ANA PAULA PINOTI PAVANELI

Effect of organic selenium on the cooled boar semen quality and reproductive performance

São Paulo

2022

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CORRECTED VERSION

Thesis submitted to the Postgraduate Program in Animal Reproduction of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Department:
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Advisor:
Prof. André Furugen Cesar de Andrade, Ph.D.

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CERTIFICADO

Certificamos que a proposta intitulada "Efeito do selênio orgânico sobre a qualidade do sêmen suíno refrigerado e a performance reprodutiva de cachaços", protocolada sob o CEUA nº 3955160419 (ID 006663), sob a responsabilidade de **André Furugen César de Andrade** *e equipe; Ana Paula Pinoti Pavaneli* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 19/06/2019.

We certify that the proposal "Effect of organic selenium on the cooled boar semen quality and reproductive performance", utilizing 69 Swines (69 males), protocol number CEUA 3955160419 (ID 006663), under the responsibility of **André Furugen César de Andrade** *and team; Ana Paula Pinoti Pavaneli* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 06/19/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 05/2019 a 08/2019 Área: Reprodução Animal

Origem:	Animais provenientes de estabelecimentos comerciais						
Espécie:	Suínos	sexo:	Machos	idade:	8 a 31 meses	N:	69
Linhagem:	DB 20; DB 30 e DB 50			Peso:	200 a 450 kg		

Local do experimento: Unidade de Difusão Genética (UDG), DB Genética Suína - Fazenda São Zeferino, Varjão de Minas/MG.

São Paulo, 19 de junho de 2019

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

Author: PAVANELI, Ana Paula Pinoti

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Date: ___/___/___

Committee Members

Prof.: _____

Institution: _____ Decision: _____

Prof.: _____

Institution: _____ Decision: _____

Prof.: _____

Institution: _____ Decision: _____

Prof.: _____

Institution: _____ Decision: _____

Prof.: _____

Institution: _____ Decision: _____

I dedicate this thesis to the most important people of my life,

my parents Eliete and Aparecido Pavaneli,

and grandparents Alice and Luiz Pinoti.

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"Do your best, in the condition you have,

while you don't have better conditions, to do even better."

Mario Sergio Cortella

(Brazilian philosopher, writer, educator, speaker, and university professor)

RESUMO

PAVANELI, A. P. P. **Efeito do selênio orgânico sobre a qualidade do sêmen suíno refrigerado e a performance reprodutiva de cachaços.** 91 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

O selênio é um micromineral essencial para o desenvolvimento e maturação dos espermatozoides, atuando como modulador da produção e qualidade espermática, e fertilidade masculina. Na espécie suína, fontes inorgânicas de selênio como o selenito de sódio (SS) têm sido ainda amplamente utilizadas para a suplementação das dietas, visando atender as exigências nutricionais de cada uma das categorias dentro do sistema de produção. Considerando a maior biodisponibilidade das fontes orgânicas, o presente estudo buscou avaliar o uso da hidróxi-selenometionina (OH-SeMet) na dieta de cachaços, uma fonte pura e orgânica de selênio, sobre as características seminais e performance reprodutiva dos animais. Avaliou-se também dois níveis de inclusão de OH-SeMet. Para isso, 42 cachaços de raça pura (Landrace e Large-White) com idades entre 8 e 31 meses foram alimentados com os seguintes tratamentos por 95 dias: 0,3 mg selênio/kg via SS (n = 14); 0,3 mg selênio/kg via OH-SeMet (n = 14); e 0,6 mg selênio/kg via OH-SeMet (n = 14). Neste período, dois experimentos foram conduzidos concomitantemente. No experimento 1, o sêmen *in natura* foi avaliado quanto ao volume, concentração, motilidade e morfologia espermática, e atividade da enzima glutationa peroxidase (GPx) em plasma seminal. Doses inseminantes foram processadas, armazenadas a 17 ºC por 72 h, e após isso, avaliadas quanto à qualidade espermática (motilidade, morfologia, integridade de membranas, e resistência ao estresse oxidativo). Quantificou-se ainda, a concentração de selênio no plasma seminal e sanguíneo dos animais. As coletas de sêmen aconteceram semanalmente, enquanto as análises experimentais foram conduzidas a cada 2 semanas, totalizando 7 pontos de avaliação durante o estudo. No experimento 2, um total de 1131 fêmeas de raça pura (Landrace e Large-White) foi inseminado com doses provenientes dos diferentes tratamentos para avaliação de taxa de prenhez (TP) e características de leitegada. As inseminações aconteceram ao longo de todo o período experimental (95 dias), com doses sendo enviadas às granjas semanalmente. Os efeitos de fonte (SS *vs.* OH-SeMet) e de nível de suplementação orgânica (0,3 *vs.* 0,6 mg selênio/kg) foram avaliados em ambos os experimentos. Cachaços alimentados com OH-SeMet apresentaram maior concentração de selênio no plasma seminal ($p < 0,05$), bem como tenderam ($p < 0,10$) para uma maior contagem espermática no ejaculado (66,6 *vs.* 56,57 × 10^9) e, consequentemente, para um maior número de doses produzidas (22,11 *vs.* 18,85). Nenhum efeito foi observado sobre a concentração de selênio no

plasma sanguíneo, atividade da GPx no plasma seminal, e qualidade espermática no sêmen *in natura* e refrigerado ($p > 0,05$). Nas granjas, doses inseminantes do grupo OH-SeMet resultaram em maior TP (99,3 *vs.* 97%) e menor porcentagem de natimortos (5,87 *vs.* 7,11%) ($p < 0,05$). Quanto ao uso de diferentes níveis de suplementação com a OH-SeMet, não foi observada qualquer diferença entre os valores estudados ($p > 0,05$). Como conclusão, a substituição de SS por OH-SeMet na dieta de cachaços aumenta a disponibilidade de selênio para o sistema reprodutor destes animais, parece melhorar a produção espermática, e resulta em melhor performance reprodutiva quando doses inseminantes são utilizadas nas granjas.

Palavras-chave: Hidróxi-selenometionina. Selenito de sódio. Qualidade seminal. Fertilidade.

ABSTRACT

PAVANELI, A. P. P. **Effect of organic selenium on the cooled boar semen quality and reproductive performance.** 91 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2022.

Selenium is an essential trace mineral for sperm development and maturation, acting as a modulator of sperm production and quality and male fertility. In pigs, inorganic selenium sources such as sodium selenite (SS) have been widely used to supplement diets, aiming to meet the nutritional requirements of each animal category within the production system. Considering the greater bioavailability of organic sources, the present study aimed to evaluate the use of hydroxy-selenomethionine (OH-SeMet) in the boars' diet, a pure and organic source of selenium, on their semen characteristics and reproductive performance. It also evaluated two supplementation levels of OH-SeMet. For this, 42 purebred boars (Large-White and Landrace) aged 8 to 31 months were fed with the following dietary treatments during 95 days: 0.3 mg selenium/kg as SS (n = 14); 0.3 mg selenium/kg as OH-SeMet (n = 14); and 0.6 mg selenium/kg as OH-SeMet (n = 14). During this period, two experiments were carried out concurrently. In experiment 1, raw semen was evaluated for volume, sperm concentration, motility and morphology, and activity of the enzyme glutathione peroxidase (GPx) in seminal plasma. Semen doses were processed, stored at 17 ºC for 72 h, and after that, evaluated for sperm quality (motility characteristics, morphology, integrity of membranes, and resistance to oxidative stress). The concentration of selenium in the seminal and blood plasma of the animals was also quantified. Semen collections occurred weekly, while experimental analyzes were carried out every 2 weeks, totaling 7 evaluation points during the study. In experiment 2, a total of 1131 purebred females (Large-White and Landrace) were inseminated with doses from the different treatments to assess the pregnancy rate (PR) and litter characteristics. Inseminations occurred throughout the experimental period (95 days), with semen doses sent to the farms weekly. The effects of the source (SS *vs.* OH-SeMet) and organic supplementation level (0.3 *vs.* 0.6 mg selenium/kg) were evaluated in both experiments. Boars fed OH-SeMet had more selenium in their seminal plasma ($p < 0.05$), as well as tended ($p < 0.10$) toward a higher total sperm count in the ejaculate (66.6 *vs.* 56.57×10^9) and, consequently, for an improvement on the number of semen doses produced (22.11 *vs.* 18.85). No effect was observed on selenium concentration in blood plasma, GPx activity in seminal plasma, and sperm quality in raw and stored semen ($p > 0.05$). On the farms, semen doses from the OH-SeMet group resulted in higher PR (99.3 *vs.* 97%) and fewer stillborn piglets (5.87 *vs.* 7.11%) ($p < 0.05$). Regarding the use of different

levels of OH-SeMet supplementation, no difference was observed between the values studied ($p > 0.05$). In conclusion, replacing SS with OH-SeMet in the boars' diet increases selenium availability in their reproductive system, seems to improve sperm production and results in better reproductive performance when semen doses are used on the farms.

Keywords: Hydroxy-selenomethionine. Sodium selenite. Semen quality. Fertility.

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SUMMARY

1 INTRODUCTION

Pig farming is an activity in constant expansion. Part of this is a result of the implementation and improvement of reproductive biotechnologies such as artificial insemination (AI) in farms. In this scenario, improving the use of boars of high genetic potential and the quality of semen AI-doses is highly relevant to the system. Herein, it is worth highlighting the importance of AI-centers. Units specialized in the semen collection, processing, and commercialization of doses, bring convenience, safety, and rapid genetic advancement to farms. In addition to factors such as genetics, animal health, semen collection, storage, and transport of semen AI-doses, providing adequate nutrition to boars has been increasingly important to obtain optimal semen quality and better reproductive results in the field (CHEAH; YANG, 2011; DONG *et al.*, 2016).

Regarding the trace minerals commonly incorporated in the mammal diet, selenium has been shown not only to meet the minimum nutritional requirements for vital functions, but also to be extremely important for reproduction in different species, both in males and females (AHSAN *et al.*, 2014; QAZI *et al.*, 2018, 2019). In the male, two selenoproteins (selenium-containing proteins) seem to best explain the link between dietary selenium and its effects on semen quality and fertility. The first of them, Selenoprotein P, is responsible for taking the mineral to the male reproductive system, specifically the testes. In this organ, the phospholipid hydroperoxide glutathione peroxidase (GPx4 or PHGPx) is the main selenoprotein produced and performs a double role: as an antioxidant enzyme during spermatogenesis and then as a structural component of spermatozoa during its maturation (URSINI *et al.*, 1999; PFEIFER *et al.*, 2001; FLOHÉ, 2007; BURK; HILL, 2015). In the seminal plasma, the prominent role of the glutathione peroxidase (GPx) as an antioxidant, as well as a possible contribution of Selenoprotein P protecting sperm during storage and transport into the female reproductive tract, have been reported in males from different species (KOZIOROWSKA-GILUN *et al.*, 2011; MICHAELIS *et al.*, 2014; BURK; HILL, 2015; QAZI *et al.*, 2019).

The role of dietary selenium on boar reproduction was first demonstrated at the end of the 20th century. Marin-Guzman *et al.* (1997, 2000a) observed that insufficient levels of selenium in the diet resulted in detrimental effects on boar sperm structure and functionality, as well as on fertilization rates obtained *in vivo.* Also, it was indicated the participation of selenium in establishing the number of Sertoli cells in the seminiferous tubules and, consequently, the number of boar sperm reserves in the testis (MARIN-GUZMAN *et al.*, 2000b). Even today,

these pieces of evidence continue to drive the work of researchers worldwide about how advances in nutrition can enhance the beneficial effects of selenium on boar reproduction.

Based on the current literature, adult boars' daily selenium requirement corresponds to 0.3 mg/kg of dry matter ingested (NRC, 2012; ROSTAGNO *et al.*, 2017). Given the insufficient levels found in primary feed ingredients used for farm animals' nutrition, and the widely known importance of this mineral for reproduction, selenium supplementation has been a routine practice in pigs, especially in males of high genetic potential in AI-centers. In this way, the mineral requirement can be met from two commercial sources: inorganic or organic. As the name suggests, organic ones are selenium accompanied by an organic molecule forming a complex. This molecule can be any amino acid, protein, or polysaccharide, which facilitates selenium absorption and its use by the organism while reducing excretion and environmental impact (POWER; HORGAN, 2000). Furthermore, organic sources allow the mineral to be stored in the body as selenium reserves, mainly in muscle tissues, so that it can be used later under stress conditions, such as a disease or a climate condition, which the animal can stop feeding while demanding even more selenium to overcome these adverse situations (SURAI; FISININ, 2015).

In pigs, when comparing the use of different selenium sources on boar reproduction, precisely sodium selenite (inorganic) *vs.* selenomethionine (SeMet; organic) via selenium-enriched yeast (Se-yeast), there are controversial results. While a range of studies reported benefits in favor of the organic form (SPEIGHT *et al.*, 2012; MARTINS *et al.*, 2014, 2018; PETRUJKIĆ *et al.*, 2014; ESTIENNE; WHITAKER, 2017), others suggested that organic and inorganic forms are equivalent (LOVERCAMP *et al.*, 2013; MARTINS *et al.*, 2015) or even that SeMet can negatively affect sperm quality (LÓPEZ *et al.*, 2010). The lack of information about the selenium concentration in the basal diet offered to the animals, and the well-known variation in SeMet concentration delivered by commercial Se-yeast (a range from 21 to 70%), may contribute to different findings (SURAI; FISININ, 2015; GERAERT *et al.*, 2015).

Among the organic selenium forms currently available, hydroxy-selenomethionine (OH-SeMet) or also known as 2-hydroxy-4-methylselenobutanoic acid (HMSeBA), has been identified as the most bioavailable (GERAERT *et al.*, 2015; SURAI *et al.*, 2018). Studies in poultry and pigs showed positive aspects for OH-SeMet, as a better tissue selenium enrichment and a greater selenium transfer to the eggs (BRIENS *et al.*, 2013, 2014; JLALI *et al.*, 2013, 2014; COULOIGNER *et al.*, 2015; CHAO *et al.*, 2019), when compared to inorganic or other organic forms. Recently, it was also demonstrated that OH-SeMet-supplemented sows had

increased litter size, better transfer of passive immunity for piglets, and an improvement in their antioxidant capacity, as well as observed for their offspring (LI *et al.*, 2020; MOU *et al.*, 2020).

Despite the good results observed so far with the use of OH-SeMet in other animal species and pig categories, nothing is reported about using this source in the diet of boars on sperm production, semen quality, and fertility. Thus, the present study is the first to evaluate the effects of OH-SeMet as dietary supplementation of boars on raw semen characteristics (volume, sperm concentration, motility, morphology, and glutathione peroxidase (GPx) activity in seminal plasma); the quality of semen AI-doses after 72h of storage at 17 ºC (sperm motility characteristics, morphology, integrity of membranes, and sperm resistance to oxidative stress); and the reproductive performance (pregnancy rate; the total number of piglets born, born alive, mummies, and stillborn). Also, the work aims to identify between two levels of organic selenium supplementation (0.3 and 0.6 mg selenium/kg), the most efficient for reproductive parameters.

2 LITERATURE REVIEW

2.1 SELENIUM REGULATION IN THE BODY

Selenium is an essential trace mineral commonly incorporated in the diet of animals and humans, performing essential roles in their health, both at the cellular and organismal levels. After its absorption by the gastrointestinal tract, selenium's biological effects are mediated by the so-called selenoproteins, a distinct protein class selenium-dependent for its structural composition (encoded by 25 genes in humans and 24 in mice) (LABUNSKYY; HATFIELD; GLADYSHEV, 2014; QAZI *et al.*, 2019). Such proteins are produced by different organs and tissues and perform structural and enzymatic roles, most of them well-known for their catalytic and redox functions, contributing to regulating the whole organism's antioxidant system (BURK; HILL, 2015; SURAI; FISININ, 2015).

The liver is the first organ encountered by selenium after its absorption and is the center responsible for the mineral regulation and distribution to extrahepatic tissues. Selenium is transported as selenocysteine residues in the form of Selenoprotein P, the only selenoprotein able to carry multiple of them in its structure. Selenoprotein P is the selenium form used to produce other selenoproteins in organs such as the kidney, heart, muscle, brain, testis, and the liver itself (LABUNSKYY; HATFIELD; GLADYSHEV, 2014; SCHWEIZER *et al.*, 2016). Two low-density lipoprotein receptor family members can bind to Selenoprotein P and facilitate its uptake in these tissues, the apolipoprotein E receptor-2 (apoER2) and megalin (BURK; HILL, 2015).

