
- Jodar, M., A. Soler-Ventura, and R. Oliva. 2017. "Semen Proteomics and Male Infertility." *Journal of Proteomics* 162: 125–134.
- King, M., H. Eubel, A. H. Millar, and B. Baer. 2011. "Proteins Within the Seminal Fluid Are Crucial to Keep Sperm Viable in the Honeybee *Apis mellifera*." *Journal of Insect Physiology* 57, no. 3: 409–414. <https://doi.org/10.1016/j.jinsphys.2010.12.011>.
- Klemm, R. D., C. E. Knight, and S. Stein. 1973. "Gross and Microscopic Morphology of the Glandula Proctodealis (Foam Gland) of *Coturnix c. japonica* (Aves)." *Journal of Morphology* 141, no. 2: 171–184. <https://doi.org/10.1002/jmor.1051410205>.
- Knowles, S. C. L., S. Nakagawa, and B. C. Sheldon. 2009. "Elevated Reproductive Effort Increases Blood Parasitaemia and Decreases



- Immune Function in Birds: A Meta-Regression Approach." *Functional Ecology* 23, no. 2: 405–415. <https://doi.org/10.1111/j.1365-2435.2008.01507.x>.
- Kobayashi, T., T. Kaneko, Y. Iuchi, et al. 2002. "Localization and Physiological Implication of Aldose Reductase and Sorbitol Dehydrogenase in Reproductive Tracts and Spermatozoa of Male Rats." *Journal of Andrology* 23, no. 5: 674–684. <https://doi.org/10.1002/j.1939-4640.2002.tb02310.x>.
- Kodama, E., T. Baba, N. Kohno, S. Satoh, H. Yokosawa, and H. Sawada. 2002. "Spermosin, a Trypsin-Like Protease From Ascidian Sperm." *European Journal of Biochemistry* 269, no. 2: 657–663. <https://doi.org/10.1046/j.0014-2956.2001.02696.x>.
- Korenman, S. G., and B. W. O'Malley. 1968. "Progesterone Action: Regulation of Avidin Biosynthesis by Hen Oviduct In Vivo and In Vitro." *Endocrinology* 83, no. 1: 11–17. <https://doi.org/10.1210/endo-83-1-11>.
- Kotrschal, A., B. Rogell, A. Bundsen, et al. 2013. "Artificial Selection on Relative Brain Size in the Guppy Reveals Costs and Benefits of Evolving a Larger Brain." *Current Biology* 23, no. 2: 168–171. <https://doi.org/10.1016/j.cub.2012.11.058>.
- Kunnas, T. A., M. J. Wallén, and M. S. Kulomaa. 1993. "Induction of Chicken Avidin and Related mRNAs After Bacterial Infection." *Biochimica et Biophysica Acta* 1216, no. 3: 441–445. [https://doi.org/10.1016/0167-4781\(93\)90012-3](https://doi.org/10.1016/0167-4781(93)90012-3).
- Labas, V., I. Grasseau, K. Cahier, et al. 2015. "Qualitative and Quantitative Peptidomic and Proteomic Approaches to Phenotyping Chicken Semen." *Journal of Proteomics* 112: 313–335. <https://doi.org/10.1016/j.jprot.2014.07.024>.
- Laflamme, B. A., F. W. Avila, K. Michalski, and M. F. Wolfner. 2014. "A *Drosophila* Protease Cascade Member, Seminal Metalloprotease-1, Is Activated Stepwise by Male Factors and Requires Female Factors for Full Activity." *Genetics* 196, no. 4: 1117–1129. <https://doi.org/10.1534/genetics.113.160101>.
- Laflamme, B. A., and M. F. Wolfner. 2013. "Identification and Function of Proteolysis Regulators in Seminal Fluid." *Molecular Reproduction and Development* 80, no. 2: 80–101. <https://doi.org/10.1002/mrd.22130>.
- Leśniak, W., Ł. P. Słomnicki, and A. Filipek. 2009. "S100A6—New Facts and Features." *Biochemical and Biophysical Research Communications* 390, no. 4: 1087–1092. <https://doi.org/10.1016/j.bbrc.2009.10.150>.
