Characterizing the impact of oocyte nutrient-sensitive genes on reproductive aging in *Caenorhabditis elegans*

by

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Abstract

Reproductive aging is the gradual decline in reproductive function over time, encompassing reductions in fertility, hormonal changes, and diminished germ cell quality. This process is highly sensitive to nutrient conditions, as sufficient energy availability is essential for maintaining reproductive function. However, nutrient surplus may accelerate aging and impair fertility. Prior studies have demonstrated that *Caenorhabditis elegans* (*C. elegans*) exposed to a high-glucose diet exhibit reduced reproductive capacity and compromised oocyte quality. Poor oocyte quality is a well-documented contributor to reproductive dysfunction in both *C. elegans* and humans, underscoring the importance of understanding the molecular mechanisms that influence reproductive health under obesogenic diets. Genes whose transcription is regulated in a nutrient-sensitive manner play a critical role in mediating the relationship between diet and reproductive function. This study aimed to determine the impact of two such genes, *icmt-1* and *psme-4,* on the reproductive capacity of *C. elegans* by using RNAi-mediated knockdown. *C. elegans* were exposed to lifelong RNAi and subject to elevated glucose exposure (20 mM glucose) starting at the fourth larval stage to coincide with establishment of the reproductive system. To assess the reproductive capacity in aging worms, *C. elegans* were mated on day five (D5) of adulthood, and their reproductive success was evaluated based on progeny production. Additionally, D5 oocytes were imaged using DIC imaging to assess morphological changes and overall oocyte quality in response to gene knockdown and high-glucose exposure. This study found that the RNAi knockdown of *icmt-1* significantly reduced reproductive success in aging *C. elegans*, potentially due to systemic effects rather than direct impact on oocyte quality. Since *icmt-1* regulates Ras protien localization and apoptotic pathways, its knockdown may cause widespread cellular stress and metabolic disruptions that impair reproduction. Conversely, *psme-4* knockdown significantly improved age-related reproductive success and oocyte quality under glucose-enriched conditions, suggesting that reducing proteasomal activity may mitigate glucose-induced reproductive decline. *psme-4* knockdown did not significantly affect reproductive capacity under normal dietary conditions, indicating that its glucose-induced upregulation may play a role in the reproductive defects observed under high-glucose conditions. These findings suggest that *icmt-1* and *psme-4* influence reproductive responses to dietary glucose, offering insight into the genetic basis of diet-induced infertility.

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1 Introduction

1.1 Female age-related decline in reproductive capacity in humans

Age-related decline in female reproductive capacity, a well-established phenomenon that significantly impacts fertility as women grow older, is prominent as early as 20 years before menopause in humans (Klein and Sauer, 2001). The greatest determinants in this reduced reproductive capacity are the noticeable decline in both quantity and quality of oocytes (Klein and Sauer, 2001). With advancing age, oocytes experience an increase in chromosomal abnormalities, such as aneuploidy, which raises the risk of miscarriage, as well as birth defects like trisomy 21 (Mikwar *et al.,* 2020). Furthermore, the observed reduction in ovarian reserve leads to fewer viable oocytes available for fertilization, which in turn contributes to lower fertility rates (Klein and Sauer, 2001). The process is gradual but accelerates after the age of 40, when the likelihood of successful pregnancy decreases substantially (Mikwar *et al.,* 2020).

Despite this age-related decline in fertility, trends in reproductive behavior have shifted significantly in many parts of the world. Over the last few decades, there has been a notable rise in the age at which women are having children (Zabak *et al.,* 2023). This shift is partly driven by social, economic, and educational factors, with women choosing to delay childbearing for career development, financial stability, or personal preferences (Zabak *et al.,* 2023). Consequently, the number of women attempting pregnancy later in life has increased, leading to more women seeking fertility treatments, such as in vitro fertilization (IVF) and cryotechnology, as means to combat the natural decline in reproductive capacity (Okhovati *et al.,* 2015). However, the success rate of IVF is reduced in older patients as a result of the decreased quality and quantity of oocytes (Klein and Sauer, 2001). Thus, while the trend of later childbearing reflects broader

societal changes, it underscores the ongoing biological limitations that women face when attempting to conceive at an older age.

With trends in childbearing shifting later in life, research is necessary to further our understanding of female reproductive preservation and strengthen reproductive autonomy (O'Brian and Wingfield, 2018). Moreover, age-related female reproductive decline affects other systems in the body which may lead to further health conditions, such as colorectal cancer and ischemic heart disease (Traub and Santoro, 2010). This highlights the necessity for additional research in the field of women's reproductive health. In conclusion, as societal trends continue to shift toward later childbearing, it is crucial to deepen our understanding of the biological mechanisms underlying age-related reproductive decline, not only to improve fertility outcomes but also to address the broader health implications that may arise with age.

1.2 Caenorhabditis elegans as a model organism

The non-parasitic nematode *Caenorhabditis elegans* shares many reproductive aging characteristics with humans. *C. elegans* are a widely used biomedical model organism that possess distinct reproductive, nervous, and digestive systems. The use of *C. elegans* as a model organism was first highlighted due to their small size and short lifecycle allowing them to be easily maintained in a laboratory setting (Brenner, 1974). When maintained at 20°C, *C. elegans* can live for up to 30 days (Athar and Templeman, 2022). The development of *C. elegans* at this temperature consists of the progression through four larval stages (L1, L2, L3, and L4) within approximately 48 hours (Athar and Templeman, 2022). When exposed to unfavourable conditions, *C. elegans* may enter a dauer larval stage which occurs between L1 and L2, allowing them to survive for two to three times their standard lifespan. Once returned to more favourable

conditions, *C. elegans* can continue their developmental progression into L4 and subsequently adulthood, allowing them to regain reproductive function (Athar and Templeman, 2022). Furthermore, *C. elegans* exhibit temperature-dependent developmental timing. When exposed to lower temperatures (*e.g.* 15°C) they exhibit a longer lifespan compared to those exposed to 20°C. Conversely, when exposed to higher temperatures (*e.g.* 25°C), *C. elegans* live for a shorter period of time (Zhang *et al.,* 2015). Furthermore, the transparent body of *C. elegans* allows for easy visualization and assessment of internal structures such as oocytes (Zhang *et al.,* 2020).

While *C. elegans* serve as a useful model for cell biology and genetic research, they also have notable limitations. One key drawback is their lack of many reproductive system organs such as breasts and placenta (Athar and Templeman, 2022). Moreover, they lack the complex tissue organization seen in mammals, along with some key physiological processes such as menstruation (Athar and Templeman, 2022). As a result, while genetic manipulation experiments in *C. elegans* can yield important insights, they do not fully capture the systemic effects of these alterations.

The reproductive system of a hermaphroditic *C. elegans* is comprised of two symmetrical U-shaped arms that are connected by a shared uterus (Scharf *et al.,* 2021). Germ cells undergo mitotic divisions in the distal tip of the gonad (Scharf *et al.,* 2021). As the germ cells move further from the distal tip cells and enter the proximal gonad, they begin meiosis and differentiate into oocytes (Scharf *et al.,* 2021). Mature oocytes are then moved through the spermatheca, the specialized reproductive structure that produces and stores sperm made during the larval stages in hermaphrodites (Scharf *et al.,* 2021; Hirsh *et al.*, 1976). As the oocytes pass through the spermatheca, they become fertilized and proceed into the uterus and eventually exit through the vulva near the center of the organism (Scharf *et al.,* 2021).

C. elegans are capable of both self-fertilization and sexual reproduction, however, males occur only approximately 0.1% of the time in the population due to non-disjunction of the X chromosome (Loxterkamp *et al.*, 2021; Athar and Templeman, 2022). The reproductive span of self-fertilizing *C. elegans* is around five days, with the limiting factor being sperm (Athar and Templeman, 2022). With the addition of males, *C. elegans* can extend their reproductive span up to around 13 days (Mendenhall *et al*., 2011). Furthermore, the typical brood size of a self-fertilizing hermaphrodite is around 300, however, sexual reproduction can drastically increase brood size to a maximum of 1400 progeny per worm (Athar and Templeman, 2022).

Many of the genetic pathways that regulate reproductive function are conserved from nematodes to humans, with available homologs for greater than 50% of protein-coding genes, making them a powerful model organism for this research (Athar and Templeman, 2022). *C. elegans* demonstrate an age-related decline in reproductive capacity that is linked to deteriorating oocyte quality; it begins in early to mid-adulthood, meaning they possess a considerable post-reproductive lifespan, comparable to humans (Scharf *et al.,* 2021). The genome of *C. elegans* has been fully sequenced and annotated, leading to the development of an RNAi library that covers approximately 80% of its genes, providing a valuable resource for genetic research (Tissenbaum, 2014). Due to the vast similarities between humans and *C. elegans* with respect to genetics and reproductive aging, I will utilize *C. elegans* as a model organism to investigate cellular pathways that impact reproductive capacity in a nutrient-dependent manner, with a broader aim of understanding fundamental, evolutionarily conserved principles underlying these mechanisms.

1.3 Nutrient conditions and reproductive function

Over the past few generations, there has been a dramatic increase in prevalence of non-infectious diseases, including but not limited to type II diabetes mellitus (T2DM), cardiovascular diseases, obesity, and cancer (Kopp, 2019). It is suggested that the Western diet, consisting of high fat, high-glycemic, and high-insulinemic foods, in combination with a lack of fruit, vegetable, and whole-grain consumption, may contribute to the observed rise in non-infectious diseases (Kopp, 2019; Rakhra *et al.,* 2020), and this may extend to impacts on functioning of the female reproductive system.

Obesity has been widely recognized as a significant factor negatively impacting female reproductive health. According to Volk *et al.* (2017), excess body weight can disrupt normal reproductive function in various ways. One major consequence is an increased risk of anovulation, a condition where ovulation fails to occur regularly, leading to menstrual irregularities and difficulties with conception (Volk *et al.,* 2017; Dağ and Dilbaz, 2015). Additionally, obesity is linked to a higher likelihood of miscarriage, as it can contribute to hormonal imbalances, inflammation, and metabolic disturbances that affect embryo implantation and pregnancy maintenance (Volk *et al.,* 2017; Dağ and Dilbaz, 2015). Furthermore, obesity is a known contributor to infertility, often due to its effects on insulin resistance, hormone dysregulation, and altered ovarian function (Volk *et al.,* 2017). Polycystic ovary syndrome is a prevalent reproductive disorder associated with obesity, affecting 8-13% of women (Witchel *et al.,* 2020). This condition is characterized by irregular menstrual cycles, excessive androgen levels, and the presence of cysts on the ovaries (Witchel *et al.,* 2020). Polycystic ovary syndrome may further exacerbate reproductive dysfunction by impairing ovulation and contributing to metabolic complications (Witchel *et al.,* 2020). Excess nutrient intake, like as seen in the

Western diet, triggers widespread metabolic disturbances, including insulin resistance, inflammation, and oxidative stress. These disruptions create a cycle of hormonal and cellular dysfunction that worsens polycystic ovary syndrome symptoms and increases the risk of long-term health complications (Witchel *et al.,* 2020).

