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Timeless or tainted? The effects of male ageing on seminal fluid

Claudia Fricke[1*†], Krish Sanghvi[2†,] Mischa Emery[3], Ina Lindenbaum[1], Stuart Wigby[3], Steven A. Ramm[4,5††], Irem Sepil[2††]

[†]shared first authors, [††]shared senior authors

Affiliations:

[1]Institute for Zoology, Halle University, Halle (Saale), Germany

[2]Department of Biology, University of Oxford, Oxford, United Kingdom

[3]Department of Ecology, Evolution and Behaviour, University of Liverpool, Liverpool, United Kingdom

[4]Evolutionary Biology, Bielefeld University, Bielefeld, Germany

[5]UMR 6553 ECOBIO, Université de Rennes 1, France

*Corresponding author: Claudia Fricke

e-mail: Claudia.Fricke@zoologie.uni-halle.de

Abstract

Reproductive ageing can occur due to the deterioration of both the soma and germline. In males, it has mostly been studied with respect to age-related changes in sperm. However, the somatic component of the ejaculate, seminal fluid, is also essential for maintaining reproductive function. Whilst we know that seminal fluid proteins (SFPs) are required for male reproductive success across diverse taxa, age-related changes in SFP quantity and composition are little understood. Additionally, only few studies have explored the reproductive ageing of the tissues that produce SFPs, and the resulting reproductive outcomes. Here we provide a systematic review of studies addressing how advancing male age affects the production and properties of seminal fluid, in particular SFPs and oxidative stress, highlighting many open questions and generating new hypotheses for further research. We additionally discuss how declines in function of different components of seminal fluid, such as SFPs and antioxidants, could contribute to age-related loss of reproductive ability. Overall, we find evidence that ageing results in increased oxidative stress in seminal fluid and a decrease in the abundance of various SFPs. These results suggest that seminal fluid contributes towards important age-related changes influencing male reproduction. Thus, it is essential to study this mostly ignored component of the ejaculate, seminal fluid, to understand male reproductive ageing, and its consequences for sexual selection and paternal age effects on offspring.

Keywords: senescence, reproduction, ejaculates, seminal fluid, germline, oxidative damage, seminal fluid proteins

Introduction

Ageing is the time-dependent decline of an organism's biological function (Monaghan et al., 2008), leading to reduced physiological abilities and ultimately death. Ageing results in numerous biological changes that include telomere shortening, accumulation of somatic mutations, loss of proteostasis, mitochondrial dysfunction, and disruption of nutrient sensing pathways (Charlesworth, 1993; Kirkwood, 2005; López-Otín et al., 2013). Organisms also tend to have a lower reproductive output at older compared to younger ages. However, the onset and rate of decline in female fertility varies considerably across taxa, depending on life-history strategies and ecologies of species (e.g. Lemaître et al., 2020b; Campos et al., 2022), making it difficult to generalise patterns of ageing across the tree of life (Jones et al., 2014).

Onset and rate of age-related biological decline and impaired reproductive function varies between males and females (Bronikowski et al., 2022). There has been a long-standing focus on females in life-history research, and studies have only recently begun to consider male reproductive ageing (e.g. Fricke and Koppik, 2019; Comizzoli and Ottinger, 2021; Archer et al., 2022). Evidence suggests that male reproductive ageing can affect male fertilising ability (Paul and Robaire, 2013; Aich et al., 2021), influence female behavior (Dean et al., 2010; Vuarin et al., 2019), and lead to paternal effects on offspring (Daxinger and Whitelaw, 2012). Male houbara bustards (*Chlamydotis undulata*), for example, produce fewer progeny as they age, and sons of old fathers have greatly reduced sperm numbers (Vuarin et al., 2019, 2021). Other studies show that male ageing can lead to lower sperm quality (Gasparini et al., 2010, 2014; Velando et al., 2011; Cornwallis et al., 2014; Selvaratnam and Robaire, 2016; Monaghan and Metcalfe, 2019; Vega-Trejo et al., 2019; Turnell and Reinhardt, 2020), and quantity (Johnson et al., 2015; Sepil et al., 2020). Additionally, sperm from older males have lower success in sperm competition and fertilise fewer eggs than sperm from younger males, as seen in guppies (*Poecilia reticulata*, Gasparini

et al., 2019), zebra fish (*Danio rerio*, Kanuga et al., 2011) and crickets (*Acheta domesticus*, Reinhardt and Siva-Jothy, 2005). Sperm ageing can also affect the quality of offspring (Gasparini et al., 2017), characterised by offspring lifespan (Xie et al., 2018; Wylde et al., 2019), telomere length (Bouwhuis et al., 2018; Noguera et al., 2018; Bauch et al., 2019), development (Preston et al., 2015), reproduction (Bouwhuis et al., 2015; Vuarin et al., 2021), and viability (Tan et al., 2013). While most studies on male ageing have focused on sperm traits, only few have tested for changes in the quality and quantity of seminal fluid with age, and its resultant fitness outcomes. Therefore, whether the reported effects of male ageing are actually driven by changes in seminal fluid rather than just sperm are yet unknown.

Ejaculated sperm are usually surrounded by a cocktail of substances collectively called the seminal fluid (Poiani, 2006; Hopkins et al., 2017). These consist of somatic cells such as immune cells; macromolecules such as carbohydrates, vitamins, minerals; hormones; and seminal fluid proteins (SFPs). Seminal fluid (SF) in most species is made in specialised accessory reproductive cells, tissues, or glands, such as the prostate, seminal vesicle, bulbourethral, and ampullary glands in humans (McGraw et al., 2015). SFPs have been shown to be especially crucial in male and female reproduction; they belong to a range of molecular classes such as antioxidants, lipases, lectins, proteases, and protease inhibitors and have been shown to have a diverse set of functions (Chapman, 2001; Avila et al., 2011; Perry et al., 2013; Ramm, 2020). For instance, SFPs facilitate normal sperm function (Wolfner, 1997), aid sperm storage and male sperm competitiveness (Fiumera et al., 2005, 2007; Goenaga et al., 2015; Patlar et al., 2020), maintain sperm viability (den Boer et al., 2008, 2009; King et al., 2011) and regulate sperm capacitation (Manjunath and Thérien, 2002). But SFPs can also act on attributes beyond sperm, for example, by affecting female reproductive behavior (Bath et al., 2017; Chapman et al., 2003; Liu and Kubli, 2003). Indeed, seminal fluid has been shown to affect female immunity modulation (Short and Lazzaro, 2010),

investment in the mating partner's male function in hermaphrodites (Nakadera et al., 2014), female egg-laying behaviour (Chapman et al., 2003; Liu and Kubli, 2003), and mating plug formation to prevent female re-mating (Stockley et al., 2020).

The germline is predicted to receive higher protection from somatic ageing (Maklakov and Immler, 2016). But seminal fluid is produced by somatic tissue and directly interacts with germ cells, thus could play an important role in facilitating interactions between somatic cells and the germline. This could have effects across the Weismann barrier (i.e. despite the germline and somatic tissue being separated early in development, changes in the soma could affect the germline or the next generation) (Sciamanna et al., 2019; Bline et al., 2020). Knowing how seminal fluid changes with age and how this can influence sperm, offspring, and female physiological and behavioural responses to mating, in addition to understanding age-related changes in sperm, is essential to gain a complete picture of male reproductive ageing. Here, we first conduct a systematic review on how advancing male age influences the non-sperm components of the ejaculate (i.e. seminal fluid) across animals, and then discuss the impacts this might have on age-specific reproductive success. While the effects of male ageing on sperm have been reviewed elsewhere (e.g. Reinhardt, 2007; Pizzari et al., 2008; Monaghan and Metcalfe, 2019), to our knowledge, this is the first systematic review of how advancing male age affects seminal fluid. As studies differ greatly in their biological and methodological factors, which can modulate or confound male ageing effects, we discuss the possible influence on the conclusions that are reached.

Systematic review

Literature search and data collection

To understand how male age affects seminal fluid, we conducted a literature search following PRISMA eco-evo guidelines (O'Dea et al., 2021). We used a search string for abstracts, titles, and keywords "*(sfp* OR seminal fluid OR seminal plasma) AND (ageing OR age OR aging OR senescence)"* to identify studies which test how advancing male age affects seminal fluid, using two search engines: SCOPUS and Web of Science (WoS), on December 14th 2021, accessed through the University of Oxford server. The searches returned a total of 738 hits from WoS (year range: 1991 to 2021) and 620 from SCOPUS (year range: 1941 to 2021). After duplicate deletion, which was done using Rayyan (Ouzzani et al., 2016), we obtained a total of 970 unique papers. We then screened the abstracts of these papers using pre-defined inclusion and exclusion criteria (see below), before screening the full-texts to obtain a final list of papers from which relevant data was extracted.

To be retained for full-text screening, the paper had to be a research article (not review or meta-analysis), on any animal, and measure a seminal fluid trait for males of different ages, judged from its abstract. We excluded studies during abstract screening if they were on the wrong topic, did not compare males of different ages, did not have clear ageing data, only covered a small proportion of lifespan (e.g. only included young males), did not measure seminal fluid traits, or only measured seminal fluid during maturation of males (i.e. during juvenile or pubertal stages). The initial screening of abstracts produced a total of 94 studies whose full texts were considered in more detail.

When assessing full texts, to be included in our analysis review, a study needed to: compare males of non-overlapping age groups, compare non-sperm components of the seminal fluid (like oxidative stress enzymes, proteins, hormones, lipids, and macro- or micronutrients), report sample sizes of males in each age group and exact ages (or range of ages) to which males in each age group belonged. We excluded studies whose full texts were not available (two studies), or which were not in English (three studies). We additionally

conducted a scoping search on Google Scholar to obtain additional papers which might have been missed in our systematic screening and search. This was done by using the keywords "seminal fluid protein + aging + ageing + senescence" for each of the following taxa: "bulls", "insects", "pigs", "rodents", "humans", "birds", "mammals", "fish", and searching the first 5 search result pages for relevant studies.

From all studies which fulfilled our inclusion criteria, we collected information on how male age affected various non-sperm characteristics of the ejaculate, as described in the paper. Additionally, we collected data on factors which could modulate the influence of seminal fluid ageing, such as: male mating history (i.e. whether males were held as virgin or not prior to testing), at which ages males were sampled, what fraction of average lifespan was covered and sampling methodology. The fraction of average lifespan covered is likely to influence whether seminal fluid ageing is detected in a study because ageing trajectories are expected to follow a non-linear pattern, with senescence being more prominent in late-adult life (e.g. Jones et al., 2014; Lemaître et al., 2020b).