When selenium intake meets required nutritional levels, selenoproteins are produced according to each organ's maintenance and export needs. The optimal expression of all selenoproteins in the body may be impaired when selenium is limited. Two hierarchical situations can occur, one among the organs for selenium utilization, and another at a cellular level, aiming to prioritize the synthesis of vital selenoproteins within each organ. For instance, hepatic cells sacrifice the production of selenoproteins for their internal use to secrete Selenoprotein P into the plasma to supply higher-ranking organs such as the brain and the testis under selenium-deficient conditions (BURK; HILL, 2015). A study with mice demonstrated there is still an exciting competition between the brain and testis for selenium availability under selenium-compromised conditions, with concomitant effects on neurodevelopment and neurodegeneration (PITTS *et al.*, 2015).

In the same way, as in the liver, kidney tissues also decrease the levels of specific selenoprotein transcripts while others are maintained when selenium is limited, which indeed are of greater importance for their proper functioning (SUNDE *et al.*, 2009). On the other hand, in the case of excess dietary selenium intake and as a control mechanism against possible toxicity, the liver wisely increases the excretion of selenium metabolites, predominantly via the urine and feces (trimethylselenonium and selenosugar forms), and in extreme cases, also by breathing (dimethyl selenide) (BURK; HILL, 2015).

2.2 SELENIUM IN THE MALE REPRODUCTIVE SYSTEM – ROLE IN SPERMATOGENESIS AND SPERM MATURATION

Although the exact concentration of selenium in the male reproductive system is unknown, it has been reported that the testes have priority for its retention and use in periods of insufficient selenium intake (BURK; HILL, 2015; PITTS *et al.*, 2015). The importance of selenium starts to become more evident during the puberty phase, when under the regulation of gonadotrophic hormones, a growing requirement of the testes for selenium was observed in rats (BEHNE *et al.*, 1982; BEHNE; DUK; ELGER, 1986). The same research group later reported the crucial participation of this mineral in the male gonad development (testicular morphology), testosterone synthesis by the Leydig cells, and consequently, in the production and normal development of sperm (BEHNE; WEILER; KYRIAKOPOULOS, 1996).

The biological role of selenium, after its absorption, is performed via selenoproteins in the body (LABUNSKYY; HATFIELD; GLADYSHEV, 2014; QAZI *et al.*, 2019). Among those produced in the male reproductive organs, the phospholipid hydroperoxide glutathione peroxidase (GPx4 or PHGPx) stands out as the main one, especially in the testes. This selenoprotein is essential for the processes of spermatogenesis and sperm maturation. The selenoprotein acts in two important ways: as an antioxidant enzyme during the differentiation of germ cells until their spermatid form and as an enzymatically inactive structural protein at the final stages of sperm development (URSINI *et al.*, 1999; FLOHÉ, 2007).

Reactive oxygen species (ROS) are molecules with one or more unpaired electrons in their outer orbit, making them unstable, short-lived, and highly reactive. This high reactivity can damage biological molecules, including nucleic acids, proteins, and lipids (PHANIENDRA *et al.*, 2015; AITKEN, 2017). ROS generation during spermatogenesis is expected due to the high mitosis and meiosis rates found within seminiferous tubules during this process. These species result from aerobic sperm metabolism, and as long as they are present at physiological

levels are beneficial for reproduction. The problem is when ROS exceed these limits and becomes destructive to the developing cells, compromising their viability and functionality. Herein, the selenoprotein GPx4 contributes to the control of ROS and allows the normal development of sperm (GUERRIERO *et al.*, 2014; AITKEN, 2020). Additionally, as the main component of the mitochondrial capsule of spermatozoa – a matrix that surrounds the spiral structure of the mitochondria in the middle piece – guarantees the normal form and function of this vital organelle, such as producing ATP – the primary energy source for sperm cells (URSINI *et al.*, 1999).

Marin-Guzman *et al.* started their studies in the ’90s about the importance of selenium for boar semen quality and fertility. The authors developed a work where a group of mature boars received a selenium-supplemented diet (0.5 mg/kg), while others were fed a non-supplemented one, containing 0.06 mg selenium/kg, exclusively from its ingredients. The males were fed these dietary treatments from weaning to 18 months of age. The first evidence they published was that males that did not receive a selenium-supplemented diet resulted, among other dysfunctions, in abnormal sperm morphology and lower motility, and also lower fertilization rate when used for AI (MARIN-GUZMAN *et al.*, 1997). Following this, it was confirmed the negative impact of low selenium diets on sperm morphology, resulting in mitochondrial alterations and abnormal tails, a lower ATP concentration in the spermatozoa, and poor contact of the plasma membrane to the helical coil of the tail middle piece (MARIN-GUZMAN *et al.*, 2000a); explaining the lower fertility observed in the previous work.

Lastly, exciting roles of selenium were observed in establishing the number of Sertoli cells and sperm reserves in boar testis (MARIN-GUZMAN *et al.*, 2000b). The authors observed that mature boars fed a selenium-supplemented diet (0.5 mg/kg) had a greater number of Sertoli cells, more secondary spermatocytes, and more round spermatids in testes when compared to those non-supplemented. Sertoli cells are present in seminiferous tubule walls and participate in germ cell development and migration, nourishing and sustaining them during spermatogenesis, controlling the passage of secretions between the tubular and interstitial compartments, synthesizing proteins and other factors (GRISWOLD, 1995).

2.3 REACTIVE OXYGEN SPECIES AND THE ANTIOXIDANT SYSTEM OF BOAR SEMEN – SELENOPROTEINS IN THE SEMINAL PLASMA

ROS generation by the spermatozoa along its trajectory is considered a physiological event, which has been reported as essential for fertilization achievement, participating in sperm

capacitation, hyperactivation, acrosome reaction, and even during sperm-oocyte fusion (DUTTA *et al.*, 2020). However, since ROS production leaves the physiological levels for some reason and overwhelms the antioxidant capacities in the medium, or when antioxidant production is diminished, it results in the event known as oxidative stress. Sperm structures are susceptible to oxidative damage, including the plasma membrane and chromatin, which may have detrimental effects on sperm viability and functionality and its fertilizing potential (AITKEN *et al.*, 2016).

Boar spermatozoa are especially sensitive to ROS-induced oxidative stress for two reasons. Firstly, the high proportion of polyunsaturated fatty acids present in the plasma membrane is an excellent substrate for lipid peroxidation (CEROLINI *et al.*, 2000). Furthermore, sperm carry a poor antioxidant reserve, which does not allow them to counteract ROS-attack alone. Herein, seminal plasma, due to its antioxidant properties, becomes the main responsible for keeping ROS levels within the physiological range, protecting sperm cells from the moment of ejaculation and during their journey in the female reproductive tract (JUYENA; STELLETTA, 2012; BARRANCO *et al.*, 2015).

The total antioxidant capacity of seminal plasma, measured from the set of antioxidant properties, is represented both for enzymatic – superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) – and non-enzymatic ones, such as L-ascorbate, urate, alpha-tocopherol, pyruvate, and taurine (KOZIOROWSKA-GILUN *et al.*, 2011). In boars, among the total GPx activity measured in raw semen, 80.7 to 90.8 % of that comes from selenium-dependent GPx molecules (CEROLINI *et al.*, 2001). In fact, of eight different enzymatic isoforms of GPx known in mammals (GPX 1–8), five are selenoproteins – or in other words, there are selenium-dependent in their composition (QAZI *et al.*, 2019).

2.4 THE ROLE OF SELENIUM IN BOAR FERTILITY

Adequate selenium levels in the male reproductive organs are important for their development and proper functioning, being essential for spermatogenesis and sperm maturation in mammals (BEHNE; WEILER; KYRIAKOPOULOS, 1996; MARIN-GUZMAN *et al.*, 2000a; FLOHÉ, 2007; CHEAH; YANG, 2011). Therefore, since semen quality and fertility are primarily dependent on the quality of sperm production, any dysfunction along the stages may result in poor-quality semen and reduced fertility in males (ASHAN *et al.*, 2014). Also, considering ROS-induced injuries to spermatozoa are one of the most important causes of a

decrease in semen quality and fertility, ensuring a selenium-adequate diet aiming to improve the production of selenoproteins with antioxidant properties in the male reproductive tissues seems a very effective action (MARIN-GUZMAN *et al.*, 1997).

In boars, Marin-Guzman *et al.* (1997) demonstrated that sperm from animals non-supplemented with selenium resulted in a lower rate of egg fertilization (73.4%) and a lower number of accessory sperm penetrating the pellucid zone (14.2) compared to animals that received a supplemented-diet, which resulted in values of 98.5% and 59.7 sperm, respectively. In this study, mature boars received a non-supplemented (0.06 mg selenium/kg) or a supplemented (0.5 mg selenium/kg) diet since weaning to 18 months of age, and mature gilts had their reproductive tracts recovered and evaluated 5-7 days after being inseminated. Confirmations that selenium is indeed crucial for the reproductive success of boars continued to be evidenced in the following decades. Petrujkić *et al.* (2014) observed that mature boars fed a selenium-supplemented diet (0.3 mg/kg) for 90 days achieved higher conception rates, expressed as farrowed gilts, compared with the group non-supplemented (0.098 mg selenium/kg) during the same period. The improvement varied from 20 to 40%, depending on the selenium supplement used (inorganic and organic, respectively).

Three years later, Estienne and Whitaker (2017) reported that cryopreserved sperm from mature boars fed selenium-supplemented diets (0.3 mg/kg) since weaning to approximately 18 months of age resulted in a higher percentage of embryos that progressed to the blastocyst stage 144 h post-IVF (*in vitro* fertilization) compared to non-supplemented boars (0.03 mg selenium/kg). This result showed that the benefits of offering adequate selenium levels in the diet could be observed even after extremely stressful processes to the boar spermatozoa, as in cryopreservation. The authors suggest that selenium supplementation may be a practical approach to improving pig farms' fertility results using cryopreserved semen.

2.5 SELENIUM SOURCES AND THEIR BIOAVAILABILITY

In nature, selenium can be found in two chemical forms, organic and inorganic. In inorganic forms, selenium can be presented as selenite, selenate, or selenide, and also in its metallic form. Plants absorb selenium in its inorganic form from the soil, mainly as selenite, and can transform it into organic compounds, such as selenomethionine (SeMet) and selenocysteine (SeCys), both are selenoaminoacids. SeMet represents about 50% of the total selenium in major feed ingredients, including forages, cereal grains, and oilseed. Therefore,

animals are used to receive selenium in the organic form, mainly as SeMet, in natural conditions (SURAI; KOCHISH; FISININ, 2021).

Although selenium is present in plants and is the base for farm animals' nutrition, selenium concentration in the soil varies significantly. At the same time, its availability to plants can also be negatively influenced by factors such as the acid pH, poor aeration, high sulfate concentration in the soil, and areas of high rainfall (SURAI; KOCHISH; FISININ, 2021). In this way, it is almost impossible to expect a constant selenium concentration in animal feed ingredients, being its supplementation in the diet an effective alternative to offer the necessary selenium levels to the different animal species and their categories (SURAI; FISININ, 2015, 2016; SURAI *et al.*, 2018). Herein, selenium supplementation can be provided in the diet from inorganic or organic sources available in the market.

Since the American agency Food and Drug Administration (FDA) approved the use of selenite or selenate for dietary selenium supplementation in poultry and pigs in the '70s, these inorganic forms have been widely used in the production units. However, some disadvantages of using these forms have accumulated over the last few decades, including the interaction between them and other nutrients in the diet, toxicity, and inability to build and maintain selenium reserves in the body (SURAI; KOCHISH; FISININ, 2021). In this way, other selenium forms were launched in the worldwide market aiming to better meet the needs of the sector – the organic sources, mainly in the form of SeMet, the same form from feed ingredients.

It is well-known that organic forms in general are more bioavailable than inorganic ones within a nutritional plan (GERAERT *et al.*, 2015; SURAI *et al.*, 2018). The bioavailability term can be defined as the efficiency with which a nutrient is absorbed from the gastrointestinal tract, and are thus available for storage or use by the cells (FORBES; ERDMAN, 1983). The facilities for organic forms begin with their absorption. As they carry an amino acid (methionine in the case of SeMet) in their composition, they are absorbed as such, by active transport, which does not occur in inorganic forms (SURAI; KOCHISH; FISININ, 2021). Although the intestinal absorption of both inorganic and organic sources can be equivalent (92% and 95%, respectively), Thompson and Stewart (1973) observed that when the excretion was measured, it was greater in animals that received the inorganic one. Herein, there is the main advantage of organic forms – animals fed this kind of supplementation can store the surplus of selenium in their tissues, mainly in muscles, while the inorganic form must be excreted to avoid toxicity. These selenium reserves can be easily activated, being very interesting in stressful situations, when more selenium is required in addition to possible loss of feed intake (SURAI; FISININ, 2015).

Surai *et al.* (2018) have an interesting way to expose the main selenium forms currently available, dividing them into three generations. Selenite and selenate were very important to first correct the problem of selenium deficiency in farms, and are present in the market since 1970. The second is represented by Se-yeast, and the pure forms SeMet and Zn-SeMet, which brought the main advantages of offering selenium in the form of SeMet and the ability to store the mineral in animal tissues for further use. The emergence of such sources was a real milestone for selenium nutrition and has made a difference over the last few decades. Se-yeast is still one of the most used worldwide. Despite great progress so far, there were still points to improve. On the one hand, there is high variation in the concentration of SeMet in commercial Se-yeast products, and the difficulty to measure and guarantee it. Besides the high instability and ease of oxidation of the pure SeMet forms make their use very scarce in the field. Lastly, the third generation was launched on the market in 2014 and is a pure synthesized organic selenium form containing hydroxy-selenomethionine (OH-SeMet) as an active substance, a precursor to SeMet. OH-SeMet brings two major improvements over the previous generation: offers >95% OH-SeMet in its molecule in comparison to 50-70% SeMet offered by Se-yeast, while demonstrating high stability under feed preparation and storage conditions, different from other pure Se forms.

Some research has been carried out comparing the use of OH-SeMet *vs.* Se-yeast or sodium selenite in poultry and pigs, with promising results. For broilers chickens, it was observed that OH-SeMet provided a higher muscle selenium enrichment (BRIENS *et al.*, 2013, 2014; COULOIGNER *et al.*, 2015), as well as a higher deposition of the mineral in eggs and breast muscle, which have been demonstrated in laying hens (JLALI *et al.*, 2013). In growing pigs, OH-SeMet demonstrated a greater bioavailability to increase selenium concentration in the plasma (+70%), hepatic (+41%), and muscular (+62 %) levels, compared to Se-yeast (JLALI *et al.*, 2014). Also, it was observed a better antioxidant status in weaned piglets when OH-SeMet was used (CHAO *et al.*, 2019), and even improvements in litters from sows supplemented with this organic source, including increased litter size, better transfer of passive immunity for piglets, and enhanced antioxidant capacity (LI *et al.*, 2020; MOU *et al.*, 2020). In fact, OH-SeMet has been characterized by researchers in the field as a pure, reliable, and stable source, besides being identified as the most bioavailable among those already described (GERAERT *et al.*, 2015).

2.6 ORGANIC SELENIUM IN BOAR REPRODUCTION

Based on previous studies demonstrating a greater bioavailability and storage of selenium in tissues when farm animals are fed supplemented-diet with organic sources (BRIENS *et al.*, 2013, 2014; JLALI *et al.*, 2013, 2014), it is possible to suggest that higher levels of this mineral could be available for the boar male reproductive system and semen. It is also expected that sperm in development may have a greater antioxidant supply, via selenoprotein GPx, positively influencing their viability and further functionality (MARIN-GUZMAN *et al.*, 1997). Still, in boars from AI-centers, which their semen is constantly obtained for the production of commercial doses, animals fed selenium organic sources could better meet the greater selenium demand for sperm production through their tissue reserves.

In this way, some studies have been performed in the last decade aiming to evaluate the impact to offer different selenium sources to boars on their reproductive performance. Speight *et al.* (2012), through three experiments, generated interesting results. The experimental design was the same. At weaning, some boars started to be fed a basal diet without selenium supplementation (0.034 mg/kg; control group), while the other two groups received selenium-supplemented diets from organic (0.3 mg/kg; SeMet as Se-yeast), or inorganic (0.3 mg/kg; sodium selenite) forms. The dietary treatments were offered to approximately 25 months of age. In the first experiment, boars of 15 months of age had their semen collected on 5 consecutive days, and negative effects on sperm motility were less pronounced in those fed organic selenium. In the second, when semen from boars of 17 months of age were collected, extended, and stored at 18 °C for 9 days, sperm from boars fed SeMet were able to better maintain motility during the storage period. Lastly, when sperm from boars of 23 months of age were evaluated at days 1 and 8 after semen collection using *in vitro* fertilization procedures, the use of the organic source of selenium resulted in a higher fertility rate (70.7%) when compared to the inorganic one (58.5%) and the control group (60.9%), although the effect was not statistically significant. Herein, it is clear that the organic form was crucial for better coping with stressful conditions and with greater demand for selenium, such as the semen storage for long periods and its consecutive collections.