- Li, P., M. Hulak, Z. H. Li, et al. 2013. "Cryopreservation of Common Carp (*Cyprinus carpio* L.) Sperm Induces Protein Phosphorylation in Tyrosine and Threonine Residues." *Theriogenology* 80, no. 2: 84–89. <https://doi.org/10.1016/j.theriogenology.2013.03.021>.
- Li, Y., Y. Sun, A. Ni, et al. 2020. "Seminal Plasma Proteome as an Indicator of Sperm Dysfunction and Low Sperm Motility in Chickens." *Molecular & Cellular Proteomics* 19, no. 6: 1035–1046. <https://doi.org/10.1074/mcp.ra120.002017>.
- Linklater, J. R., B. Wertheim, S. Wigby, and T. Chapman. 2007. "Ejaculate Depletion Patterns Evolve in Response to Experimental Manipulation of Sex Ratio in *Drosophila melanogaster*." *Evolution* 61, no. 8: 2027–2034. <https://doi.org/10.1111/j.1558-5646.2007.00157.x>.
- Liu, H., and E. Kubli. 2003. "Sex-Peptide Is the Molecular Basis of the Sperm Effect in *Drosophila melanogaster*." *Proceedings of the National Academy of Sciences* 100, no. 17: 9929–9933. <https://doi.org/10.1073/pnas.1631700100>.
- Lu, J., J. Jing, Q. Yao, et al. 2016. "Relationship Between Lipids Levels of Serum and Seminal Plasma and Semen Parameters in 631 Chinese Subfertile Men." *PLoS One* 11, no. 1: e0146304. <https://doi.org/10.1371/journal.pone.0146304>.
- Lüpold, S., R. A. de Boer, J. P. Evans, J. L. Tomkins, and J. L. Fitzpatrick. 2020. "How Sperm Competition Shapes the Evolution of Testes and Sperm: A Meta-Analysis." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 375, no. 1813: 20200064. <https://doi.org/10.1098/rstb.2020.0064>.
- Macartney, E. L., A. J. Crean, S. Nakagawa, and R. Bonduriansky. 2019. "Effects of Nutrient Limitation on Sperm and Seminal Fluid: A Systematic Review and Meta-Analysis." *Biological Reviews* 94, no. 5: 1722–1739. <https://doi.org/10.1111/brv.12524>.
- Macartney, E. L., V. Zeender, A. Meena, A. N. De Nardo, R. Bonduriansky, and S. Lüpold. 2021. "Sperm Depletion in Relation to Developmental Nutrition and Genotype in *Drosophila melanogaster*." *Evolution* 75, no. 11: 2830–2841. <https://doi.org/10.1111/evo.14373>.
- Manjunath, P., and I. Thérien. 2002. "Role of Seminal Plasma Phospholipid-Binding Proteins in Sperm Membrane Lipid Modification That Occurs During Capacitation." *Journal of Reproductive Immunology* 53, no. 1–2: 109–119. [https://doi.org/10.1016/s0165-0378\(01\)00098-5](https://doi.org/10.1016/s0165-0378(01)00098-5).
- Marshall, J. L., D. L. Huestis, Y. Hiromasa, et al. 2009. "Identification, RNAi Knockdown, and Functional Analysis of an Ejaculate Protein That Mediates a Postmating, Prezygotic Phenotype in a Cricket." *PLoS One* 4, no. 10: e7537. <https://doi.org/10.1371/journal.pone.0007537>.
- Marson, J., S. Meuris, F. Moysan, D. Gervais, R. W. Cooper, and P. Jouannet. 1988. "Cellular and Biochemical Characteristics of Semen Obtained From Pubertal Chimpanzees by Masturbation." *Reproduction* 82, no. 1: 199–207. <https://doi.org/10.1530/jrf.0.0820199>.
- Martin, M. U., and W. Falk. 1997. "The Interleukin-1 Receptor Complex and Interleukin-1 Signal Transduction." *European Cytokine Network* 8, no. 1: 5–17.
