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RESEARCH ARTICLE

Social dominance, but not parasite load, affects sperm quality and sperm redox status in house sparrows

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ABSTRACT

Sperm performance is an important component of male reproductive success. However, sperm production is costly and males need to optimize their investment in sperm quality versus the somatic traits involved in mating success, e.g. their social status. As oxidative stress affects both sperm performance and somatic functions, it has been hypothesized to mediate such a trade-off. According to the oxidation-based soma/germline trade-off hypothesis, dominant males should favour the antioxidant protection of their somatic tissues, and subordinate males should favour the antioxidant protection of their sperm. We tested this hypothesis by experimentally infecting wild-caught house sparrows *Passer domesticus* with Coccidia *Isopora* sp., an internal parasite known to deplete antioxidant resources. We predicted that (i) increased parasite load affects sperm oxidative status and sperm performance and that (ii) males with experimentally high parasite load adjust the antioxidant protection of their soma versus their sperm according to their social status. Despite a 5400% increase in parasite load, sperm performance and somatic and spermatic oxidative status remained unaffected, irrespective of male social status. Nevertheless, males increased their sperm performance over time, a pattern mirrored by an increase in the antioxidant protection of their sperm. Moreover, males at the lower end of the hierarchy always produced sperm of lower velocity, suggesting that they were constrained and privileged their soma over their germline. To conclude, high parasite loads do not necessarily affect sperm performance and oxidative status. In contrast, social hierarchy and the relative investment in soma versus sperm antioxidant protection are determinants of sperm performance.

KEY WORDS: Antioxidants, Coccidia, Oxidative stress, *Passer domesticus*, Reproductive success, Soma/germline trade-off, Sperm competition

INTRODUCTION

Male reproductive success is a key fitness component that reflects a male's mating and fertilization success (Trivers, 1972; Birkhead and Møller, 1998; Fitzpatrick and Lüpold, 2014). In promiscuous species, females exert selection on male secondary sexual traits (e.g. ornaments, behavioural traits), but also on male sperm performance by inciting sperm competition (i.e. the competition among sperm of rival males to fertilize a common set of eggs; Parker, 1970), ultimately creating substantial variation in male reproductive success (Anderson, 1994; Birkhead and Møller, 1998; Andersson and Simmons, 2006; Parker and Birkhead, 2013; Fitzpatrick and Lüpold, 2014). In many species, because males must invest resources in both pre-mating (i.e. soma) and post-mating (i.e. germline) sexually selected traits, they face an evolutionary trade-off in the allocation of resources between somatic and germline functions (Lüpold et al., 2014; Dines et al., 2015; Simmons et al., 2017). Empirical and recent theoretical developments generally suggest that increasing the costs of access to matings (e.g. lower social status) should select for higher investment in sperm performance (Preston et al., 2001; Tazzyman et al., 2009; Lemaître et al., 2012; Parker et al., 2013).

Pathogen infections exert strong selection pressure on their hosts in response to which vertebrates have evolved the ability to build immune responses as defence mechanisms (e.g. Pancer and Cooper, 2006; Litman et al., 2010). However, mounting an immune response incurs costs due to both the immune agents themselves (e.g. macrophages, neutrophils) and the resources it requires, which are in turn diverted from other functions (von Schantz et al., 1999; Lochmiller and Deerenberg, 2000). Given these costs, immunity is traded-off against other fitness-related traits such as growth, reproduction or the expression of secondary sexual traits (Lochmiller and Deerenberg, 2000; Peters et al., 2004; Lawniczak et al., 2007; López et al., 2009; van der Most et al., 2011). Studies have also evidenced a cost of immunity to sperm performance, suggesting that sperm performance is condition dependent (Losdat et al., 2011; Ardia et al., 2012; Simmons, 2012; Devigili et al., 2017). One important potential physiological consequence of immune activation is the increased production of reactive oxygen species (ROS), which commonly leads to oxidative stress (Apel and Hirt, 2004; Costantini and Møller, 2009; Sorci and Faivre, 2009). Oxidative stress, the imbalance between the production of ROS and the antioxidant defences in favour of the former (Sies, 1991), arises through the deleterious effects of ROS on lipids, proteins and DNA, ultimately impacting fitness-related traits (Bize et al., 2008; Costantini et al., 2010; Noguera et al., 2011; Agarwal et al., 2012; Stier et al., 2012; Losdat et al., 2013; Costantini, 2014). Sperm cells are particularly susceptible to oxidative damage; because of the large proportion of polyunsaturated fatty acids in their membranes (Hulbert et al., 2007), spermatozoa are prone to oxidation by ROS, which can impair their performance (Helfenstein et al., 2010; Losdat et al., 2011), and ultimately reduce fertilization rates (Aitken and Roman, 2009) and embryo survival (Velando et al., 2008; Lane et al., 2014). Previous studies have shown that oxidative stress may mediate the condition dependence of sperm performance in several species (Helfenstein et al., 2010; Losdat et al., 2011; Rojas Mora et al., 2017a,b). Moreover, oxidative stress has recently been shown to constrain the trade-off in the allocation of resources between somatic and germline functions (the oxidation-based soma/germline

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allocation trade-off hypothesis; Rojas Mora et al., 2017a,b). However, it remains to be investigated whether immunity generates an oxidative cost that affects sperm performance and thereby affects how resources involved in the antioxidant protection of the soma and the germline are traded off.

In species where the social hierarchy determines access to fertile females, a male's social status might predict his sperm performance and/or reproductive tactics (Pizzari and Parker, 2009; Parker and Pizzari, 2010). Such status-dependent investment, whereby males at the lower end of the social hierarchy (e.g. satellite or sneaker males) exhibit better sperm performance than dominant males, has been observed in captive and wild vertebrates (Stockley et al., 1994; Froman et al., 2002; Neff et al., 2003; Fitzpatrick et al., 2007; Fasel et al., 2016). Similarly, in house sparrows, which display a linear hierarchy, males at the top and males at the bottom of the hierarchy have been found to produce lower quality ejaculates than males in the middle of the hierarchy (Rojas Mora et al., 2017a). In addition, oxidative status has also been shown to depend on social hierarchy during the breeding period, with high-ranking individuals suffering increased oxidative damage (Beaulieu et al., 2014; Cram et al., 2015b). Consequently, following a parasite infection, one might expect resource investment in somatic versus germline functions to differ according to male hierarchical position. Whether a parasite infection can affect and/or shift this balance is the focus of this study.