Other authors also contributed to this question. Martins *et al.* (2014, 2015) showed that the PHGPx concentration in spermatozoa was increased when mature boars were fed organic selenium (0.5 mg/kg; SeMet as Se-yeast) instead of inorganic selenium (0.5 mg/kg; sodium selenite) for 10 weeks. However, no effect of the selenium source was observed on the quality of raw semen and AI-doses stored for 72 h at 17 ºC. On the other hand, the same authors

demonstrated these works from an economic point of view and showed that providing the organic source for the animal is an excellent cost-benefit ratio. It was observed an increase of 23% in semen AI-doses production, resulting in a 37% reduction in the cost of diet per dose produced by boars fed SeMet compared to that fed sodium selenite. The authors pointed out that the total revenue produced by the organic group was 26% higher than the inorganic one (MARTINS *et al.*, 2014, 2018).

Concurrent with this, Petrujkić *et al.* (2014) demonstrated that mature boars fed a supplemented diet with organic selenium (0.3 mg/kg; SeMet as Se-yeast) for 90 days had a higher concentration of the mineral in their semen compared to the group that received inorganic selenium (0.3 mg/kg; sodium selenite) or no supplementation (0.098 mg selenium/kg). However, the authors observed that both inorganic and organic selenium sources similarly increased the GPx activity in boar semen, although only the values obtained for the inorganic form differed significantly from the control treatment. In addition, it was observed a higher farrowing rate when used semen doses from boars fed SeMet (88.33%) than that fed sodium selenite (66.67%), although the values were not statistically different. Lastly, Estienne and Whitaker (2017) observed that the use of frozen-thawed sperm from boars fed SeMet as Se-yeast (0.3 mg selenium/kg) since weaning to 18 months of age resulted in a greater percentage of embryos cleaved by 48 h post IVF when compared to those fed the inorganic form sodium selenite at the same supplementation level and for the same period.

Conversely, other research groups brought results, that were not so encouraging. While Lovercamp *et al.* (2013) reported that both selenium sources produced equivalents results for semen production and sperm quality in raw ejaculates, as well as for liquid-stored semen (6 days), López *et al.* (2010) observed that although SeMet can increase sperm concentration in the semen, it can reduce some sperm motility parameters, as well as their resistance to oxidative stress. The last authors further state that no improvements were observed when boars were fed selenium-supplemented diets (from both the sources studied) when compared to the group non-supplemented. Mature boars received a selenium-supplemented diet either from SeMet (as Se-yeast) or sodium selenite in both studies, but the supplementation level was different (0.3 mg selenium/kg in the first, and 0.4 mg/kg in the second). The dietary treatments were offered from weaning to approximately 12 months of age in the study presented by Lovercamp *et al.* (2013) and for 120 days in López *et al.* (2010).

Herein, it is important to remember the well-known variability existing among the Se-yeast commercial products regarding the concentration of SeMet derived by their forms, which can difficult the correct interpretation and comparison of results. Also, the lack of information

about the selenium levels in the basal diet used in the studies is another factor that can generate confusion (SURAI; FISININ, 2015; SURAI *et al*., 2018).

In general, most studies have suggested strong evidence of the improvements obtained by using organic selenium sources in place of inorganic ones on boar reproduction. With the development of increasingly efficient and bioavailable molecules, it remains to investigate how the most current form of organic selenium, via OH-SeMet, can affect boar semen quality and fertility.

3 OBJECTIVES

The present study aimed to compare the use of a pure synthesized form of organic selenium, hydroxy-selenomethionine (OH-SeMet), as dietary supplementation, with the commonly used inorganic source, sodium selenite (SS), on semen quality and reproductive performance of boars. For this, raw semen characteristics and the quality of semen AI-doses stored at 17 °C for 72 h were assessed, employing physical and functional tests. Besides, an extensive data analysis from AI programs was performed to correlate the different selenium sources with boar fertility. Finally, the present study aimed to identify at which level (0.3 or 0.6 mg selenium/kg) OH-SeMet could be more efficient from a reproductive perspective.

4 HYPOTHESES

1. Boars fed an OH-SeMet supplemented diet present:

a) greater selenium availability in their seminal plasma, which indicates more selenium delivered for the male reproductive system;
b) greater sperm production in the testis and hence, more semen AI-doses produced per ejaculate;
c) higher GPx activity in their seminal plasma, offering greater antioxidant protection to sperm;
d) better sperm motility and morphology in raw semen;
e) better sperm quality after storage at 17 °C for 72 h;
f) higher pregnancy rates and litter size when used in AI programs.

2. Boars fed a diet supplemented with a higher level of selenium as OH-SeMet (0.6 mg/kg) present sperm production, semen quality, and reproductive performance even better than when consuming this organic source at 0.3 mg/kg level.

5 MATERIALS AND METHODS

5.1 LOCAL AND ANIMALS

A total of 42 purebred boars (Large-White and Landrace) and 1131 purebred females (Large-White and Landrace) from DB Genética Suína (DanBred Brasil) were used for this study. Boars initiated the study aged 8 to 31 months of age and the females with parities from 0 to 5. Boars were housed in a commercial AI-center (Unit of Gene Diffusion, DB Genética Suína – DanBred Brasil) located in the São Zeferino farm in Varjão de Minas, Minas Gerais, Brazil, and were submitted to the same environmental, nutritional, and sanitary conditions. The animals were housed in individual pens or crates in a temperature-controlled room (21 to 25 °C) and had access to a nipple drinker and an automatic feeder (Figure 1).

Females were housed in five farms, three of which were nucleus units and two multipliers (DB Genética Suína – DanBred Brasil, Minas Gerais, Brazil). Two farms had collective pens with electronic sow feeders in the gestation phase, while the others housed the females in individual crates, where animals were fed by automatic or manual feeders once a day. Regardless of the farm, females were housed in farrowing crates with automatic or manual feeders near the expected farrowing date. Three farms had temperature-controlled facilities, and two farms did not. All females received a specific diet for each reproductive phase (gestation, lactation), with the quantity supplied adjusted individually.

Semen initial analysis and the processing of AI-doses were performed in the laboratory of the AI-center. Semen doses stored at 17 ºC for 72 h were evaluated for sperm motility characteristics, sperm morphology, and integrity of sperm membranes in the Laboratory of Andrology and Technology of Swine Embryos (LATES), Pirassununga, SP, Brasil. The analyses of GPx activity in seminal plasma and sperm resistance to oxidative stress in liquid-stored semen AI-doses (17 ºC for 72 h) were conducted in the Laboratory of Andrology, São Paulo, SP, Brasil. Both laboratories are from the Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo.

Selenium concentration and macrominerals levels were assessed in the Laboratory of Minerals, while bromatological analysis of diet was conducted in the Laboratory of Bromatology. Both laboratories are from the Department of Animal Science, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil.

Figure 1 – Local and animals

Source: Pavaneli (2022).
Legend: A) Unit of Gene Diffusion, DB Genética Suína – DanBred Brasil. B) Boars' shed. C) Animals housed in pens. D) Animals housed in creates.

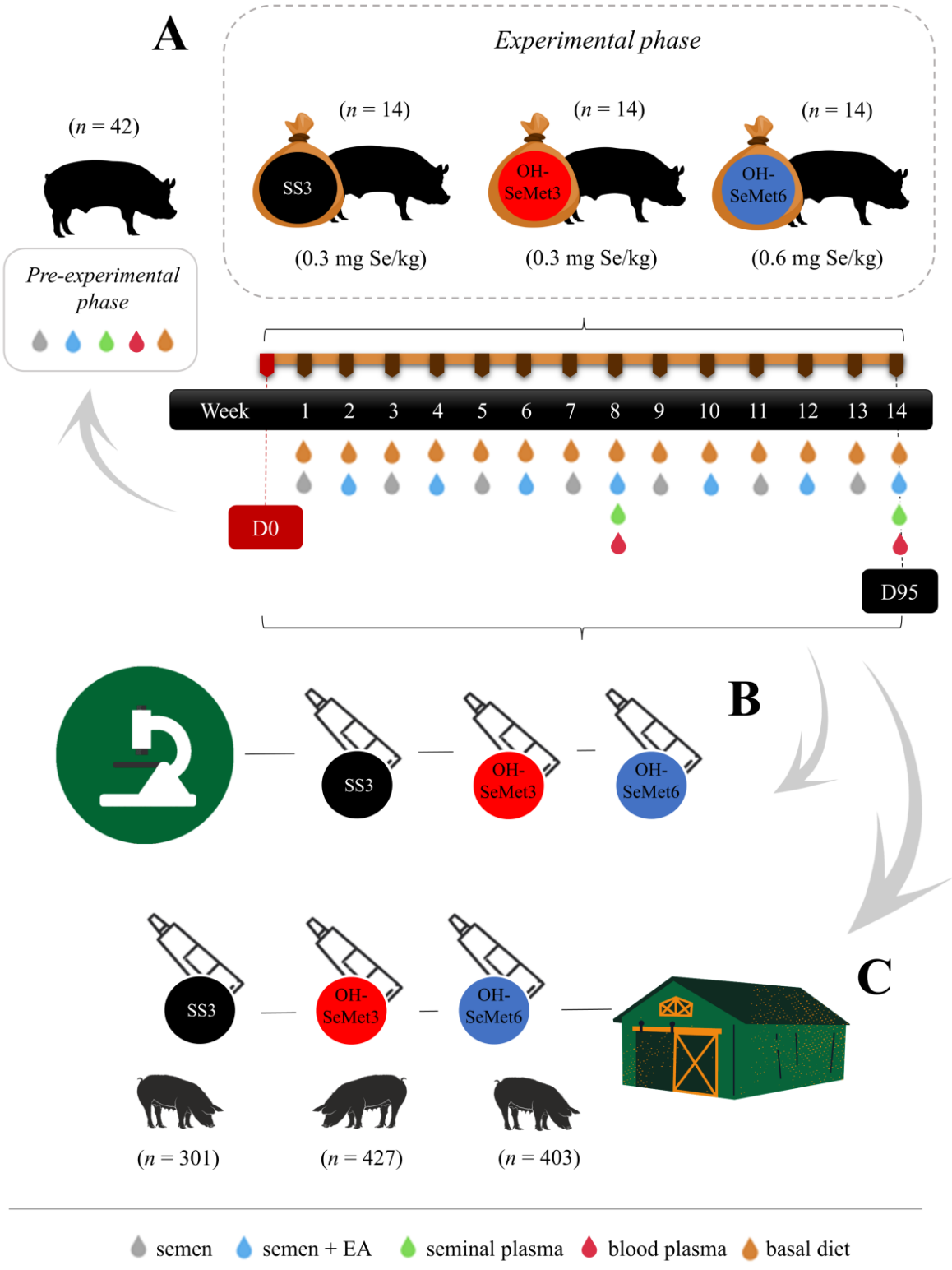
5.2 EXPERIMENTAL DESIGN

A previous ranking of the boars was performed before their distribution in the different treatments. It was based on the average semen quality (total sperm motility and sperm morphology) and resulted in a score for each male. In addition to boars' score, their age and breed were also considered to the uniform distribution into the three experimental groups: SS3 (0.3 mg selenium/kg as sodium selenite; *n* = 14); OH-SeMet3 (0.3 mg selenium/kg as hydroxy-selenomethionine; *n* = 14); and OH-SeMet6 (0.6 mg selenium/kg as hydroxy selenomethionine; *n* = 14). The animals fed the dietary treatments for approximately 14 weeks (95 days). Throughout this period, semen collections occurred weekly, while experimental analyzes were carried out every 2 weeks, totaling 7 evaluation points during the study. In addition, feed, blood, and seminal plasma samples were collected before and during the experimental phase to access the selenium concentration, and other measurements in the case of feed (Figure 2A).

After semen collection, an initial analysis of raw semen was performed before the processing AI-doses (volume, sperm motility, concentration, morphology and GPx activity in seminal plasma). Semen doses were produced as routine in AI-center, followed the regular commercialization routes, including deliveries to farms participating in the study. In addition, one dose of each boar was delivered to the LATES, University of São Paulo, Pirassununga, SP, Brasil. There, semen AI-doses were kept at 17 °C for the total storage period to be completed (72 h) before being analyzed for sperm quality (Figure 2B).

For *in vivo* fertility assay, semen AI-doses from boars fed the different dietary treatments were employed weekly in AI programs throughout the experimental phase (14 weeks), and a total of 1131 females were inseminated: (SS3, *n* = 301; OH-SeMet3, *n* = 427; and OH-SeMet6, *n* = 403). Thus, at the end of the experimental phase, an extensive data analysis was carried out for reproductive parameters within each experimental group (Figure 2C). This study was carried out between April and August in 2019 (autumn/winter in Brazil).

Figure 2 – Experimental design



Source: Pavaneli (2022).
Legend: A) Schedule for semen and sample collections before and during the experimental phase. B) Every 2 weeks, one semen dose from each boar was evaluated after 72 h at 17 °C. C) Semen doses from the three experimental groups were employed weekly in AI programs at different farms throughout the experimental phase (14 weeks). EA, experimental analyses; Se, selenium.

5.3 DISTRIBUTION OF ANIMALS INTO EXPERIMENTAL GROUPS

Before the experimental phase, two ejaculates of each animal were evaluated *in natura* within 15 days (one semen collection/week) for sperm motility and morphology. Ejaculates were collected using the semi-automatic system BoarMatic® (Minitüb GmbH, Tiefenbach, Germany). Sperm motility was evaluated by computer-assisted sperm analysis (CASA) system AndroVision® (Minitüb GmbH, Tiefenbach, Germany) while sperm morphology was assessed with phase contrast microscopy. Sperm defects were classified into major and minor according to Blom (1973). From the results obtained in two semen evaluations, an average per characteristic was calculated. An indicator (score) represented these values' sum and allowed the animal ranking (I) according to Martins *et al.* (2018).

(I) $$\textbf{Score Boar} = (3 \times \text{SMAD}) + (2 \times \text{SMID}) + (1 \times \text{IM})$$

Legend: SMAD – Sperm Major Defects; SMID – Sperm Minor Defects;
IM – Inverse Motility (total sperm motility).

For animal ranking, the lower the animal's score, the better its semen quality was considered (MARTINS *et al.*, 2018). Once boars were ranked, the individual score together with age and breed factors were used to distribute the animals into the experimental groups (Appendix A).

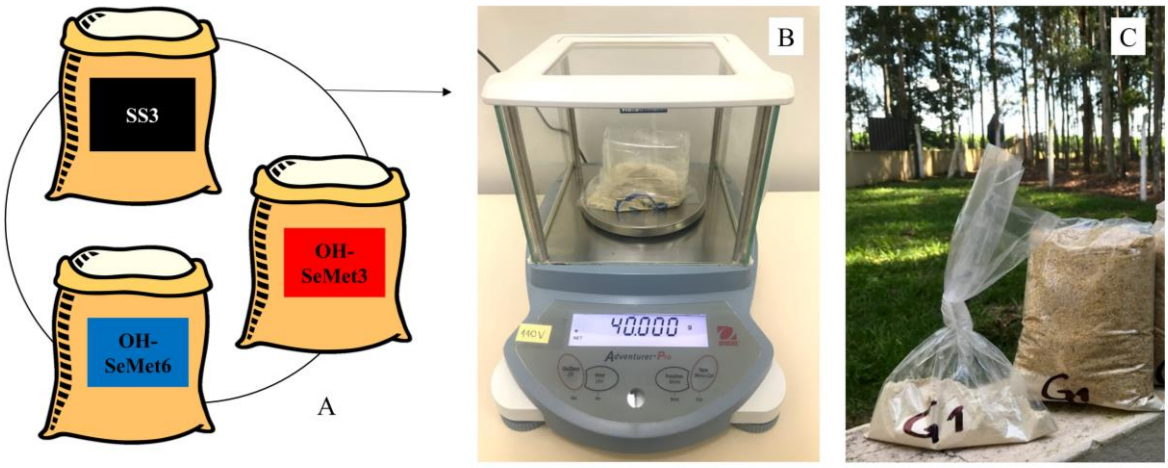
5.4 PREPARATION AND PROVISION OF DIETARY TREATMENTS

The dietary treatments were prepared in the form of premixtures within which a specified amount (0.04 kg) contained the ideal selenium levels for each day in each of the experimental groups (Figure 3A). These treatment premixtures were prepared at the DB Company Feed Mill and a protein concentrate was used as a carrier to extend both selenium from organic and inorganic sources. Once premixtures were prepared, samples were collected and sent to the Laboratory of Minerals, CBO Laboratorial Analysis, Valinhos, São Paulo, Brazil for checking the selenium levels.