- Mason, C., B. Tschirren, and N. Hemmings. 2024. "Effects of Female-Specific Selection for Reproductive Investment on Male Fertility Traits." *Journal of Evolutionary Biology* 37, no. 9: 1113–1124. <https://doi.org/10.1093/jeb/voae095>.
- Mendeluk, G., F. L. G. Flecha, P. R. Castello, and C. Bregni. 2000. "Factors Involved in the Biochemical Etiology of Human Seminal Plasma Hyperviscosity." *Journal of Andrology* 21, no. 2: 262–267. <https://doi.org/10.1002/j.1939-4640.2000.tb02104.x>.
- Miyata, H., C. D. Thaler, L. T. Haimo, and R. A. Cardullo. 2012. "Protease Activation and the Signal Transduction Pathway Regulating Motility in Sperm From the Water Strider *Aquarius remigis*." *Cytoskeleton* 69, no. 4: 207–220. <https://doi.org/10.1002/cm.21012>.
- Moazamian, R., A. Polhemus, H. Connaughton, et al. 2015. "Oxidative Stress and Human Spermatozoa: Diagnostic and Functional Significance of Aldehydes Generated as a Result of Lipid Peroxidation." *MHR: Basic Science of Reproductive Medicine* 21, no. 6: 502–515. <https://doi.org/10.1093/molehr/gav014>.
- Mohan, J., K. V. H. Sastry, J. S. Tyagi, and D. K. Singh. 2004. "Isolation of *E. coli* From Foam and Effects of Fluoroquinolones on *E. coli* and Foam Production in Male Japanese Quail." *Theriogenology* 62, no. 8: 1383–1390. <https://doi.org/10.1016/j.theriogenology.2003.12.033>.
- Møller, A. P. 1991. "Sperm Competition, Sperm Depletion, Parental Care, and Relative Testis Size in Birds." *American Naturalist* 137, no. 6: 882–906. <https://doi.org/10.1086/285199>.
- Mousseau, T. A., and C. W. Fox. 1998. "The Adaptive Significance of Maternal Effects." *Trends in Ecology & Evolution* 13, no. 10: 403–407. [https://doi.org/10.1016/S0169-5347\(98\)01472-4](https://doi.org/10.1016/S0169-5347(98)01472-4).
- Napodano, C., K. Pocino, D. Rigante, et al. 2019. "Free Light Chains and Autoimmunity." *Autoimmunity Reviews* 18, no. 5: 484–492. <https://doi.org/10.1016/j.autrev.2019.03.003>.
- Neupane, P., S. Bhuju, N. Thapa, and H. K. Bhattarai. 2019. "ATP Synthase: Structure, Function and Inhibition." *Biomolecular Concepts* 10, no. 1: 1–10. <https://doi.org/10.1515/bmc-2019-0001>.
- Nichols, C. R. 1991. "A Comparison of the Reproductive and Behavioural Differences of Feral and Domestic Japanese Quail." Master's thesis, the

- University of British Columbia. UBS Theses and Dissertations. <http://hdl.handle.net/2429/30169>.
- Odet, F., S. A. Gabel, J. Williams, R. E. London, E. Goldberg, and E. M. Eddy. 2011. "Lactate Dehydrogenase C and Energy Metabolism in Mouse Sperm." *Biology of Reproduction* 85, no. 3: 556–564. <https://doi.org/10.1095/biolreprod.111.091546>.
- Ogawa, K., H. Nakanishi, and M. Imanishi. 1974. "Effect of Frothy Fluid From the Cloacal Gland on Fertility in the Japanese Quail (*Coturnix coturnix Japonica*)." *Bulletin of the Faculty of Agriculture, Kagoshima University* 24: 35–40.
- Perez-Riverol, Y., C. Bandla, D. J. Kundu, et al. 2025. "The PRIDE Database at 20 Years: 2025 Update." *Nucleic Acids Research* 53, no. D1: D543–D553. <https://doi.org/10.1093/nar/gkaf1011>.
- Perl, A. 2007. "The Pathogenesis of Transaldolase Deficiency." *IUBMB Life* 59, no. 6: 365–373. <https://doi.org/10.1080/15216540701387188>.