Male mating history could influence the ageing of seminal fluid, such that if males are kept virgins, old males would have stored seminal fluid for longer durations, thus have more degraded SFPs and higher accumulation of oxidative damage than mated old males or virgin young males. On the other hand, old virgin males would accumulate higher quantities of SFPs than younger virgin males (Koppik et al., 2018; Sepil et al., 2020). If previously mated males are tested the quantity of seminal fluid produced would depend on the timing of the last mating, number of times the male mated in succession and its rate of replenishment, given that the abundance of SFPs within accessory tissues/glands decreases significantly immediately after a mating event (Hopkins et al., 2019a; Sepil et al., 2019). Furthermore, if mating history is not controlled for, then older males would have mated more times over their life (e.g. Aich et al, 2021), and thus have undergone more rounds of SFP replenishment and

thus potentially a higher turnover of the glandular tissue producing the SFP than young males.

Male sampling methodology (if samples are collected longitudinally or cross-sectionally) can also have a large impact on the study outcome. Cross-sectional sampling of males makes individual-level deterioration in ejaculate traits with advancing age harder to detect (Nussey et al, 2008), especially if low-quality males selectively disappear (Bouwhuis et al, 2009; Hamalainen et al, 2014). This non-random age-dependent mortality could lead to biased sampling of males, where younger age classes would have higher variance and might bias estimates of averages in seminal fluid traits compared to old age classes. Thus, cross-sectional studies might underestimate male reproductive senescence, compared to longitudinal sampling measuring the same individuals at different ages.

Summary of studies from the systematic review

Overall, we obtained data from 27 papers through our systematic searches, and 7 additional papers from Google Scholar (see table S1 for the full list of included studies). Out of these 34 studies, 14 reported how male age affected SFPs (see table 1), although some of these studies reported changes in total protein content, while others, changes in specific SFPs only. 10 studies reported data on oxidative damage levels or anti-oxidants present in seminal fluid (henceforth collectively called "oxidative stress", see table 2). Apart from these two components of the seminal fluid, a smaller fraction of studies assessed the concentration of lipids or lipoproteins (4 studies), minerals/vitamins content (4 studies), sugar content (2 studies), or hormone concentrations (4 studies) in the seminal plasma/ejaculate.

The low number of studies dedicated to male age-related changes in the seminal fluid is also reflected in the limited taxonomic breadth, with a strong focus on mammals (see Fig.

1). Within mammals, studies were conducted on farm animals, humans, and laboratory rodents (see table S1). For most studies, males were sampled up to around 80% of their average adult lifespan and 50% of their maximum adult lifespan (see tables 1 and 2 for lifespan sampled by studies; table S2 for sources of lifespan measurements). Another caveat is that non-significant results might go unpublished and it is difficult to estimate this extent, though a number of studies in our set of papers report no changes with age, so we hope the bias is not strong. In the following review, we restricted our discussion to studies that tested for male age-related changes in SFPs and oxidative stress response, as these aspects of the seminal fluid were better represented compared to other ejaculatory components.

Age-dependent changes in SFPs

Many studies that measured accessory tissue/gland protein content found an overall decline in SFPs with male ageing (Rezaei et al., 2015; Fraser et al., 2016; Koppik et al., 2018; see also Table 1). However, this pattern becomes less clear when considering studies that quantified individual SFPs or overall compositional changes. Here, some SFPs increased (Santhosh and Krishna, 2013; Simmons et al., 2014; Borziak et al., 2016; Inyawilert et al., 2019; Kant et al., 2019; Westfalewicz et al., 2021), while others decreased (Marshall et al., 2009; Rezaei et al., 2015; Koppik and Fricke, 2017; Herrera-Cruz et al., 2018; Ruhmann et al., 2018; Sepil et al., 2020; Westfalewicz et al., 2021) with male age. Furthermore, in studies which analysed the full proteome of the seminal fluid only a small proportion of SFPs changed with age (e.g. Sepil et al., 2020).

Methodologies differed widely between studies, ranging from estimating changes in overall SFP content to reporting individual protein changes. Generally, studies which tended to report increases in SFPs with age (e.g. in *Homo sapiens*, *Bos taurus* and *Teleogryllus*

oceanicus) sampled <50% of the average lifespan of the species (e.g. Simmons et al., 2014; Kant et al., 2019; Westfalewicz et al., 2021). Hence, extending sampling to cover the entire average lifespan is crucial, especially when ageing trajectories are expected to follow a non-linear pattern, with senescence being more prominent in late-adult life.

Most studies on non-human mammals did not report male mating history (virgin or mated) prior to testing. In farm animal studies, older males are likely to have been mated as part of a breeding program, although this was not always explicitly stated. For studies on insects, males were primarily kept virgin prior to testing. It is known that in *D. melanogaster*, age-related changes in SFPs depend on male mating history (Koppik and Fricke, 2017; Koppik et al., 2018; Sepil et al., 2020). Old unmated males transfer a lower abundance of SFPs in a first mating relative to young males, despite having a higher abundance of SFPs in storage, whereas old frequently mated males show no change in either transfer or storage(Sepil et al., 2020). Thus, mating history has the potential to influence the results reported in studies which do not control for it. We suggest future studies should adopt a fully factorial design to test for effects of mating history on seminal fluid ageing and use young and old males both as virgin and mated males, and ideally control for mating number.

In most studies, samples were acquired from the male directly (e.g. via dissection or masturbation), but whether this correctly represents what would be transferred to females in a natural ejaculate is uncertain, especially when males have the potential for strategic ejaculation (Wedell et al., 2002). The vast majority of studies were cross-sectional and thus for future studies to employ a longitudinal approach would be promising if males do not need to be sacrificed to extract an ejaculate/ the seminal fluid and a large cohort of males can be followed across their lifetime.

Age-dependent changes in oxidative stress responses

Overall, the enzymes involved in protecting against oxidative damage decreased significantly in the seminal fluid with advancing male age (see table 2). The three studies that measured both enzyme abundance and oxidative stress in the seminal fluid found oxidative stress markers increased in older males. Specifically, all studies which measured antioxidant content in the seminal fluid (e.g. TSOD, MnSOD, CuZnSOD, TGSH, CAT) consistently reported a decline in older males compared to younger or middle-aged males. Additionally, an oxidative stress marker was found in higher quantities in older male seminal fluid compared to younger males in two studies (El-Gindy and Zeweil, 2017; Kara et al., 2019). Notably, all these oxidative stress studies used mammals, so we cannot judge whether this is a pattern also seen in other animal groups. None of these studies reported the mating history of the males, and only one study sampled males longitudinally (Fraser et al., 2016).

Discussion

Here, we systematically reviewed how the non-sperm components of the ejaculate (i.e. seminal fluid) changed with male age. Sperm ageing has been a major focus of previous studies, while seminal fluid has not been studied as extensively. This is highlighted by the limited number of studies and taxa found in our systematic review, with the majority of studies either probing at age-related changes in SFPs or oxidative stress. Below, we discuss how the age-dependent changes in seminal fluid components found in our systematic review might influence male reproductive ageing, suggest some hypotheses, and discuss why the omission of seminal fluid and its associated somatic tissue is an important oversight in evolutionary and ecological research.

Seminal fluid protein ageing

We found some heterogeneity between studies in age-related SFP changes. This could be due to studies reporting quantitative changes in a set of proteins only (rather than all the proteins), or due to specific proteins responding differently to age based on their function or tissue-of-origin (Borziak et al., 2016; Sepil et al., 2020). Proteomics techniques that quantify the whole ejaculate are needed to better elucidate these biological patterns and with the advance of molecular approaches and particularly proteomics, this will become ever more feasible for a range of taxa.

Confounding factors could also explain some of the inconsistencies observed between studies. For instance, male mating history could have a large influence on SFP quantity changes as explained in the methods section above. Another caveat in comparing studies is that males are not always sampled up to old age and so an important fraction of the ageing trajectory is missed. This could be a serious bias as selection to maintain functionality is expected to be strong during early life and the reported changes could represent compensatory adaptative responses to ageing mediated through seminal fluid. Differentiating age-related tainted effects from these compensatory responses would require sampling beyond the 50% average lifespan into older ages, when these latter responses are expected to wane.

While studies in our review rarely directly discuss the functional importance of the changes in observed SFPs, below, we suggest testable hypotheses for how male seminal fluid ageing might have functional consequences. Overall, studies in our review show changes in specific SFP abundances which are known to influence male fertilisation success as well as a variety of female responses. For instance, older male *D. melanogaster* are less able to delay female remating and stimulate egg laying compared to younger males (Koppik and Fricke,

2017; Ruhmann et al., 2018; Sepil et al., 2020). Similarly in *Aedes aegypti* mosquitoes, older males are less able to prevent female remating (Agudelo et al., 2021). These responses are largely mediated by SFPs in *D. melanogaster* (Chapman et al., 2003) and the expression of functionally important SFPs declines with age (Koppik and Fricke, 2017). Therefore, it is possible that the decline in SFPs are driving the changes in female post-mating behaviour. For instance, Sepil et al. (2020) found a significant age-related increase in SFP abundances in the accessory glands of unmated males, but no change in SFP abundances in the accessory glands of frequently-mated males. Yet, the authors also found that female egg laying behaviour and remating affinity changed as a function of male age following matings with spermless males, hence the seminal fluid alone does contribute to the decline in reproductive function with male age. SFP transfer data can partially explain these findings. While there is no age-related decline in SFP abundances in the accessory glands, old unmated males transferred a lower quantity of SFPs to females compared to younger unmated males. There was no age-related change in the quantity of SFPs transferred to females for frequently-mated males, so it is likely that changes in SFP quality rather than quantity explain the decline in reproductive function with age in this group of males.

Apart from affecting male ability to induce female post-mating responses, age-related changes in seminal fluid might also affect sperm traits. For example, in the jungle fowl *Gallus gallus*, age-related changes in proteins which affect sperm velocity were detected (Borziak et al., 2016). Thus, the decreased ability of older males to gain paternity under sperm competition and fertilise eggs may be driven by changes in SFPs rather than changes in sperm per se.