We experimentally infected wild-caught house sparrows (*Passer domesticus*) with Coccidia *Isospora* sp., a bird internal parasite that drains antioxidant reserves (Hõrak et al., 2004), to test the hypothesis (i) that the condition dependence of sperm performance is mediated by oxidative stress, (ii) that an immune challenge affects the soma/germline resource allocation trade-off, and (iii) that males differentially invest their resources in the antioxidant protection of their soma versus their germline according to their position in the social hierarchy. To this aim, we assessed the oxidative status of both soma (blood) and germline (sperm) and assessed sperm performance after a full spermatogenesis episode. We also recorded the males' social hierarchy established through agonistic interactions. We predicted infested males to generally show increased oxidative stress and lower sperm performance than controls. Further, we predicted dominant males to strategically invest oxidative protection preferentially into their somatic functions, resulting in an increase in sperm oxidative damage and reduced sperm performance. In contrast, we predicted middle-ranking males to shift their soma versus germline balance towards the protection of their sperm to minimize the deleterious effects on their sperm performance and maximize fertilization success, resulting in elevated oxidative damage in their soma. Lastly, low-ranking males were expected to suffer from elevated oxidative stress in both their soma and germline and to produce sperm of lower performance.

MATERIALS AND METHODS

Experimental setup and parasite infection

In April 2015, we captured 56 male and 56 female house sparrows, *Passer domesticus* (Linnaeus 1758), using mist-nets in various farms of western Switzerland. Birds were trapped and ringed under permit no. 2565 granted by the Swiss Federal Office for Environment. The experiment was examined and approved (permit no. BE24/15) by the Ethical Committee for Animal Experimentation of the Veterinary Office of the Canton Bern. Birds were detained under permit no. WTH/g-525/14 granted by the Hunting and Wildlife Service of the Canton Bern. All birds were kept in 14 outdoor aviaries (four males and four females per aviary) at the research station Hasli (University of Bern, Switzerland) for 2 months (mid-April to mid-June 2015). The aviary dimensions were 2 m×2.5 m×4 m (height×width×length) and each of them included a 2 m^2 pond, partially grassy soil and plastic rain protection covering half of the ceiling. After a 3 week acclimatization period, all birds were treated against parasites by adding an antibiotic to their drinking water (1 ml l^{-1} of 2.5% Baycox®, Bayer AG Germany) during four consecutive days. Toltrazuril, the active molecule of Baycox, was shown to effectively eliminate coccidian parasites in birds after 2 days of treatment (Greif, 2000; Mathis et al., 2004; Krautwald-Junghanns et al., 2009), a pattern that was also observed here (see Results). Males and females were then infected with Coccidia *Isospora* sp. in seven randomly chosen aviaries, while all birds in the remaining seven aviaries received a control treatment (see below). *Isospora* sp. are obligate intracellular intestinal parasites that infect their hosts through ingestion via the faeces of another host (Hõrak et al., 2004). After ingestion, the oocysts produce sporozoites that penetrate the epithelium cells of the host's small intestine, where they reproduce asexually and further induce cell destruction in the small intestine, liver, spleen and lungs (Hõrak et al., 2004). We used *Isospora* sp. because they occur naturally in passerine birds and because *Isospora* sp. infections can lead to substantial decreases in antioxidant levels (e.g. carotenoids, vitamin E; Hõrak et al., 2004), therefore potentially exposing individuals to oxidative stress. The infection was conducted following the protocol described by Hõrak et al. (2004). Oocysts of *Isospora* sp. were extracted from faeces obtained from sparrows we kept in captivity before the experiment started. After mixing faeces with tap water, we verified the presence of *Isospora* sp. oocysts under a microscope, mixed the sample 1:1 with potassium dichromate ($K_2Cr_2O_7$) and kept the final solution for 7 days at 4°C until oocysts sporulated. We then removed the potassium dichromate using 2500 rpm centrifugation and mixed the remaining solution at 1:1 with NaCl. Finally, birds were orally dosed with either 0.1 ml of a 20,000 oocysts ml^{-1} solution (parasitized group, each bird received ca. 2000 oocysts) or 0.1 ml of NaCl only (control group).

We blood sampled (ca. 80 µl using a heparinized capillary) and sperm sampled (see details below) males before parasite infection (day 0, hereafter referred to as 'pre-infection') and then sampled all males again after 9 days and after 18 days, the latter being chosen such that males had completed at least one full spermatogenesis cycle (Bhat and Maiti, 1988). Two days prior to each male blood-/sperm-sampling event, we separated females and males and took a sperm sample from each male such that males' differential access to females could not have influenced sperm performance at the time of sampling (see details in Rojas Mora et al., 2018). We reintroduced females into their respective aviaries immediately after sperm/blood sampling.

Social hierarchy

After a 3 week acclimation period, we established the social hierarchy of the males in each aviary by video recording social interactions at the feeders (i.e. there was one feeder per aviary) every third day for a total of 10 h of recording per aviary. Each aviary was provided with a tower feeder mounted on a plastic plate that collected all the spilt seeds through a plastic mesh, making food only accessible at the two feeder openings. Feeders were topped up with a mix of canary grass seeds (*Phalaris canariensis*; 80%) and Quicko® egg food supplement (20%) every second day such that they were never left empty. For each recording event, we removed the feeders for 90 min, after which we reintroduced the feeders in the aviaries and recorded all the antagonistic interactions at the feeders during 1 h (8284 dyads in total). Within each aviary, we computed each male's David score, a measurement of individual

social rank (Gammell et al., 2003). In each aviary, there was a dominant male, a first subordinate male, a second subordinate male and a third subordinate male at the lower end of the hierarchy. Although we did not test for hierarchy stability in 2015, we did so in a previous study conducted in 2013 under similar conditions and found that hierarchies assessed every week over 3 weeks remained stable (A. Rojas Mora and F. Helfenstein, unpublished data).

Sperm performance

Sperm samples (ca. 2–3 µl) were obtained by cloacal massage (Wolfson, 1952); 0.2 µl of semen was immediately diluted in 40 µl of DMEM (Dulbecco's modified Eagle medium) that was preheated at 40°C. Then, 2 µl of the mixture was deposited in a microscope chamber slide and video recorded using a Toshiba CMOS HD camera mounted on an Olympus BX43 microscope (×100 magnification, 25 frames s^{-1}). Sperm videos were then analysed using the Computer Assisted Sperm Analyzer (CASA) plug-in in ImageJ software (Wilson-Leedy and Ingermann, 2007). CASA computed the proportion of motile sperm, curvilinear velocity (VCL, the total distance traced by a spermatozoon), straight-line velocity (VSL, the straight distance between the initial and the final point travelled by a spermatozoon) and average path velocity (VAP, a smooth trajectory of the total travelled distance). We used the proportion of motile sperm and sperm velocity as predictors of sperm performance because they are key determinants of the outcome of sperm competition (e.g. Froman and Feltmann, 1998; Gage et al., 2004; Malo et al., 2005; reviewed in Fitzpatrick and Lüpold, 2014). Sperm velocity is often depicted as the principal component (PC) scores of a PC analysis conducted on VCL, VAP and VSL (e.g. Bennison et al., 2016), or as VCL itself in passerine birds (e.g. Rojas Mora et al., 2017a) because passerine spermatozoa swim linearly. Here, as our results on sperm velocity were similar when using PC scores or VCL itself, we only present data using VCL as the metric of sperm velocity. Studies have also shown that velocity calculated across a given percentage of the fastest spermatozoa could provide a more biologically relevant measurement of sperm swimming ability (e.g. Bennison et al., 2016). Therefore, besides considering VCL calculated across 100% of the motile spermatozoa, we also ran our analyses on VCL calculated across the fastest 10% and 5% spermatozoa.