- Perl, A., Y. Qian, K. R. Chohan, et al. 2006. "Transaldolase Is Essential for Maintenance of the Mitochondrial Transmembrane Potential and Fertility of Spermatozoa." *Proceedings of the National Academy of Sciences* 103, no. 40: 14813–14818. <https://doi.org/10.1073/pnas.0602678103>.
- Perry, J. C., L. Sirot, and S. Wigby. 2013. "The Seminal Symphony: How to Compose an Ejaculate." *Trends in Ecology & Evolution* 28, no. 7: 414–422. <https://doi.org/10.1016/j.tree.2013.03.005>.
- Pick, J. L., P. Hutter, C. Ebner, A.-K. Ziegler, M. Giordano, and B. Tschirren. 2016. "Artificial Selection Reveals the Energetic Expense of Producing Larger Eggs." *Frontiers in Zoology* 13, no. 1: 38. <https://doi.org/10.1186/s12983-016-0172-y>.
- Pick, J. L., P. Hutter, and B. Tschirren. 2016. "In Search of Genetic Constraints Limiting the Evolution of Egg Size: Direct and Correlated Responses to Artificial Selection on a Prenatal Maternal Effector." *Heredity* 116, no. 6: 542–549. <https://doi.org/10.1038/hdy.2016.16>.
- Pick, J. L., P. Hutter, and B. Tschirren. 2017. "Divergent Artificial Selection for Female Reproductive Investment Has a Sexually Concordant Effect on Male Reproductive Success." *Evolution Letters* 1, no. 4: 222–228. <https://doi.org/10.1002/evl3.21>.
- Poiani, A. 2006. "Complexity of Seminal Fluid: A Review." *Behavioral Ecology and Sociobiology* 60, no. 3: 289–310. <https://doi.org/10.1007/s00265-006-0178-0>.
- R Core Team. 2024. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Rae, M. T., C. E. Kyle, D. W. Miller, A. J. Hammond, A. N. Brooks, and S. M. Rhind. 2002. "The Effects of Undernutrition, In Utero, on Reproductive Function in Adult Male and Female Sheep." *Animal Reproduction Science* 72, no. 1–2: 63–71. [https://doi.org/10.1016/S0378-4320\(02\)00068-4](https://doi.org/10.1016/S0378-4320(02)00068-4).
- Ram, K. R., and M. F. Wolfner. 2009. "A Network of Interactions Among Seminal Proteins Underlies the Long-Term Postmating Response in *Drosophila*." *Proceedings of the National Academy of Sciences* 106, no. 36: 15384–15389. <https://doi.org/10.1073/pnas.0902923106>.
- Ramm, S. A. 2020. "Seminal Fluid and Accessory Male Investment in Sperm Competition." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 375, no. 1813: 20200068. <https://doi.org/10.1098/rstb.2020.0068>.
- Ramm, S. A., L. McDonald, J. L. Hurst, R. J. Beynon, and P. Stockley. 2009. "Comparative Proteomics Reveals Evidence for Evolutionary Diversification of Rodent Seminal Fluid and Its Functional Significance in Sperm Competition." *Molecular Biology and Evolution* 26, no. 1: 189–198. <https://doi.org/10.1093/molbev/msn237>.
- Reinhardt, K., R. Naylor, and M. T. Siva-Jothy. 2011. "Male Mating Rate Is Constrained by Seminal Fluid Availability in Bedbugs, *Cimex lectularius*." *PLoS One* 6, no. 7: e22082. <https://doi.org/10.1371/journal.pone.0022082>.
- Retes, P. L., D. G. das Neves, L. F. Bernardes, et al. 2019. "Reproductive Characteristics of Male and Female Japanese Quails (*Coturnix coturnix Japonica*) Fed Diets With Different Levels of Crude Protein During the Growth and Production Phases." *Livestock Science* 223: 124–132. <https://doi.org/10.1016/j.livsci.2019.03.011>.
- Rhind, S. M., M. T. Rae, and A. N. Brooks. 2001. "Effects of Nutrition and Environmental Factors on the Fetal Programming of the Reproductive Axis." *Reproduction* 122, no. 2: 205–214. <https://doi.org/10.1530/rep.0.1220205>.