While it can be difficult to pinpoint the precise changes responsible, it is becoming increasingly possible to manipulate the expression of individual SFPs to better understand how particular SFPs affect female post-mating behaviour and sperm competition. For

instance, using a combination of proteomics and RNAi, Marshall et al. (2009) identified a single accessory gland-derived ejaculate protein in the ground cricket *Allonemobius socius* that influences female egg-laying and declines in expression with male age. Hence, this protein is a prime candidate to explain the waning ability of males to induce female egg laying as the male ages. However, the link between seminal fluid expression and female responses is not necessarily straightforward. RNAi knockdown studies in other taxa have demonstrated that suppressing the expression of individual SFPs can have both positive and negative impacts on fitness-relevant traits such as female fecundity (Xu et al., 2013; Weber et al., 2019), though this may in part reflect the difficulty of measuring fitness components under realistic conditions. A further limitation of many such knockdown or knockout studies is that they tend (often of necessity) to consider only one or a few seminal fluid proteins, whereas in reality the seminal fluid proteome is a highly integrated unit whose individual components co-vary in their expression (Mohorianu et al., 2018; Patlar et al., 2019).

Effects of male age on seminal fluid quantity versus quality

In addition to changes in the abundance of individual proteins or changes to the composition of the seminal proteome, ageing can potentially impact seminal fluid through alterations to protein quality. A loss of protein homeostasis – proteostasis – is a well-known feature of ageing, characterised by a failure of chaperones, stress-response factors, and protein degradation machinery to respond to stress and prevent protein misfolding (Labbadia and Morimoto, 2014). The role of failing proteostasis in loss of SFP quality in ageing males is currently unclear but has the potential to impact ejaculate function.

Work in *D. melanogaster* suggests that factors other than SFP *quantity* may be responsible for the decline in seminal fluid-mediated functions with male age (Sepil et al.,

2020). Aged males, that are known to have compromised fertility and reduced seminal fluid function, still appear capable of levels of seminal proteome production and transfer that are similar to young males (Sepil et al., 2020). However, several proteins in aged males show evidence of qualitative changes via mass shifts on Western blots (Sepil et al., 2020). While it remains to be investigated how widespread age-related changes in SFP quality are and what the functional consequences are, it nonetheless raises the possibility that a decline in SFP functionality with age is primarily related to proteostasis loss, rather than diminishing amounts of SFPs.

Ageing of seminal fluid producing reproductive tissues

While our systematic review showed general age-related declines in SFPs, how the somatic tissues which produce SFPs are affected by ageing across taxa still remains unclear. Generally, the size of prostates/accessory glands tends to increase as males grow older (Jin et al., 1996; Atalan et al., 1999; Rezaei et al., 2015; Reyes-Hernández and Pérez-Staples, 2017), while also shrinkage with age was reported in a few studies (Mazeed and Mohanny, 2010; Santhosh and Krishna, 2013). However, the overall size of the organ does not necessarily predict protein content, as found in *A. ludens* (Herrera-Cruz et al., 2018) and *D. bipectinata* (Santhosh and Krishna, 2013). In humans, the increase in prostate size is known as benign prostatic hyperplasia (Berges and Oelke, 2011; Zhang et al., 2013), however prostate size varies among ethnic groups and so does the rate of change with age (i.e. Bolivian Tsimane, Trumble et al., 2015) or the occurrence of enlarged prostates in older males (Mubenga et al., 2020). Some theory predicts that the enlargement of the prostate is a side-effect of cellular hyperfunction that causes ageing of this tissue (Blagosklonny, 2021). The hyperfunction theory of ageing proposes that suboptimal nutrient-sensing molecular signalling in late-life causes ageing via excessive biosynthesis, as opposed to energy-tradeoffs (Lind et al., 2019).

Impact of male age on oxidative stress

The studies we reviewed consistently found that antioxidant quantity in the seminal fluid decreases with increasing male age, while oxidative stress markers tend to increase in the seminal fluid as males age. Reactive oxygen species (ROS) are unstable, free radical compounds and are required for vital cellular processes (Finkel and Holbrook, 2000; Hajam et al., 2022), but can also be deleterious to cells. For instance ROS play a role in sperm activation and changing sperm motility, e.g. in humans (Aitken et al., 2022) with the potential to influence male reproduction (Mannucci et al., 2022). However, work in *D. melanogaster* shows that while older males have higher metabolic rates in their sperm, ROS production is actually lower in these sperm (Turnell and Reinhardt, 2020).

Antioxidants, on the other hand, play a key role in stabilising free radicals generated as part of cellular processes (Hood et al., 2019), and an imbalance between antioxidants and ROS causes oxidative stress. Oxidative stress has been shown to influence sperm homeostasis and can cause sperm DNA damage thus affecting male fertility (Mannucci et al., 2022) and has been shown to be key in regulating various intracellular pathways related to sperm, and activation of various sperm transcription factors (Aitken and Baker, 2006; Sabeti et al., 2016; Aitken, 2017). Our review suggest that older males have lower antioxidant levels but higher oxidative stress markers in their seminal fluid, and thus may overall have higher oxidative stress than young males. The decline in antioxidants might indicate a tradeoff where ageing males cannot maintain optimal antioxidant levels if these are energetically costly.

The mechanisms for why older males have higher oxidative stress could be several. For instance, ROS from sperm could “leak” into seminal fluid or somatic cells which produce SF could accumulate more ROS damage over time in old versus young males. This increase

in oxidative stress in older males could have severe hypothesised functional consequences, such as higher oxidative damage to sperm, or the fertilized egg, and reduced sperm performance, which can be tested by future studies. More studies are needed to disentangle the origin/cause of age-dependent changes in ROS production in seminal fluid, the consequences of scavenging by SF antioxidants, and the overall effects on sperm, male and female reproduction.

Factors that could influence seminal fluid ageing rates

Studies identified in our systematic literature review included only a few factors such as proportional lifespan sampled, male mating history and sampling of males to explain differences in seminal fluid ageing.

Besides these, other factors could be predicted to influence SF ageing. For instance, evidence for reproductive ageing has been shown to be stronger in laboratory and captive animals compared to wild ones (Nussey et al., 2013; Zajitschek et al., 2020; Kappeler et al., 2022). Additionally, domestic animals, which were used in a majority of studies found in our systematic review, are kept in semi-controlled conditions and are killed off prior to reaching a senescent age (i.e. post their "prime"). Thus, evidence for reproductive senescence in the seminal fluid may be weaker in domestic animals, although in our review, we found evidence for seminal fluid senescence in both lab and domestic animals.

Other abiotic and biotic factors could influence seminal fluid ageing, such as a male's social environment. Both sperm and seminal fluid are highly plastic in their expression (reviewed in Perry et al., 2013; and Ramm, 2020). Males are known to invest more in seminal fluid production under more competitive environments, such as under high sperm competition (Hopkins et al., 2019b), possibly at the cost of reduced later-life investment in reproduction (Lemaître et al., 2020a). The costs of ejaculate plasticity have been discussed

before (see e.g. Ramm, 2020), but to our knowledge have not been tested, and whether these costs differ for old versus young males can be investigated in the future. Knowledge of costs could be one factor predicting ageing trajectories of seminal fluid. If costly and its continued production causes damage then strong selection on early reproduction might be favored despite the damage caused and we would expect rapid ageing as a consequence. However, if seminal fluid production is cheap then factors such a sperm competition, male dominance and/ or female preferences might have more scope to shape ageing patterns. For example if old males are socially dominant and preferred by females they might face little competition and selection on seminal fluid is relaxed and thus ageing might arise. Conversely, if older males are more likely to experience sperm competition or female ejaculate rejection then there might be relatively high selection for seminal fluid competency late in life. Additionally, sperm production patterns, i.e. continuously versus one bout early in life might be important too and thus whether sperm might become a limiting factor and thus supply and demand needs to be balance through reproductive lifespan. To test these ideas knowledge of ejaculate ageing across a broad range of taxa with different reproductive patterns are necessary.

Mating systems can also influence ageing of seminal fluid. Ageing effects are expected to be more pronounced in polyandrous species where males are likely to invest more in their ejaculates (Veltsos et al., 2022), due to facing a higher risk of sperm competition. However, the influence of sperm competition on male reproductive senescence likely depends on the life-history of a species. For example, in some species, males may preferentially invest resources in producing more SFPs early in life, and suffer faster rates of reproductive senescence later in life (Lemaitre et al, 2020). Older males in such species are often inferior in both pre- and postcopulatory competition (Johnson and Gemmell, 2012; Gasparini et al., 2019) and are discriminated against by females (Velando et al., 2011; Rezaei

et al., 2015). Alternatively, species with increased levels of sperm competition may evolve increased investment in SF (Immler et al, 2011; Lupold et al, 2020), which may reduce the rate of senescence in these ejaculate traits (Delbarco-Trillo et al, 2018).

Abiotic factors such as nutrition could also impact the trajectory of ageing of the seminal fluid. Studies on male rats showed that both over- and undernutrition during pregnancy seem to lead to premature male reproductive ageing (reviewed in Zambrano et al., 2021). This is because, at least in mammals, the early stages of development have an overall impact on health and quality of life during adulthood (developmental programming) with endocrine disruptors and maternal nutrition impacting developmental programming.

Male ageing effects on offspring fitness via seminal fluid

The impact of male age is not limited to his own and his mates' reproductive success, but potentially extends to offspring fitness as well. Males are known to influence the fitness of their offspring through mechanisms other than the transmission of DNA (Curley et al., 2011; Crean and Bonduriansky, 2014). Advanced paternal age has been shown to shorten offspring lifespan, exacerbate ageing-related pathology and to alter offspring social behaviour (Kong et al., 2012; Brenman-Suttner et al., 2018; Xie et al., 2018). Classically, these impacts were believed to be due to the accumulation of de novo mutations in ageing germ cells. However, recent work suggests non-genetic mechanisms such as changes in methylation patterns or small non-coding RNA populations that are more likely to drive the intergenerational effects of ageing (Xie et al., 2018). Importantly, it was recently suggested that seminal fluid might be an under-appreciated mediator of paternal effects (Simmons, 2011; Watkins et al., 2018; Evans et al., 2019; Simmons and Lovegrove, 2019, 2020; Kekäläinen et al., 2020), yet this has not yet been tested in a paternal ageing context.