For each 0.04 kg premixture, 0.3 kg of the basal diet (no selenium supplementation) was added, followed by its provision to the animals. It was only to increase the volume provided at the first moment to the animals and avoid losses of the dietary treatment (Figure 3B-C). Once

that first portion was consumed, the rest of the daily feed (basal diet; 2.4 kg) was offered to all the animals. The basal diet was in according to the Nutritional Requirements of Swine (NRC, 2012), without, however, meeting the selenium requirements, which were met by the different dietary treatments. The selenium levels of the basal diet were exclusively derived from the ingredients used (Appendix B). Feeding was carried out only once a day in the morning, in which all the animals received an approximate total of 2.7 kg of food/day (Figure 4). The animals' food consumption was checked daily before supplying a new treatment.

Figure 3 – Preparation of dietary treatments



Source: Pavaneli (2022).
Legend: A) Premixtures containing the desired mineral sources and levels for each experimental group. B) Premixture being weighed for later mixing with the basal diet. C) Premixture (0.04 kg) + basal diet (0.3 kg) = 0.340 kg (dietary treatment). G1, experiment group SS3.

Figure 4 – Provision of dietary treatments

Source: Pavaneli (2022).
Legend: Daily feed ready for animals of different treatments: dietary treatment (0.340 kg; small bag) + 2.4 kg basal diet (big bag). G1, experimental group SS3; G2, experimental group OH-SeMet3; G3, experimental group OH-SeMet6.

5.5 SEMEN COLLECTION AND INITIAL ANALYSES

Ejaculates were collected using the semi-automatic system BoarMatic® (Minitüb GmbH, Tiefenbach, Germany) (Figure 5A). After collection, the semen was weighted to estimate its volume (Figure 5B), and the total sperm motility and sperm concentration were assessed by computer-assisted sperm analysis (CASA) system AndroVision® (Minitüb GmbH, Tiefenbach, Germany) (Figure 5C). Total sperm motility was defined as the percentage of spermatozoa with an amplitude of lateral head displacement (ALH) > 4 µm and a beat cross frequency (BCF) > 4 Hz. With these semen characteristics known, it was possible to calculate the total sperm count in the ejaculate and the number of AI-doses produced per ejaculate. Raw semen was also evaluated for sperm morphology (item 5.5.1) and GPx activity in seminal plasma (item 5.5.2).

After raw semen initial evaluations, the ejaculate was extended in a long-term preservation extender (VITASEM®, Magapor; Zaragoza, Spain) to produce the AI-doses (1.5×10^9 sperm; 45 mL) (Figure 7A). Semen doses produced were initially kept in the AI-center (17 °C) (Figure 7B) until the moment of being transported by semen transport vehicles (controlled temperature to 17 °C) to the farms, and the LATES, University of São Paulo, Pirassununga, SP, Brasil.

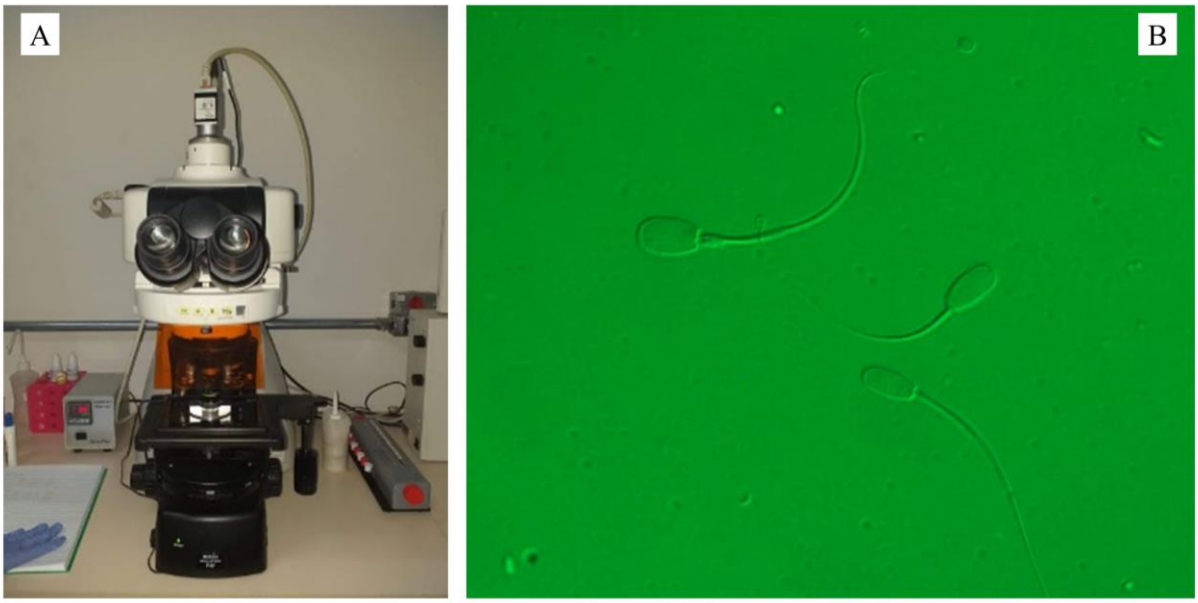
Figure 5 – Semen collection and initial analyses

Source: Pavaneli (2022).
Legend: A) Semen collection by the semi-automatic system. B) Weighing the ejaculate. C) Computer-assisted sperm analysis (CASA) system to assess total sperm motility and sperm concentration.

5.5.1 Sperm morphology

For sperm morphology assessment, a drop of semen previously fixed in buffered formaldehyde saline solution 4% was deposited on a slide, covered by a coverslip, and analyzed by differential interference contrast (DIC) microscopy (Nikon, Eclipse NI-U model) in 1000x magnification under immersion oil (Figure 6). In this evaluation, 200 sperm/sample were counted and classified according to sperm morphology as normal, or abnormal (defects in the acrosome, head, neck, midpiece, and tail regions; the presence of proximal and distal cytoplasmic droplets; and teratological forms) (GARCIA, 1971; RAO, 1971) (Appendix C).

Figure 6 – Sperm morphology analysis



Source: LATES, University of São Paulo.
Legend: A) Differential interference contrast (DIC) microscopy. B) DIC image.

5.5.2 Glutathione peroxidase activity

The protocol used to determine the GPx activity in seminal plasma was based on the consumption of NADPH, according to Nichi *et al.* (2006). In this method, the reaction between a hydroperoxide and reduced glutathione (GSH) is induced. This reaction is catalyzed by the GPx together with the enzyme glutathione reductase (GR) and causes the conversion of glutathione disulfide (GSSG—glutathione oxidized) to GSH, which in turn consumes NADPH (measured with a spectrophotometer). A volume of 100 µL of seminal plasma was used for

sample evaluation. For this, raw semen was initially centrifuged at 2,400× *g* for 5 min to obtain this semen portion, which was stored at 196 °C until the analysis. The assay mixture consisted of NADPH (0.12 mM, 1 mL), GSH (1 mM, 100 µL), GR (0.25 U/mL, 20 µL), and sodium azide (0.25 mM, 20 µL). The spectrophotometer cell was brought up to a volume of 1.9 mL with phosphate buffer 143 mM, EDTA 6.3 mM (pH 7.5), which was also used to dissolve the NADPH. The GSH was dissolved in 5% metaphosphoric acid. Sodium azide was used to inhibit the action of catalase. This reaction was initiated with the addition of 1.2 mM of tert-butyl hydroperoxide (TBHP, 100 µL), and the consumption of NADPH was detected at a wavelength of 340 nm for 10 min at 37 °C (measurements performed every 5 s). The results of GPx were expressed as units of GPx/mL of semen, and calculations used 6.22 mM^{-1} cm^{-1} as the extinction coefficient of NADPH (BEUTLER, 1975).

Figure 7 – Semen doses produced and stored at 17 °C

Source: Pavaneli (2022).
Legend: A) Semen doses ready to be sent to the farms. B) Semen fridges in the AI-center.

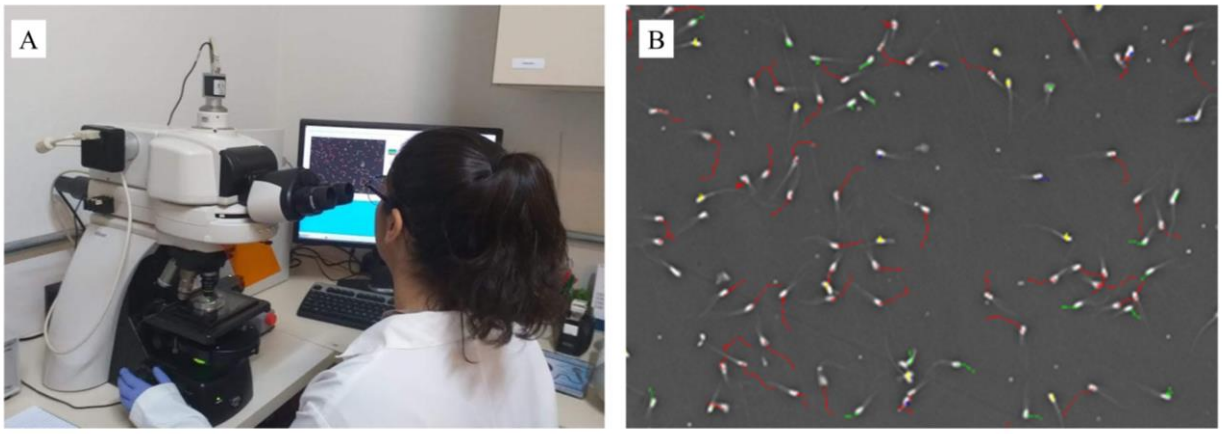
5.6 SPERM EVALUATIONS AFTER STORAGE IN LIQUID STATE

5.6.1 Computer-assisted sperm analysis

Sperm motility characteristics were assessed in semen AI-doses after storage at 17 ºC for 72 h. Samples were analyzed by computer-assisted sperm analysis (CASA) system (SCA Microptic®, Microptic SL, Barcelona, Spain) mounted on the epifluorescence microscopy

(Nikon, Eclipse NI- U) (Figure 8). The characteristics evaluated were total motility (TMOT, %), progressive motility (PMOT, %), average path velocity (VAP, μm/s), straight linear velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s), the amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz), straightness (STR, %), linearity (LIN, %) and wobble coefficient (WOB, %). With the aid of the Edit/Sort tool offered by the software, the percentage of hyperactivated sperm in each sample was also evaluated (ANDRADE *et al.*, 2017; PAVANELI *et al.*, 2017).

Figure 8 – Motility sperm analysis



Source: Pavaneli (2022).
Legend: A) Computer-assisted sperm analysis (CASA) system. B) CASA image.

5.6.2 Sperm morphology

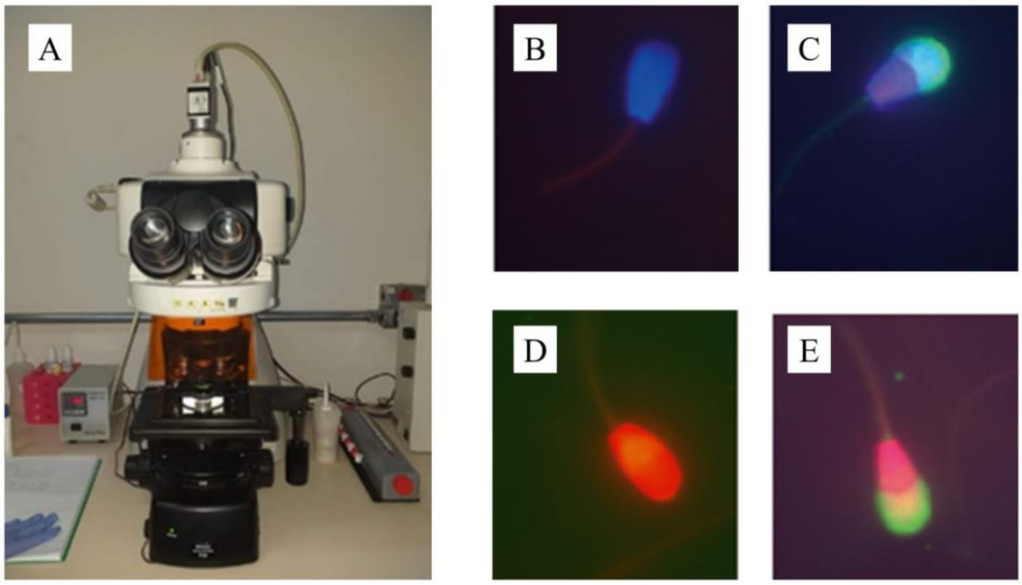
For sperm morphology assessment in liquid-stored semen AI-doses (17 ºC for 72 h), the same method described for raw semen (item 5.5.1) was applied.

5.6.3 Integrity of plasma and acrosomal membranes

For assessment of sperm membranes integrity, a previous dilution of the liquid-stored semen AI-doses (17 ºC for 72 h) was carried out in the TALP medium (BAVISTER; LEIBFRIED; LIEBERMAN, 1983) to obtain a final concentration equal to 25×10^6 sperm/mL. An aliquot of 150 µL of this simultaneously received the addition of 2 µL of Hoechst 33342 (40 µg/mL), 3 µL of propidium iodide (PI, 0.5 mg/mL), and 50 µL of *Pisum sativum* agglutinin conjugated to fluorescein (FITC-PSA, 100 µg/mL) (CELEGHINI *et al.*, 2007; ANDRADE *et*

al., 2007). Afterward, the samples were incubated at 37 °C for 8 min, sheltered from the light. For analysis, a drop of 8 µL was placed between a pre-heated slide and coverslip, with immediate reading under epifluorescence microscopy (Nikon, Eclipse NI-U model; Figure 9A), in a triple filter (D/F/R, C58420), showing a set: UV-2E/C (excitation 340–380 nm and emission 435–485 nm), B-2E/C (excitation 465–495 nm and emission 515–555 nm), and G-2E/C (excitation 540–525 nm and emission 605– 655 nm), at 1000x magnification. Each sample had 200 sperm counted, which were classified into four categories: 1) Spermatozoa with intact plasma membrane and intact acrosome (PI and FITC-PSA negative); 2) Spermatozoa with intact plasma membrane and damaged acrosome (PI negative and FITC-PSA positive); 3) Spermatozoa with damaged plasma membrane and intact acrosome (PI positive and FITC-PSA negative); and 4) Spermatozoa with damaged plasma acrosome and damaged acrosome (PI and FITC-PSA positive) (Figure 9B-E).

Figure 9 – Evaluation of plasma and acrosome membranes



Sources: LATES, University of São Paulo; Celeghini *et al*., 2007.
Legend: A) Epifluorescence microscopy used for evaluation. B) Spermatozoa with intact plasma membrane and intact acrosome. C) Spermatozoa with intact plasma membrane and damaged acrosome. D) Spermatozoa with damaged plasma membrane and intact acrosome. E) Spermatozoa with damaged plasma acrosome and damaged acrosome.