- Romero-Haro, A., L. Pérez-Rodríguez, and B. Tschirren. 2023. "Increased Male-Induced Harm in Response to Female-Limited Selection: Interactive Effects Between Intra- and Interlocus Sexual Conflict?" *Proceedings of the Royal Society B: Biological Sciences* 290, no. 1997: 20230140. <https://doi.org/10.1098/rspb.2023.0140>.
- Sachs, B. 1969. "Photoperiodic Control of Reproductive Behavior and Physiology of the Male Japanese Quail (*Coturnix coturnix Japonica*)." *Hormones and Behavior* 1, no. 1: 7–24. [https://doi.org/10.1016/0018-506X\(69\)90002-6](https://doi.org/10.1016/0018-506X(69)90002-6).
- Saeed, B., R. Baban, and U. Al-Nasiri. 2021. "Lactate Dehydrogenase C4 (LDH-C4) is Essential for the Sperm Count and Motility: A Case Control Study." *Baghdad Journal of Biochemistry and Applied Biological Sciences* 2, no. 3: 146–159. <https://doi.org/10.47419/bjabs.v2i03.65>.
- Sakaguchi, M., H. Sonegawa, H. Murata, et al. 2008. "S100A11, an Dual Mediator for Growth Regulation of Human Keratinocytes." *Molecular Biology of the Cell* 19, no. 1: 78–85. <https://doi.org/10.1091/mbc.e07-07-0682>.
- Santiago-Moreno, J., and E. Blesbois. 2020. "Functional Aspects of Seminal Plasma in Bird Reproduction." *International Journal of Molecular Sciences* 21, no. 16: 5664. <https://doi.org/10.3390/ijms21165664>.
- Sasanami, T., S. Izumi, N. Sakurai, et al. 2015. "A Unique Mechanism of Successful Fertilization in a Domestic Bird." *Scientific Reports* 9, no. 5: 7700. <https://doi.org/10.1038/srep07700>.
- Schjenken, J., and S. Robertson. 2014. "Seminal Fluid and Immune Adaptation for Pregnancy—Comparative Biology in Mammalian Species." *Reproduction in Domestic Animals* 49, no. s3: 27–36. <https://doi.org/10.1111/rda.12383>.
- Schjenken, J. E., and S. A. Robertson. 2020. "The Female Response to Seminal Fluid." *Physiological Reviews* 100, no. 3: 1077–1117. <https://doi.org/10.1152/physrev.00013.2018>.
- Schon, S. B., L. J. Luense, X. Wang, et al. 2019. "Histone Modification Signatures in Human Sperm Distinguish Clinical Abnormalities." *Journal of Assisted Reproduction and Genetics* 36, no. 2: 267–275. <https://doi.org/10.1007/s10815-018-1354-7>.
- Seiwert, C. M., and E. Adkins-Regan. 1998. "The Foam Production System of the Male Japanese Quail: Characterization of Structure and Function." *Brain, Behavior and Evolution* 52, no. 2: 61–80. <https://doi.org/10.1159/000006553>.
- Sepil, I., B. R. Hopkins, R. Dean, et al. 2020. "Male Reproductive Aging Arises via Multifaceted Mating-Dependent Sperm and Seminal Proteome Declines, but Is Postponable in *Drosophila*." *Proceedings of the National Academy of Sciences* 117, no. 29: 17094–17103. <https://doi.org/10.1073/pnas.2009053117>.
- Sharma, R., A. Agarwal, G. Mohanty, et al. 2013. "Proteomic Analysis of Human Spermatozoa Proteins With Oxidative Stress." *Reproductive Biology and Endocrinology* 11, no. 1: 48. <https://doi.org/10.1186/1477-7827-11-48>.
- Sherman, B. T., M. Hao, J. Qiu, et al. 2022. "DAVID: A Web Server for Functional Enrichment Analysis and Functional Annotation of Gene

- Lists (2021 Update)." *Nucleic Acids Research* 50, no. W1: W216–W221. <https://doi.org/10.1093/nar/gkac194>.