Oxidative stress

We first quantified malondialdehyde (MDA) levels to assess the level of lipid peroxidation in sperm, red blood cells (RBCs) and blood plasma. MDA is a reliable marker of past oxidative damage to lipids due to ROS whereby high MDA levels indicate high oxidative damage (e.g. Hõrak and Cohen, 2010). MDA in sperm (hereafter 'MDA_{sperm}') and MDA in RBCs (hereafter 'MDA_{RBC}') were quantified using a derivatization with 2-thiobarbituric acid quantification with ultra-high performance liquid chromatography (UHPLC) with fluorescence detection, a method that is fully described in Rojas Mora et al. (2016). MDA in plasma (hereafter 'MDA_{plasma}') was quantified using a newly developed more sensitive method that uses derivatization with 2,4-dinitrophenylhydrazine and UHPLC high-resolution mass spectrometry, a method that is fully described in Mendonça et al. (2017).

We quantified the amount of reduced (GSH) and oxidized (GSSG) glutathione in sperm and RBCs. GSH is an endogenous intracellular peptide that can scavenge ROS in a reaction catalysed by glutathione peroxidase, eventually being oxidized to glutathione disulfide (GSSG; Halliwell and Gutteringe, 2007). We quantified the ratio between oxidized glutathione and reduced glutathione (GSSG/GSH), which provides an accurate measurement of a cell's oxidative balance, with high ratio values indicating high oxidation in the considered tissue (Cnubben et al., 2001). We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) following the method described in Rojas Mora et al. (2016). Repeatability of the method was high (coefficient of variation CV=0.072, intra-class correlation coefficient=0.966, n=8, P<0.001).

Finally, we also assessed superoxide dismutase (SOD) activity (U ml^{-1}) in sperm and in RBCs. SOD is an endogenous antioxidant that catalyses the dismutation of superoxide anions into molecular oxygen or hydrogen. Increasing levels of SOD therefore indicate increasing antioxidant potential. We used Cayman Chemical's SOD assay kit, which is based on the detection of superoxide radicals generated by xanthine oxidase and neutralized by SOD. In all analyses, samples were processed blind with regard to bird identity and parasite infection.

Statistical analyses

We used repeated measures linear mixed-effect models to test the effect of the parasite infection and social rank on oxidative stress and sperm performance. We used R version 3.1.2 and the packages 'lme4' (https://CRAN.R-project.org/package=lme4), 'lmerTest' (https://CRAN.R-project.org/package=lmerTest) and 'lsmeans' (Lenth, 2016). Oxidative stress was quantified as MDA_{sperm}, MDA_{plasma}, MDA_{RBC}, GSH in sperm and RBCs, GSSG in sperm and RBCs, the GSSG/GSH ratio in sperm and RBCs, and SOD activity in sperm and RBCs. To quantify male investment in sperm versus soma, we computed three additional indexes: the proportion of reduced glutathione in sperm relative to reduced glutathione in sperm and soma ($GSH_{sperm}/GSH_{sperm+RBC}$), the proportion of MDA in sperm relative to MDA in sperm and soma ($MDA_{sperm}/MDA_{sperm+RBC}$), and the proportion of SOD activity in sperm relative to SOD activity in

Table 1. Oxidative stress in soma

	MDA_{plasma}		MDA_{RBC}		$GSSG_{RBC}/GSH_{RBC}$		SOD_{RBC}	
Effect	$F_{d.f.}$	P	$F_{d.f.}$	P	$F_{d.f.}$	P	$F_{d.f.}$	P
Parasite infection	$0.03_{1,44}$	0.86	$0.99_{1,44}$	0.33	$1.11_{1,44}$	0.30	$0.64_{1,43}$	0.43
Rank	$0.32_{3,44}$	0.82	$0.37_{3,44}$	0.77	$0.43_{3,44}$	0.73	$0.89_{3,43}$	0.46
Day	**$15.7_{2,89}$**	**<0.001**	**$18.2_{2,89}$**	**<0.001**	**$97.7_{2,89}$**	**<0.001**	$0.51_{2,89}$	0.60
Infection×rank	$0.02_{3,44}$	0.99	$0.19_{3,44}$	0.91	$0.21_{3,44}$	0.89	$2.53_{3,43}$	0.07
Infection×day	$0.90_{2,89}$	0.41	$1.52_{2,89}$	0.23	$1.90_{2,89}$	0.15	$1.08_{2,89}$	0.34
Rank×day	$0.36_{6,89}$	0.90	$1.80_{6,89}$	0.11	$0.97_{6,89}$	0.45	$0.96_{6,89}$	0.46
Infection×rank×day	$0.30_{6,89}$	0.93	$1.92_{6,89}$	0.09	$1.34_{6,89}$	0.25	$1.41_{6,89}$	0.22

Linear mixed-effect models testing for effects of parasite infection, sampling day and social rank on lipid peroxidation levels (malondialdehyde, MDA) in plasma, lipid peroxidation levels (MDA) in red blood cells (RBCs), the ratio between oxidized and reduced glutathione ($GSSG_{soma}/GSH_{soma}$) and superoxide dismutase (SOD) activity in RBCs. Each male was sampled after 0, 9 and 18 days of infection. Significant terms are highlighted in bold.