5.6.4 Sperm resistance to oxidative stress

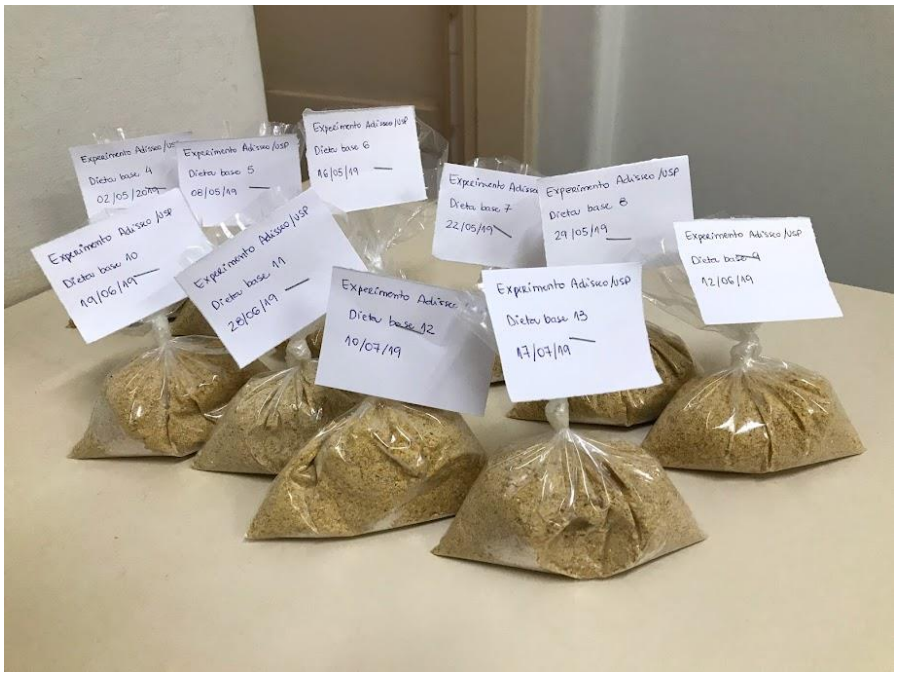
The indirect measurement of sperm resistance to oxidative stress was also assessed in semen AI-doses after storage at 17 ºC for 72 h. This analysis was performed by quantifying the concentration of malondialdehyde (MDA) in the sample, a final product of lipid peroxidation. For this, the TBARS test (thiobarbituric acid reactive substances) was performed according to the methodology adapted by Nichi *et al.* (2006). Firstly, sperm lipid peroxidation was induced by adding iron sulfate (50 μL; 4 mM) and ascorbic acid (50 μL; 20 mM) to 200 μL of stored semen; subsequently, the mixture was incubated for 90 min at 37 °C. After this, 600 μL of ice-cold trichloroacetic acid 10% was added and mixed following the sample storage (-20 °C) until its evaluation. Samples were centrifuged (20,800× g for 15 min, 5 °C) to precipitate protein and debris. Following this, 800 μL of the supernatant was recovered and incubated with 800 μL of thiobarbituric acid 1% at 95 °C in a water bath for 15 min. The reaction was stopped by placing samples on ice. This method is based on the reaction of two molecules of thiobarbituric acid with one molecule of MDA at high temperatures and low pH, resulting in a pink-colored complex that can be quantified in a spectrophotometer (Ultrospec 3300 Pro, Amersham Biosciences, USA) at a wavelength of 532 nm. The values obtained were compared with a standard curve for MDA concentration. The lipid peroxidation index was described in nanograms of TBARS/10^6 sperm.

5.7 FEED, BLOOD, AND SEMINAL PLASMA SAMPLES

Before the experimental phase, a feed sample previously consumed by the animals was collected and identified (a selenium-supplemented diet). Throughout the experimental period, a sample of the basal diet (no selenium supplementation) was collected weekly whenever a new batch arrived in the AI-center. All the samples were packed in plastic bags and stored at -20 °C until analysis (Figure 10). Blood and seminal plasma were collected at three points: 1) 15 days before the start of the experiment; 2) during it (week 8) and; 3) at the final period (week 14). Blood was collected using physical restraint and puncture of the external jugular vein using a 10 mL syringe and a 40 x 12 mm needle (Figure 11A). Blood samples were collected in tubes containing EDTA (Figure 11B), centrifuged at 1509× g for 10 min, and stored at -20 °C until analysis (Figure 11C). Seminal plasma was obtained after centrifugation of raw semen (2400× g for 5 min) and stored at -196 °C until analysis (Figure 11D).

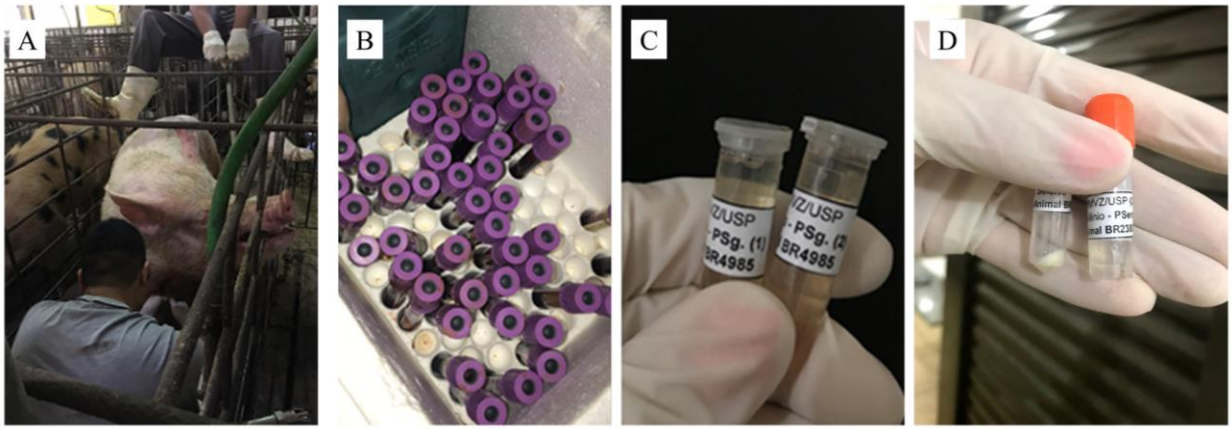
Feed, blood, and seminal plasma samples were evaluated for selenium concentration (item 5.7.1). Feed samples were also submitted to a bromatological evaluation and had the macrominerals calcium and phosphorus quantified (item 5.7.2).

Figure 10 – Samples of the basal diet collected and stored weekly



Source: Pavaneli (2022).

Figure 11 – Seminal and blood plasma



Source: Pavaneli (2022).
Legend: A) Physical restraint and blood collection through puncture of the external jugular vein. B) Tubes containing EDTA + blood collected. C) Blood plasma samples identified after blood centrifugation. D) Seminal plasma identified after raw semen centrifugation.

5.7.1 Selenium concentration

Selenium concentration was evaluated from 0.5 g of the basal diet (pool of weekly samples) and 2 mL of blood and seminal plasma. The referred samples were added 5 mL of HNO3 and 3 mL of HClO3, and the mixture was forwarded to the digest block until it reaches a temperature of 210 °C. After cooling the solution, 2.5 mL of HCl 1:9 was added, which remained for 30 minutes in a water bath with boiling water. After cooling, 5 mL of hydroxylamine hydrochloride and three drops of red cresol were added. The pH was previously regulated using HCl 1:4 and NH3 1:1 until the salmon color (pH 0.5). Subsequently, 5 mL of DAN (2-3 Diaminonaphthalene) was added and the solution was taken once more to the water bath (80 °C), for 30 minutes (in the dark). After cooling, 10 mL of cyclohexane was added, and careful stirring was performed. Then, the supernatant was collected, and selenium was determined by the fluorimetric method (OLSON; PALMER; CARY, 1975).

5.7.2 Bromatological and macrominerals analyses

Diets were analyzed for dry matter, crude protein, crude fiber, nitrogen-free extract, extract, and mineral matter based on procedures previously described by Silva and Queiroz (2002). Calcium levels were determined by inductively coupled plasma optical emission spectrometry (ICP OES) (SINDIRAÇÕES, 2013), while phosphorus levels were measured by the colorimetric analysis (AOAC, 1996).

5.8 *IN VIVO* FERTILITY ASSAY

Semen doses from 34 of the boars used (SS3, n = 11; OH-SeMet3, n = 12 and OH-SeMet6, n = 11) were employed in AI programs during the experimental phase, and a total of 1131 females were inseminated: SS3 (n = 301); OH-SeMet3 (n = 427) and OH-SeMet6 (n = 403). The choice of which boars would be used in the AI programs and the semen doses' distribution to the different farms were entirely made by the commercial AI-center, according to its interests and needs. In the farms, gilts and sows were randomly inseminated according to each production unit (Appendix D). Sows were inseminated by the post-cervical AI (1.5×10^9 sperm; 45 mL) and gilts by the intracervical AI (3×10^9 sperm; 90 mL). Only semen AI-doses with a maximum of 72 h of storage were used in the AI programs, and each female received semen doses from the same male throughout the estrus. Estrus detection was carried out twice

daily by walking a sexually mature male and observing the female reflex of tolerance to the male and the man. Once the signs of estrus were observed, females received the first AI at 0 h (gilts) or 12 h (sows), and from then on, they were inseminated at 24-hour intervals until the end of estrus is detected. The pregnancy diagnosis was carried out indirectly by estrus detection, conducted between days 17-25 after insemination to detect possible estrus returns. The ratio between the number of inseminated females and the number of females pregnant corresponded to the pregnancy rate in each group studied. The total number of piglets born, born alive, mummies, and stillborn was counted. At the end of the experimental phase, an extensive data analysis was carried out for reproductive indexes within each of the experimental groups.

5.9 STATISTICAL ANALYSIS

Data were analyzed using the MIXED and GLIMMIX procedure (SAS 9.3; Institute Inc., Cary, NC, USA) according to a randomized block design containing treatments as main factors. The boars were random effects factor while the treatment and the time were a fixed-effects factor. Treatments were evaluated using orthogonal contrast to analyze treatment effects; contrast 1 (C1) referred to the effect of source (0.3 mg selenium/kg as SS × 0.3 mg selenium/kg as OH-SeMet), and contrast 2 (C2) referred to the effect of organic level (0.3 mg selenium/kg as OH-SeMet × 0.6 mg selenium/kg as OH-SeMet). The Tukey-Kramer test evaluated the effect of time. For semen analysis and sperm characteristics, as well as for selenium concentration in blood plasma and seminal plasma, the interval between semen collections and the age of the boars were used as a covariate. In the analysis of pregnancy rate and the litter size characteristics, the farm was considered as a random-effects factor, the treatment as a fixed-effects factor, and parity was used as a covariate. The total number of piglets born was also used as a covariate for the litter size characteristics. Data from inseminated females who were culled or died during the study were removed before the pregnancy rate analysis. The following variables were analyzed using the MIXED procedure: all sperm characteristics, the total number of pigs born, and the number of piglets born alive. The pregnancy rate was considered a binary distribution and the percentage of stillborn, mummies, and teratological forms as a Poisson distribution. The binary and Poisson distributions were analyzed using the SAS GLIMMIX procedure. Differences were considered significant when $p < 0.05$, and a tendency when $p \leq 0.10$. Results were expressed as means and standard error of the mean (SEM).

6 RESULTS

No significant interaction was observed between dietary treatments and weeks for all variables studied. So, the main-effect analysis could be performed.

6.1 RAW SEMEN ASSESSMENTS

6.1.1 Initial analyses

No effect of source or organic supplementation level was observed on volume and sperm total motility ($p > 0.05$) (Table 1). However, it was observed that sperm concentration, TSCE, and NSDP tended to be higher for boars fed diet supplemented with organic selenium ($p \leq 0.10$). Except for total sperm motility, all the other characteristics presented a week effect, or in other words, were influenced in some way by the course of the experiment regardless of the treatment studied. However, there is no established pattern of increase or decrease in values as the experiment progressed. The data appears to behave randomly (Table 2).

Table 1 – Characteristics of raw semen from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

	Dietary Treatment				*p-value*			
	SS3 (n = 14)	OH-SeMet3 (n = 14)	OH-SeMet6 (n = 14)	SEM	C1	C2	W	T*W
VOL	234.37	223.86	220.81	5.75	0.824	0.926	0.034	0.232
SC	263.10	315.79	301.84	7.21	0.070	0.548	0.012	0.120
TSCE	56.57	66.60	60.55	1.39	0.098	0.292	<.001	0.511
NSDP	18.85	22.11	20.18	0.46	0.102	0.308	<.001	0.638
TMOT	87.21	89.16	87.69	0.30	0.346	0.349	0.158	0.662

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs*. 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; VOL, volume (mL); SC, sperm concentration ($\times 10^6$ sperm/mL); TSCE, total sperm count in ejaculate ($\times 10^9$ sperm); NSDP, number of semen doses produced per ejaculate (3×10^9 sperm/dose); TMOT, total motility (%).

Table 2 – Characteristics of raw semen from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1]

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
VOL	SS3	229.00 ± 22.73	250.86 ± 31.56	212.29 ± 15.49	198.14 ± 28.73	271.86 ± 26.37	256.29 ± 34.59	222.14 ± 33.71
	OH-SeMet3	180.57 ± 16.50	235.86 ± 27.31	241.71 ± 27.01	205.43 ± 16.32	221.00 ± 20.90	234.29 ± 18.83	248.14 ± 24.12
	OH-SeMet6	190.43 ± 23.36	262.57 ± 35.73	196.71 ± 28.83	214.71 ± 28.42	208.46 ± 19.81	231.71 ± 32.04	241.69 ± 28.13
	Mean	200.00 ± 12.32^{b}	249.76 ± 17.94^{a}	216.90 ± 14.10^{ab}	206.10 ± 14.21^{ab}	234.39 ± 13.48^{ab}	240.76 ± 16.60^{ab}	237.22 ± 16.40^{ab}
SC	SS3	240.30 ± 37.73	264.94 ± 24.02	275.54 ± 29.60	194.01 ± 23.43	242.93 ± 22.35	299.99 ± 42.42	319.17 ± 37.05
	OH-SeMet3	340.04 ± 34.38	325.90 ± 23.05	279.17 ± 26.61	314.50 ± 44.39	326.49 ± 31.14	331.08 ± 27.75	294.14 ± 22.66
	OH-SeMet6	305.16 ± 35.41	301.00 ± 42.70	272.37 ± 26.22	229.38 ± 27.92	348.18 ± 39.51	365.95 ± 32.81	285.97 ± 25.78
	Mean	294.93 ± 21.26^{ab}	298.07 ± 18.21^{ab}	275.78 ± 15.54^{ab}	247.68 ± 20.73^{b}	304.29 ± 19.11^{ab}	332.34 ± 20.08^{a}	300.10 ± 16.66^{ab}
TSCE	SS3	50.87 ± 6.91	63.88 ± 5.76	54.46 ± 5.23	33.36 ± 3.15	60.79 ± 4.23	68.35 ± 8.02	62.60 ± 6.81
	OH-SeMet3	57.58 ± 5.93	73.27 ± 7.49	62.41 ± 6.36	57.44 ± 5.66	73.32 ± 7.44	72.64 ± 4.52	69.58 ± 5.12
	OH-SeMet6	56.81 ± 6.42	63.36 ± 4.06	51.14 ± 5.70	46.68 ± 4.13	66.20 ± 5.07	74.36 ± 6.42	66.12 ± 8.69
	Mean	55.09 ± 3.65^{cd}	66.84 ± 3.42^{ab}	56.00 ± 3.34^{bcd}	46.13 ± 2.96^{d}	66.78 ± 3.35^{abc}	71.78 ± 3.67^{a}	66.10 ± 3.93^{abc}
NSDP	SS3	16.95 ± 2.30	21.29 ± 1.92	18.15 ± 1.74	11.12 ± 1.05	20.26 ± 1.41	22.78 ± 2.67	20.87 ± 2.27
	OH-SeMet3	19.19 ± 1.98	24.42 ± 2.50	20.81 ± 2.12	18.25 ± 1.80	24.44 ± 2.48	24.21 ± 1.51	23.19 ± 1.71
	OH-SeMet6	18.94 ± 2.14	21.12 ± 1.35	17.05 ± 1.90	15.56 ± 1.38	22.07 ± 1.69	24.78 ± 2.14	22.04 ± 2.90
	Mean	18.36 ± 1.22^{cd}	22.28 ± 1.14^{ab}	18.67 ± 1.11^{bcd}	14.99 ± 0.93^{d}	22.26 ± 1.12^{abc}	23.93 ± 1.22^{a}	22.03 ± 1.31^{abc}
TMOT	SS3	88.81 ± 1.11	88.79 ± 0.81	90.36 ± 1.04	87.59 ± 1.52	84.54 ± 2.02	89.43 ± 1.32	88.13 ± 1.83
	OH-SeMet3	87.84 ± 1.28	88.54 ± 1.31	89.42 ± 1.06	89.86 ± 1.24	89.43 ± 1.30	89.67 ± 1.11	89.40 ± 1.41
	OH-SeMet6	86.92 ± 1.22	87.05 ± 1.13	87.72 ± 1.41	88.29 ± 1.66	87.28 ± 1.73	87.90 ± 1.36	88.80 ± 0.96
	Mean	87.84 ± 0.69	88.11 ± 0.64	89.13 ± 0.69	88.58 ± 0.85	87.07 ± 1.02	89.03 ± 0.72	88.76 ± 0.84

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. VOL, volume (mL); SC, sperm concentration ($\times 10^6$ sperm/mL); TSCE, total sperm count in ejaculate ($\times 10^9$ sperm); NSDP, number of semen doses produced per ejaculate (3×10^9 sperm/dose); TMOT, total motility (%). $^{a\text{-}d}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.1.2 Sperm morphology

No significant effect of source or organic supplementation level was observed on sperm morphology in raw semen ($p > 0.05$) (Table 3). However, it was observed a high prevalence of tail defects for all the treatments, predominantly marked by folded tail. These alterations consequently resulted in a drop of normal cells in the ejaculates, presenting percentages below that recommended by the CBRA (2013) for their use to semen AI-doses processing (≥ 70%). Although it was observed a time effect for most sperm forms, there is no established pattern of increase or decrease in values as the experiment progressed (Table 4).