Conclusion

Despite the low number of studies found, our review is crucial in highlighting the gaps in our knowledge due to the lack of attention paid to seminal fluid ageing. Our review generates hypotheses on how ageing of seminal fluid could affect male and female fitness, and makes predictions for how various biological and methodological factors could modulate the effects of seminal fluid ageing. It further shows that ageing impacts the level of oxidative stress in the seminal fluid, and to some extent the abundance of SFPs in the ejaculate. We highlight how the age-dependent changes observed in the seminal fluid profile can affect male fitness. Additionally, we find that male ageing can alter expression or abundance of specific SFPs that regulate female post-mating behaviour (Koppik and Fricke, 2017; Sepil et al., 2020), oviposition rate (Marshall et al., 2009), male sperm competition (Ruhmann et al., 2018; Sepil et al., 2020), response to oxidative stress (Kant et al., 2019; Westfalewicz et al., 2021), immune and antimicrobial function (Borziak et al., 2016), and sperm velocity (Borziak et al., 2016). Most research to date has been done on mammals and insects, specifically on species important for animal husbandry or biomedicine. Hence, broadening the taxonomic spread of future studies in general, and the inclusion of species with different mating systems in particular should be a priority.

We highlight how understanding reproductive ageing of sperm, but also of the seminal fluid and the tissues producing them can provide a better picture of male reproductive ageing. Any future research agenda must therefore include a more focused assessment of the downstream consequences of seminal fluid ageing on fitness-related traits, encompassing impacts on fertility, sperm competitive ability and effects on the resulting offspring. Future work should ideally study the non-sperm ejaculate components as a whole,

together with changes in sperm as this will be key to advance our understanding of male reproductive ageing.

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Literature cited

Agudelo, J., Alfonso-Parra, C., and Avila, F. W. (2021). Male Age Influences Re-mating Incidence and Sperm Use in Females of the Dengue Vector Aedes aegypti. *Front. Physiol.* 12, 691221. doi: 10.3389/fphys.2021.691221.

Ahmad, I., Ahmad, I., Ahmad, N., Qureshi, Z., Jamil, H., and Rahman, Z. (2020). Determination of physiological biomarkers in the semen and peripheral blood of sahiwal bulls of two age groups. *Pak. J. Agric. Sci.* 57, 263–268.

Aich, U., Head, M. L., Fox, R. J., and Jennions, M. D. (2021). Male age alone predicts paternity success under sperm competition when effects of age and past mating effort are experimentally separated. *Proc. R. Soc. B Biol. Sci.* 288, 20210979. doi: 10.1098/rspb.2021.0979.

Aitken, R. J. (2017). Reactive oxygen species as mediators of sperm capacitation and pathological damage. *Mol. Reprod. Dev.* 84, 1039–1052. doi: 10.1002/mrd.22871.

Aitken, R. J., and Baker, M. A. (2006). Oxidative stress, sperm survival and fertility control. *Mol. Cell. Endocrinol.* 250, 66–69. doi: 10.1016/j.mce.2005.12.026.

Aitken, R. J., Drevet, J. R., Moazamian, A., and Gharagozloo, P. (2022). Male Infertility and Oxidative Stress: A Focus on the Underlying Mechanisms. *Antioxidants* 11, 306. doi: 10.3390/antiox11020306.

Archer, C. R., Paniw, M., Vega-Trejo, R., and Sepil, I. (2022). A sex skew in life-history research: the problem of missing males. *Proc. R. Soc. B Biol. Sci.* 289, 20221117. doi: 10.1098/rspb.2022.1117.

Atalan, G., Holt, P. E., and Barr, F. J. (1999). Ultrasonographic estimation of prostate size in normal dogs and relationship to bodyweight and age. *J. Small Anim. Pract.* 40, 119–122.

Avila, F. W., Sirot, L. K., LaFlamme, B. A., Rubinstein, C. D., and Wolfner, M. F. (2011). Insect Seminal Fluid Proteins: Identification and Function. *Annu. Rev. Entomol.* 56, 21–40. doi: 10.1146/annurev-ento-120709-144823.

Bath, E., Bowden, S., Peters, C., Reddy, A., Tobias, J. A., Easton-Calabria, E., et al. (2017). Sperm and sex peptide stimulate aggression in female Drosophila. *Nat. Ecol. Evol.* 1, 0154. doi: 10.1038/s41559-017-0154.

Bauch, C., Boonekamp, J. J., Korsten, P., Mulder, E., and Verhulst, S. (2019). Epigenetic inheritance of telomere length in wild birds. *PLOS Genet.* 15, e1007827. doi: 10.1371/journal.pgen.1007827.

Berges, R., and Oelke, M. (2011). Age-stratified normal values for prostate volume, PSA, maximum urinary flow rate, IPSS, and other LUTS/BPH indicators in the German male community-dwelling population aged 50 years or older. *World J. Urol.* 29, 171–178. doi: 10.1007/s00345-010-0638-z.

Blagosklonny, M. V. (2021). The hyperfunction theory of aging: three common misconceptions. *Oncoscience* 8, 103–107. doi: 10.18632/oncoscience.545.

Bline, A. P., Le Goff, A., and Allard, P. (2020). What Is Lost in the Weismann Barrier? *J. Dev. Biol.* 8, 35. doi: 10.3390/jdb8040035.

Borziak, K., Álvarez-Fernández, A., L. Karr, T., Pizzari, T., and Dorus, S. (2016). The Seminal fluid proteome of the polyandrous Red junglefowl offers insights into the molecular basis of fertility, reproductive ageing and domestication. *Sci. Rep.* 6, 35864. doi: 10.1038/srep35864.

Bouwhuis, S., Vedder, O., and Becker, P. H. (2015). Sex-specific pathways of parental age effects on offspring lifetime reproductive success in a long-lived seabird: SEX-SPECIFIC PARENTAL EFFECTS ON OFFSPRING FITNESS. *Evolution* 69, 1760–1771. doi: 10.1111/evo.12692.

Bouwhuis, S., Verhulst, S., Bauch, C., and Vedder, O. (2018). Reduced telomere length in offspring of old fathers in a long-lived seabird. *Biol. Lett.* 14, 20180213. doi: 10.1098/rsbl.2018.0213.

Brenman-Suttner, D. B., Long, S. Q., Kamesan, V., de Belle, J. N., Yost, R. T., Kanippayoor, R. L., et al. (2018). Progeny of old parents have increased social space in Drosophila melanogaster. *Sci. Rep.* 8, 3673. doi: 10.1038/s41598-018-21731-0.

Bronikowski, A. M., Meisel, R. P., Biga, P. R., Walters, J. R., Mank, J. E., Larschan, E., et al. (2022). Sex-specific aging in animals: Perspective and future directions. *Aging Cell* 21. doi: 10.1111/acel.13542.

549 Campos, F. A., Altmann, J., Cords, M., Fedigan, L. M., Lawler, R., Lonsdorf, E. V., et al. (2022). Female
550 reproductive aging in seven primate species: Patterns and consequences. *Proc. Natl. Acad.*
551 *Sci.* 119, e2117669119. doi: 10.1073/pnas.2117669119.

552 Chapman, T. (2001). Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity* 87, 511–521.

553 Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., et al. (2003). The sex
554 peptide of *Drosophila melanogaster*: Female post-mating responses analyzed by using RNA
555 interference. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9923–9928.

556 Charlesworth, B. (1993). Evolutionary mechanisms of senescence. *Genetica* 91, 11–19. doi:
557 10.1007/BF01435984.

558 Comizzoli, P., and Ottinger, M. A. (2021). Understanding Reproductive Aging in Wildlife to Improve
559 Animal Conservation and Human Reproductive Health. *Front. Cell Dev. Biol.* 9, 1–8. doi:
560 10.3389/fcell.2021.680471.

561 Cornwallis, C. K., Dean, R., and Pizzari, T. (2014). Sex-specific patterns of aging in sexual ornaments
562 and gametes. *Am. Nat.* 184, E66–E78.

563 Crean, A. J., and Bonduriansky, R. (2014). What is a paternal effect? *Trends Ecol. Evol.* 29, 554–559.
564 doi: 10.1016/j.tree.2014.07.009.

565 Curley, J. P., Mashoodh, R., and Champagne, F. A. (2011). Epigenetics and the origins of paternal
566 effects. *Horm. Behav.* 59, 306–314. doi: 10.1016/j.yhbeh.2010.06.018.

567 Daxinger, L., and Whitelaw, E. (2012). Understanding transgenerational epigenetic inheritance via
568 the gametes in mammals. *Nat. Rev. Genet.* 13, 153–162. doi: 10.1038/nrg3188.

569 Dean, R., Cornwallis, C. K., Løvlie, H., Worley, K., Richardson, D. S., and Pizzari, T. (2010). Male
570 reproductive senescence causes potential for sexual conflict over mating. *Curr. Biol.* 20,
571 1192–1196. doi: 10.1016/j.cub.2010.04.059.

572 den Boer, S. P. A., Boomsma, J. J., and Baer, B. (2008). Seminal fluid enhances sperm viability in the
573 leafcutter ant *Atta colombica*. *Behav. Ecol. Sociobiol.* 62, 1843–1849.

574 den Boer, S. P. A., Boomsma, J. J., and Baer, B. (2009). Honey bee males and queens use glandular
575 secretions to enhance sperm viability before and after storage. *J. Insect Physiol.* 55, 538–
576 543.

577 El-Gindy, Y. M., and Zeweil, H. S. (2017). Effects of parsley supplementation on the seminal quality,
578 blood lipid profile and oxidant status of young and old male rabbits. *World Rabbit Sci.* 25,
579 215. doi: 10.4995/wrs.2017.6532.

580 Evans, J. P., Wilson, A. J., Pilastro, A., and Garcia-Gonzalez, F. (2019). Ejaculate-mediated paternal
581 effects: evidence, mechanisms and evolutionary implications. *Reproduction* 157, R109–R126.
582 doi: 10.1530/REP-18-0524.

583 Finkel, T., and Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature*
584 408, 239–247. doi: 10.1038/35041687.

585 Fiumera, A. C., Dumont, B. L., and Clark, A. G. (2005). Sperm competitive ability in *Drosophila*
586 *melanogaster* associated with variation in male reproductive proteins. *Genetics* 169, 243–
587 257.