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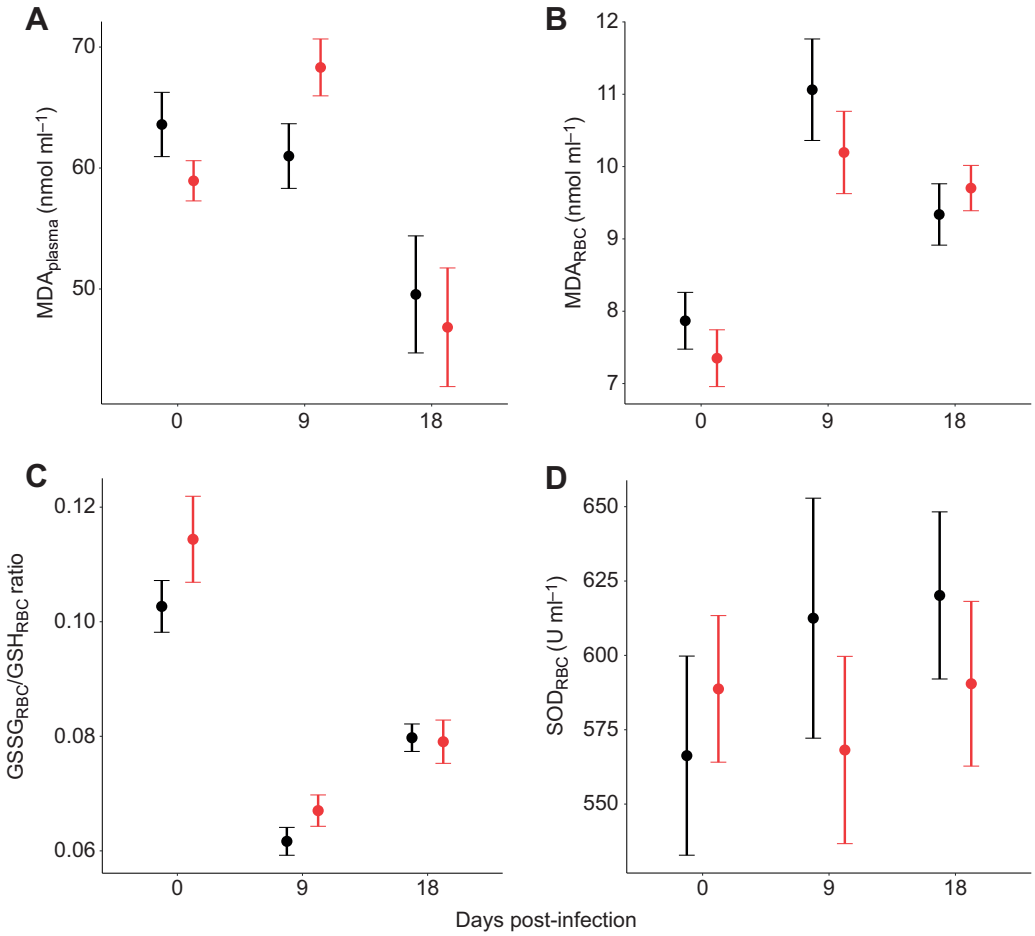

Fig. 1. Oxidative stress in soma. Mean (±s.e.m.) values of (A) malondialdehyde (MDA) levels in plasma, (B) MDA levels in red blood cells (RBCs), (C) glutathione ratio (GSH/GSSG) in RBCs and (D) superoxide dismutase (SOD) activity in RBCs according to parasite infection (black: control, red: infected) and sampling day for all 56 male house sparrows.

sperm and soma ($SOD_{sperm}/SOD_{sperm+RBC}$). The parasite infection (two-level factor, infected or control), sampling day (three-level factor; pre-infection, day 9 and day 18 post-infection), male social rank (four-level factor: dominant, and subordinate 1, 2 and 3) and the three-way interactions were fitted as fixed effects.

For sperm motility and sperm velocity, models included fixed effects of parasite infection, sampling day, male social rank and the three-way interaction. For sperm velocity, the analysis was conducted on all motile sperm and also on the fastest 10% and 5% of sperm. We also quantified correlations between each of the two metrics of sperm performance and each marker of oxidative stress in sperm using mixed-effect models.

All models included random male identity nested within aviary identity to control for the non-independence among males being kept in the same aviary and for the three measurements per male. To achieve normality of the residuals, we log-transformed MDA, SOD and the GSSG/GSH ratio, and we logit-transformed all proportions. Models were fitted with restricted maximum likelihood estimation of parameters, and degrees of freedom for fixed effects were calculated using the Kenward–Roger approximation. *Post hoc*

Table 2. Oxidative stress in sperm

	MDA_{sperm}		$GSSG_{sperm}/GSH_{sperm}$		SOD_{sperm}	
Effect	$F_{d.f.}$	*P*	$F_{d.f.}$	*P*	$F_{d.f.}$	*P*
Parasite infection	$0.53_{1,39}$	0.47	$0.003_{1,41}$	0.95	$0.69_{1,41}$	0.41
Rank	$0.47_{3,39}$	0.71	$0.26_{3,41}$	0.86	$0.96_{3,41}$	0.42
Day	**$4.61_{2,78}$**	**0.01**	$2.58_{2,80}$	0.08	**$4.82_{2,82}$**	**0.01**
Infection×rank	**$3.39_{3,39}$**	**0.03**	$2.44_{3,41}$	0.08	$0.20_{3,41}$	0.90
Infection×day	$0.35_{2,78}$	0.71	$0.64_{2,80}$	0.53	$1.85_{2,82}$	0.16
Rank×day	$1.36_{6,78}$	0.24	$1.07_{6,80}$	0.39	$2.03_{6,82}$	0.07
Infection×rank×day	$1.37_{6,78}$	0.24	$0.62_{6,80}$	0.71	$1.12_{6,82}$	0.36

Linear mixed-effects models testing for effects of parasite infection, sampling day and social rank on lipid peroxidation levels (MDA) in sperm, the ratio between oxidized and reduced glutathione ($GSSG_{sperm}/GSH_{sperm}$) in sperm and SOD activity in sperm. Each individual was sampled after 0, 9 and 18 days of infection. Significant terms are highlighted in bold.

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comparisons were conducted using *t*-tests based on least square means with Tukey correction for multiple tests (R package 'lsmeans'; Lenth, 2016).

RESULTS

Data structure

Our experiment included 56 males (28 parasitized, 28 control) that were blood and sperm sampled three times: immediately before infection (day 0), 9 days after infection and 18 days after infection. We also measured the intensity of coccidian infection before infection and after 18 days of infection; immediately before infection (after all individuals had received the antibiotic), the intensity of infection was close to zero across all males (0±3 oocysts g^{-1}, only two males had oocysts) whereas 18 days post-infection, infected males had 58 times more oocysts in their faeces than control males (infected: 334±565 oocysts g^{-1}; control: 6±13 oocysts g^{-1}, Wilcoxon matched-pairs test: V=17.0, P<0.0001). Our antibiotic treatment and coccidian infection were hence effectively conducted. Across all 56 males, body mass did not differ between experimental groups before or after infection ($F_{2,108}$=0.99, P=0.37). Male size, measured as tarsus length, also did not differ between experimental groups ($F_{1,54}$=0.002, P=0.97).