One key molecular consequence of an obesogenic diet is the overproduction of reactive oxygen species (ROS), which are byproducts of mitochondrial energy metabolism (Kopp, 2019). In moderate amounts, ROS function as signaling molecules, but when produces in excess, they contribute to oxidative stress and cellular damage (Kopp, 2019). Elevated ROS levels interfere with key pathways, including insulin signaling, by disrupting downstream effectors like Akt, leading to insulin resistance (Kopp, 2019). Since insulin plays a role in regulating ovarian function, this disruption exacerbates the hormonal imbalances seen in polycystic ovary syndrome, stimulating excess androgen production and further impairing ovulation (Witchel *et al.,* 2020). Additionally, oxidative stress triggers chronic inflammation, which perpetuates metabolic dysfunction, by increasing cytokine production and lipid peroxidation. This increase in inflammation can contribute to insulin resistance by disrupting by interfering with the insulin signaling pathway, negatively impacting glucose homeostasis (Yadav, 2014). These combined cellular stressors may increase the risk of type two diabetes (Yadav, 2014).

In response to these shifts in nutrient levels, nutrient-sensing pathways, which are cellular signaling networks, play a crucial role in regulating metabolic balance and health (Lal *et al.,* 2022). These pathways regulate key physiological processes such as growth, metabolism, reproduction, and aging by integrating signals from macronutrients such as glucose (Lal *et al.,* 2022). Nutrient-sensing pathways such as insulin and insulin-like growth factor-1 signaling (IIS) and mechanistic target of rapamycin (mTOR) play a fundamental role in tissue maintenance and

somatic growth, as they mediate the effects of diet on aging, reproduction, and overall health (Templeman and Murphy, 2018). These pathways are largely conserved in mammals and *C. elegans*, with the IIS pathway playing a crucial role in modulating the aging process through its involvement in functions such as proteostasis, environmental stressor responses, growth and development (Altintas *et al.,* 2016; Molière *et al.,* 2024). Activation of the IIS pathway by insulin-like peptide ligands initiates multiple signaling cascades such as the Ras/MAPK pathway, and the phosphoinositide 3-kinase (PI3K)/Akt pathway (Templeman and Murphy, 2018). Activation of PI3K/Akt pathway leads to the phosphorylation of downstream target proteins including forkead box O (FoxO) transcription factors (*e.g.* DAF-16) by Akt. As a result, these transcription factors are prevented from entering the nucleus, thereby blocking the activation of their target genes (Templeman and Murphy, 2018). The IIS pathway is downregulated in the presence of adverse conditions that threaten cellular homeostasis, such as caloric restriction or oxidative stress, leading to the activation and movement of FoxO into the nucleus where it activates genes involved in promoting longevity (Altintas *et al.,* 2016). Reducing IIS pathway activity also leads to preservation of oocyte quality and delayed age-related reproductive cessation (Luo *et al.,* 2010). Conversely, overactivation of the IIS pathway leads to reduced activities of FoxO transcriptions factors. This results in decreased elimination of ROS, contributing to phenotypes such as T2D and cancer (Melink *et al.,* 2011).

As previously noted, mTOR is a key nutrient-sensing pathway and a highly conserved serine/threonine kinase that regulates cellular metabolism, growth, and survival in response to nutrient availability and insulin signaling (Templeman and Murphy, 2018). mTOR exists in two protein complexes mTOR Complex 1 (mTORC1) and mTOR Complex 2. Under obesogenic conditions and overnutrition, mTORC1 is activated through the insulin/PI3K/Akt pathway due to

increased circulating insulin (Templeman and Murphy, 2018; Saxton and Sabatini, 2017). When insulin binds to its receptor, it initiates a signaling cascade causing PI3K activation. PI3K recruits Akt which inhibits a negative regulator of mTORC1, activating mTORC1 (Saxton and Sabatini, 2017). mTORC1 may now promote further processes such as protein synthesis, lipid biosynthesis, and cell growth (Saxton and Sabatini, 2017). Excessive mTORC1 activity for a prolonged period is correlated with pancreatic β-cell death, negatively impacting glucose tolerance and resulting in conditions such as hyperinsulinemia and insulin resistance (Saxton and Sabatini, 2017). Research has indicated that inhibiting mTORC1 under obesogenic conditions may improve glucose uptake (Saxton and Sabatini, 2017). Under standard conditions, it is suggested that mTORC1 inhibition may promote an extended lifespan in *C. elegans* (Saxton and Sabatini, 2017). However, when nutrient availability is low, mTORC1 is inhibited leading to reduced protein synthesis and increased autophagy contributing to accelerated aging, impaired ovarian function and, thus, impaired fertility (Templeman and Murphy, 2018). Interestingly, prolonged mTORC1 inhibition is also associated with negative pancreatic β-cell phenotypes, indicating that mTORC1 plays an important regulatory role in the body's reaction to glucose consumption (Saxton and Sabatini, 2017).

For the model organism *C. elegans*, glucose enrichment is an overnutrition paradigm that induces some similar physiological implications as mammals consuming a Western diet. Elevated glucose exposure is associated with increased IIS pathway activity and is shown to result in a decreased lifespan in *C. elegans* in part through the inhibition of DAF-16/FOXO, increased apoptosis, and the downregulation of aquaporin glycerol channel activity (Lee *et al.,* 2009). Glucose enrichment can lead to mitochondrial dysfunction, resulting in the swelling of germ and muscle cells, increased cellular stress, and altered concentrations of enzymes required

for energy production in *C. elegans* (Alcántar-Fernández *et al.,* 2019). Furthermore, glucose enrichment in *C. elegans* may lead to lipid accumulation similarly to diet-induced obesity in mammals (Alcántar-Fernández *et al.,* 2019). When exposed to high-glucose diets, *C. elegans* show a marked decrease in oocyte production, indicating a decrease in reproductive capacity (Wang *et al.,* 2020; Athar, unpublished observations). Furthermore, glucose enrichment worsens oocyte quality in aging *C. elegans* (Athar, unpublished observations). The impact of overnutrition on the reproductive system highlights the need for further research into the underlying metabolic and molecular mechanisms that drive reproductive decline, providing insight into how nutrient excess accelerates reproductive aging.

1.4 Genetic response to elevated glucose

In an ongoing study (Karmani, unpublished), RNA sequencing was performed on isolated oocytes from young, day-2 adult worms to identify genes that are differentially expressed in wild-type (N2) oocytes exposed to glucose enrichment, compared to control dietary conditions. From these differentially expressed genes, I selected two candidates in order to investigate their influence on the oocyte quality and reproductive capacity of aging worms. Results from the RNA sequencing determined that *T28B8.4* has a 1.75-fold increase in expression in glucose-exposed oocytes, while *F21F3.3* has the greatest glucose-induced decrease in expression in *C. elegans* oocytes, with a 9.9-fold change (Karmani, unpublished). *T28B8.4* encodes an ortholog to the mammalian proteosome activator complex 4 (*psme-4*), which, at normal levels, enables the activity of proteosome binding, acetylated histone binding, and the peptidase activator, all of which are essential for progression of meiosis and progeny viability (Yazgili *et al.,* 2022; Fernando *et al.,* 2022). *F21F3.3* is an ortholog to the mammalian isoprenylcysteine carboxyl O-

methyltransferase (*icmt-1*) gene, which is involved in the carboxyl methylation of isoprenylated proteins and leads to increased apoptosis when expression is reduced (Appleton *et al.,* 2018).

In humans, *icmt-1* encodes the ICMT enzyme that functions to post translationally modify Ras, which is essential for maintaining proper function in cell signaling (Kramer *et al.,* 2003). ICMT methylates the carboxyl group of the prenylated cysteine on Ras. This methylation causes Ras to localize to the plasma membrane where it activates signaling pathways such as the PI3K/Akt pathway (Kramer *et al.,* 2003). The PI3K/Akt pathway functions in multiple capacities to inhibit pro-apoptotic factors (He *et al.,* 2021). For example, the phosphorylation of FoxO transcription factors through the activated PI3K/Akt pathway inhibits activation of FoxO dependent pro-apoptotic genes (He *et al.,* 2021). Furthermore, Akt phosphorylates and thus inhibits pro-apoptotic factors like Bad and procaspase-9, leading to cell proliferation (He *et al.,* 2021). As shown in Figure 1, reduced ICMT function may lead to the mislocalization of Ras in the cytoplasm rather than the plasma membrane, meaning that it cannot activate the PI3K/Akt pathway, and will result in increased expression of pro-apoptotic factors, promoting cell death (He *et al.,* 2021; Wang *et al.,* 2010). In mammals, *icmt-1* is expressed at higher levels in structures such as the brain, testis, and liver (Bergo *et al.,* 2001).

The *psme-4* gene functions as a regulator of protein degradation by encoding the PA200 protein (Ge *et al.,* 2022). This mediates proteasomal activity and is expressed at higher levels in the testis and sperm of mammals (Ge *et al.,* 2022). When DNA damage occurs, histone proteins are often modified and require removal. *psme-4* binds to the acetylated histones, activating the 20S proteasome which leads to the targeted degradation of the histone (Saxton and Sabatini, 2017). *psme-4* is also involved in the activity of mTOR which is involved in the physiological aging processes across many organisms (Ge *et al.,* 2022; Saxton and Sabatini, 2017). It is

suggested that PA200 indirectly enhances mTORC1 activity by promoting protein degradation, releasing free amino acids into the cytoplasm, as well as preventing stress-induced mTORC1 suppression (Yazgili *et al.,* 2022). As described in Figure 2, this increase in mTORC1 may lead to dysregulation of various processes. As both of these candidate genes have roles in key nutrient-sensing signaling pathways, it is important to further investigate their roles in the context of reproductive aging in *C. elegans*.

Figure 1. Schematic representation of the ICMT-Ras-PI3K-Foxo signaling pathway and its proposed role in apoptosis under glucose enrichment. Reduced ICMT levels lead to the suppression of Ras, which in turn leads to reduced PI3K activity. This allows for the activation of Foxo, increasing pro-apoptotic signals.