588 Fiumera, A. C., Dumont, B. L., and Clark, A. G. (2007). Associations between sperm competition and
589 natural variation in male reproductive genes on the third chromosome of *Drosophila*
590 *melanogaster*. *Genetics* 176, 1245–1260.

591 Fraser, L., Strzeżek, J., Filipowicz, K., Mogielnicka-Brzozowska, M., and Zasiadczyk, L. (2016). Age and
592 seasonal-dependent variations in the biochemical composition of boar semen.
593 *Theriogenology* 86, 806–816. doi: 10.1016/j.theriogenology.2016.02.035.

594 Fricke, C., and Koppik, M. (2019). Male reproductive ageing: A tale of the whole ejaculate.
595 *Reproduction* 158, R219–R229. doi: 10.1530/REP-18-0579.

596 Froy, H., Lewis, S., Nussey, D. H., Wood, A. G., and Phillips, R. A. (2017). Contrasting drivers of
597 reproductive ageing in albatrosses. *J. Anim. Ecol.* 86, 1022–1032. doi: 10.1111/1365-
598 2656.12712.

599 Gasparini, C., Devigili, A., and Pilastro, A. (2019). Sexual selection and ageing: interplay between pre-
600 and post-copulatory traits senescence in the guppy. *Proc. R. Soc. B Biol. Sci.* 286, 20182873–
601 20182873. doi: 10.1098/rspb.2018.2873.

602 Gasparini, C., Dosselli, R., and Evans, J. P. (2017). Sperm storage by males causes changes in sperm
603 phenotype and influences the reproductive fitness of males and their sons. *Evol. Lett.* 1, 16–
604 25.

605 Gasparini, C., Kelley, J. L., and Evans, J. P. (2014). Male sperm storage compromises sperm motility in
606 guppies. *Biol. Lett.* 10, 20140681.

607 Gasparini, C., Marino, I. A. M., Boschetto, C., and Pilastro, A. (2010). Effect of male age on sperm
608 traits and sperm competition success in the guppy (Poecilia reticulata). *J. Evol. Biol.* 23, 124–
609 135. doi: 10.1111/j.1420-9101.2009.01889.x.

610 Goenaga, J., Yamane, T., Rönn, J., and Arnqvist, G. (2015). Within-species divergence in the seminal
611 fluid proteome and its effect on male and female reproduction in a beetle. *BMC Evol. Biol.*
612 15, 266. doi: 10.1186/s12862-015-0547-2.

613 Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., et al. (2022). Oxidative Stress
614 in Human Pathology and Aging: Molecular Mechanisms and Perspectives. *Cells* 11, 552. doi:
615 10.3390/cells11030552.

616 Herrera-Cruz, M., Abraham, S., Nuñez-Beverido, N., Flores-Estévez, N., Reyes-Hernández, M.,
617 Alvarado, M., et al. (2018). Male age and strain affect ejaculate quality in the Mexican fruit
618 fly: Age and strain affect Mexican fly ejaculates. *Insect Sci.* 25, 703–711. doi: 10.1111/1744-
619 7917.12446.

620 Hood, W. R., Williams, A. S., and Hill, G. E. (2019). An Ecologist's Guide to Mitochondrial DNA
621 Mutations and Senescence. *Integr. Comp. Biol.* 59, 970–982. doi: 10.1093/icb/icz097.

622 Hopkins, B. R., Sepil, I., Bonham, S., Miller, T., Charles, P. D., Fischer, R., et al. (2019a). BMP signaling
623 inhibition in *Drosophila* secondary cells remodels the seminal proteome and self and rival
624 ejaculate functions. *Proc. Natl. Acad. Sci. U. S. A.* 116, 24719–24728.

625 Hopkins, B. R., Sepil, I., Thézénas, M.-L., Craig, J. F., Miller, T., Charles, P. D., et al. (2019b). Divergent
626 allocation of sperm and the seminal proteome along a competition gradient in *Drosophila*
627 *melanogaster*. *Proc. Natl. Acad. Sci.* 116, 17925–17933. doi: 10.1073/pnas.1906149116.

628 Hopkins, B. R., Sepil, I., and Wigby, S. (2017). Seminal fluid. *Curr. Biol.* 27, R404–R405.

629 Inyawilert, W., Rungruangsak, J., Chanthi, S., Liao, Y., Phinyo, M., Tang, P., et al. (2019). Age-related
630 difference changes semen quality and seminal plasma protein patterns of Thai native
631 rooster. *Int. J. Agric. Technol.* 15, 287–296.

632 Jin, B., Turner, L., Crawford, B., Birrell, A., and Handelsman, D. J. (1996). The development of the
633 Baboon prostate: ultrasound methodology, modelling, and natural history. *J. Androl.* 17,
634 342–352.

635 Johnson, S. L., Dunleavy, J., Gemmell, N. J., and Nakagawa, S. (2015). Consistent age-dependent
636 declines in human semen quality: A systematic review and meta-analysis. *Ageing Res. Rev.*
637 19, 22–33. doi: 10.1016/j.arr.2014.10.007.

638 Johnson, S. L., and Gemmell, N. J. (2012). Are old males still good males and can females tell the
639 difference?: Do hidden advantages of mating with old males off-set costs related to fertility,
640 or are we missing something else. *BioEssays* 34, 609–619. doi: 10.1002/bies.201100157.

641 Jones, O. R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C. G., Schaible, R., Casper, B. B., et al.
642 (2014). Diversity of ageing across the tree of life. *Nature* 505, 169–173. doi:
643 10.1038/nature12789.

644 Kant, K., Tomar, A. K., Singh, S., and Yadav, S. (2019). Ageing associated proteomic variations in
645 seminal plasma of Indian men. *J. Proteins Proteomics* 10, 83–89. doi: 10.1007/s42485-019-
646 00013-x.

647 Kanuga, M. K., Benner, M. J., Doble, J. A., Wilson-Leedy, J. G., Robison, B. D., and Ingermann, R. L.
648 (2011). Effect of aging on male reproduction in zebrafish (Danio rerio). *J. Exp. Zool. Part Ecol.*
649 *Genet. Physiol.* 315A, 156–161. doi: 10.1002/jez.661.

650 Kappeler, P. M., Pethig, L., Prox, L., and Fichtel, C. (2022). Reproductive Senescence in Two Lemur
651 Lineages. *Front. Ecol. Evol.* 10, 894344. doi: 10.3389/fevo.2022.894344.

652 Kara, H., Orem, A., Yulug, E., Yucesan, F. B., Kerimoglu, G., Yaman, S. O., et al. (2019). Hazelnut
653 consumption improves testicular antioxidant function and semen quality in young and old
654 male rats. *Food Chem.* 294, 1–8. doi: 10.1016/j.foodchem.2019.04.087.

655 Kekäläinen, J., Jokiniemi, A., Janhunen, M., and Huuskonen, H. (2020). Offspring phenotype is shaped
656 by the nonsperm fraction of semen. *J. Evol. Biol.* 33, 584–594. doi: 10.1111/jeb.13592.

657 Kelso, K. A., Redpath, A., Noble, R. C., and Speake, B. K. (1997). Lipid and antioxidant changes in
658 spermatozoa and seminal plasma throughout the reproductive period of bulls. *Reproduction*
659 109, 1–6. doi: 10.1530/jrf.0.1090001.

660 King, M., Eubel, H., Millar, A. H., and Baer, B. (2011). Proteins within the seminal fluid are crucial to
661 keep sperm viable in the honeybee *Apis mellifera*. *J. Insect Physiol.* 57, 409–414.

662 Kirkwood, T. B. L. (2005). Understanding the Odd Science of Aging. *Cell* 120, 437–447. doi:
663 10.1016/j.cell.2005.01.027.

664 Kong, A., Frigge, M. L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., et al. (2012). Rate of
665 de novo mutations and the importance of father's age to disease risk. *Nature* 488, 471–475.
666 doi: 10.1038/nature11396.

667 Koppik, M., and Fricke, C. (2017). Gene expression changes in male accessory glands during ageing
668 are accompanied by reproductive decline in *Drosophila melanogaster*. *Mol. Ecol.* 26, 6704–
669 6716. doi: 10.1111/mec.14384.

670 Koppik, M., Ruhmann, H., and Fricke, C. (2018). The effect of mating history on male reproductive
671 ageing in Drosophila melanogaster. *J. Insect Physiol.* 111, 16–24. doi:
672 10.1016/j.jinsphys.2018.10.003.

673 Labbadia, J., and Morimoto, R. I. (2014). Proteostasis and longevity: when does aging really begin?
674 *F1000Prime Rep.* 6. doi: 10.12703/P6-07.

675 Lemaître, J. F., Gaillard, J. M., and Ramm, S. A. (2020a). The hidden ageing costs of sperm
676 competition. *Ecol. Lett.* doi: 10.1111/ele.13593.

677 Lemaître, J.-F., Ronget, V., and Gaillard, J.-M. (2020b). Female reproductive senescence across
678 mammals: A high diversity of patterns modulated by life history and mating traits. *Mech.*
679 *Ageing Dev.* 192, 111377. doi: 10.1016/j.mad.2020.111377.

680 Lind, M. I., Ravindran, S., Sekajova, Z., Carlsson, H., Hinas, A., and Maklakov, A. A. (2019).
681 Experimentally reduced insulin/IGF-1 signaling in adulthood extends lifespan of parents and
682 improves Darwinian fitness of their offspring. *Evol. Lett.* 3, 207–2016.

683 Liu, H., and Kubli, E. (2003). Sex-peptide is the molecular basis of the sperm effect in *Drosophila*
684 *melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 9929–9933.

685 López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The Hallmarks of
686 Aging. *Cell* 153, 1194–1217. doi: 10.1016/j.cell.2013.05.039.

687 Majić Balić, I., Milinković-Tur, S., Samardžija, M., and Vince, S. (2012). Effect of age and
688 environmental factors on semen quality, glutathione peroxidase activity and oxidative
689 parameters in simmental bulls. *Theriogenology* 78, 423–431. doi:
690 10.1016/j.theriogenology.2012.02.022.

691 Maklakov, A. A., and Immler, S. (2016). The Expensive Germline and the Evolution of Ageing. *Curr.*
692 *Biol.* 26, R577–R586. doi: 10.1016/j.cub.2016.04.012.

693 Manjunath, P., and Thérien, I. (2002). Role of seminal plasma phospholipid-binding proteins in sperm
694 membrane lipid modification that occurs during capacitation. *J. Reprod. Immunol.* 53, 109–
695 119. doi: 10.1016/S0165-0378(01)00098-5.