- Short, S. M., and B. P. Lazzaro. 2010. "Female and Male Genetic Contributions to Post-Mating Immune Defence in Female *Drosophila melanogaster*." *Proceedings of the Royal Society B: Biological Sciences* 277, no. 1700: 3649–3657. <https://doi.org/10.1098/rspb.2010.0937>.
- Shortall, K., A. Djeghader, E. Magner, and T. Soulimane. 2021. "Insights Into Aldehyde Dehydrogenase Enzymes: A Structural Perspective." *Frontiers in Molecular Biosciences* 8: 659550. <https://doi.org/10.3389/fmolb.2021.659550>.
- Sias, B., F. Ferrato, M. Pellicer-Rubio, et al. 2005. "Cloning and Seasonal Secretion of the Pancreatic Lipase-Related Protein 2 Present in Goat Seminal Plasma." *Biochimica et Biophysica Acta (BBA)—Molecular and Cell Biology of Lipids* 1686, no. 3: 169–180. <https://doi.org/10.1016/j.bbalip.2004.09.008>.
- Singh, A., S. Verma, S. B. Modak, M. M. Chaturvedi, and J. S. Purohit. 2022. "Extra-Nuclear Histones: Origin, Significance and Perspectives." *Molecular and Cellular Biochemistry* 477, no. 2: 507–524. <https://doi.org/10.1007/s11010-021-04300-4>.
- Singh, B., M. F. Sundbom, U. Muthukrishnan, et al. 2025. "Extracellular Histones as Exosome Membrane Proteins Regulated by Cell Stress." *Journal of Extracellular Vesicles* 14, no. 2: e70042. <https://doi.org/10.1002/jev2.70042>.
- Singh, R. P., K. V. H. Sastry, N. K. Pandey, et al. 2012. "The Role of the Male Cloacal Gland in Reproductive Success in Japanese Quail (*Coturnix japonica*)."
- Reproduction, Fertility and Development* 24, no. 2: 405. <https://doi.org/10.1071/RD11057>.
- Singh, R. P., K. V. H. Sastry, N. Shit, et al. 2011. "Cloacal Gland Foam Enhances Motility and Disaggregation of Spermatozoa in Japanese Quail (*Coturnix japonica*)."
- Theriogenology* 75, no. 3: 563–569. <https://doi.org/10.1016/j.theriogenology.2010.09.028>.
- Sirot, L. K., M. F. Wolfner, and S. Wigby. 2011. "Protein-Specific Manipulation of Ejaculate Composition in Response to Female Mating Status in *Drosophila melanogaster*." *Proceedings of the National Academy of Sciences* 108, no. 24: 9922–9926. <https://doi.org/10.1073/pnas.1100905108>.
- Siva, A. B., D. B. Kameshwari, V. Singh, et al. 2010. "Proteomics-Based Study on Asthenozoospermia: Differential Expression of Proteasome Alpha Complex." *MHR: Basic Science of Reproductive Medicine* 16, no. 7: 452–462. <https://doi.org/10.1093/molehr/gaq009>.
- Ślōmnicki, Ł. P., B. Nawrot, and W. Leśniak. 2009. "S100A6 Binds p53 and Affects Its Activity." *International Journal of Biochemistry & Cell Biology* 41, no. 4: 784–790. <https://doi.org/10.1016/j.biocel.2008.08.007>.
- Śłowińska, M., J. Nynca, G. J. Arnold, et al. 2017. "Proteomic Identification of Turkey (*Meleagris gallopavo*) Seminal Plasma Proteins." *Poultry Science* 96, no. 9: 3422–3435. <https://doi.org/10.3382/ps/pex132>.
- Soggiu, A., C. Piras, H. A. Hussein, et al. 2013. "Unravelling the Bull Fertility Proteome." *Molecular BioSystems* 9, no. 6: 1188. <https://doi.org/10.1039/c3mb25494a>.
- Sorci, G., G. Giovannini, F. Riuzzi, et al. 2011. "The Danger Signal s100b Integrates Pathogen– And Danger–Sensing Pathways to Restrain Inflammation." *PLoS Pathogens* 7, no. 3: e1001315. <https://doi.org/10.1371/journal.ppat.1001315>.