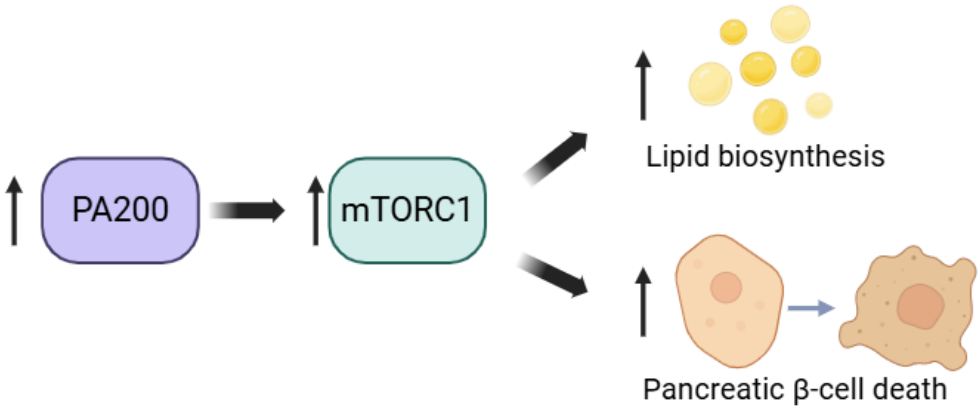

Figure 2. Schematic representation of the PA200-mTORC1 signaling pathway, and its proposed role in lipid biosynthesis and pancreatic β-cell death under glucose enrichment. Increased PA200 leads to the indirect activation of mTORC1.

1.5 Objectives

My honours project will investigate the role of the two genes whose transcription is regulated in a nutrient-sensitive manner, *psme-4* and *icmt-1,* on the reproductive capacity of *C. elegans*, quantified using a late-mating assay, and a visual assessment of oocyte deterioration using differential interference contrast microscopy (DIC). I will do so by exposing the N2 strain of *C. elegans* to the *L4440* plasmid as a control in comparison to worms exposed to *icmt-1* RNAi and *psme-4* RNAi, all of which will also be examined in response to a high-glucose diet.

I hypothesize that the wild-type *C. elegans* will show a deterioration in oocyte quality and reproductive capacity in response to the high-glucose diet. I hypothesize that the knockdown of *icmt-1* will cause a reduction in reproductive capacity and oocyte quality in the 0 mM glucose condition, due to its protective effects being lost. I predict to see no significant change in reproductive capacity and oocyte quality in the 20 mM glucose condition in response to *icmt-1* RNAi, as this gene is already downregulated in response to glucose, and it is possible that the

RNAi may not effectively induce further gene knockdown. Conversely, I hypothesize that the knockdown of *psme-4* will improve reproductive capacity and oocyte quality in the 20 mM glucose condition. The upregulation of this gene in response to glucose may be contributing to the phenotypes observed under glucose exposure, therefore, reducing the expression of this gene under these conditions may reduce the severity of these detrimental effects on oocytes. Lastly, I predict to see no significant change in reproductive capacity and oocyte quality in the 0 mM glucose condition in response to the knockdown of *psme-4*, while considering the possibility that the normal expression of this gene is functionally important, and a loss of this function could result in a decreased reproductive capacity and oocyte quality. Collectively, this project will offer a deeper understanding of the intricate interplay between nutrient surplus, reproductive capacity and age.

2 Materials and Methods

2.1 Maintenance and synchronisation

Two strains of *C. elegans* were used in these experiments: N2 Bristol (wild-type) as the experimental strain exposed to their respective experimental plasmid, and *fog-2(q71)* males used for mating (Table. 1). *fog-2(q71)* males were used because they occur at a much higher frequency than wild-type males, so these males may be readily collected to ensure sufficient fertilization of older hermaphrodites (Stewart and Phillips, 2002). *fog-2(q71)* and non-experimental N2 populations were maintained on OP50 *Escherichia coli* (*E. coli*) lawns grown on nematode growth medium (NGM), made of3 g/L NaCl, 2.5 g/L Bacto-peptone, 17 g/L agar, dissolved in Milli-Q water and autoclaved. The solution was cooled to approximately 55°C, followed by the addition of 25 mL/L 1M KPO_4, 1 mL/L 1M $CaCl_2$, 1 mL/L $MgSO_4$, and 1 mL/L cholesterol, at 15°C or 20°C, according to the standard conditions outlined by Brenner (1974). Carbenicillin-IPTG-NGM plates used for growing up HT115 bacteria for RNAi exposure; these consisted of NGM plus 1 mL 100 mg/mL Carbenicillin and 1 mL 1M Isopropyl β-D-1-thiogalactopyranoside (IPTG) added after the cooling period. For the 20 mM glucose plates, 20 mL/L 1M glucose was also added after the cooling period. OP50 was cultured in Luria Broth (LB) (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) for approximately 12 hours at 37°C and 180 rpm and seeded onto NGM plates at least one day prior to use (350 µL of cultured LB per 60 mm plate). HT115 *E. coli* was also cultured in LB but with the addition of 50 µL 12.5 mg/mL Tetracycline and 25 µL 100 mg/mL Carbenicillin and inoculated with a single colony from either empty or transformed plasmid stock plates. After the *E. coli* lawn had dried, each 60 mm plate was spotted with 30 µL 0.1 M IPTG approximately one hour prior to the addition of N2 worms.

The L4440 vector is a plasmid used to deliver RNAi in *C. elegans.* An empty L4440 vector was used as a control (vector-control) in this study. In the experimental groups, the L4440 vector contained a DNA fragment encoding dsRNA complementary to the gene of interest flanked between two T7 promoters (Conte *et al.,* 2015). This dsRNA encoding vector was obtained from the Ahringer RNAi library (Kamath and Ahringer, 2003). This vector was transformed into HT115 bacteria (Kamath and Ahringer, 2003). Upon induction of this bacteria by IPTG, IPTG binds to the lac repressor protein allowing it to dissociate from the T7 promoter, leading to the production of dsRNA which is consumed by *C. elegans* (Kamath and Ahringer, 2003; Conte *et al.,* 2015). This consumption leads to gene knockdown (Kamath and Ahringer, 2003).

Hypochlorite synchronization was used to synchronize *C. elegans* populations. To do so, worms were rinsed using 1 mL M9 Buffer (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5 g/L NaCl, 1 mL/L $MgSO_4$, all dissolved in Milli-Q H_2O and filter sterilized) into 1.5 mL centrifuge tubes that were centrifuged for one minute at 1300 g. Worms were then exposed to hypochlorite solution (80 mL Milli-Q H_2O, 5 mL 5M KOH, and 15 mL sodium hypochlorite), dissolving the adult worm bodies and releasing the eggs from the adult hermaphrodites. After a second round of centrifugation, the pellet was resuspended and rinsed three times using M9 Buffer. The egg pellet was then resuspended one last time with M9 Buffer and plated onto 60 mm Carbenicillin-IPTG-NGM plates seeded with its respective experimental plasmid. The eggs were then incubated at 20˚C. *C. elegans* were maintained at 20˚C for the duration of the experiment.

Whole-life RNAi was used for these experiments, meaning that directly following synchronization, eggs were plated onto their respective RNAi conditions, and N2 worms were maintained on these respective L4440, *psme-4*, or *icmt-1* HT115 *E. coli* lawns for the durations of the experiment. Glucose exposure began at the L4 stage and was maintained for the

proceeding duration of the experiment. The introduction of glucose at the L4 stage was done to target the impact of glucose to the development of the reproductive system of the worms.

Table 1: *Caenorhabditis elegans* strains, identifiers, and sources.

Strain	Identifier	Source
C. elegans strain N2 var. Bristol: wild-type	N2	Caenorhabditis Genetics Center
C. elegans strain *fog-2(q71)*	CB4108	Caenorhabditis Genetics Center

2.2 RNAi assay

In a previous set of experiments, oocytes from six biological replicates belonging to D2 adult worms exposed to either non-glucose or glucose conditions for 48 hours beginning at the L4 stage were sent to the Michael Smith Genomic Centre for RNA sequencing (Karmani, unpublished). These results were then analyzed to observe differential gene expression in response to these conditions (Karmani, unpublished).

Based on the initial results, I selected two genes: one that displayed a glucose-induced increase in expression (*psme-4*), and another that showed the greatest glucose-induced reduction in expression (*icmt-1*) (Karmani, unpublished). I selected *E. coli* corresponding to each gene from the Ahringer RNAi library and cultured them overnight at 37°C and 180 rpm in 50 mL LB with 25 μL carbenicillin and 50 μL tetracycline. I then streaked this over an RNAi LB agar plate and left to grow overnight at 37°C and 180 rpm. I selected three colonies per condition and sent them for sequencing using the Plasmidsaurus RNA sequencing kit. I aligned the returned RNA sequences and selected those with the fewest mutations and closest match for this experiment.

2.3 RNAi late mating assay

A population of N2 worms was synchronized, and eggs were plated onto Carbenicillin-IPTG-NGM plates. These worms were then incubated at 20°C for approximately 48 hours until they reached the fourth and last larval stage (L4) indicated by visual observation. At this stage, worms were transferred onto 60 mm Carbenicillin-IPTG-NGM plates consisting of six different conditions and split accordingly based on the plasmid they were plated onto after the hypochlorite synchronization as indicated in Figure 3. The conditions include: L4440, L4440 with 20 mM glucose, *psme-4*, *psme-4* with 20 mM glucose, *icmt-1*, or *icmt-1*with 20 mM glucose. Approximately 15-20 worms were maintained per 60 mm plate.

Worms were then transferred every 48 hours to their corresponding conditions, until they reached D5 of adulthood. At D5, hermaphrodites were transferred to individual 35 mm Carbenicillin-IPTG-NGM or Carbenicillin-IPTG-NGM 20 mM glucose NGM plates seeded with their respective plasmid and according to their experimental conditions. At this stage, Day 1 (D1) *fog-2(q71)* worms were added to the 35 mm plates in a 3:1 ratio with three males per one N2.

Worms were left to mate for 48-72 hours. Successful mating was determined by the presence of male progeny two to three days after mating. They were then assessed for the presence of progeny and scored as either reproductive, non-reproductive, or censored. The presence of greater than three *fog-2(q71)* on the plate was deemed ‘reproductive’. If three or fewer males were present on the plate, it was scored as ‘non-reproductive’. The presence of a dead hermaphrodite, or the absence of the hermaphrodite, or all three males was considered ‘censored’ and not included in statistical analysis.