Table 3 – Sperm morphology in raw semen from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

Defects (%)	Dietary Treatment			SEM	*p-value*			
	SS3 (*n* = 14)	OH-SeMet3 (*n* = 14)	OH-SeMet6 (*n* = 14)		C1	C2	W	T*W
Acrosome	1.92	1.92	1.71	0.10	0.985	0.553	0.377	0.605
Head	1.46	1.23	1.37	0.07	0.411	0.738	0.007	0.874
Neck	0.07	0.02	0.05	0.09	0.187	0.419	<.001	0.444
Midpiece	0.34	0.40	0.35	0.03	0.542	0.537	0.004	0.162
PCD	3.62	3.16	2.50	0.27	0.215	0.993	0.019	0.116
DCD	2.03	1.58	1.49	0.10	0.119	0.836	0.205	0.070
Tail	19.50	18.27	16.63	0.56	0.948	0.352	0.004	0.828
TF	0.14	0.09	0.12	0.01	0.970	0.973	0.694	1.000
Normal Sperm	62.52	66.40	67.03	1.03	0.834	0.916	0.003	0.955

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; PCD, proximal cytoplasmic droplet; DCD, distal cytoplasmic droplet; TF, teratological forms.

Table 4 – Sperm morphology in raw semen from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(It continues)*

Defects (%)	Dietary Treatment	Week						
		2	4	6	8	10	12	14
Acrosome	SS3	2.00 ± 0.53	2.23 ± 0.51	2.04 ± 0.41	2.12 ± 0.49	1.37 ± 0.26	1.87 ± 0.43	1.75 ± 0.45
	OH-SeMet3	2.12 ± 0.35	2.88 ± 0.61	1.50 ± 0.45	2.12 ± 0.50	1.69 ± 0.55	0.72 ± 0.26	2.00 ± 0.62
	OH-SeMet6	2.38 ± 0.44	1.73 ± 0.35	1.62 ± 0.30	1.82 ± 0.45	1.58 ± 0.25	1.69 ± 0.39	1.12 ± 0.25
	Mean	2.17 ± 0.25	2.28 ± 0.29	1.73 ± 0.22	2.03 ± 0.27	1.55 ± 0.22	1.50 ± 0.23	1.62 ± 0.27
Head	SS3	1.33 ± 0.26	1.96 ± 0.39	1.54 ± 0.29	1.57 ± 0.28	1.18 ± 0.14	1.14 ± 0.20	1.46 ± 0.31
	OH-SeMet3	1.12 ± 0.25	1.23 ± 0.43	1.79 ± 0.46	1.57 ± 0.31	0.92 ± 0.20	1.04 ± 0.21	0.88 ± 0.17
	OH-SeMet6	1.32 ± 0.25	1.42 ± 0.33	1.79 ± 0.30	1.85 ± 0.39	1.25 ± 0.23	0.85 ± 0.18	1.00 ± 0.26
	Mean	1.25 ± 0.14^{ab}	1.53 ± 0.22^{ab}	1.71 ± 0.21^{a}	1.66 ± 0.19^{ab}	1.11 ± 0.11^{ab}	1.01 ± 0.12^{b}	1.12 ± 0.15^{ab}
Neck	SS3	0.00 ± 0.00	0.05 ± 0.05	0.23 ± 0.09	0.12 ± 0.06	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00
	OH-SeMet3	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.05	0.05 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	OH-SeMet6	0.00 ± 0.00	0.00 ± 0.00	0.25 ± 0.08	0.04 ± 0.04	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
	Mean	0.00 ± 0.00^{b}	0.02 ± 0.02^{b}	0.18 ± 0.04^{a}	0.07 ± 0.03^{b}	0.03 ± 0.02^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Midpiece	SS3	0.42 ± 0.15	0.31 ± 0.09	0.32 ± 0.10	0.25 ± 0.10	0.57 ± 0.12	0.15 ± 0.07	0.35 ± 0.12
	OH-SeMet3	0.50 ± 0.16	0.43 ± 0.10	0.17 ± 0.07	0.29 ± 0.09	0.61 ± 0.13	0.43 ± 0.10	0.36 ± 0.12
	OH-SeMet6	0.58 ± 0.19	0.17 ± 0.07	0.25 ± 0.08	0.57 ± 0.16	0.50 ± 0.15	0.09 ± 0.06	0.25 ± 0.12
	Mean	0.50 ± 0.09^{ab}	0.31 ± 0.05^{ab}	0.25 ± 0.05^{b}	0.37 ± 0.07^{ab}	0.56 ± 0.07^{a}	0.24 ± 0.05^{ab}	0.32 ± 0.07^{ab}
PCD	SS3	3.82 ± 1.42	3.32 ± 0.97	2.67 ± 1.00	1.86 ± 0.58	8.18 ± 2.98	2.55 ± 0.75	1.60 ± 0.50
	OH-SeMet3	2.46 ± 0.79	3.04 ± 1.08	3.35 ± 1.01	4.23 ± 1.20	4.23 ± 1.58	3.08 ± 0.94	1.59 ± 0.43
	OH-SeMet6	2.87 ± 1.04	1.33 ± 0.53	2.14 ± 0.75	3.19 ± 1.19	3.58 ± 1.14	2.08 ± 0.61	2.19 ± 0.73
	Mean	3.03 ± 0.62^{ab}	2.54 ± 0.52^{bc}	2.75 ± 0.54^{bc}	3.10 ± 0.61^{ab}	5.40 ± 1.23^{a}	2.56 ± 0.44^{bc}	1.82 ± 0.34^{c}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. PCD, proximal cytoplasmic droplet. $^{a-c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

Table 4 – Sperm morphology in raw semen from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(Conclusion)*

Defects (%)	Dietary Treatment	Week						
		2	4	6	8	10	12	14
DCD	SS3	1.64 ± 0.45	2.67 ± 0.67	1.91 ± 0.40	2.73 ± 0.63	2.37 ± 0.64	1.14 ± 0.22	1.73 ± 0.38
	OH-SeMet3	1.46 ± 0.50	1.65 ± 0.28	1.68 ± 0.34	1.65 ± 0.34	2.00 ± 0.53	1.58 ± 0.26	1.04 ± 0.24
	OH-SeMet6	1.14 ± 0.43	1.25 ± 0.41	1.09 ± 0.27	1.73 ± 0.47	1.77 ± 0.51	1.61 ± 0.18	1.90 ± 0.83
	Mean	1.41 ± 0.26	1.85 ± 0.28	1.57 ± 0.20	2.01 ± 0.28	2.06 ± 0.32	1.44 ± 0.14	1.51 ± 0.27
Tail	SS3	22.18 ± 3.58	18.79 ± 2.54	20.75 ± 3.00	24.71 ± 3.04	16.42 ± 1.95	16.35 ± 1.55	16.69 ± 2.05
	OH-SeMet3	21.86 ± 3.53	15.09 ± 1.60	19.50 ± 2.97	22.14 ± 3.61	14.54 ± 1.76	16.96 ± 2.77	16.77 ± 2.01
	OH-SeMet6	16.54 ± 2.35	18.29 ± 1.77	14.42 ± 1.92	18.79 ± 2.41	14.68 ± 2.00	15.96 ± 2.04	17.50 ± 2.15
	Mean	20.19 ± 1.85^{ab}	17.56 ± 1.20^{ab}	18.38 ± 1.60^{ab}	21.88 ± 1.76^{a}	15.20 ± 1.08^{b}	16.41 ± 1.22^{b}	16.99 ± 1.16^{b}
TF	SS3	0.07 ± 0.05	0.14 ± 0.08	0.19 ± 0.09	0.19 ± 0.09	0.23 ± 0.09	0.08 ± 0.05	0.12 ± 0.06
	OH-SeMet3	0.07 ± 0.07	0.00 ± 0.00	0.11 ± 0.06	0.21 ± 0.07	0.07 ± 0.05	0.07 ± 0.05	0.07 ± 0.05
	OH-SeMet6	0.07 ± 0.05	0.14 ± 0.06	0.15 ± 0.09	0.18 ± 0.07	0.14 ± 0.06	0.11 ± 0.06	0.08 ± 0.05
	Mean	0.07 ± 0.03	0.10 ± 0.04	0.15 ± 0.04	0.20 ± 0.04	0.15 ± 0.04	0.09 ± 0.03	0.09 ± 0.03
Normal Sperm (%)	SS3	59.57 ± 4.74	63.46 ± 3.18	63.86 ± 3.88	56.96 ± 4.14	63.79 ± 4.57	62.75 ± 5.12	67.65 ± 2.94
	OH-SeMet3	61.54 ± 6.76	65.62 ± 5.25	68.77 ± 4.86	59.36 ± 6.34	72.08 ± 3.83	67.65 ± 4.58	70.69 ± 3.41
	OH-SeMet6	64.81 ± 5.43	72.04 ± 2.33	68.90 ± 4.91	59.57 ± 6.23	68.23 ± 5.16	67.92 ± 4.26	69.15 ± 3.91
	Mean	61.90 ± 3.24^{bc}	66.82 ± 2.23^{abc}	66.95 ± 2.57^{abc}	58.63 ± 3.19^{c}	67.92 ± 2.62^{ab}	66.02 ± 2.67^{abc}	69.17 ± 1.95^{a}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. DCD, distal cytoplasmic droplet; TF, teratological forms. $^{a-c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.1.3 GPx activity

As a result of GPx activity in the seminal plasma, no source or organic supplementation level affected this variable ($p > 0.05$) (Table 5). However, a time week was observed for this variable throughout the 14 weeks of study. Herein, it was observed that GPx activity started very high in the second week, showing a considerable drop in the following weeks ($p < 0.05$) (Table 6).

Table 5 – Glutathione peroxidase activity in raw semen from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

	Dietary Treatment			SEM	*p-value*			
	SS3 (*n* = 14)	OH-SeMet3 (*n* = 14)	OH-SeMet6 (*n* = 14)		C1	C2	W	T*W
GPx (units/mL)	38.61	42.59	39.11	1.50	0.479	0.564	<.001	0.140

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; GPx, glutathione peroxidase.

Table 6 – Glutathione peroxidase activity in raw semen from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1]

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
GPx (units/mL)	SS3	74.25 ± 8.57	35.14 ± 2.99	36.54 ± 2.36	35.16 ± 1.98	22.88 ± 0.91	24.06 ± 1.25	39.06 ± 2.89
	OH-SeMet3	92.05 ± 12.18	35.77 ± 4.67	35.42 ± 2.81	45.81 ± 1.66	24.76 ± 1.50	24.25 ± 1.57	33.84 ± 3.61
	OH-SeMet6	75.84 ± 10.16	31.73 ± 2.19	39.76 ± 1.70	41.08 ± 1.20	24.90 ± 1.27	25.15 ± 1.01	33.71 ± 3.31
	Mean	80.72 ± 6.00^{a}	34.24 ± 1.94^{c}	37.24 ± 1.35^{bc}	40.25 ± 1.20^{b}	24.18 ± 0.71^{d}	24.51 ± 0.73^{d}	35.58 ± 1.88^{c}

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. GPx, glutathione peroxidase. $^{a-d}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.2 SPERM EVALUATIONS AFTER STORAGE IN LIQUID STATE

6.2.1 Computer-assisted sperm analysis

Sperm motility characteristics evaluated in liquid-stored semen AI-doses were not affected by any source or organic supplementation level tested in this study ($p > 0.05$) (Table 7). On the other hand, all of them were affected by time. In this context, most variables behaved similarly. In general, the values for total and progressive motility, amplitude of lateral head displacement, hyperactive sperm, beat cross frequency, as well as for curvilinear, straight linear, and average path velocities, increased over the last weeks of the study, ending up with higher values compared to the first evaluation performed ($p < 0.05$). Conversely, the characteristics linearity, straightness, and wobble coefficient had their values reduced over the weeks, which could already be observed from the first evaluation times ($p < 0.05$) (Table 8).

Table 7 – Sperm motility characteristics in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

	Dietary Treatment			SEM	*p-value*			
	SS3 (n = 14)	OH-SeMet3 (n = 14)	OH-SeMet6 (n = 14)		C1	C2	W	T*W
TMOT	93.19	92.66	91.32	0.30	0.637	0.214	<.001	0.320
PMOT	69.07	65.18	64.71	0.74	0.211	0.901	<.001	0.434
VCL	48.27	46.07	46.02	0.55	0.605	0.863	<.001	0.818
VSL	31.25	29.11	29.23	0.30	0.198	0.915	<.001	0.792
VAP	37.97	35.81	35.73	0.39	0.363	0.917	<.001	0.625
BCF	6.13	5.99	6.10	0.01	0.160	0.113	0.006	0.500
ALH	1.87	1.88	1.84	0.02	0.664	0.473	<.001	0.356
STR	82.54	81.66	82.60	0.20	0.195	0.278	<.001	0.215
LIN	65.22	63.97	64.97	0.35	0.263	0.457	<.001	0.569
WOB	79.03	78.33	78.84	0.25	0.542	0.601	<.001	0.470
HYP	1.40	1.26	1.20	0.06	0.859	0.641	<.001	0.372

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; TMOT, total motility (%); PMOT, progressive motility (%); VCL, curvilinear velocity (µm/s); VSL, straight linear velocity (µm/s); VAP, average path velocity (µm/s); BCF, beat cross frequency (Hz); ALH, amplitude of lateral head displacement (µm); STR, straightness (%); LIN, linearity (%); WOB, wobble coefficient (%); HYP, hyperactive sperm (%).