- Speakman, J. R., and M. Garratt. 2014. "Oxidative Stress as a Cost of Reproduction: Beyond the Simplistic Trade-Off Model." *BioEssays* 36, no. 1: 93–106. <https://doi.org/10.1002/bies.201300108>.
- Stephens, K., R. A. Cardullo, and C. D. Thaler. 2018. "*Culex pipiens* Sperm Motility Is Initiated by a Trypsin-Like Protease From Male Accessory Glands." *Molecular Reproduction and Development* 85, no. 5: 440–448. <https://doi.org/10.1002/mrd.22980>.
- Stockley, P., C. Franco, A. J. Claydon, et al. 2020. "Revealing Mechanisms of Mating Plug Function Under Sexual Selection." *Proceedings of the National Academy of Sciences* 117, no. 44: 27465–27473. <https://doi.org/10.1073/pnas.1920526117>.
- Tang, B., G. Xie, X. Hu, et al. 2022. "A Comparative Proteomic Study of High and Low Semen Quality Seminal Plasma in Drakes." *Poultry Science* 101, no. 11: 102130. <https://doi.org/10.1016/j.psj.2022.102130>.
- Tao, H. P., T. F. Lu, S. Li, et al. 2023. "Pancreatic Lipase-Related Protein 2 Is Selectively Expressed by Peritubular Myoid Cells in the Murine Testis and Sustains Long-Term Spermatogenesis." *Cellular and Molecular Life Sciences* 80: 217. <https://doi.org/10.1007/s00018-023-04872-y>.
- Thankamoni, R. K., D. W. Tamgire, P. Dasari, et al. 2024. "The Association of Sperm DNA Fragmentation With Serum Lipid Profile Among Males With Abnormal Semen Parameters: An Analytical Cross-Sectional Study." *Cureus* 16, no. 5: e60243. <https://doi.org/10.7759/cureus.60243>.
- The UniProt Consortium. 2025. "UniProt: The Universal Protein Knowledgebase." *Nucleic Acids Research* 53, no. D1: D609–D617. <https://doi.org/10.1093/nar/gkae1010>.
- Thélie, A., S. Rehault-Godbert, J. C. Poirier, M. Govoroun, S. Fouchécourt, and E. Blesbois. 2019. "The Seminal Acrosin-Inhibitor CIT1/SPINK2 Is a Fertility-Associated Marker in the Chicken." *Molecular Reproduction and Development* 86, no. 7: 762–775.
- Tyler, N. C., M. Namntu, and M. Ciacciariello. 2021. "The Effect of Crude Protein and Calcium Intake on Fertility of Male Broiler Breeders." *Poultry Science* 100, no. 9: 101284. <https://doi.org/10.1016/j.psj.2021.101284>.
- Vedder, O., and M. Beccardi. 2025. "Poor Developmental Conditions Decrease Adult Body Size and Egg Size, but Not Egg Laying Rate and Survival Throughout Adulthood: A Long-Term Experiment in a Precocial Bird." *Journal of Animal Ecology* 94: 1231–1243. <https://doi.org/10.1111/1365-2656.70043>.
- Vedder, O., B. Tschirren, E. Postma, and M. Moiron. 2023. "Rapid Decline of Prenatal Maternal Effects With Age Is Independent of Postnatal Environment in a Precocial Bird." *Evolution* 77, no. 11: 2484–2491. <https://doi.org/10.1093/evolut/qpaa159>.
- Verkhatskii, A. N. 2023. "Microglial Physiology and Function." In *Neuroglia: Function and Pathology*, 347–408. Academic Press. <https://doi.org/10.1016/B978-0-12-821565-4.00002-X>.
- Vilagran, I., M. Castillo-Martin, N. Prieto-Martínez, S. Bonet, and M. Yeste. 2016. "Triosephosphate Isomerase (TPI) and Epididymal Secretory Glutathione Peroxidase (GPX5) Are Markers for Boar Sperm Quality." *Animal Reproduction Science* 165: 22–30. <https://doi.org/10.1016/j.anireprosci.2015.12.001>.