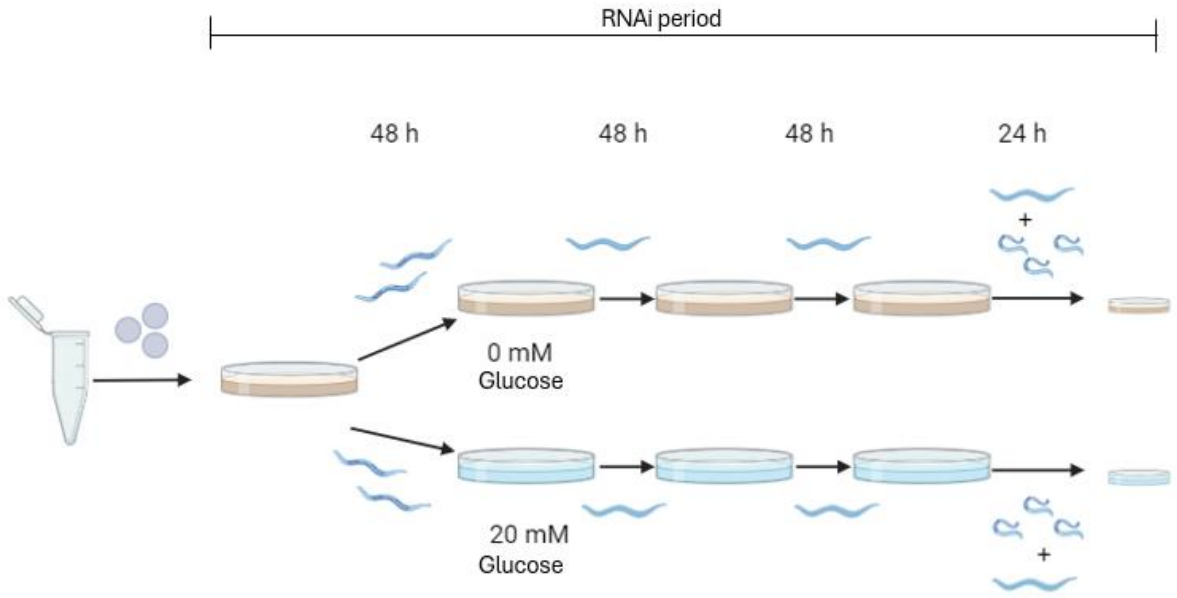

Figure 3. Illustration of the RNAi late mating protocol followed for two conditions. "RNAi period" comprised of three different treatments *(icmt-1* knockdown vs *psme-4* knockdown vs L4440 empty vector control), each of which was exposed to 0 mM glucose or 20 mM glucose, leading to a total of six experimental groups tested together. There was a total of three independent replicates of this design. Independent replicate refers to a unique experimental trial with a separate population of worms.

2.4 RNAi DIC imaging and oocyte quality analysis

A population of *fog-2(q71)* worms was synchronized, followed by the synchronization of a population of N2 worms the following day. The populations were incubated at 20°C for 78 and 48 hours, respectively. After 48 hours, N2 L4 larvae were transferred onto 60 mm Carbenicillin-IPTG-NGM plates consisting of six different conditions, split accordingly based on the plasmid they were plated onto after the hypochlorite synchronization as indicated in Figure 4 (L4440, L4440 with 20 mM glucose, *psme-4*, *psme-4* with 20 mM glucose, *icmt-1*, or *icmt-1* with 20 mM glucose). Fifteen N2 worms were transferred to each 60 mm plate, followed by the addition of 45 *fog-2(q71)* males. The following day, hermaphrodites were transferred to individual 35 mm

plates. Successful mating was determined by the presence of male progeny two to three days after mating.

The worms were left to mate for 48 hours, and then day 2 (D2) hermaphrodites were transferred to new Carbenicillin-IPTG-NGM plates according to their experimental conditions. N2 worms were transferred one final time at the D4 stage to their respective conditions. On D5 of adulthood, worms were mounted on microscope slides with 2% agar pads and anesthetized with 0.4% levamisole. Ten worms were mounted per microscope slide, and approximately 20 worms were imaged per experimental condition, using differential interference contrast (DIC) microscopy on a Nikon Eclipse-Ti2 microscope at 40X magnification.

The images were then blinded and randomized prior to evaluation. Images were scored as either normal, mild, or severe, for phenotypes including being abnormally small, misshapen, unfertilized, or the presence of cavities, as described in Templeman *et al*. (2018). The shape of the oocyte was deemed normal when it appeared cuboidal without visible irregularities. Minor deviations for the cuboidal shape were considered mild, and strong deviations resulting in a non-cuboidal shape were labelled as severe. For the size phenotype, large oocytes were given a normal score, moderately sized oocytes or the presence of a few small oocytes was considered mild, and the oocytes were considered severe when the majority of the oocytes were small. in terms of cavities, oocytes were considered normal when there were no spaces between the oocytes or between the oocytes and the side of the worm. Mild indicated the presence of some small gaps between oocytes or between the oocytes and the side of the worm, and severe indicated minor contact between the oocytes, or between the oocytes and side of the worm.

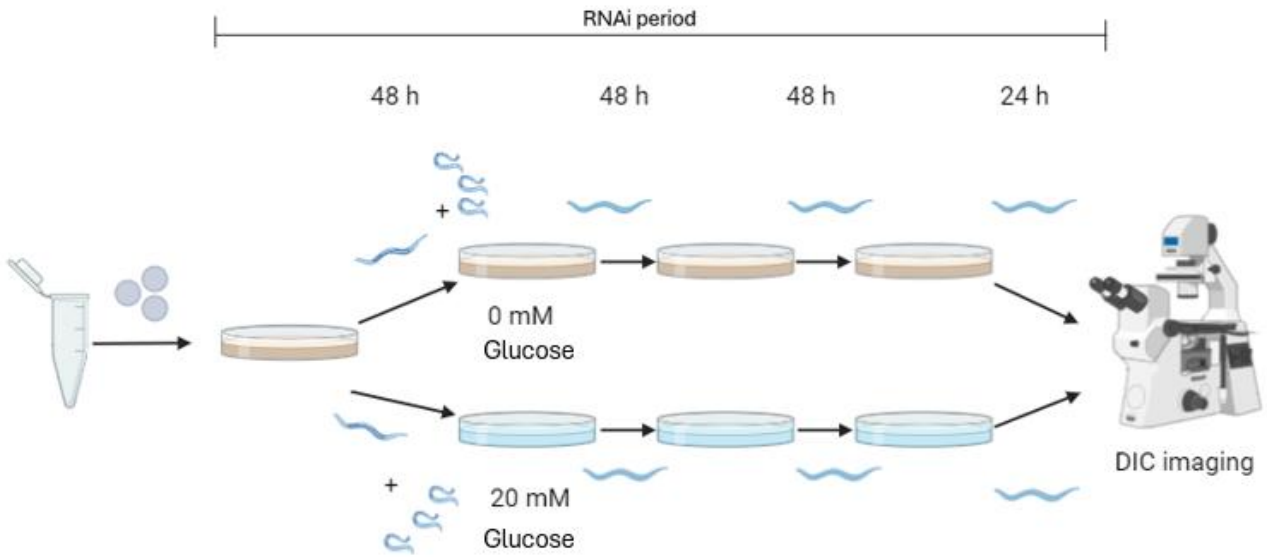

Figure 4. Schematic of the RNAi oocyte quality protocol followed for two conditions. "RNAi period" comprised of three different treatments *(icmt-1* knockdown vs *psme-4* knockdown vs L4440 empty vector control), each of which was exposed to 0 mM glucose or 20 mM glucose, leading to a total of six experimental groups tested together. There was a total of three independent replicates of this design. Independent replicate refers to a unique experimental trial with a separate population of worms.

2.5 Statistical analysis

Statistical analysis was conducted using RStudio version 2023.12.1+402 for the late mating analysis, and GraphPad Prism for the image analysis, and graphs were generated using GraphPad Prism software.

A Cochran-Mantel-Haenszel Test was used to analyze the late mating assay by comparing the percentage of worms able to produce progeny and analyze the significance of the results. The Cochran-Mantel-Haenszel Test was used to compare two-experimental groups and was repeated to compare seven combinations of groups including: vector-control to vector-control with

glucose exposure, vector-control to *icmt-1* knockdown, vector-control to *psme-4* knockdown, vector-control with glucose to *icmt-1* knockdown with glucose, vector-control with glucose to *psme-4* with glucose, *icmt-1* knockdown to *icmt-1* knockdown with glucose, and *psme-4* knockdown to *psme-4* knockdown with glucose. Censored plates were not included in statistical analysis. The Bonferroni correction as then used to account for the multiple replicates and determine significance. The Bonferroni correction works by adjusting the significance level by dividing the initial significance by the number of replicates. This provided an adjusted p-value of 0.0167.

For imaging analysis, a Fisher's Exact Test was used to compare the scores of the experimental and vector-control groups. Seven combinations were compared including: vector-control to vector-control with glucose exposure, vector-control to *icmt-1* knockdown, vector-control to *psme-4* knockdown, vector-control with glucose to *icmt-1* knockdown with glucose, vector-control with glucose to *psme-4* with glucose, *icmt-1* knockdown to *icmt-1* knockdown with glucose, and *psme-4* knockdown to *psme-4* knockdown with glucose. The Bonferroni correction as then used to account for the multiple replicates and determine significance, providing an adjusted p-value of 0.0167.

3 Results

To characterize the impact of the knockdown of nutrient-sensitive genes on the reproductive capacity and oocyte quality of aging *C. elegans*, I performed late mating assays along with the analysis of oocyte quality (Fig. 3 and Fig. 4 respectively). Worms were exposed to knockdown of either *icmt-1* or *psme-4* in response to glucose enrichment or NGM. Through these experiments I aimed to further understand the relationship between nutrition and reproductive success in aging organisms.

3.1 Effects of RNAi and glucose enrichment on reproductive capacity

I hypothesized that *C. elegans* exposed to glucose would have a decreased late-mating capacity compared to those not exposed to glucose. Furthermore, I hypothesized that *C. elegans* with *icmt-1* knockdown under non-glucose conditions would display a reduced reproductive capacity compared to the *L4440* (vector-control) condition. Lastly, I hypothesized that *C. elegans* with *psme-4* knockdown would demonstrate an improved reproductive capacity when exposed to glucose compared to the vector-control group that was exposed to glucose. In support of these hypotheses, I observed these differences consistently across all three biological replicates (Fig. 5).