Table 8 – Sperm motility characteristics in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(It continues)*

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
TMOT (%)	SS3	90.86 ± 1.61	93.16 ± 1.32	91.84 ± 1.20	93.35 ± 0.82	93.68 ± 1.10	94.24 ± 1.20	95.17 ± 0.79
	OH-SeMet3	88.69 ± 2.36	91.94 ± 0.97	93.12 ± 0.80	95.60 ± 0.87	91.54 ± 1.19	92.38 ± 1.57	95.64 ± 0.84
	OH-SeMet6	89.16 ± 1.64	90.62 ± 1.34	92.67 ± 0.70	92.00 ± 1.42	89.47 ± 1.27	90.76 ± 1.68	95.46 ± 0.87
	Mean	89.57 ± 1.08^{b}	91.79 ± 0.72^{b}	92.54 ± 0.53^{b}	93.60 ± 0.65^{b}	91.51 ± 0.73^{b}	92.46 ± 0.87^{b}	95.42 ± 0.47^{a}
PMOT (%)	SS3	64.21 ± 3.09	66.76 ± 3.65	63.79 ± 3.44	71.92 ± 2.08	70.42 ± 2.60	73.74 ± 2.09	72.09 ± 3.34
	OH-SeMet3	60.14 ± 3.76	60.89 ± 4.16	60.76 ± 3.68	72.83 ± 2.63	64.38 ± 2.58	65.06 ± 4.60	72.45 ± 2.36
	OH-SeMet6	60.09 ± 3.43	58.34 ± 4.44	64.35 ± 2.43	68.49 ± 2.84	63.85 ± 2.19	65.62 ± 3.96	73.53 ± 3.04
	Mean	61.48 ± 1.96^{c}	61.65 ± 2.42^{c}	62.95 ± 1.83^{c}	71.08 ± 1.46^{ab}	66.27 ± 1.47^{bc}	68.06 ± 2.21^{abc}	72.65 ± 1.67^{a}
VCL (µm/s)	SS3	41.62 ± 1.92	44.77 ± 1.98	45.26 ± 2.55	52.61 ± 1.69	50.97 ± 1.46	49.99 ± 2.47	52.15 ± 3.59
	OH-SeMet3	42.86 ± 1.47	42.42 ± 1.74	43.49 ± 2.37	51.37 ± 2.03	46.85 ± 1.50	46.31 ± 2.95	49.19 ± 2.39
	OH-SeMet6	41.49 ± 1.92	41.42 ± 2.63	45.59 ± 2.03	48.16 ± 2.72	47.89 ± 2.73	49.63 ± 3.21	48.51 ± 3.64
	Mean	41.99 ± 1.01^{c}	42.78 ± 1.25^{c}	44.78 ± 1.32^{bc}	50.72 ± 1.27^{a}	48.59 ± 1.13^{ab}	48.62 ± 1.64^{ab}	50.00 ± 1.86^{a}
VSL (µm/s)	SS3	28.98 ± 1.11	29.62 ± 1.45	30.19 ± 1.67	32.71 ± 1.13	31.80 ± 0.94	32.59 ± 1.49	32.64 ± 2.16
	OH-SeMet3	27.89 ± 1.18	27.56 ± 1.16	27.14 ± 1.33	31.74 ± 1.07	28.83 ± 0.68	29.59 ± 1.63	31.03 ± 1.27
	OH-SeMet6	27.76 ± 1.01	27.28 ± 1.35	29.17 ± 1.37	28.95 ± 1.20	29.46 ± 1.03	30.89 ± 1.52	31.38 ± 1.93
	Mean	28.21 ± 0.63^{c}	28.09 ± 0.76^{c}	28.83 ± 0.85^{bc}	31.19 ± 0.69^{ab}	30.04 ± 0.54^{abc}	31.02 ± 0.89^{ab}	31.71 ± 1.04^{a}
VAP (µm/s)	SS3	34.09 ± 1.37	35.49 ± 1.62	36.31 ± 2.05	40.88 ± 1.34	39.21 ± 1.10	39.37 ± 1.85	40.06 ± 2.72
	OH-SeMet3	33.85 ± 1.18	33.38 ± 1.37	33.61 ± 1.74	39.78 ± 1.36	35.87 ± 0.67	36.14 ± 2.08	38.05 ± 1.64
	OH-SeMet6	33.41 ± 1.37	32.93 ± 1.87	35.75 ± 1.51	35.68 ± 1.48	36.72 ± 1.57	38.15 ± 2.07	37.90 ± 2.54
	Mean	33.78 ± 0.74^{c}	33.87 ± 0.94^{c}	35.22 ± 1.02^{bc}	38.86 ± 0.86^{a}	37.28 ± 0.69^{ab}	37.88 ± 1.15^{ab}	38.71 ± 1.34^{a}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. TMOT, total motility; PMOT, progressive motility; VCL, curvilinear velocity; VSL, straight linear velocity; VAP, average path velocity. $^{a\text{-}c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

Table 8 – Sperm motility characteristics in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(It continues)*

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
BCF (Hz)	SS3	6.09 ± 0.06	6.10 ± 0.08	6.03 ± 0.07	6.05 ± 0.05	6.25 ± 0.07	6.19 ± 0.07	6.21 ± 0.07
	OH-SeMet3	5.95 ± 0.05	5.97 ± 0.04	5.94 ± 0.07	5.94 ± 0.03	6.00 ± 0.09	6.11 ± 0.10	6.07 ± 0.05
	OH-SeMet6	6.16 ± 0.05	6.01 ± 0.05	6.01 ± 0.06	6.19 ± 0.09	6.07 ± 0.09	6.12 ± 0.09	6.22 ± 0.08
	Mean	6.07 ± 0.03^{ab}	6.03 ± 0.03^{ab}	5.99 ± 0.04^{b}	6.06 ± 0.04^{ab}	6.11 ± 0.05^{ab}	6.14 ± 0.05^{ab}	6.16 ± 0.04^{a}
ALH (µm)	SS3	1.56 ± 0.04	1.82 ± 0.05	1.80 ± 0.06	2.00 ± 0.05	1.98 ± 0.05	1.89 ± 0.08	2.04 ± 0.09
	OH-SeMet3	1.72 ± 0.05	1.78 ± 0.05	1.87 ± 0.06	1.99 ± 0.07	1.91 ± 0.07	1.89 ± 0.08	1.97 ± 0.08
	OH-SeMet6	1.67 ± 0.06	1.71 ± 0.08	1.84 ± 0.07	1.92 ± 0.07	1.92 ± 0.10	1.95 ± 0.11	1.89 ± 0.11
	Mean	1.65 ± 0.03^{c}	1.77 ± 0.03^{bc}	1.84 ± 0.04^{ab}	1.97 ± 0.04^{a}	1.94 ± 0.04^{a}	1.91 ± 0.05^{ab}	1.97 ± 0.05^{a}
STR (%)	SS3	85.90 ± 0.68	83.37 ± 0.74	83.20 ± 0.78	79.96 ± 0.57	81.11 ± 0.78	82.90 ± 0.71	81.71 ± 0.75
	OH-SeMet3	83.32 ± 0.65	82.55 ± 0.86	80.93 ± 0.94	79.94 ± 1.15	80.34 ± 1.20	83.22 ± 0.81	81.63 ± 0.71
	OH-SeMet6	84.09 ± 0.43	83.24 ± 1.02	83.45 ± 0.62	81.19 ± 0.62	81.77 ± 1.02	81.39 ± 1.24	83.16 ± 0.85
	Mean	84.44 ± 0.38^{a}	83.05 ± 0.51^{ab}	82.48 ± 0.49^{bc}	80.36 ± 0.47^{d}	81.04 ± 0.58^{cd}	82.51 ± 0.54^{bc}	82.16 ± 0.45^{bcd}
LIN (%)	SS3	71.06 ± 1.33	66.19 ± 1.34	66.84 ± 1.30	62.16 ± 0.93	62.60 ± 1.47	65.55 ± 1.25	63.14 ± 1.44
	OH-SeMet3	67.44 ± 1.21	65.18 ± 1.50	62.78 ± 1.44	62.36 ± 1.75	62.24 ± 2.14	64.57 ± 1.82	63.51 ± 1.41
	OH-SeMet6	67.41 ± 1.22	66.58 ± 1.55	66.12 ± 1.61	62.89 ± 1.09	62.75 ± 2.19	63.38 ± 2.08	65.49 ± 1.64
	Mean	68.54 ± 0.75^{a}	65.99 ± 0.84^{ab}	65.22 ± 0.86^{bc}	62.47 ± 0.74^{c}	62.52 ± 1.10^{c}	64.53 ± 0.99^{bc}	64.02 ± 0.86^{bc}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. BCF, beat cross frequency; ALH, amplitude of lateral head displacement; STR, straightness; LIN, linearity. $^{a-d}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

Table 8 – Sperm motility characteristics in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(Conclusion)*

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
WOB (%)	SS3	83.11 ± 0.86	79.32 ± 0.97	80.26 ± 0.88	77.68 ± 0.75	77.06 ± 1.17	78.99 ± 0.88	77.14 ± 1.10
	OH-SeMet3	81.47 ± 0.80	78.82 ± 1.11	77.44 ± 0.98	77.78 ± 1.19	77.15 ± 1.71	78.39 ± 1.11	77.69 ± 1.08
	OH-SeMet6	80.81 ± 0.75	79.83 ± 0.96	79.95 ± 1.01	77.39 ± 0.79	77.48 ± 1.51	77.64 ± 1.46	78.65 ± 1.18
	Mean	81.78 ± 0.48^{a}	79.33 ± 0.57^{b}	79.20 ± 0.58^{b}	77.61 ± 0.53^{b}	77.22 ± 0.83^{b}	78.36 ± 0.66^{b}	77.81 ± 0.64^{b}
HYP (%)	SS3	0.64 ± 0.12	1.07 ± 0.15	1.05 ± 0.16	1.81 ± 0.23	1.82 ± 0.31	1.29 ± 0.20	2.01 ± 0.39
	OH-SeMet3	0.83 ± 0.19	0.95 ± 0.14	1.27 ± 0.25	1.75 ± 0.35	1.18 ± 0.26	1.15 ± 0.24	1.69 ± 0.33
	OH-SeMet6	0.48 ± 0.08	0.54 ± 0.10	0.89 ± 0.17	1.36 ± 0.21	1.76 ± 0.42	1.67 ± 0.31	1.54 ± 0.39
	Mean	0.66 ± 0.08^{c}	0.86 ± 0.08^{c}	1.08 ± 0.11^{bc}	1.64 ± 0.16^{a}	1.59 ± 0.19^{a}	1.37 ± 0.15^{ab}	1.75 ± 0.21^{a}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. WOB, wobble coefficient; HYP, hyperactive sperm. $^{a-c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.2.2 Sperm morphology

Sperm morphology evaluated in liquid-stored semen AI-doses was not affected by any source or organic supplementation level tested in this study ($p > 0.05$) (Table 9). However, it was observed a high prevalence of acrosome defects for all the treatments, predominantly marked by swollen acrosome. These alterations consequently resulted in a drop of normal cells in the ejaculates, presenting percentages below that recommended by the CBRA (2013) for liquid-stored semen AI-doses (≥ 80%). Also, it was observed a time effect for most sperm forms. Interestingly, the percentage of sperm with midpiece defects was reduced throughout the experiment, reaching their lowest value in the fourteenth week compared to the first three evaluations ($p < 0.05$). On the other hand, sperm tail defects increased over time, mainly after the sixth week ($p < 0.05$), but still within the values allowed (≤ 10%) by CBRA (2013) (Table 10).

Table 9 – Sperm morphology in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

Defects (%)	Dietary Treatment			SEM	*p-value*			
	SS3 (*n* = 14)	OH-SeMet3 (*n* = 14)	OH-SeMet6 (*n* = 14)		C1	C2	W	T*W
Acrosome	15.31	16.85	17.26	0.45	0.274	0.990	<.001	0.085
Head	1.53	1.32	1.62	0.06	0.321	0.256	0.025	0.716
Neck	0.14	0.14	0.18	0.02	0.684	0.614	0.068	0.158
Midpiece	0.28	0.24	0.35	0.02	0.455	0.388	0.005	0.420
PCD	2.19	2.50	1.93	0.18	0.639	0.492	0.094	0.194
DCD	2.34	1.89	1.99	0.12	0.433	0.681	0.002	0.544
Tail	4.55	5.35	4.10	0.17	0.366	0.122	<.001	0.687
TF	0.21	0.12	0.11	0.02	0.138	0.979	0.756	0.964
Normal Sperm	63.39	67.91	65.18	0.91	0.270	0.589	<.001	0.222

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; PCD, proximal cytoplasmic droplet; DCD, distal cytoplasmic droplet; TF, teratological forms.

Table 10 – Sperm morphology in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(It continues)*

Defects (%)	Dietary Treatment	Week						
		2	4	6	8	10	12	14
ACRO	SS3	11.89 ± 1.50	13.50 ± 1.27	13.96 ± 2.18	11.43 ± 1.23	22.29 ± 1.52	20.39 ± 2.92	13.46 ± 1.02
	OH-SeMet3	13.23 ± 0.97	17.82 ± 1.62	20.08 ± 1.99	13.62 ± 1.43	23.32 ± 1.84	16.82 ± 1.22	12.46 ± 1.78
	OH-SeMet6	12.12 ± 1.36	12.00 ± 1.34	20.50 ± 2.27	16.75 ± 2.37	23.46 ± 2.33	18.68 ± 2.18	15.91 ± 1.85
	Mean	12.41 ± 0.74^{d}	14.52 ± 0.90^{cd}	18.13 ± 1.30^{bc}	13.94 ± 1.05^{d}	23.02 ± 1.09^{a}	18.63 ± 1.27^{b}	13.84 ± 0.91^{d}
HEAD	SS3	1.25 ± 0.26	1.86 ± 0.19	1.15 ± 0.29	1.50 ± 0.33	1.42 ± 0.34	1.62 ± 0.32	1.92 ± 0.30
	OH-SeMet3	0.85 ± 0.23	1.82 ± 0.32	1.57 ± 0.30	1.21 ± 0.27	0.96 ± 0.19	1.36 ± 0.19	1.46 ± 0.30
	OH-SeMet6	1.25 ± 0.26	1.59 ± 0.26	1.71 ± 0.32	1.62 ± 0.29	1.75 ± 0.31	1.62 ± 0.38	1.77 ± 0.29
	Mean	1.10 ± 0.14^{b}	1.76 ± 0.16^{a}	1.47 ± 0.18^{ab}	1.44 ± 0.17^{ab}	1.36 ± 0.17^{ab}	1.52 ± 0.17^{ab}	1.72 ± 0.17^{ab}
NECK	SS3	0.04 ± 0.04	0.15 ± 0.09	0.21 ± 0.09	0.25 ± 0.11	0.00 ± 0.00	0.18 ± 0.10	0.15 ± 0.15
	OH-SeMet3	0.04 ± 0.04	0.08 ± 0.05	0.23 ± 0.07	0.12 ± 0.06	0.12 ± 0.07	0.23 ± 0.09	0.15 ± 0.09
	OH-SeMet6	0.12 ± 0.06	0.14 ± 0.11	0.04 ± 0.04	0.25 ± 0.11	0.32 ± 0.12	0.18 ± 0.11	0.21 ± 0.10
	Mean	0.06 ± 0.03	0.12 ± 0.05	0.16 ± 0.04	0.21 ± 0.06	0.16 ± 0.05	0.20 ± 0.06	0.17 ± 0.07
MP	SS3	0.54 ± 0.13	0.29 ± 0.11	0.38 ± 0.16	0.25 ± 0.09	0.12 ± 0.06	0.25 ± 0.11	0.18 ± 0.07
	OH-SeMet3	0.25 ± 0.09	0.27 ± 0.09	0.46 ± 0.15	0.31 ± 0.11	0.14 ± 0.08	0.14 ± 0.06	0.11 ± 0.06
	OH-SeMet6	0.31 ± 0.11	0.69 ± 0.19	0.43 ± 0.12	0.36 ± 0.11	0.29 ± 0.10	0.31 ± 0.12	0.08 ± 0.06
	Mean	0.36 ± 0.06^{a}	0.41 ± 0.08^{a}	0.43 ± 0.08^{a}	0.30 ± 0.06^{ab}	0.18 ± 0.05^{ab}	0.23 ± 0.06^{ab}	0.12 ± 0.03^{b}
PCD	SS3	2.83 ± 1.09	3.46 ± 1.45	3.31 ± 1.20	1.45 ± 0.35	1.64 ± 0.50	0.80 ± 0.23	1.36 ± 0.28
	OH-SeMet3	2.62 ± 0.95	3.00 ± 0.95	2.50 ± 0.89	3.54 ± 1.16	1.77 ± 0.49	2.00 ± 0.62	1.86 ± 0.53
	OH-SeMet6	3.33 ± 1.48	1.42 ± 0.43	1.42 ± 0.42	2.35 ± 0.95	1.54 ± 0.54	1.81 ± 0.61	1.65 ± 0.45
	Mean	2.93 ± 0.67	2.64 ± 0.59	2.43 ± 0.53	2.47 ± 0.53	1.64 ± 0.29	1.57 ± 0.32	1.63 ± 0.25

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. ACRO, acrosome; MP, middle piece; PCD, proximal cytoplasmic droplet. $^{a-d}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

Table 10 – Sperm morphology abnormalities liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1] *(Conclusion)*