Oxidative stress in soma

Parasite infection and male social rank did not affect MDA_{plasma}, MDA_{RBC}, the $GSSG_{RBC}/GSH_{RBC}$ ratio or SOD_{RBC} (Table 1, Fig. 1). GSH_{RBC} and $GSSG_{RBC}$ tested independently were both significantly affected by the interaction social rank×sampling day (Table S1). *Post hoc* analyses revealed that after 9 days, dominant males tended to have higher GSH_{RBC} and $GSSG_{RBC}$ values than lowest ranking subordinate-3 males (GSH_{RBC}: t=4.06, P<0.001; $GSSG_{RBC}$: t=3.36, P=0.06). Other pairwise comparisons between differently ranked males were not significant for both GSH_{soma} and $GSSG_{soma}$ (all t<2.70, P>0.24).

We observed a significant effect of sampling day on MDA_{plasma}, MDA_{RBC} and $GSSG_{RBC}/GSH_{RBC}$ (but not on SOD_{RBC}; Table 1, Fig. 1). MDA_{plasma} did not differ between 0 and 9 days of infection (t=0.44, P=0.70) but then generally decreased (9 versus 18 days post-infection: t=−5.11, P<0.001; 0 versus 18 days post-infection: t=−4.68, P<0.001; Fig. 1A). MDA_{RBC} generally increased during the experiment (0 versus 9 days post-infection: t=5.59, P<0.001; 0 versus 18 days post-infection: t=4.53, P<0.001; Fig. 1B). The $GSSG_{RBC}/GSH_{RBC}$ ratio decreased after 9 days (t=−14.0, P<0.001) and then increased between day 9 and day 18 post-infection (t=−5.60, P<0.001; Fig. 1C). The $GSSG_{RBC}/GSH_{RBC}$ ratio was significantly lower at the end of the experiment than at the start (t=−8.38, P<0.0001; Fig. 1C).

Oxidative stress in sperm

Parasite infection and male social rank did not affect MDA_{sperm}, the $GSSG_{sperm}/GSH_{sperm}$ ratio or SOD_{sperm} (Table 2, Fig. 2). GSH_{sperm} and $GSSG_{sperm}$ tested independently also remained unaffected (Table S1). A significant effect of sampling day was found for MDA_{sperm} and SOD_{sperm} (Table 2, Fig. 2). MDA_{sperm} remained stable between 0 and 9 days post-infection (t=0.95, P=0.35) and then decreased between days 9 and 18 post-infection (t=2.02, P=0.047), remaining lower after 18 days of infection compared with that at the start of the experiment (t=2.91, P=0.005; Fig. 2A). The significant infection×social rank interaction (Table 2) compares the effect of the coccidian infection among social ranks without accounting for time. *Post hoc* tests revealed that MDA_{sperm} of males with the same social rank did not differ between infected versus control birds and

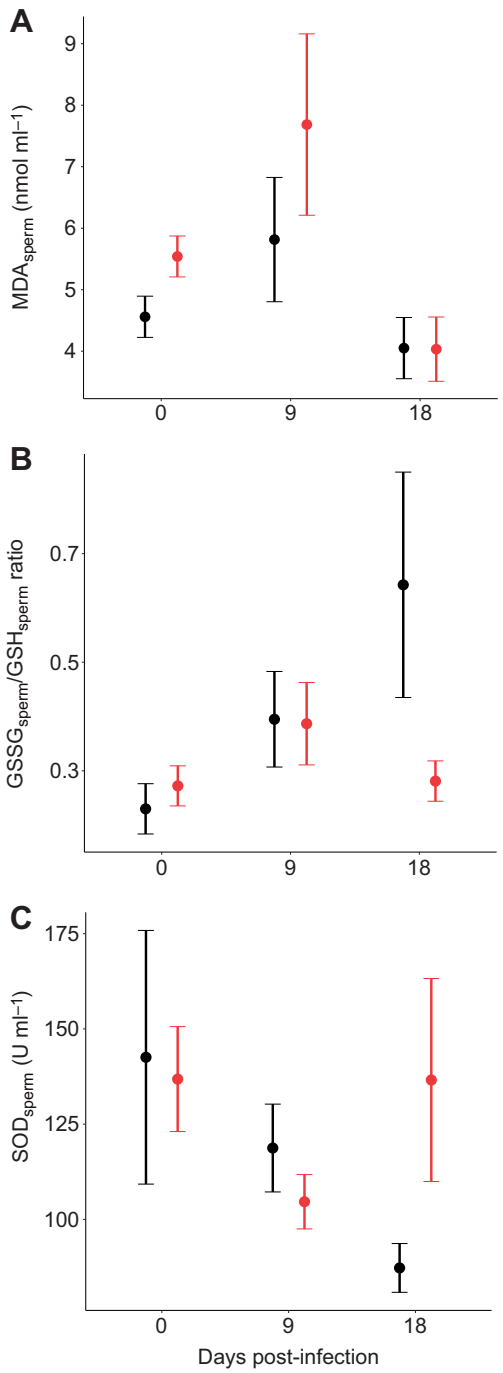

Fig. 2. Oxidative stress in sperm. Mean (±s.e.m.) values of (A) MDA levels, (B) GSH/GSSG ratio and (C) SOD activity in sperm according to parasite infection (black: control, red: infected) and sampling day for all 56 male house sparrows.

MDA_{sperm} of males with the same infection treatment did not differ across social rank (all t<2.68, P>0.14). Consequently, this interaction will not be further discussed. SOD_{sperm} generally decreased (0 versus 18 days post-infection: t=3.06, P=0.003; Fig. 2C).

Proportion of oxidative stress in sperm versus sperm and soma

The proportion $MDA_{sperm}/MDA_{sperm+RBC}$ was generally not affected by the infection but there was a marginally non-significant effect of the interaction between infection and social

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Table 3. Proportion of oxidative stress in sperm

	$MDA_{sperm}/MDA_{sperm+RBC}$		$GSH_{sperm}/GSH_{sperm+RBC}$		$SOD_{sperm}/SOD_{sperm+RBC}$	
Effect	$F_{d.f.}$	P	$F_{d.f.}$	P	$F_{d.f.}$	P
Parasite infection	$1.19_{1,39}$	0.28	$0.05_{1,42}$	0.83	$1.39_{1,41}$	0.24
Rank	$0.18_{3,39}$	0.91	$0.96_{3,42}$	0.42	$0.43_{3,41}$	0.73
Day	**$8.93_{2,78}$**	**<0.001**	$2.60_{2,81}$	0.08	**$5.29_{2,82}$**	**0.007**
Infection×rank	$2.78_{3,39}$	0.054	$2.24_{3,42}$	0.10	$0.35_{3,41}$	0.79
Infection×day	$0.48_{2,78}$	0.62	$2.94_{2,81}$	0.058	$1.27_{2,82}$	0.29
Rank×day	$1.71_{6,78}$	0.13	$1.65_{6,81}$	0.14	$1.04_{6,82}$	0.41
Infection×rank×day	$1.17_{6,78}$	0.33	$0.73_{6,81}$	0.63	$0.71_{6,82}$	0.64