C. elegans exposed to 20 mM glucose had a significantly decreased reproductive capacity compared to those exposed to 0 mM glucose ($p=0.003403$; Fig. 5.). Populations with *icmt-1* knockdown that were exposed to 0 mM glucose demonstrated a significantly worsened reproductive capacity compared to the vector-control condition that was exposed to 0 mM glucose ($p=0.0000006819$; Fig. 5.). Conversely, *C. elegans* with *icmt-1* knockdown that were exposed to 20 mM glucose, did not demonstrate a significantly different reproductive capacity

compared to those in the vector-control conditions, exposed to 20 mM glucose (p=0.876; Fig. 5.). There was also no significant difference in reproductive capacity between *icmt*-1 RNAi-treated worms exposed to 0 mM versus 20 mM glucose (p=0.1469; Fig. 5.). Worms exposed to 0 mM glucose with *psme-4* knockdown did not demonstrate a significantly different reproductive capacity in comparison to those in the vector-control conditions exposed to 0 mM glucose (p=0.3308; Fig. 5.). However, *C. elegans* exposed to 20 mM glucose with knockdown of psme-*4* demonstrated a significantly improved reproductive capacity compared to those in the vector-control condition exposed to 20 mM glucose (p=0.000000264; Fig. 5.). Lastly, in worms with the *psme-4* knockdown, populations exposed to 0 mM glucose demonstrated a significantly reduced reproductive capacity compared to those exposed to 20 mM glucose (p=0.0007887; Fig. 5.). Overall, this experiment suggests that glucose exposure negatively impacts *C. elegans* reproductive capacity, while *psme-4* knockdown mitigates this effect under high-glucose conditions, and *icmt-1* knockdown impairs reproduction under standard conditions.

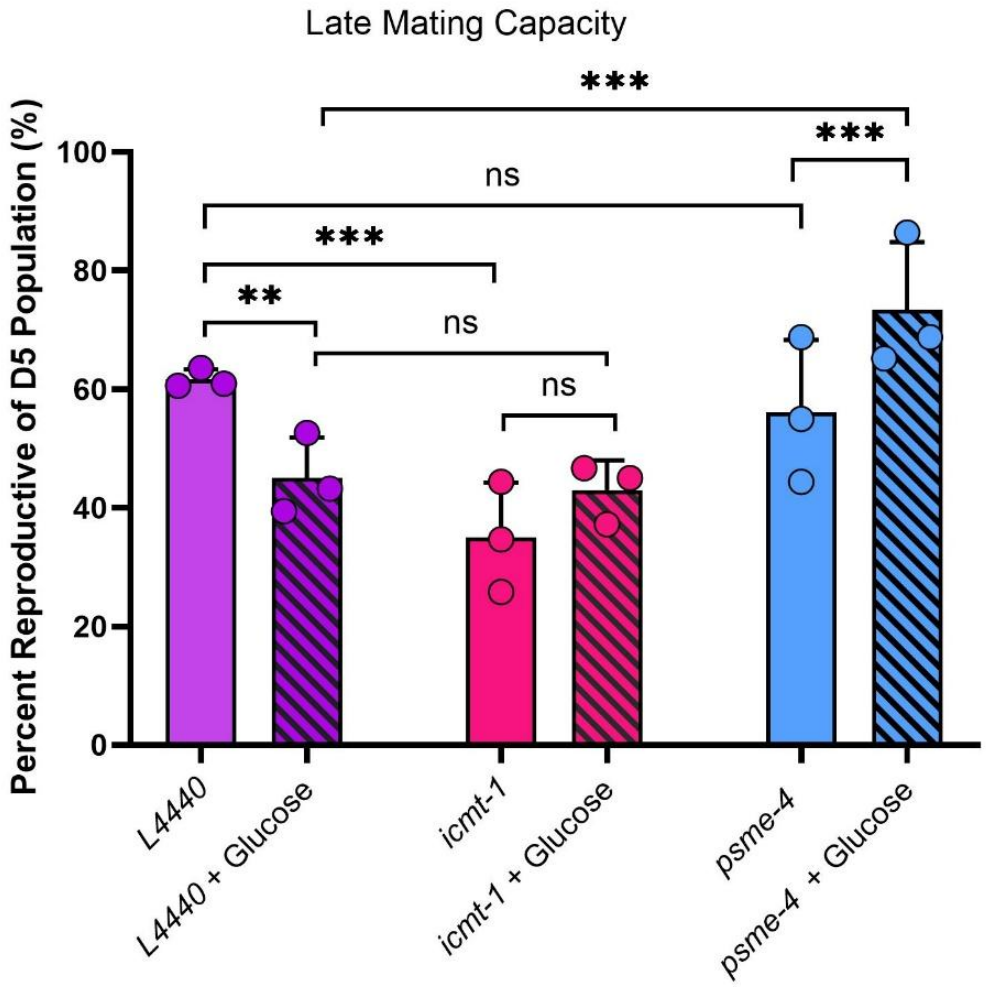

Figure 5. The late mating capacity of day 5 adult hermaphrodites showed a change in reproductive capacity in response to gene knockdown and glucose enrichment. Whole life RNAi exposure was used for this experiment. At the fourth larval stage, worms were split into 0 mM and 20 mM subgroups. At day 5 of adulthood worms were mated with *fog-2(q71)* males. The presence of greater than three males on the plate indicated the worm was reproductive. If more than two hermaphrodites were present, the plate was considered reproductive only if one was clearly the progeny of the experimental worm. If the hermaphrodite was dead or absent, the plate was censored and not included in the statistical analysis. Each point represents the percent reproductive per condition for one replicate. N= 44-77 per group, per replicate. A Cochran-Mantel-Haenszel test was used to determine statistical significance across all biological replicates. A p=value of 0.0167 was considered statistically significant due to a Bonferroni correction, accounting for multiple comparisons. ***$p<0.001$ and **$p<0.01$ as indicated in the comparisons.

3.2 Effects of RNAi and glucose enrichment on oocyte quality

C. elegans oocytes were scored on the severity of three phenotypes including being abnormally small, misshapen, or the presence of cavities (Fig. 6). Consistent with the late-mating capacity results, I hypothesized that *C. elegans* exposed to glucose enrichment would have a worsened oocyte quality compared to *C. elegans* not exposed to glucose enrichment. Additionally, I hypothesized that *C. elegans* with *icmt-1* knockdown would demonstrate worsened oocyte phenotypes compared to the vector-control under non-glucose conditions. Furthermore, I hypothesized to see no significant difference in oocyte quality between *C. elegans* exposed to glucose enrichment in the *icmt-1* knockdown condition and the vector-control glucose conditions. I hypothesized that *C. elegans* with *psme-4* knockdown would not possess any notable changes in oocyte quality compared to the vector-control under non-glucose conditions. Lastly, I hypothesized that compared to the vector-control glucose condition, *C. elegans* exposed to glucose enrichment with the *psme-4* knockdown would have improved oocyte quality.

I performed this experiment in two biological replicates (Fig. 6 for representative images, Fig. 7 for replicate one quantification, Fig. 8 for replicate two quantification). In the second of two replicates, *C. elegans* exposed to 20 mM glucose demonstrated worsened oocyte morphology as depicted by an increase in small oocytes, misshapen oocytes, and cavities ($p<0.0001$, $p=0.0154$, $p=0.028$ respectively; Fig. 8). These observations were expected based on previous literature outlining the detrimental effect of glucose enrichment on oocyte morphology (Athar, unpublished observations). This trend was observed across both replicates for the phenotype of oocyte size ($p=0.0078$; Fig. 7). In terms of shape, worms exposed to 20 mM glucose demonstrated more misshapen oocytes, however, this trend was not statistically

significant in the first replicate (p=0.0591; Fig. 7). As for increased presence of cavities, the first replicate did not demonstrate the same trend seen in the second replicate (p=0.3254).

Contrary to late-mating results, *icmt-1* knockdown did not consistently worsen oocyte quality under control dietary conditions. Worms with *icmt-1* knockdown appear to have worsened oocyte morphology compared to the vector-control exposed to 0 mM glucose, as indicated by the increased presence of small oocytes (Fig. 8: p=0.0130). However, this trend was not consistent among replicates (Fig. 7: p=0.0078). Under control dietary conditions, *icmt-1* knockdown did not consistently show a difference in shape (Fig. 7: p=0.1707; Fig. 8: p=0.0332) and cavities (Fig. 7: p=0.1707; Fig. 8: p=0.0332) compared to the vector-control. Between *icmt-1* knockdown and vector-control worms both exposed to 20 mM glucose, there was consistently no difference in the presence of small oocytes (Fig. 7: 0.1638; Fig. 8: p=0.8099). Conversely, there was a consistent significant difference in shape and cavities between *icmt-1* knockdown and vector-control worms exposed to 20 mM glucose, with the exception of shape in replicate two still trending towards significance (Fig. 7: p=0.0087 and p=0.0083; Fig. 8: p=0.0623 and p<0.0001 respectively). In replicate one *icmt-1* knockdown with 20 mM glucose exposure exhibited worsened oocyte quality in terms of size and cavities compared to *icmt-1* knockdown with 0 mM glucose (p<0.0001 and p<0.0001 respectively; Fig. 7). No difference was observed for shape (p=0.2255; Fig. 7). These results were not consistent among replicates (Size: p=0.5452, Shape: p=0.0161, Cavities: p=0.0003; Fig. 8). Overall, *icmt-1* knockdown largely resulted in inconsistent oocyte phenotypes.

Similarly, some of the oocyte quality results from the *psme-4* knockdown conditions also demonstrated inconsistencies. Results from replicate one showed no significant difference between worms with *psme-4* knockdown and the vector-control, both exposed to 0 mM glucose,

for size, shape, or the presence of cavities (p=0.6798, p=0.4588, and p=0.3272 respectively; Fig. 7). This was inconsistent, with replicate two demonstrating a significant difference between all three phenotypes when compared to the vector-control (Fig. 8: size: p=0.0052, shape: p=0.0001, cavities: p=0.0205). In glucose-enriched conditions, *psme-4* knockdown improves all parameters of oocyte quality consistently across both replicates, with the exception of incidence of cavities in replicate two. (Fig. 7: $p<0.0001$ for all; Fig. 8: size: p=0.0036, shape: p=0.0010, cavities: p=0.1758). Between worms with *psme-4* knockdown, populations exposed to 20 mM glucose demonstrated significantly improved phenotypes for size, shape, and the presence of cavities compared to 0 mM glucose exposure, as indicated in replicate one (p=0.0035, $p<0.0001$, and $p<0.0001$ respectively; Fig. 7). Results from the second replicate demonstrate inconsistencies compared to the first replicate. When comparing worms with *psme-4* knockdown exposed to 0 mM and 20 mM glucose in the second replicate, those exposed to 0 mM glucose exhibit significantly fewer small oocytes (p=0.0006; Fig. 8). Conversely, those exposed to glucose enrichment exhibit fewer severely misshapen oocytes (p=0.0011; Fig. 8). When comparing the presence of cavities between these two conditions, no significant difference was observed (p=0.1313; Fig. 8). Overall, *psme-4* knockdown also resulted in many inconsistent oocyte phenotypes, with the exception of *psme-4* knockdown compared to the vector-control on glucose enrichment improving oocyte quality.