696 Mannucci, A., Argento, F. R., Fini, E., Coccia, M. E., Taddei, N., Becatti, M., et al. (2022). The Impact of
697 Oxidative Stress in Male Infertility. *Front. Mol. Biosci.* 8, 799294. doi:
698 10.3389/fmolb.2021.799294.

699 Marshall, J. L., Huestis, D. L., Hiromasa, Y., Wheeler, S., Oppert, C., Marshall, S. A., et al. (2009).
700 Identification, RNAi Knockdown, and Functional Analysis of an Ejaculate Protein that
701 Mediates a Postmating, Prezygotic Phenotype in a Cricket. *PLoS ONE* 4, e7537. doi:
702 10.1371/journal.pone.0007537.

703 Mazeed, A. M., and Mohanny, K. M. (2010). Some reproductive characteristics of honeybee drones
704 in relation to their ages: Reproductive characters of honeybee drones. *Entomol. Res.* 40,
705 245–250. doi: 10.1111/j.1748-5967.2010.00297.x.

706 McGraw, L. A., Suarez, S. S., and Wolfner, M. F. (2015). On a matter of seminal importance: Insights
707 & Perspectives. *BioEssays* 37, 142–147. doi: 10.1002/bies.201400117.

708 Mohorianu, I., Fowler, E. K., Dalmay, T., and Chapman, T. (2018). Control of seminal fluid protein
709 expression via regulatory hubs in *Drosophila melanogaster*. *Proc. R. Soc. B Biol. Sci.* 285,
710 20181681. doi: 10.1098/rspb.2018.1681.

711 Monaghan, P., Charmantier, A., Nussey, D. H., and Ricklefs, R. E. (2008). The evolutionary ecology of
712 senescence. *Funct. Ecol.* 22, 371–378. doi: 10.1111/j.1365-2435.2008.01418.x.

713 Monaghan, P., and Metcalfe, N. B. (2019). The deteriorating soma and the indispensable germline:
714 gamete senescence and offspring fitness. *Proc. R. Soc. B Biol. Sci.* 286, 20192187. doi:
715 10.1098/rspb.2019.2187.

716 Mubenga, L. E., Hermans, M. P., Chimanuka, D., Muhindo, L., Bwenge, E., and Tombal, B. (2020).
717 Prostate volume and its relationship with anthropometric variables among different ethnic
718 groups of South-Kivu, DR Congo. *Afr. J. Urol.* 26, 32. doi: 10.1186/s12301-020-00040-x.

719 Nakadera, Y., Swart, E. M., Hoffer, J. N. A., den Boon, O., Ellers, J., and Koene, J. M. (2014). Receipt of
720 Seminal Fluid Proteins Causes Reduction of Male Investment in a Simultaneous
721 Hermaphrodite. *Curr. Biol.* 24, 859–862. doi: 10.1016/j.cub.2014.02.052.

722 Noguera, J. C., Dean, R., Isaksson, C., Velando, A., and Pizzari, T. (2012). Age-specific oxidative status
723 and the expression of pre- and postcopulatory sexually selected traits in male red
724 junglefowl, Gallus gallus. *Ecol. Evol.* 2, 2155–2167. doi: 10.1002/ece3.300.

725 Noguera, J. C., Metcalfe, N. B., and Monaghan, P. (2018). Experimental demonstration that offspring
726 fathered by old males have shorter telomeres and reduced lifespans. *Proc. R. Soc. B Biol. Sci.*
727 285, 20180268. doi: 10.1098/rspb.2018.0268.

728 Nussey, D. H., Froy, H., Lemaitre, J.-F., Gaillard, J.-M., and Austad, S. N. (2013). Senescence in natural
729 populations of animals: Widespread evidence and its implications for bio-gerontology.
730 *Ageing Res. Rev.* 12, 214–225. doi: 10.1016/j.arr.2012.07.004.

731 O'Dea, R. E., Lagisz, M., Jennions, M. D., Koricheva, J., Noble, D. W. A., Parker, T. H., et al. (2021).
732 Preferred reporting items for systematic reviews and meta-analyses in ecology and
733 evolutionary biology: a PRISMA extension. *Biol. Rev.* 96, 1695–1722. doi: 10.1111/brv.12721.

734 Ouzzani, M., Hammady, H., Fedorowicz, Z., and Elmagarmid, A. (2016). Rayyan—a web and mobile
735 app for systematic reviews. *Syst. Rev.* 5, 210. doi: 10.1186/s13643-016-0384-4.

736 Patlar, B., Weber, M., and Ramm, S. A. (2019). Genetic and environmental variation in transcriptional
737 expression of seminal fluid proteins. *Heredity* 122, 595–611. doi: 10.1038/s41437-018-0160-
738 4.

739 Patlar, B., Weber, M., Temizyürek, T., and Ramm, S. A. (2020). Seminal Fluid-Mediated Manipulation
740 of Post-mating Behavior in a Simultaneous Hermaphrodite. *Curr. Biol.* 30, 143-149.e4. doi:
741 10.1016/j.cub.2019.11.018.

742 Paul, C., and Robaire, B. (2013). Ageing of the male germ line. *Nat. Rev. Urol.* 10, 227–234. doi:
743 10.1038/nrurol.2013.18.

744 Perry, J. C., Sirot, L., and Wigby, S. (2013). The seminal symphony: how to compose an ejaculate.
745 *Trends Ecol. Evol.* 28, 414–422. doi: 10.1016/j.tree.2013.03.005.

746 Pizzari, T., Dean, R., Pacey, A., Moore, H., and Bonsall, M. B. (2008). The evolutionary ecology of pre-
747 and post-meiotic sperm senescence. *Trends Ecol. Evol.* 23, 131–140. doi:
748 10.1016/j.tree.2007.12.003.

749 Poiani, A. (2006). Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* 60, 289–310. doi:
750 10.1007/s00265-006-0178-0.

751 Preston, B. T., Saint Jalme, M., Hingrat, Y., Lacroix, F., and Sorci, G. (2015). The sperm of aging male
752 bustards retards their offspring's development. *Nat. Commun.* 6, 6146. doi:
753 10.1038/ncomms7146.

754 Ramm, S. A. (2020). Seminal fluid and accessory male investment in sperm competition. *Philos.*
755 *Trans. R. Soc. B Biol. Sci.* 375, 20200068. doi: 10.1098/rstb.2020.0068.

756 Reinhardt, K. (2007). Evolutionary Consequences of Sperm Cell Aging. *Q. Rev. Biol.* 82, 375–393. doi:
757 10.1086/522811.

758 Reinhardt, K., and Siva-Jothy, M. T. (2005). An Advantage for Young Sperm in the House Cricket
759 *Acheta domesticus*. *Am. Nat.* 165, 718–723. doi: 10.1086/430010.

760 Reyes-Hernández, M., and Pérez-Staples, D. (2017). Mating senescence and male reproductive organ
761 size in the Mexican fruit fly: Age effects on mating in Mexican fruit fly. *Physiol. Entomol.* 42,
762 26–35. doi: 10.1111/phen.12160.

763 Rezaei, A., Krishna, M. S., and Santhosh, H. T. (2015). Male Age Affects Female Mate Preference,
764 Quantity of Accessory Gland Proteins, and Sperm Traits and Female Fitness in D.
765 melanogaster. *Zoolog. Sci.* 32, 16. doi: 10.2108/zs140121.

766 Ruhmann, H., Koppik, M., Wolfner, M. F., and Fricke, C. (2018). The impact of ageing on male
767 reproductive success in Drosophila melanogaster. *Exp. Gerontol.* 103, 1–10. doi:
768 10.1016/j.exger.2017.12.013.

769 Sabeti, P., Pourmasumi, S., Rahiminia, T., Akyash, F., and Talebi, A. R. (2016). Etiologies of sperm
770 oxidative stress. *Int. J. Reprod. Biomed.* 14, 231–240.

771 Santhosh, H. T., and Krishna, M. S. (2013). Relationship between male age, accessory gland, sperm
772 transferred, and fitness traits in *Drosophila bipectinata*. *J. Insect Sci.* 13, 159.

773 Sciamanna, I., Serafino, A., Shapiro, J. A., and Spadafora, C. (2019). The active role of spermatozoa in
774 transgenerational inheritance. *Proc. R. Soc. B Biol. Sci.* 286, 20191263. doi:
775 10.1098/rspb.2019.1263.

776 Selvaratnam, J., and Robaire, B. (2016). Overexpression of catalase in mice reduces age-related
777 oxidative stress and maintains sperm production. *Exp. Gerontol.* 84, 12–20. doi:
778 10.1016/j.exger.2016.08.012.

Sepil, I., Hopkins, B. R., Dean, R., Bath, E., Friedman, S., Swanson, B., et al. (2019). Ejaculate deterioration with male age, and its amelioration in *Drosophila*. Evolutionary Biology doi: 10.1101/624734.

Sepil, I., Hopkins, B. R., Dean, R., Bath, E., Friedman, S., Swanson, B., et al. (2020). Male reproductive aging arises via multifaceted mating-dependent sperm and seminal proteome declines, but is postponable in *Drosophila*. *Proc. Natl. Acad. Sci.* 117, 17094–17103. doi: 10.1073/pnas.2009053117.

Short, S. M., and Lazzaro, B. P. (2010). Female and male genetic contributins to post-mating immune defence in female *Drosophila melanogaster*. *Proc. R. Soc. Lond. B* 277, 3649–3657.

Simmons, L. W. (2011). Allocation of maternal- and ejaculate-derived proteins to reproduction in female crickets, Teleogryllus oceanicus: Allocation of maternal- and ejaculate-derived proteins. *J. Evol. Biol.* 24, 132–138. doi: 10.1111/j.1420-9101.2010.02158.x.

Simmons, L. W., Beveridge, M., Li, L., Tan, Y., and Millar, A. H. (2014). Ontogenetic changes in seminal fluid gene expression and the protein composition of cricket seminal fluid. *Evol. Dev.* 16, 101–109. doi: 10.1111/ede.12068.

Simmons, L. W., and Lovegrove, M. (2019). Nongenetic paternal effects via seminal fluid. *Evol. Lett.* 3, 403–411. doi: 10.1002/evl3.124.