Linear mixed models testing for effects of parasite infection, sampling day and male social rank on the proportion of MDA, GSH and SOD in sperm versus sperm and soma. All 56 individuals were sampled after 0, 9 and 18 days of infection. Significant terms are highlighted in bold.

rank ($F_{3,39}$=2.78, P=0.054; Table 3). *Post hoc* comparisons revealed that among subordinate-3 males, $MDA_{sperm}/MDA_{sperm+RBC}$ was higher in infected males than in controls (t=2.76, P=0.009). Other higher ranked males did not show a similar pattern (all t<1.04, P>0.30). $MDA_{sperm}/MDA_{sperm+RBC}$ significantly decreased over the experiment (Table 3, Fig. 3B), indicating a relative reduction in sperm oxidative damage compared with damage in RBCs over the course of the experiment.

The proportion $GSH_{sperm}/GSH_{sperm+RBC}$ was not generally affected by the infection but there was a marginally non-significant effect of the interaction between infection and sampling day ($F_{2,81}$=2.94, P=0.058; Table 3). *Post hoc* comparisons revealed a significant decrease in $GSH_{sperm}/GSH_{sperm+RBC}$ between day 9 and day 18 post-infection in the control group only (t=−2.75, P=0.007; Fig. 3A). However, infected and control groups did not differ from each other within sampling days, hence confirming an absence of treatment effect (all t<1.38, P>0.17; Fig. 3A).

The proportion $SOD_{sperm}/SOD_{sperm+RBC}$ was not affected by parasite infection or social rank but it generally decreased over the experiment (pre-infection versus day 18: t=3.12, P=0.03; Table 3, Fig. 3C), indicating a relative reduction in sperm SOD activity.

Sperm velocity and motility

Sperm velocity measured across all motile sperm was significantly affected by a three-way interaction among parasite infection, social rank and sampling day (Table 4, Fig. 4A). However, separate analyses, as well as *post hoc* analyses, showed that within each sampling day, sperm velocity was not affected by the infection for any of the hierarchical ranks (pre-infection: all t<1.42, all P>0.84; 9 days post-infection: all t<1.65, all P>0.72; 18 days post-infection: all t<1.70, all P>0.69). Yet, at pre-infection, irrespective of the infection treatment, sperm velocity was lower for subordinate-3 males than for subordinate-2 (t=2.81, P=0.007) and subordinate-1 males (t=2.32, P=0.024; Fig. 4A). In addition, after 18 days of infection, subordinate-3 males had significantly lower sperm velocity than subordinate-1 males (t=2.17, P=0.035). Other pairwise comparisons between similarly ranked males from different experimental groups or between similarly treated males with different ranks were not significant (all t<1.68, P>0.10; Fig. 4A). Across all males, sperm velocity was greater after 18 days of infection compared with pre-infection values (t=12.0, P<0.001; Fig. 4A). Analyses conducted on sperm velocity measured across the 10% and 5% fastest sperm gave similar results (Table S2).

The percentage of motile sperm was significantly affected by sampling day; it increased over the course of the experiment (Table 4, Fig. 4B), as observed for sperm velocity. However, sperm motility remained unaffected by parasite infection or social rank (Table 4).

Sperm velocity was negatively correlated with the levels of MDA in sperm (β=−0.97±0.42, $F_{1,135}$=4.98, P=0.03), indicating that high levels of sperm MDA are associated with reduced sperm velocity. Sperm motility did not show such a pattern (β=−0.003±0.020, $F_{1,138}$=0.03, P=0.87). Sperm velocity and motility were not significantly correlated with the $GSSG_{sperm}/GSH_{sperm}$ ratio (motility: β=0.007±0.013, $F_{1,144}$=0.32, P=0.57; velocity: β=0.10±0.31, $F_{1,144}$=0.08, P=0.77) or with sperm SOD activity (motility: β=−0.10±0.17, $F_{1,143}$=0.36, P=0.55; velocity: β=−5.75±4.01, $F_{1,141}$=1.92, P=0.17).

DISCUSSION

Using wild-caught house sparrows, we experimentally quantified the effect of a parasite infection on sperm performance and on the soma/germline oxidative trade-off. We also tested whether such effects were mediated by social hierarchy. We observed no effect of parasite infection on sperm performance or oxidative stress, and no interactive effect of male social status. In the course of the experiment, males generally shifted their soma/germline oxidative ratio towards oxidative protection of sperm, which went along with increased sperm performance. Further, males at the lower end of the social hierarchy generally had lower sperm velocity.

Our study shows that a mild parasite infection (body condition was unaffected), which commonly leads to a systemic immune response (e.g. Hõrak et al., 2004), does not affect sperm performance and hence may not reduce male fertilization success. Because our parasite infection was successfully conducted, with a 5467% increase in parasite load, our results may appear surprising. Indeed, immune challenges have been shown to impact sperm performance in some species (Losdat et al., 2011; Simmons, 2012; Kekäläinen et al., 2014). However, such deleterious effects of immunity on male reproductive function are not always observed. For example, a recent study on house sparrows showed no negative effect of immunity on sperm performance despite diminishing testosterone levels (Needham et al., 2017). Because sperm performance is supposedly condition dependent (Simmons and Kotiaho, 2002; Gasparini and Pilastro, 2011), one explanation for our negative result could be that the immune challenge triggered by the experimental infection was not energetically demanding enough to affect individual ‘condition’ and in turn to impact condition-dependent traits such as sperm performance. Corroborating this assumption, Lee et al. (2005) showed that house sparrows’ immune system is more plastic and induces milder systemic reactions in comparison to that of other passerine species. Additionally, the level of coccidian infection, which we experimentally induced, was almost 5 times lower than the one that significantly affected the health of another passerine species, *Cardualis chloris* (Hõrak et al., 2004). Moreover, the favourable captive conditions under which the experiment was conducted could also have contributed to limiting the extent of the sperm versus soma trade-off. Alternatively, because males of polygynandrous species are under strong selective pressure