In summary, these findings suggest that *icmt-1* is essential for reproductive function under normal conditions, and its knockdown may decrease reproductive function due to system effects beyond oocyte quality. Furthermore, while *psme-4* contributes to glucose-induced reproductive decline, its knockdown may improve reproductive capacity potentially by improving oocyte quality.

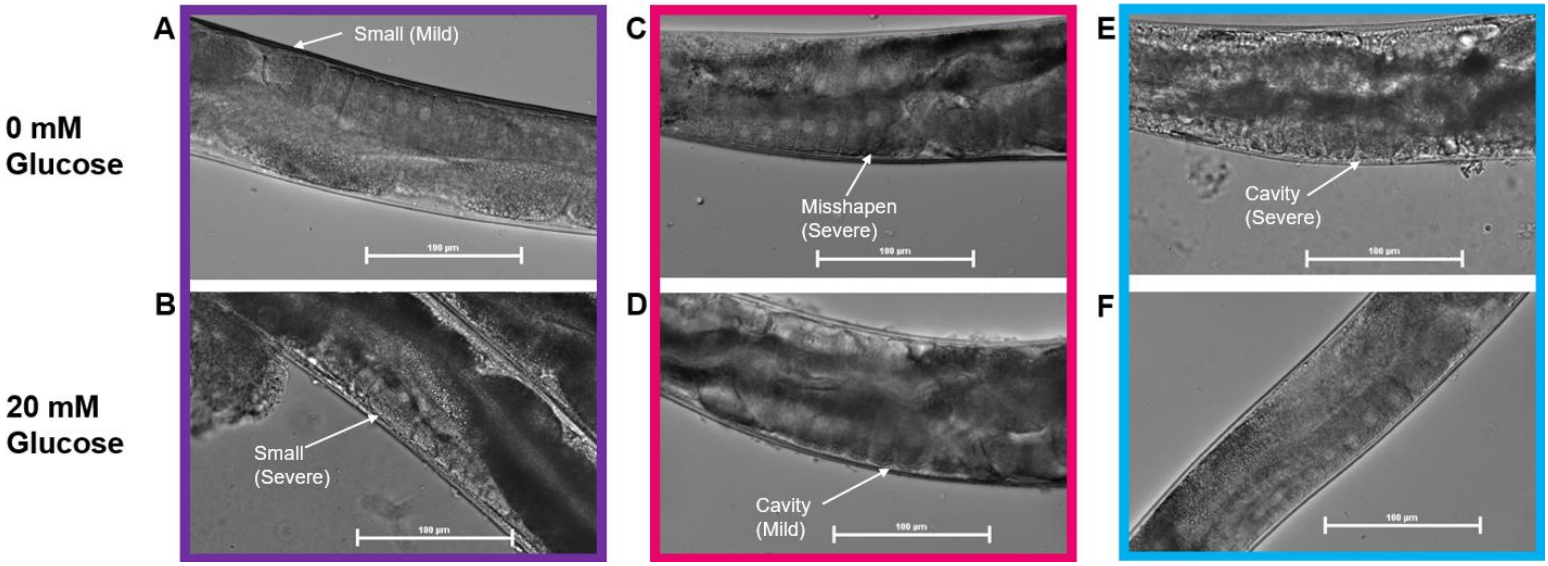

Figure 6. Representative images of mated day 5 hermaphrodites taken using DIC imaging. Whole life RNAi exposure was used for this experiment. At the fourth larval stage, worms were split into 0 mM and 20 mM subgroups and mated with *fog-2(q71)* males. Worms were considered reproductive based on the presence of male progeny on the plate. Hermaphrodites were mounted onto 2% agar pads and anesthetized using 0.4% levamisole. Imaging was conducted using a Nikon Eclipse-Ti2 Camera at 40X magnification. **A)** Representative image of day 5 vector-control worm on 0 mM glucose. **B)** Representative image of day 5 vector-control worm on 20 mM glucose. **C)** Representative image of day 5 *icmt-1* knockdown worm on 0 mM glucose. **D)** Representative image of day 5 *icmt-1* knockdown worm on 20 mM glucose. **E)** Representative image of day 5 *psme-4* knockdown worm on 0 mM glucose. **F)** Representative image of day 5 *psme-4* knockdown worm on 20 mM glucose.

Replicate 1

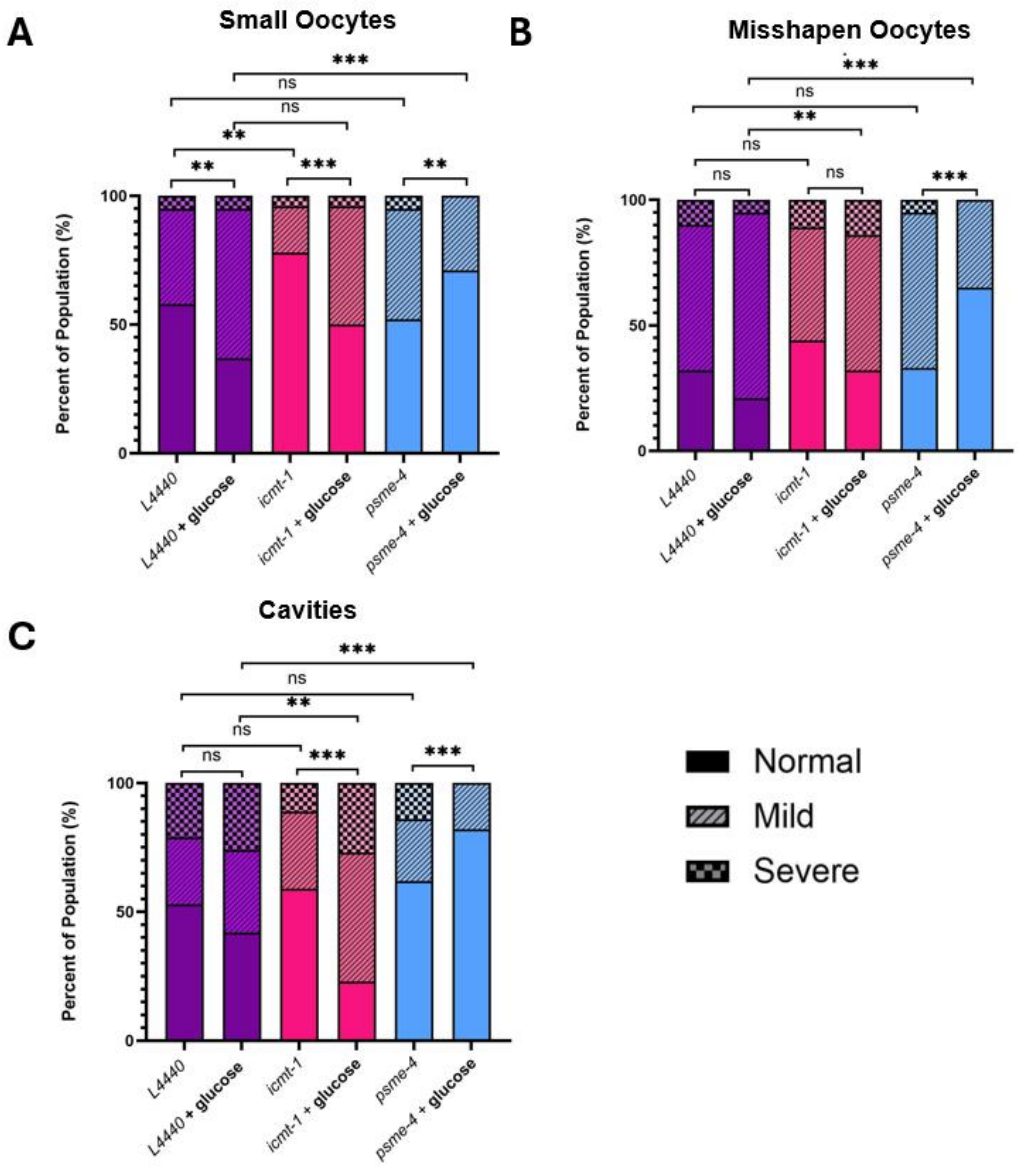

Figure 7. Analysis of oocyte quality of mated day 5 hermaphrodites. Results are from the first biological replicate. Whole life RNAi exposure was used for this experiment. At the fourth larval stage, worms were split into 0 mM and 20 mM subgroups and mated with *fog-2(q71)* males. Worms were considered reproductive based on the presence of male progeny on the plate. Hermaphrodites were mounted onto 2% agar pads and anesthetized using 0.4% levamisole. Imaging was conducted using a Nikon Eclipse-Ti2 Camera at 40X magnification. Oocyte quality was scored based the size, irregularity of shape, and presence of cavities. The degree of severity is indicated based on the variation of shading. **A)** Presence of small oocytes. **B)** The irregularity of shape. **C)** the presence of cavities. Fisher's Exact Test comparisons were performed for all phenotypes. ***$p<0.001$ and **$p<0.01$.