Simmons, L. W., and Lovegrove, M. (2020). Can paternal effects via seminal fluid contribute to the evolution of polyandry? *Biol. Lett.* 16, 20200680. doi: 10.1098/rsbl.2020.0680.

Stockley, P., Franco, C., Claydon, A. J., Davidson, A., Hammond, D. E., Brownridge, P. J., et al. (2020). Revealing mechanisms of mating plug function under sexual selection. *Proc. Natl. Acad. Sci.* 117, 27465–27473. doi: 10.1073/pnas.1920526117.

Takemura, S., Ichikawa, H., Naito, Y., Takagi, T., Yoshikawa, T., and Minamiyama, Y. (2014). S-allyl cysteine ameliorates the quality of sperm and provides protection from age-related sperm dysfunction and oxidative stress in rats. *J. Clin. Biochem. Nutr.* 55, 155–161. doi: 10.3164/jcbn.14-39.

Tan, C. K. W., Pizzari, T., and Wigby, S. (2013). PARENTAL AGE, GAMETIC AGE, AND INBREEDING INTERACT TO MODULATE OFFSPRING VIABILITY IN *DROSOPHILA MELANOGASTER*: BRIEF COMMUNICATION. *Evolution*, n/a-n/a. doi: 10.1111/evo.12131.

Trumble, B. C., Stieglitz, J., Rodriguez, D. E., Linares, E. C., Kaplan, H. S., and Gurven, M. D. (2015). Challenging the Inevitability of Prostate Enlargement: Low Levels of Benign Prostatic Hyperplasia Among Tsimane Forager-Horticulturalists. *J. Gerontol. A. Biol. Sci. Med. Sci.* 70, 1262–1268. doi: 10.1093/gerona/glv051.

Turnell, B. R., and Reinhardt, K. (2020). Metablic rate and oxygen radical levels increase but radical generation rate decreases with male age in *Drosophila melanogaster* sperm. *J. Gerontol. Biol. Sci.*, glaa078.

Vega-Trejo, R., Fox, R. J., Iglesias-Carrasco, M., Head, M. L., and Jennions, M. D. (2019). The effects of male age, sperm age and mating history on ejaculate senescence. *Funct. Ecol.* in press, 1–13.

817 Velando, A., Noguera, J. C., Drummond, H., and Torres, R. (2011). Senescent males carry
818 premutagenic lesions in sperm. *J. Evol. Biol.* 24, 693–697. doi: 10.1111/j.1420-
819 9101.2010.02201.x.

820 Veltsos, P., Porcelli, D., Fang, Y., Cossins, A. R., Ritchie, M. G., and Snook, R. R. (2022). Experimental
821 sexual selection reveals rapid evolutionary divergence in sex-specific transcriptomes and
822 their interactions following mating. *Mol. Ecol.* 31, 3374–3388. doi: 10.1111/mec.16473.

823 Vince, S., Zaja, I. Z., Samardzija, M., Balic, I. M., Vilic, M., Duricic, D., et al. (2018). Age-related
824 differences of semen quality, seminal plasma, and spermatozoa antioxidative and oxidative
825 stress variables in bulls during cold and warm periods of the year. *ANIMAL* 12, 559–568. doi:
826 10.1017/S1751731117001811.

827 Vuarin, P., Bouchard, A., Lesobre, L., Levêque, G., Chalah, T., Jalme, M. S., et al. (2019). Post-
828 copulatory sexual selection allows females to alleviate the fitness costs incurred when
829 mating with senescing males. *Proc. Biol. Sci.* 286, 20191675–20191675. doi:
830 10.1098/rspb.2019.1675.

831 Vuarin, P., Lesobre, L., Levêque, G., Saint Jalme, M., Lacroix, F., Hingrat, Y., et al. (2021). Paternal age
832 negatively affects sperm production of the progeny. *Ecol. Lett.*, 1–9. doi: 10.1111/ele.13696.

833 Waheed, M. M., El-Bahr, S. M., and Al-haider, A. K. (2013). Influence of Seminal Plasma Antioxidants
834 and Osteopontin on Fertility of the Arabian Horse. *J. Equine Vet. Sci.* 33, 705–709. doi:
835 10.1016/j.jevs.2012.11.006.

836 Watkins, A. J., Dias, I., Tsuro, H., Allen, D., Emes, R. D., Moreton, J., et al. (2018). Paternal diet
837 programs offspring health through sperm- and seminal plasma-specific pathways in mice.
838 *Proc. Natl. Acad. Sci.* 115, 10064–10069. doi: 10.1073/pnas.1806333115.

839 Weber, M., Giannakara, A., and Ramm, S. A. (2019). Seminal fluid-mediated fitness effects in the
840 simultaneously hermaphroditic flatworm *Macrostomum lignano*. *Ecol. Evol.* 9, 13889–13901.
841 doi: 10.1002/ece3.5825.

842 Wedell, N., Gage, M. J. G., and Parker, G. A. (2002). Sperm competition, male prudence and sperm-
843 limited females. *Trends Ecol. Evol.* 17, 313–320.

844 Westfalewicz, B., Słowińska, M., Judycka, S., Ciereszko, A., and Dietrich, M. A. (2021). Comparative
845 Proteomic Analysis of Young and Adult Bull (Bos taurus) Cryopreserved Semen. *Animals* 11,
846 2013. doi: 10.3390/ani11072013.

847 Wolfner, M. F. (1997). Tokens of love: Functions and regulation of *Drosophila* male accessory gland
848 products. *Insect Biochem. Mol. Biol.* 27, 179–192.

849 Wylde, Z., Spagopoulou, F., Hooper, A. K., Maklakov, A. A., and Bonduriansky, R. (2019). Parental
850 breeding age effects on descendants' longevity interact over 2 generations in matrilines and
851 patrilines. *PLOS Biol.* 17, e3000556. doi: 10.1371/journal.pbio.3000556.

852 Xie, K., Ryan, D. P., Pearson, B. L., Henzel, K. S., Neff, F., Vidal, R. O., et al. (2018). Epigenetic
853 alterations in longevity regulators, reduced life span, and exacerbated aging-related
854 pathology in old father offspring mice. *Proc. Natl. Acad. Sci.* 115. doi:
855 10.1073/pnas.1707337115.

Xu, J., Baulding, J., and Palli, S. R. (2013). Proteomics of Tribolium castaneum seminal fluid proteins: Identification of an angiotensin-converting enzyme as a key player in regulation of reproduction. *J. Proteomics* 78, 83–93. doi: 10.1016/j.jprot.2012.11.011.

Zajitschek, F., Zajitschek, S., and Bonduriansky, R. (2020). Senescence in wild insects: Key questions and challenges. *Funct. Ecol.* 34, 26–37. doi: 10.1111/1365-2435.13399.

Zambrano, E., Nathanielsz, P. W., and Rodríguez-González, G. L. (2021). Developmental programming and ageing of male reproductive function. *Eur. J. Clin. Invest.* 51. doi: 10.1111/eci.13637.

Zhang, S.-J., Qian, H.-N., Zhao, Y., Sun, K., Wang, H.-Q., Liang, G.-Q., et al. (2013). Relationship between age and prostate size. *Asian J. Androl.* 15, 116–120. doi: 10.1038/aja.2012.127.

Figure 1: Phylogenetic distribution of all species in our review across 34 studies along with the number of studies on each. Species which had data reported for age-dependent changes in SFPs and oxidative stress are marked.

Table 1: Summary of studies testing the effect of male age on seminal fluid proteins across different taxa as found in the systematic search. Proportion lifespan (LS) sampled is given in relation to reported average lifespan (avg) or maximum (max) recorded lifespan (sources for those numbers can be found in table S2) for each species.