- von Hellfeld, R., R. Konietzny, P. D. Charles, et al. 2025. "The Development Environment Mediates Adult Seminal Proteome Allocation in Male *Drosophila melanogaster*." *bioRxiv*. <https://doi.org/10.1101/2025.01.09.632097do>.
- Wang, C., R. Yan, D. Luo, K. Watabe, D.-F. Liao, and D. Cao. 2009. "Aldo-Keto Reductase Family 1 Member b10 Promotes Cell Survival by Regulating Lipid Synthesis and Eliminating Carbonyls." *Journal of Biological Chemistry* 284, no. 39: 26742–26748. <https://doi.org/10.1074/jbc.m109.022897>.
- Wigby, S., J. C. Perry, Y. H. Kim, and L. K. Sirot. 2016. "Developmental Environment Mediates Male Seminal Protein Investment in *Drosophila melanogaster*." *Functional Ecology* 30, no. 3: 410–419. <https://doi.org/10.1111/1365-2435.12515>.
- Williams, T. D. 1994. "Intraspecific Variation in Egg Size and Egg Composition in Birds: Effects on Offspring Fitness." *Biological Reviews of the Cambridge Philosophical Society* 69, no. 1: 35–59. <https://doi.org/10.1111/j.1469-185x.1994.tb01485.x>.
- Wolyniak, M. J., R. H. Frazier, P. K. Gemborys, and H. E. Loehr. 2024. "Malate Dehydrogenase: A Story of Diverse Evolutionary Radiation."



*Essays in Biochemistry* 68, no. 2: 213–220. <https://doi.org/10.1042/ebc20230076>.

Xiao, X., and W. X. Yang. 2007. “Actin-Based Dynamics During Spermatogenesis and Its Significance.” *Journal of Zhejiang University Science. B* 8, no. 7: 498–506. <https://doi.org/10.1631/jzus.2007.b0498>.

Xiong, H., Z. Chen, J. Zhao, W. Li, and S. Zhang. 2022. “TNF- $\alpha$ /ENO1 Signaling Facilitates Testicular Phagocytosis by Directly Activating Elmo1 Gene Expression in Mouse Sertoli Cells.” *FEBS Journal* 289, no. 10: 2809–2827. <https://doi.org/10.1111/febs.16326>.

Zakowski, J. J., and D. E. Bruns. 1985. “Biochemistry of Human Alpha Amylase Isoenzymes.” *CRC Critical Reviews in Clinical Laboratory Sciences* 21, no. 4: 283–322. <https://doi.org/10.3109/10408368509165786>.

Zendeer, V., S. Pfammatter, B. Roschitzki, S. Dorus, and S. Lüpold. 2023. “Genotype-By-Environment Interactions Influence the Composition of *Drosophila* Seminal Proteome.” *Proceedings of the Royal Society B: Biological Sciences* 290, no. 2006: 20231313. <https://doi.org/10.1098/rspb.2023.1313>.

Zhao, J., and C. J. Zhong. 2009. “A Review on Research Progress of Transketolase.” *Neuroscience Bulletin* 25, no. 2: 94–99. <https://doi.org/10.1007/s12264-009-1113-y>.

Zorn, B., A. Sešek-Briški, J. Osredkar, and H. Meden-Vrtovec. 2003. “Semen Polymorphonuclear Neutrophil Leukocyte Elastase as a Diagnostic and Prognostic Marker of Genital Tract Inflammation—A Review.” *Clinical Chemistry and Laboratory Medicine* 41, no. 1: 2–12. <https://doi.org/10.1515/cclm.2003.002>.

Zorn, B., I. Virant-Klun, and H. Meden-Vrtovec. 2000. “Semen Granulocyte Elastase: Its Relevance for the Diagnosis and Prognosis of Silent Genital Tract Inflammation.” *Human Reproduction* 15, no. 9: 1978–1984. <https://doi.org/10.1093/humrep/15.9.1978>.

### Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** mec70257-sup-0001-TablesS1-S15.xlsx. **Figure S1:** mec70257-sup-0002-FigureS1.pdf.