Replicate 2

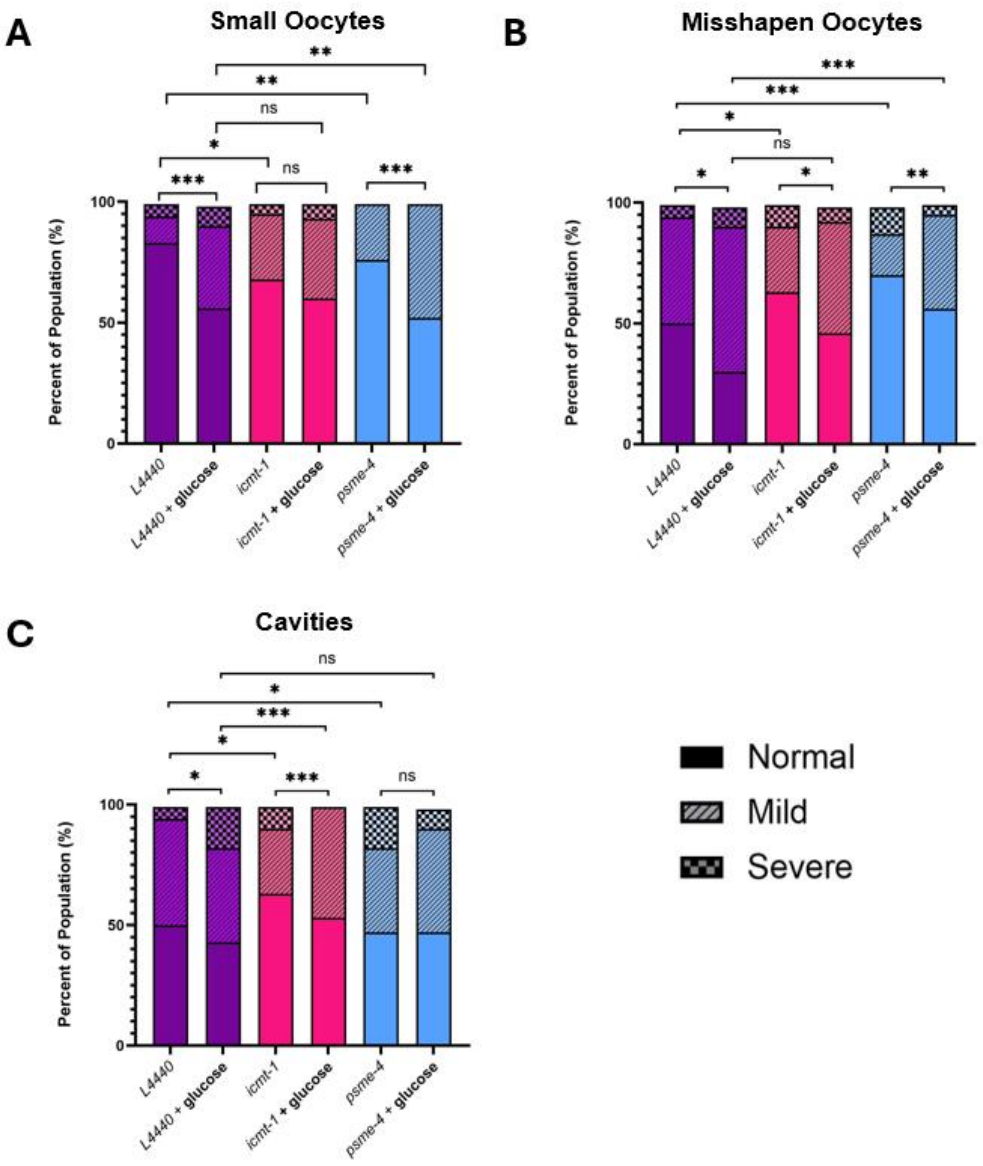

Figure 8. Analysis of oocyte quality of mated day 5 hermaphrodites. Results are from the second biological replicate. Whole life RNAi exposure was used for this experiment. At the fourth larval stage, worms were split into 0 mM and 20 mM subgroups and mated with *fog-2(q71)* males. Worms were considered reproductive based on the presence of male progeny on the plate. Hermaphrodites were mounted onto 2% agar pads and anesthetized using 0.4% levamisole. Imaging was conducted using a Nikon Eclipse-Ti2 Camera at 40X magnification. Oocyte quality was scored based the size, irregularity of shape, and presence of cavities. The degree of severity is indicated based on the variation of shading. **A)** Presence of small oocytes. **B)** The irregularity of shape. **C)** the presence of cavities. Fisher's Exact Test comparisons were performed for all phenotypes. ***p<0.001, **p<0.01, and *p<0.05.

4 Discussion

Reproductive aging is a cumulation of many complex processes, influences by genetic, metabolic, and environmental factors. Understanding the mechanisms that regulate reproductive capacity and oocyte quality is crucial, given the increasing trend of delayed childbearing (Zabak *et al.,* 2023), Western diet consumption (Rakhara *et al.,* 2020), and rising prevalence of metabolic disorders (Rakhara *et al.,* 2020) in human populations. Similarly, dietary factors, particularly high-glucose intake, have been linked to reproductive dysfunction in both mammals and invertebrate models (Mondoux *et al.,* 2011; Sinclair and Watkins, 2013). To further investigate the interconnected relationship between diet and reproductive capacity in aging individuals, I exposed vector-control, *icmt-1*-knockdown, and *psme-4* knockdown worms to glucose enrichment and control dietary conditions. I found that glucose enrichment is associated with worsened reproductive capacity, in line with previous literature suggesting the detrimental role of prolonged glucose enrichment on reproductive function (Wang *et al.,* 2020; Mondoux *et al.,* 2011; Athar, unpublished observations). Furthermore, I found that knockdown of *icmt-1* under control dietary conditions resulted in a decreased reproductive capacity. Conversely, under glucose-enriched conditions, those with *psme-4* knockdown revealed an improved reproductive capacity. These results indicate that normal expression of genes whose transcription is regulated in a nutrient-sensitive manner, such and *icmt-1* and *psme-4,* may play a role in preserving reproductive capacity. This study demonstrated a trend in decreased oocyte quality when exposed to elevated glucose levels. Conversely, a consistent trend was not observed when examining the results of *icmt-1* knockdown on oocyte morphology. Interestingly, results from this study indicate a trend in improved oocyte quality in worms with *psme-4* knockdown under elevated glucose conditions, however, this trend was not observed consistently when examining oocyte size.

Results from this study suggest that while expression of these nutrient-sensitive genes may play a role in reproductive capacity, it may not necessarily be due to their influence on oocyte quality.

4.1 Glucose enrichment worsens reproductive capacity and oocyte quality

The results of this study demonstrate that glucose enrichment significantly impacts reproductive capacity and oocyte quality in *C. elegans* as indicated by a reduced ability to produce progeny in late-mating assays, and worsened phenotypes in oocyte analysis. These results are consistent with previous studies demonstrating that glucose exposure was associated with a decline in reproductive success (Athar, unpublished observations; Wang *et al.,* 2020). This decline may be attributed to glucose-induced metabolic stress, which can lead to mitochondrial dysfunction, oxidative stress, and disruptions in signaling pathways essential for oocyte development. High glucose levels have been shown to affect insulin signaling, a key regulator of reproductive longevity in *C. elegans* (Lee *et al.,* 2009). Excess glucose may accelerate age-related decline in germ cell quality by interfering with these pathways, ultimately reducing fecundity.

Another potential explanation for this reduced reproductive capacity is a glucose-induced decrease in oocyte quality. High quality oocytes are cuboidal, connected to neighbouring oocytes, and large. With age, these qualities begin to diminish, resulting in small and misshapen oocytes, with cavities separating neighbouring oocytes. Similarly, previous studies have observed that worms exposed to elevated glucose levels tend to have decreased oocyte quality. Previous studies have demonstrated that small oocytes in aged *C. elegans* may be indicative of a reduced reproductive capacity and decreased oocyte quality (Andux and Ellis, 2008; Luo *et al.,* 2010). It is thought that small oocytes may be associated with decreased fertility because they

may lack adequate resources required to sustain embryogenesis (Andux and Ellis, 2008). Cavities between neighbouring oocytes and their surrounding somatic tissue are also associated with worsened oocyte quality. It is proposed that this may be due to a disruption the in molecular uptake and signal transduction required for proper oocyte development, as seen in younger individuals (Luo and Murphy, 2010). This is consistent with the result of my study demonstrating that glucose negatively influenced oocyte morphology. This was demonstrated through vector-control worms exposed to high-glucose conditions exhibiting smaller, misshapen oocytes with increased cavities between oocytes. Insulin signaling disruptions caused by prolonged glucose exposure may impair oocyte maturation and reduce overall reproductive capacity (Alcántar-Fernández *et al.,* 2019). In *C. elegans,* glucose-induced metabolic alteration may also lead to an accumulation of reactive oxygen species by overwhelming cellular antioxidant defenses and increasing oxidative damage, which impairs structural integrity and the developmental potential of oocytes (Volk *et al.,* 2017). Similarly, in mammals hyperglycemia has been linked to many negative health outcomes including insulin resistance and disruptions in metabolic homeostasis, both of which negatively impact oocyte quality, decreasing an individual's capacity to reproduce effectively (Volk *et al.,* 2017).

Oocyte morphology has been previously established as a predictor of oocyte quality and is consistently used as a proxy for reproductive capacity; however, I did not directly measure the functional viability of these oocytes. Other *C. elegans* studies have assessed oocyte quality through additional functional assays, such as cytoplasmic texture to visually assess the internal oocyte status or quantifying the number of unhatched eggs as an indicator or embryonic lethality (Imakubo *et al.,* 2021). Incorporating such direct analyses in future research would provide a

more comprehensive understanding of how glucose affects not only the structural characteristics of oocytes, but also their ability to support embryonic development.

The significant decline in reproductive capacity, along with the increased incidence of cavities and small, misshapen oocytes in *C. elegans* exposed to glucose enrichment indicates that a diet consisting of elevated glucose levels may accelerate reproductive decline by exacerbating oocyte quality deterioration. This highlights the importance of metabolic regulation in reproductive health and suggest that dietary interventions could play a role in mitigating fertility decline associated with glucose-induced metabolic stress.

4.2 Nutrient-sensitive genes transcripts influence reproductive ability

4.2.1 icmt-1

To examine the relationship between nutrient sensitive genes and reproductive aging, I performed RNAi on two genes seen to be differentially expressed in *C. elegans* oocytes under glucose enrichment, *icmt-1* and *psme-4*. Genes whose transcription is regulated in a nutrient-sensitive manner play a critical role the regulation of metabolism, stress responses, and reproductive function. While these genes are not necessarily established components of the insulin/IIS and mTOR signaling pathways, their regulation may interact with these pathways influencing how organisms adapt to fluctuating energy availability (Templeman and Murphy, 2018). In *C. elegans,* genes involved in nutrient sensing are tightly linked to oocyte quality and reproductive longevity, as they regulate mitochondrial function, proteostasis, and cellular repair mechanisms necessary for maintaining germline integrity (Alcántar-Fernández *et al.,* 2019; Molière *et al.,* 2024).