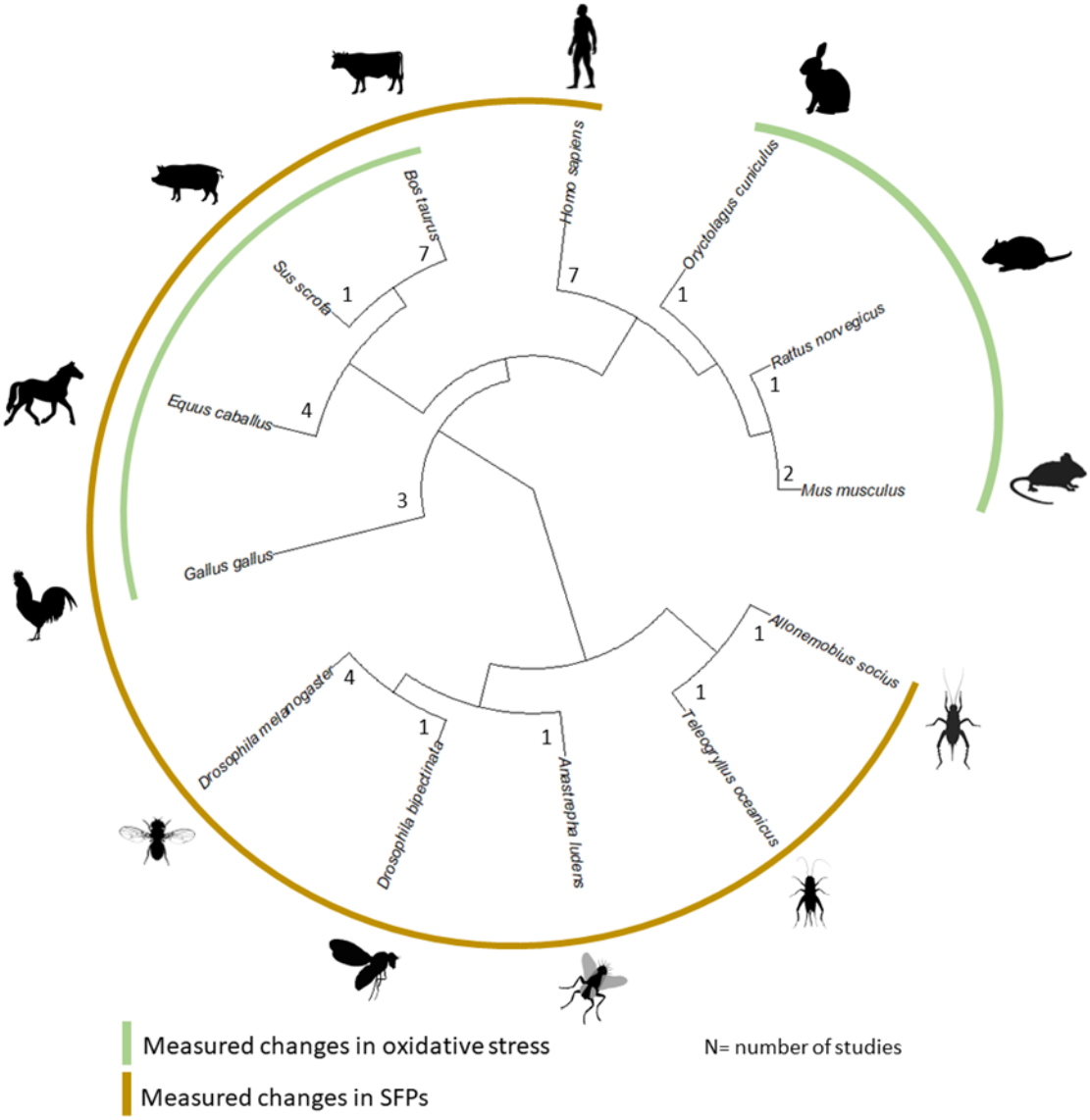
Study	**Species**	**Proportion LS sampled**	**Sample sizes**	**Changes observed in SFPs**	**Sampling**	**Mating history**
Borziak et al, 2016	*Gallus gallus*	1 to 7 years out of 5.5 (avg in wild) and 18 (max)	16 total	Total of 1141 SFPs identified, out of which 9 change with age*velocity, and 4 with age only. Protein tyrosine phosphatase type IVA 1 was present in old males only. Young males had more of SPARC precursor, acetyl-CoA acetyltransferase cytosolic, and ras-related protein Rab-11B compared to old males.	Cross-sectional	Mated but sexually rested
Inyawilert et al, 2019	*Gallus gallus domesticus*	7 to 24 months out of 60 (avg) and 112 (max) months	18 total	Proteins with light (72kDa) molecular weights decreased with increasing age. Mid-weight proteins (90 kDa) increased with increasing age. Heavy proteins (140 kDa) showed no significant change.	Cross-sectional	Unreported
Abou-Ahmed et al, 1993	*Equus caballus*	7 to 25 years out of 25 (avg) and 47 (max) years	53 total	Total seminal fluid protein content was highest in middle aged males, and lowest in the youngest and oldest age groups	Cross-sectional	Mated
Westfalewicz et al, 2021	*Bos taurus*	2 to 4 years out of 10 (avg) and 25 (max)	6 total	Seventeen SFPs differed between young and old males. Older bulls had higher proteins of: glutathione; S-transferase omega 2 (GSTO2); PRDX5; PARK7; superoxide dismutase (SODC), compared to younger males. Younger bulls had higher amounts of: keratin, type II cytoskeletal 59 kDa, component IV (K2C4); outer dense fiber protein 2 (ODF2); tektin-5 (TEKT5) and TBB2B compared to older bulls	Longitudinal	Unreported
Fraser et al, 2016	*Sus scrofa*	19 to 42 months out of 66 (avg) and 264 (max) months	4 total	Overall content of seminal fluid proteins decline with age. Did not identify specific SFPs.	Longitudinal	Unreported
Kant et al, 2019	*Homo spaiens*	20 to 40 years out of 72 (avg) and 120 (max) years	6 per age group	Seventeen protein spots and 10 proteins differed between young and old groups (humans are known to contain ~3000 SFPs). Glutaredoxin domain containing cysteine-rich protein-2, clusterin, serum albumin, translation initiation factor IF-2 like, ecto-ADP-ribosyltransferase 4, CB1 cannabinoid receptor-interacting protein 1, serotransferrin were found in higher abundance in older males compared to younger males. Alternative protein RRT-34 and protein Unc-119 homolog A were found in lower abundance in older age samples compared younger males.	Cross-sectional	Mated
Simmons et al, 2014	*Teleogryllus oceanicus*	4 to 20 days out of 74 (avg) and 135 (max) days	57 total	Total of 27 distinct SFPs identified. Total protein content did not vary with age. ToSfp014, ToSfp025, ToSfp007 (Trypsin-like serine protease), ToSfp017, ToSfp011, ToSfp026, ToSfp005 (Dipeptidase), ToSfp027 (apyrase), ToSfp001, ToSfp024 (carbonic anhydrase) increased with age. Other SFPs did not change significantly with age.	Cross-sectional	Virgins
Koppik and Fricke, 2017	*Drosophila melanogaster*	7 to 42 days out of 45 (avg) and 110 (max) days	10 per age group	All 5 SFP genes tested, decreased in expression with age: Acp26Aa, Acp29AB, Acp36DE, SP and Acp62F.	Cross-sectional	Mated and unmated treatments

Sepil et al, 2020	*D. melanogaster*	7 to 35 days out of 45 (avg) and 110 (max) days	80 per age group	117 SFPs identified, out of which 40 changed with age. Focused on 6 functionally important SFPs. Acp62F, Semp1, and Acp26Aa decreased with age. Acp70A [sex peptide], Acp36DE, and CG9997 showed no change with age. Age-related accumulation of SFPs in unmated males, but reduced transfer. No change in SFP abundance or transfer with age in frequently-mating males Evidence of age related Post-translational modifications in some SFPs	Cross-sectional	Mated and unmated treatments
Rezaei et al, 2015	*D. melanogaster*	2 to 53 days out of 45 (avg) and 110 (max) days	20 per age group	Overall seminal fluid amount decreases with age. Did not measure specific SFPs.	Cross-sectional	Virgins
Ruhmann et al, 2018	*D. melanogaster*	4 d - 42 d out of 45 (avg) and 110 (max) days	18 per age group	Measure two SFPs: sex peptide and ovulin. Sex peptide decreased in old males, ovulin levels did not change with age.	Cross-sectional	Mated
Herrera-Cruz et al, 2018	*Anastrepha ludens*	8 d to 72 days out of 50 days (avg), 1 year (max)	20 per age group	Old males had lower overall protein content in their testis (but not accessory glands) compared to young males.	Cross-sectional	Virgins
Marshall et al, 2009	*Allonemobius socius*	5 to 40 days out of 35 days (avg) and 100 days (max)	42 total	Protein X (trypsin like serine protein) reduces with male age.	Cross-sectional	Virgins
Santhosh and Krishna, 2013	*Drosophila bipectinata*	2 to 47 days out of 58 days (avg) and 200 days (max)	50 per age group	Overall SFP quantity increases with male age	Cross-sectional	Virgins

Table 2: Summary of studies found in the systematic literature search that focus on male-age dependent changes in antioxidants, oxidative stress biomarkers and reactive oxygen species in male ejaculates/ seminal plasma. Proportion lifespan (LS) sampled is given in relation to reported average lifespan (avg) or maximum (max) recorded lifespan (sources for those numbers can be found in table S2) for each species.

Study	**Species**	**Proportion LS sampled**	**Sample sizes**	**Changes observed in oxidative stress**	**Sampling**	**Mating history**
Vince et al., 2018	*Bos taurus*	2 to 10 years out of 10 (avg) and 25 (max)	9 young, 9 old	Antioxidants such as TSOD, MnSOD, CuZnSOD, TGSH, CAT all higher in young males. Oxidative stress was higher in old males.	Cross-sectional	Unreported
Ahmad et al., 2020	*Bos taurus*	3 to 10 years out of 10 (avg) and 25 (max)	6 young 6 old	Younger bulls have higher total antioxidants. For catalase and malondialdehyde, there is no sig. difference.	Cross-sectional	Unreported
Majić Balić et al., 2012	*Bos taurus*	2 to 10 years out of 10 (avg) and 25 (max)	9 young, 10 old	Seasonal dependent changes in antioxidants: For total glutathione peroxidase (T-GSH-Px), young bulls have more in all seasons. For glutathione peroxidase (Se-GSH-Px), protein carbonyl content (PCC), young males have more in 3/4 seasons.	Cross-sectional	Unreported
Kelso et al., 1997	*Bos taurus*	2 to 9 years out of 10 (avg) and 25 (max)	4 in each of the three classes	For both antioxidants measured, Glutathione peroxidase and Superoxide dismutase, younger males had more than older males	Cross-sectional	Unreported
Noguera et al., 2012	*Gallus gallus*	1 to 4 years out of 5.5 (avg in wild) and 18 (max)	6 young, 15 old	Decrease in antioxidants such as -SH group of proteins, uric acid, vitA, vit C, vit E in old males	Cross-sectional	Unreported
El-Gindy et al, 2017	*Oryctolagus cuniculus*	9 to 42 months out of 24 months (avg) and 150 months (max)	18 young, 18 old	Aspartate transaminase showed no significant change with age Antioxidants decreased in old males Oxidative stress marker Malondialdehyde increased sig in old males	Cross-sectional	Unreported
Kara et al, 2019	*Mus musculus*	3 to 24 months out of 24 (avg) and 48 (max) months	14 young, 21 old	Antioxidants glutathione peroxidase and reductive glutathione decreased in older males. Oxidative stress marker malondialdehyde increased in old males	Cross-sectional	Unreported
Fraser et al, 2016	*Sus scrofa*	19 to 42 months out of 66 (avg) and 264 (max) months	4 in total	Antiperoxidant activity lower in older animals. Antioxidant L-glutathione concentration peaks at mid age 19-30 mo, and declines in older animals	Longitudinal	Unreported

Waheed et al., 2013	*Equus caballus*	4 to 22 years out of 25 (avg) and 47 (max) years	6 in each age group	Antioxidant Glutathioneperoxidase highest in middle aged males, and lower in oldest and youngest males	Cross-sectional	Unreported
Takemura et al., 2014	*Rattus norvegicus*	15 to 75 weeks out of 124 (avg) and 187 (max) weeks	4 to 5 in each group	DJ-1 antioxidant decreased with age. Cu/ZnSOD antioxidant decreased with age	Cross-sectional	Unreported


Bos taurus
7
Sus scrofa
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Equus caballus
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Gallus gallus
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Homo sapiens
7
Oryctolagus cuniculus
1
Rattus norvegicus
1
Mus musculus
2
Allonemobius socius
1
Teleogryllus oceanicus
1
Anastrepha ludens
1
Drosophila bipectinata
1
Drosophila melanogaster
4
Measured changes in oxidative stress
Measured changes in SFPs
N= number of studies

Figure 1