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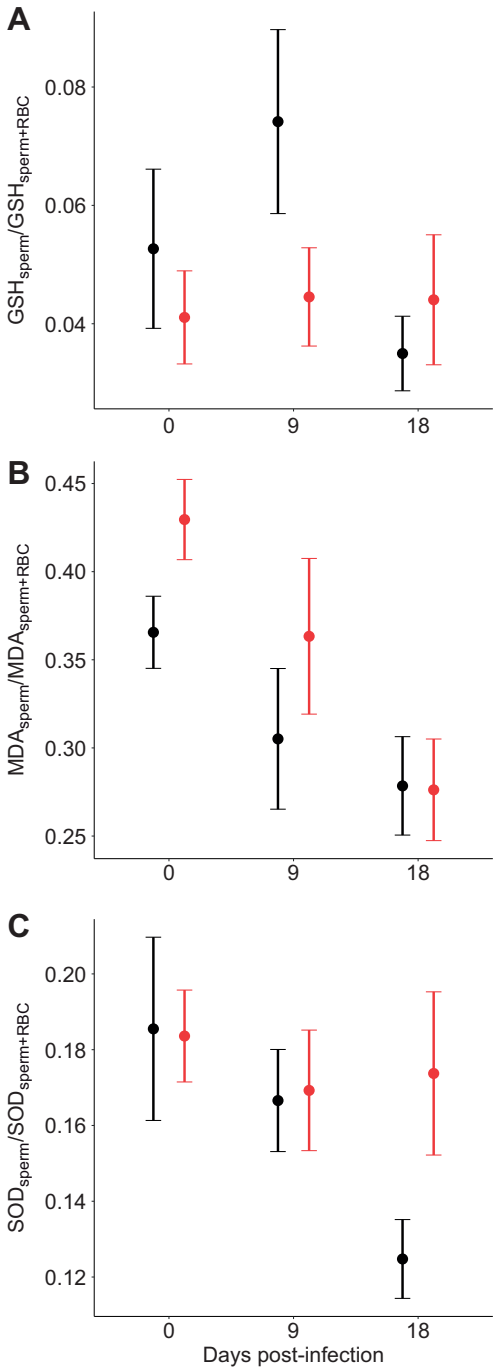

Fig. 3. Proportion of oxidative stress in sperm. Mean (±s.e.m.) values of (A) the proportion of glutathione (GSH) levels in sperm versus sperm and RBCs, (B) the proportion MDA levels in sperm versus sperm and RBCs and (C) the proportion SOD activity in sperm versus sperm and RBCs according to parasite infection (black: control, red: infected) and sampling day.

to produce high-quality sperm during the mating season (to maximize their within-pair and extra-pair fertilization success; Parker and Birkhead, 2013; Fitzpatrick and Lüpold, 2014), our results may have reflected a reallocation of energy and/or resources towards the germline, at the expense of other non-measured traits (e.g. long-term survival).

Male social rank was partly related to sperm performance, with males at the lower end of the hierarchy displaying lower sperm velocity, even before the onset of the experiment. Consequently,

Table 4. Sperm velocity and motility

	Sperm velocity (all sperm)		Sperm motility	
Effect	$F_{d.f.}$	P	$F_{d.f.}$	P
Intercept	–	–	–	–
Parasite infection	$2.08_{1,44}$	0.16	$0.72_{1,18}$	0.50
Rank	$2.28_{3,44}$	0.09	$0.82_{3,58}$	0.63
Day	**$144.0_{1,41}$**	**<0.001**	**$15.1_{1,43}$**	**<0.001**
Infection×rank	$0.73_{3,44}$	0.33	$0.49_{3,31}$	0.69
Infection×day	$0.22_{1,41}$	0.43	$0.01_{1,43}$	0.92
Rank×day	**$3.32_{3,41}$**	**0.03**	$1.00_{3,43}$	0.40
Infection×rank×day	**$2.98_{3,41}$**	**0.042**	$0.74_{3,43}$	0.53

Linear mixed-effects models testing for effects of parasite infection, sampling day and social rank on sperm velocity measured across all motile sperm and on sperm motility (the proportion of motile sperm). All 56 individuals were sampled 0, 9 and 18 days after the parasite infection. Significant terms are highlighted in bold.

males at the lower end of the hierarchy might achieve lower fitness not only due to reduced access to fertile females (Clutton-Brock and Huchard, 2013) but also due to reduced sperm velocity, a key predictor of male fertilization success (Malo et al., 2005; Pizzari and Parker, 2009; Fitzpatrick and Lüpold, 2014). In contrast, previous studies showed that subordinate males generally produce higher quality sperm than dominant males (Rudolfsen et al., 2006; Cornwallis and Birkhead, 2007; Haugland et al., 2009). However, this pattern was only revealed in species with discrete social roles, a model that does not apply to our study species. In house sparrows, which develop more complex hierarchies (Hegner and Wingfield, 1987), males at both the lower and higher ends of the hierarchy seem to produce lower quality sperm compared with males in the middle

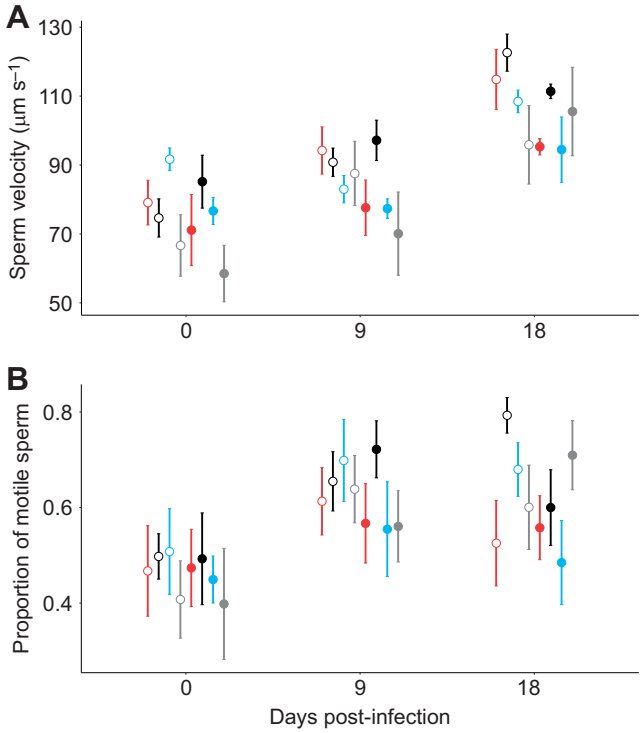

Fig. 4. Sperm velocity and motility. Mean (±s.e.m.) values of (A) sperm velocity and (B) sperm motility according to parasite infection (open circles, control; solid circles, infected) and male social rank (red, dominant; black, subordinate 1; blue, subordinate 2; grey, subordinate 3). Sperm traits were measured after 0, 9 and 18 days of infection.

of the hierarchy (Rojas Mora et al., 2017a). Overall, the effect of social hierarchy on sperm performance observed here suggests that males could have either strategically invested in their sperm performance differently depending on their rank and/or were limited by their access to resources as a result of their social rank (e.g. males at the lower end having more limited access to food sources).