The RNAi knockdown of *icmt-1* caused a significant reduction in reproductive success in reproductively aged worms. This result was observed when comparing *icmt-1* knockdown to the vector-control on 0 mM glucose (Fig. 5). This observation is in line with my hypothesis, proposing that *icmt-1* may function to protect reproductive function and, therefore, a loss of this protection may worsen reproductive success. One potential reason for this could be due to oocyte quality, however, *icmt-1* knockdown was not consistently associated with a decrease in any of the three oocyte phenotypes (Fig. 7; Fig. 8). The imaging results indicate that the observed decrease in late-mating capacity in worms with *icmt-1* knockdown is likely not due to changes in oocyte quality, and potentially due to its impact on other bodily functions.

A plausible explanation for this outcome could involve systemic effects beyond the reproductive system, due to whole-body RNAi effects, rather than targeting RNAi effects to the reproductive system. To control for this, tissue specific RNAi mutants could be used to isolate the RNAi to the germline. Since *icmt-1* is involved in post-translational protein modification and regulation of apoptotic pathways, its knockdown may disrupt cellular processes in somatic tissues (Kramer *et al.,* 2003). *icmt-1* facilitates proper localization and activation of Ras proteins, which are critical regulators of cell growth, proliferation, and survival (Kramer *et al.,* 2003). Without sufficient *icmt-1* activity, Ras proteins may mislocalize, leading to impaired cellular signaling and increase apoptotic pathway (Kramer *et al.,* 2003). In somatic tissues, this could manifest as widespread cellular stress, tissue dysfunction, or premature aging, which could divert energy and resources away from reproductive processes (Kramer *et al.,* 2003). Additionally, *icmt-1* knockdown may influence endocrine signalling pathways that regulate reproductive timing and internal gamete quality unobservable through DIC imaging and the phenotypes assessed, further contributing to the observed decline in reproductive success (He *et al.,* 2021).

Ras signaling is known to influence endocrine pathways, including insulin/IIS pathways, which regulate metabolism, stress responses, and reproductive timing (He *et al.,* 2021; Templeman and Murphy, 2018). Disruption of these pathways could lead to hormonal imbalances that negatively impact reproductive health (Templeman and Murphy, 2018). Furthermore, hormonal dysregulation could impair communication between tissues, further contributing to reduced reproductive success (He *et al.,* 2021). Notably, the absence of further reproductive decline in *icmt-1* knockdown worms exposed to glucose may be due to this gene is already severely downregulated under glucose enriched conditions, rendering additional knockdown ineffective.

To determine if the changes in late-mating capacity are due to decreased Ras activity, loss-of-function experiments targeting Ras could be performed. These experiments would involve comparing control worms, *icmt-1* knockdown alone, Ras loss-of-function alone, and the combined *icmt-1* knockdown with Ras loss-of-function. If the reproductive defect caused by *icmt-1* knockdown is primarily due to impaired Ras signaling, then the combined knockdown should not show a greater effect than either knockdown or loss-of-function alone. In other words, if Ras is the main pathway mediating the reproductive phenotype, the double knockdown would resemble the single knockdowns, indicating they act in the same pathway. ROS accumulation in the oocyte is another possible explanation for the reproductive phenotype observed. To test this hypothesis, worms could be stained with a ROS-sensitive fluorescent dye. Their gonad could then be imaged to check for increased fluorescence in oocytes, which would indicate higher ROS levels, and increased cellular stress (Maremonti *et al.,* 2019).

4.2.2 psme-4

Results from this study demonstrate that *psme-4* knockdown improved reproductive success during aging under glucose enriched conditions. The observed improvement in reproductive success upon *psme-4* knockdown suggests that reducing proteasomal activity may mitigate the glucose-induced acceleration in reproductive decline. *psme-4* is a proteasome activator that enhances protein degradation, particularly under stress conditions (Ge *et al.,* 2022). This hypothesis is in line with results from Anderson *et al.* (2022), demonstrating that proteasomal overactivity resulted in a severe decrease in fecundity in *C. elegans.* During glucose enrichment, increased proteasomal activity may accelerate the breakdown of key regulatory proteins involved in maintaining oocyte quality and reproductive function (VerPlank and Goldberg, 2017). By reducing *psme-4* levels, the excessive degradation of these proteins could be limited, preserving cellular homeostasis and improving reproductive outcomes.

Moreover, I found that *psme-4* knockdown did not significantly affect reproductive capacity under normal dietary conditions, indicating that its glucose-induces upregulation may play a role in the observed phenotypic defects normally seen under glucose enrichment. In the absence of metabolic stress, baseline proteasomal activity may be sufficient to maintain cellular proteostasis without causing harm to reproductive tissues (Karabinova *et al.,* 2011). However, under glucose-enriched conditions, elevated *psme-4* expression could contribute to excessive protein degradation (Kodroń *et al*., 2020). Proteasomal overactivation may lead to degradation of proteins necessary for oocyte development and maturation, contributing to the worsened oocyte quality observed under vector-control glucose enriched conditions (Karabinova *et al.,* 2011). Therefore, the protective effect of *psme-4* knockdown in glucose-exposed worms may reflect a restoration of balance in proteasomal activity, preventing further reproductive decline.

To test for proteasomal activity, a fluorescence approach could be used where the rate of fluorescence over time is measured in control and *psme-4* knockdown worms in standard and glucose enriched conditions (Anderson *et al.,* 2022). Proteasomal activity would be indicated by increased fluorescence, as a caged reporter becomes detectable through cleavage (Anderson *et al.,* 2022). In parallel, a Western blot could be performed to assess if *psme-4* knockdown prevents degradation of specific proteins involved in germline signaling pathways (Anderson *et al.,* 2022). Protein levels could be compared between control and *psme-4* knockdown worms under standard and glucose enriched conditions.

It is also worth noting that during the course of the late-mating experiments, I observed potential changes in growth development that could be relevant. Specifically, both *psme-4* and *icmt-1* knockdown worms appeared to take longer to reach the L4 stage, compared to control worms. Although this trend was not statistically assessed for significance, it suggests a possible delay in development. Further studies would be required to explore whether these developmental delays are consistent and statistically significant, and how they might relate to the observed reproductive phenotypes.

In summary, this study demonstrates the distinct roles and potential mechanisms of *icmt-1* and *psme-4* in regulating reproductive success under glucose-enriched conditions. While *icmt-1* knockdown exacerbates reproductive decline by potentially disrupting systems cellular processes, *psme-4* knockdown mitigates the detrimental effects of glucose exposure, suggesting a protective role in maintaining reproductive function.

4.3 Experimental limitations and prospective research avenues

This study demonstrated significant trends with respects to late-mating capacity and oocyte quality, however, improvements could be made to further validate these findings. First, while RNAi-mediated knockdown is a powerful tool for investigating gene function, it may not completely eliminate gene expression. Residual gene activity may persist, leading to partial phenotypes that may not fully represent the consequences of complete gene loss. This limitation could mask the full extent of *icmt-1* and *psme-4* involvement in reproductive aging. Techniques such as reverse transcription polymerase chain reaction could be used to measure the amount of mRNA transcripts present after RNAi treatment, to gauge the extent of gene silencing. The timing of RNAi application also presents a consideration. In this study, RNAi exposure occurred throughout the worm's lifespan. While this approach captures long-term effects, it may inadvertently influence other physiological systems. Implementing stage-specific knockdown, particularly at the fourth larval stage, could minimize off-target effects and provide a clearer view of how these genes impact reproductive aging. Alternatively, tissue-specific RNAi could be implemented to target knockdown to the reproductive system, as mentioned previously. This is done using mutant worms that only possess the molecular machinery for RNAi-mediated gene knockdown in specific tissues, under the control of a tissue-specific promoter in otherwise RNAi-deficient mutant worms (Watts *et al.,* 2020)

Moreover, this study used oocyte phenotyping as a proxy for oocyte quality. While informative, further exploration of cytoplasmic texture and embryonic lethality could provide deeper insights into the functionality of these oocytes, rather than the morphology alone. Alterations in cytoplasmic organization may indicate disruptions in oocyte maturation or defective meiotic processes, which may not be apparent from external phenotype alone.

Investigating embryonic lethality rates could also reveal the direct influence of glucose on progeny viability. For example, a higher proportion of unhatched embryos from worms exposed to elevated glucose would indicate poorer oocyte quality in this condition. Sample size constraints may have also influenced the imaging-based assessments. Only two successful replicates were included in this study resulting in a relatively low sample size per condition. This means that the image analysis had relatively low statistical power. Increasing the sample size would enhance the statistical power and provide more robust conclusions.

While *C. elegans* serve as a valuable model for studying conserved pathways, their physiological differences from mammals limit direct translation to human biology. To address this, future research could explore *icmt-1* and *psme-4* homologs in mammalian models. It would be important to first investigate whether *imct-1* and *psme-4* homologs are expressed in the mammalian reproductive system, and if their expression is altered in response to changes in metabolic state. If expression is present, the roles of these genes could be further investigated in terms of their influence on oocyte morphology and capacity to produce viable progeny. If their expression is influenced by changes in metabolic state, these observations could also be made under altered dietary conditions. Investigating their roles in reproductive aging using mice or other mammalian systems would provide crucial insights into the applicability of these findings to human fertility.

4.4 Conclusions

Previous findings have established that a glucose-enriched diet negatively affects reproductive capacity and oocyte quality in *C. elegans,* supporting the concept that nutrient availability is a key regulator of reproductive health. This research highlights the negative effects

of glucose enrichment on reproductive capacity and oocyte quality in *C. elegans* and provides evidence that nutrient-sensitive genes play a crucial role in modulating these effects. These findings suggest that *icmt-1* is essential for maintaining oocyte integrity under normal conditions, while *psme-4* contributes to glucose-induced reproductive decline.

By elucidating the molecular mechanisms linking diet, metabolism, and fertility, this research provides valuable insights into reproductive aging and offers potential targets for future research investigating the application of these results across species. Furthermore, these findings contribute to the growing body of evidence supporting the importance of diet in reproductive health. In a broader context, this research underscores the relevance of using *C. elegans* as a model organism to study conserved genetic pathways affecting reproductive health. While direct translation to humans requires further validation, these insights may ultimately inspire research into its applicability in more complex organisms. Lastly, in light of the increasing trend of delayed childbearing, understanding the molecular underpinnings of reproductive decline is particularly relevant. By contributing to this field, this research supports efforts to develop interventions that preserve fertility, enhance reproductive autonomy, and provide individuals with greater agency in family planning decisions.

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