Contrary to expectations, our parasite infection did not increase oxidative stress (measured as glutathione peroxidase, SOD and lipid peroxidation levels) in both soma and sperm, suggesting that immune challenges do not necessarily increase oxidative stress. Our results contradict previous work (e.g. Alonso-Alvarez et al., 2004; Costantini and Møller, 2009; Schneeberger et al., 2013) but are in line with some recent studies that also observed no effect of immunity on oxidative stress (Costantini et al., 2015; Cram et al., 2015a), potentially highlighting a more complex interplay between immunity and oxidative stress than previously thought. For example, as ROS are toxic to Coccidia (Min et al., 2004), a potential increase in ROS production due to coccidian infection may not be fully deleterious to an individual because those additional ROS may be used to fight off the parasites, which may mask the negative effects of immunity on oxidative status. In general, however, as immunity has clearly been shown to incur costs to fitness-related traits (Ashley et al., 2012; Hasselquist and Nilsson, 2012), the absence of deleterious effects on male resistance to oxidative stress observed here and elsewhere (Costantini et al., 2015; Cram et al., 2015a) might have come at the expense of other fitness traits that were not measured (e.g. long-term survival).

Experimentally immune-challenging males also did not influence the soma versus germline oxidative ratio, irrespective of male social rank. This is probably due to the fact that experimentally increasing the parasite load affected neither the blood or sperm redox balance nor sperm quality. Nevertheless, an experiment similar to ours recently reported an immune-induced shift of the soma versus germline ratio; lipopolysaccharide injection reduced guppies' sperm quality but did not impair their somatic functions, an effect attributed to differential antioxidant allocation (Devigili et al., 2017). It may therefore seem that immunity has some potential to influence the soma/germline balance and in turn reproductive strategies, but such a pattern may be context and/or species dependent.

In this experiment, we also witnessed a relative decrease in the amount of oxidative damage in sperm versus RBCs, and a concomitant increase in sperm performance over time. This 'time effect' probably reflects a seasonal effect due to some unidentified season-related changes in several potential uncontrolled factors (e.g. length of daylight, temperature, vegetation growth, etc.). Nevertheless, this general shift in the soma/germline balance, irrespective of the parasite treatment, with oxidative damage in sperm (relative to total oxidative damage) diminishing over the course of the experiment may reflect the fact that males progressively invested relatively more in the antioxidant protection of their germline along the reproductive period, which may have been enabled by captivity itself (i.e. *ad libitum* food, absence of predators). Interestingly, sperm performance mirrored this pattern, with sperm motility and velocity found to increase over the course of the experiment, confirming previously observed negative correlations between germline oxidative damage and sperm swimming performance (Losdat et al., 2011; Rojas Mora et al., 2017a,b). These results, together with our previous studies, strongly suggest that male reproductive tactics may be constrained by oxidative stress and may be physiologically modulated by plastic allocation of resources to the antioxidant protection of germline versus somatic functions.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.R.M., F.H.; Methodology: A.R.M., F.H.; Validation: F.H.; Formal analysis: S.L., C.B.; Investigation: A.R.M., R.C., V.F.; Resources: G.G., A.V.; Data curation: S.L., A.R.M.; Writing - original draft: S.L., C.B., F.H.; Writing - review & editing: S.L., F.H.; Visualization: S.L.; Supervision: F.H.; Project administration: F.H.; Funding acquisition: F.H.

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Electronic Supplementary Material for "Social dominance, but not parasite load, affects sperm quality and sperm redox status in house sparrows."

Table S1. Linear mixed-effect models testing for effects of the parasite infection, sampling day and the social rank on the oxidized and reduced glutathione levels in red blood cells ($GSSG_{RBC}$ and GSH_{RBC}) and in sperm ($GSSG_{sperm}$ and GSH_{sperm}). Each male was sampled after 0, 9 and 18 days of infection.

	$GSSG_{RBC}$		GSH_{RBC}		$GSSG_{sperm}$		GSH_{sperm}	
Effect	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P
Parasite infection[a]	0.81 $_{1,43}$	0.37	0.07 $_{1,43}$	0.78	0.48 $_{1,41}$	0.49	0.04 $_{1,41}$	0.84
Rank	0.17 $_{3,43}$	0.91	0.66 $_{3,43}$	0.58	1.49 $_{3,41}$	0.23	1.02 $_{3,41}$	0.39
Day	**97.1** $_{2,88}$	**< 0.001**	**24.6** $_{2,88}$	**< 0.001**	1.12 $_{2,81}$	0.33	0.60 $_{2,81}$	0.55
Infection × Rank	0.88 $_{3,43}$	0.46	1.69 $_{3,43}$	0.18	0.06 $_{3,41}$	0.98	1.75 $_{3,41}$	0.17
Infection × Day	**4.65** $_{2,88}$	**0.01**	3.06 $_{2,88}$	0.052	1.52 $_{2,81}$	0.22	2.33 $_{2,81}$	0.10
Rank x Day	**3.73** $_{6,88}$	**0.002**	**2.66** $_{6,88}$	**0.02**	1.55 $_{6,81}$	0.17	1.47 $_{6,81}$	0.20
Infection × Rank x Day	0.91 $_{6,88}$	0.49	1.80 $_{6,88}$	0.11	0.83 $_{6,81}$	0.55	1.06 $_{6,81}$	0.40

Table S2. Linear mixed-effect models testing for effects of the parasite infection, sampling day and social rank on sperm velocity measured across the 5% and across the 10% faster sperm. All 56 males were sampled 0 and 18 days after the parasite infection.

	Sperm velocity (10% faster)		**Sperm velocity (5% faster)**	
Effect	F_{df}	P	F_{df}	P
Intercept	-	-	-	-
Parasite treatment[a]	$2.08_{1, 44}$	0.16	$0.72_{1,18}$	0.50
Rank	$2.28_{3, 44}$	0.09	$0.82_{3, 58}$	0.63
Day[b]	**$144.0_{1, 41}$**	**< 0.001**	**$15.1_{1, 43}$**	**<0.001**
Treatment[a] × Rank	$0.73_{3, 44}$	0.33	$0.49_{3, 31}$	0.69
Treatment[a] × Day[b]	$0.22_{1, 41}$	0.43	$0.01_{1, 43}$	0.92
Rank x Day[b]	**$3.32_{3, 41}$**	**0.03**	$1.00_{3, 43}$	0.40
Treatment[a] × Rank x Day[b]	**$2.98_{3, 41}$**	**0.042**	$0.74_{3, 43}$	0.53