Defects (%)	Dietary Treatment	Week						
		2	4	6	8	10	12	14
DCD	SS3	1.71 ± 0.42	2.37 ± 0.78	2.93 ± 0.72	1.85 ± 0.45	3.68 ± 0.73	1.96 ± 0.59	1.58 ± 0.31
	OH-SeMet3	1.62 ± 0.44	3.23 ± 0.85	1.57 ± 0.37	1.93 ± 0.37	2.21 ± 0.48	1.57 ± 0.29	1.18 ± 0.29
	OH-SeMet6	2.59 ± 0.75	2.75 ± 0.64	1.81 ± 0.55	1.68 ± 0.54	2.17 ± 0.63	1.42 ± 0.47	1.55 ± 0.32
	Mean	1.96 ± 0.32^{ab}	2.80 ± 0.43^{a}	2.11 ± 0.33^{ab}	1.83 ± 0.25^{ab}	2.71 ± 0.37^{a}	1.64 ± 0.26^{ab}	1.42 ± 0.18^{b}
TAIL	SS3	3.04 ± 0.64	3.19 ± 0.66	6.69 ± 0.86	5.93 ± 0.90	3.73 ± 0.71	6.08 ± 0.64	4.50 ± 0.86
	OH-SeMet3	3.90 ± 0.78	4.12 ± 0.94	5.78 ± 0.61	6.23 ± 0.68	5.58 ± 0.64	5.41 ± 0.56	6.15 ± 0.60
	OH-SeMet6	1.83 ± 0.54	3.27 ± 0.77	5.12 ± 0.54	4.71 ± 0.58	4.12 ± 0.64	5.06 ± 0.80	4.32 ± 0.86
	Mean	2.97 ± 0.40^{b}	3.51 ± 0.45^{b}	5.76 ± 0.38^{a}	5.59 ± 0.41^{a}	4.53 ± 0.39^{ab}	5.56 ± 0.38^{a}	5.09 ± 0.45^{a}
TF	SS3	0.14 ± 0.06	0.07 ± 0.07	0.15 ± 0.07	0.14 ± 0.06	0.29 ± 0.07	0.46 ± 0.14	0.21 ± 0.07
	OH-SeMet3	0.04 ± 0.04	0.18 ± 0.07	0.07 ± 0.05	0.14 ± 0.06	0.11 ± 0.06	0.21 ± 0.11	0.07 ± 0.05
	OH-SeMet6	0.08 ± 0.08	0.00 ± 0.00	0.14 ± 0.10	0.07 ± 0.05	0.21 ± 0.09	0.11 ± 0.06	0.19 ± 0.07
	Mean	0.09 ± 0.03	0.08 ± 0.03	0.12 ± 0.04	0.12 ± 0.03	0.20 ± 0.04	0.26 ± 0.07	0.16 ± 0.04
Normal Sperm (%)	SS3	68.96 ± 4.83	67.14 ± 4.55	65.96 ± 3.32	62.61 ± 4.79	57.43 ± 3.89	58.36 ± 4.59	63.29 ± 4.77
	OH-SeMet3	71.81 ± 3.57	66.35 ± 2.83	64.62 ± 2.11	68.85 ± 4.06	62.50 ± 3.24	68.00 ± 2.63	73.27 ± 3.14
	OH-SeMet6	66.92 ± 5.38	72.00 ± 4.00	64.21 ± 2.46	66.12 ± 4.85	59.50 ± 5.40	62.77 ± 5.39	65.35 ± 4.72
	Mean	69.28 ± 2.62^{a}	68.37 ± 2.23^{ab}	64.97 ± 1.54^{abc}	65.77 ± 2.62^{ab}	59.75 ± 2.42^{c}	62.92 ± 2.54^{bc}	67.20 ± 2.52^{ab}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. DCD, distal cytoplasmic droplet; TF, teratological forms. $^{a-c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.2.3 Integrity of plasma and acrosomal membranes

The integrity of plasma and acrosome membranes evaluated in liquid-stored semen AI-doses were not affected by any source or organic supplementation level tested in this study ($p > 0.05$) (Table 11). However, this variable increased over the weeks of study, presenting its highest values in the last two assessments, which represented more than double the first three ($p < 0.05$) (Table 12).

Table 11 – Percentages of sperm with intact plasma membrane and intact acrosome in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

	Dietary Treatment			SEM	*p-value*			
	SS3 (n = 14)	OH-SeMet3 (n = 14)	OH-SeMet6 (n = 14)		C1	C2	W	T*W
IPIA	45.56	43.21	41.27	1.31	0.657	0.598	<.001	0.475

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; IPIA, intact plasma membrane and intact acrosome.

Table 12 – Percentages of sperm with intact plasma membrane and intact acrosome in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1]

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
IPIA	SS3	35.39 ± 6.24	17.75 ± 2.22	26.23 ± 4.50	47.36 ± 4.41	52.57 ± 3.46	63.62 ± 2.68	67.96 ± 2.17
	OH-SeMet3	31.11 ± 4.88	24.23 ± 4.55	22.29 ± 4.12	53.82 ± 3.65	44.46 ± 3.47	61.57 ± 2.06	65.19 ± 3.12
	OH-SeMet6	26.07 ± 4.12	23.00 ± 3.32	16.25 ± 3.02	50.75 ± 2.56	48.73 ± 3.13	62.87 ± 4.11	67.00 ± 3.56
	Mean	30.86 ±19.20^{c}	21.96 ± 12.58^{c}	21.48 ± 14.66^{c}	50.64 ± 13.49^{b}	48.58 ± 12.47^{b}	62.65 ± 10.46^{a}	66.74 ±10.40^{a}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. IPIA, intact plasma membrane and intact acrosome. $^{a-c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.2.4 Sperm resistance to oxidative stress

Sperm resistance to oxidative stress evaluated in liquid-stored semen AI-doses were not affected by any source or organic supplementation level tested in this study ($p > 0.05$) (Table 13). Regarding its behavior over the weeks of study regardless of the treatment studied, there is no established pattern of increase or decrease in values as the experiment progressed (Table 14).

Table 13 – Sperm resistance to oxidative stress in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

	Dietary Treatment			SEM	*p-value*			
	SS3 (*n* = 14)	OH-SeMet3 (*n* = 14)	OH-SeMet6 (*n* = 14)		C1	C2	W	T*W
TBARS (ng/10^6 sperm)	1767.59	1911.74	1724.74	28.89	0.353	0.133	<.001	0.836

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; TBARS, thiobarbituric acid reactive substances.

Table 14 – Sperm resistance to oxidative stress in liquid-stored semen AI-doses (at 72 h) from boars fed diet supplemented with inorganic or organic sources of selenium evaluated every 2 weeks[1]

	Dietary Treatment	Week						
		2	4	6	8	10	12	14
TBARS ($ng/10^6$ sperm)	SS3	1480.78 ± 94.94	1706.70 ± 63.47	1734.23 ± 69.11	1959.58 ± 105.43	1858.82 ± 151.83	2055.27 ± 170.12	1598.38 ± 99.22
	OH-SeMet3	1656.42 ± 97.74	1772.18 ± 88.12	1736.42 ± 92.83	2116.48 ± 77.38	1944.75 ± 141.68	2489.99 ± 234.54	1624.95 ± 94.95
	OH-SeMet6	1554.28 ± 103.12	1659.75 ± 76.37	1679.27 ± 97.68	1804.26 ± 79.16	1591.49 ± 119.73	2095.06 ± 159.60	1654.46 ± 146.73
	Mean	1565.85 ± 56.80^{c}	1712.88 ± 43.76^{c}	1716.64 ± 49.41^{bc}	1960.15 ± 52.82^{ab}	1803.40 ± 81.87^{bc}	2213.44 ± 111.82^{a}	1621.91 ± 61.83^{c}

Source: Pavaneli (2022).

[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. TBARS, thiobarbituric acid reactive substances. $^{a-c}$ Different superscript letters indicate a significant difference among the experimental weeks ($p < 0.05$; Tukey-Kramer test).

6.3 FEED, BLOOD AND SEMINAL PLASMA SAMPLES

6.3.1 Selenium concentration in feed samples

The selenium concentration found in feed samples collected before (a selenium-supplemented diet) and during the study (a non-supplemented basal diet) was 0.226 mg/kg and 0.133 mg/kg, respectively. The last value refers to a pool of weekly samples. The concentrations obtained week by week are presented in Appendix E.

6.3.2 Selenium concentration in biological fluids

The results for selenium concentration in biological samples showed that boars fed a diet supplemented with the organic source presented higher concentration of the mineral in their seminal plasma when compared to those fed the inorganic form ($p < 0.05$), while no difference was observed in the blood plasma. There was no effect of the organic supplementation levels on these evaluations ($p > 0.05$) (Table 15). On the other hand, it was observed an expressive week effect, demonstrated by a continuous increase in selenium concentration in both seminal plasma and blood plasma throughout the experiment ($p < 0.05$) (Table 16).

Table 15 – Selenium concentration in blood and seminal plasma from boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

Se (ng/mL)	Dietary Treatment			SEM	*p-value*			
	SS3 (n = 9)	OH-SeMet3 (n = 7)	OH-SeMet6 (n = 8)		C1	C2	W	T*W
Blood Plasma	162.00	155.90	167.74	2.71	0.779	0.381	<.001	0.216
Seminal Plasma	44.70	52.38	52.04	1.09	0.026	0.844	<.001	0.442

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; Se, selenium.

Table 16 – Selenium concentration in blood and seminal plasma from boars fed diet supplemented with inorganic or organic sources of selenium evaluated both in pre-experimental and experimental phases[1]

Se (ng/mL)	Dietary Treatment	Week		
		0	8	14
Seminal plasma	SS3	39.56 ± 1.65	45.00 ± 1.95	49.56 ± 2.72
	OH-SeMet3	47.14 ± 1.72	50.57 ± 1.84	59.43 ± 3.00
	OH-SeMet6	44.63 ± 2.07	50.62 ± 2.95	62.14 ± 3.20
	Mean	43.46 ± 1.21^{c}	48.50 ± 1.40^{b}	56.39 ± 2.02^{a}
Blood plasma	SS3	146.56 ± 7.50	161.44 ± 5.63	180.00 ± 7.24
	OH-SeMet3	141.14 ± 6.07	157.86 ± 2.99	168.71 ± 4.37
	OH-SeMet6	143.00 ± 4.51	169.12 ± 3.48	194.43 ± 7.99
	Mean	143.79 ± 3.52^{c}	162.96 ± 2.64^{b}	181.00 ± 4.35^{a}

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. Se, selenium. $^{a\text{-}c}$ Different superscript letters indicate a significant difference among the weeks ($p < 0.05$; Tukey-Kramer test).

6.3.3 Bromatological and macrominerals analyses

Feed samples collected before and during the study were submitted to a bromatological evaluation and macrominerals quantification. Results are shown in the Appendix F.

6.4 *IN VIVO* FERTILITY ASSAY

Of the 1131 females that were inseminated in the experiment, 1095 were pregnant and farrowed. In the litter size analysis, fifteen sows from SS3 were removed, six of them by abortion (1.99%) and nine by a regular return to estrus (2.99%); twelve sows from OH-SeMet3 also were removed, nine of them by abortion (2.11%) and three sows by a regular return to estrus (0.70%), and nine sows from OH-SeMet6 were removed, two of them by abortion (0.50%) and seven sows by a regular return to estrus (1.74%). The percentages of abortion and regular return to estrus were within limits considered acceptable for swine farms.

As a result of the *in vivo* fertility assay, it was demonstrated better reproductive performance for boars fed organic selenium when compared to those fed the inorganic one, taking into account the pregnancy rate and stillborn piglets ($p < 0.05$; Table 17). Our results also indicate a trend towards a significant interaction between week and treatment factors, which is shown in the Table 18.

Table 17 – Reproductive performance of boars fed diet supplemented with inorganic or organic sources of selenium for 95 days[1]

	Dietary Treatment			SEM	*p-value*			
	SS3	OH-SeMet3	OH-SeMet6		C1	C2	W	T*W
Fertility	(*n* = 301)	(*n* = 427)	(*n* = 403)					
Pregnancy rate (%)	97.00	99.30	98.26	0.38	0.029	0.198	0.640	0.064
Litter Size[2]	(*n* = 286)	(*n* = 415)	(*n* = 394)					
NBA	14.40	14.65	14.47	0.10	0.159	0.424	0.852	0.716
TNB	16.04	15.82	15.95	0.11	0.545	0.757	0.580	0.971
Stillborn (%)	7.11	5.87	6.92	0.25	0.008	0.146	0.391	0.124
Mummies (%)	2.25	1.90	2.27	0.12	0.328	0.665	0.886	0.728

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; W, week; T*W, interaction between treatment and week; NBA, number of piglets born alive; TNB, total number of pigs born.
[2]Non-pregnant females, those who regular return to estrus or miscarried were removed for analysis of litter size.

Table 18 – Weekly pregnancy rates obtained from the use of semen AI-doses of boars fed diet supplemented with inorganic or organic sources of selenium[1]

PR (%)	Dietary Treatment			*p-value*	
	SS3	OH-SeMet3	OH-SeMet6	C1	C2
W1	95.00 ± 5.00	100	100	0.121	1.000
W2	100	97.30 ± 2.70	95.35 ±3.25	0.573	0.615
W3	89.47 ±7.23	100	100	0.014	1.000
W4	94.74 ± 5.26	100	100	0.076	1.000
W5	88.89 ±7.62	100	100	0.048	1.000
W6	100	92.31 ±7.69	100	0.067	0.050
W7	100	100	96.97 ± 3.03	1.00	0.370
W8	92.86 ±4.02	100	100	0.084	1.000
W9	100	100	96.67 ±3.33	1.000	0.139
W10	100	100	100	1.000	1.000
W11	100	97.22 ±2.78	92.86 ± 4.96	0.604	0.360
W12	100	100	100	1.000	1.000
W13	100	100	100	1.000	1.000
W14	100	100	96.67 ± 3.33	1.000	0.394

Source: Pavaneli (2022).
[1]Values represent means and standard error of mean. Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. C1, contrast of source = inorganic *vs.* organic; C2, contrast of organic supplementation level = 0.3 *vs.* 0.6 mg selenium/kg; PR, pregnancy rate; W, week.

7 DISCUSSION

Selenium is an important trace element for mammalian reproduction and it is not a recent discovery. In males, the mineral is considered essential for the proper sperm development and function, acting as a modulator of sperm production, semen quality, and fertility (AHSAN *et al.*, 2014; QAZI *et al.*, 2019). Given the intense use of boars to produce commercial doses in semen processing centers, it has been supposed that these animals are constantly demanding greater selenium levels for ensuring their good reproductive performance in the system (MARIN-GUZMAN *et al.*, 2000b). In this way, some authors suggest that selenium supplementation of diet from organic forms could be a better choice due to their ability to build selenium reserves in the body (SURAI; FISININ, 2015). Among the organic selenium sources currently available in the market, OH-SeMet has been pointed out as the more bioavailable to the animals, improving the productivity of pigs and poultry (BRIENS *et al.*, 2013, 2014; JLALI *et al.*, 2013, 2014; COULOIGNER *et al.*, 2015; CHAO *et al.*, 2019; LI *et al.*, 2020; MOU *et al.*, 2020). To our knowledge, this is the first study evaluating the effects of OH-SeMet as dietary supplementation on boar reproduction. As expected, our results demonstrated that the use of this organic form improves the selenium availability for the male reproductive system and tends toward increasing sperm production while maintaining good semen quality before and after storage in the liquid state (17 ºC) for 72 h. Moreover, boars fed OH-SeMet had a better reproductive performance when used in AI programs, even in highly efficient farms.

It is widely known that organic selenium forms (SeMet) have greater bioavailability than inorganic ones, which can be explained by their facilitated absorption via intestinal methionine transporters and the unique ability to build selenium reserves in the body (BURK; HILL, 2015). Although the current study did not show any difference for the dietary treatments regarding selenium concentration in blood plasma, it was greater in the seminal plasma of boars fed OH-SeMet when compared to those fed sodium selenite, different from that previously observed when it was tested the Se-yeast form, and no difference was found (LÓPEZ *et al.*, 2010; LOVERCAMP *et al.*, 2013; MARTINS *et al.*, 2014). This result clearly shows that the same selenium level in the blood plasma does not always indicate the same situation in seminal plasma when OH-SeMet is used and proves that boars of high usage into AI-centers are better supplied with selenium to meet their reproductive functions when fed this organic source.

Marin-Guzman *et al.* (1997) reported an increase of GPx activity in boar tissues such as serum, liver, and testes as a response to selenium supply. Once the present study demonstrated a higher selenium concentration in the seminal plasma of boars fed OH-SeMet, it seems natural

also expect a great GPx activity in the seminal fluid of these animals. Nonetheless, our results could not show this direct relation between the measurements, similar to observed by other authors in boar semen when tested the Se-yeast form (PETRUJKIĆ *et al.*, 2014). These findings are in agreement with previous literature that showed these two factors do not appear to be directly correlated in boar semen, suggesting both are regulated by independent mechanisms (LASOTA *et al.*, 2004). Furthermore, it is important to note that in the study conducted by Marin-Guzman *et al.* (1997), boars fed a selenium-supplemented diet (sodium selenite at 0.5 mg/kg) were compared to animals that received no supplement in their diet. In this work and that presented by Petrujkić *et al.* (2014), all the animals had their nutritional selenium requirement met (0.3 mg/kg), receiving a supplemented diet either from the organic or inorganic source.

In addition, not increasing GPx activity in seminal plasma is not necessarily a bad thing. Our result suggests there was not an oxidative stress situation, which would justify the increase of GPx activity to overcome it and thus, boars fed OH-SeMet could do better. In fact, our study reinforces this assumption when evaluated the results obtained for sperm resistance to oxidative stress (TBARS assay), and no difference was observed between the experimental groups for lipid peroxidation.

Although in our study it was not possible to assess the levels of selenium directly in the testis and so to affirm that a greater input of the mineral occurred in this organ due to some dietary treatment, our results show that the use of OH-SeMet in the diet of boars tends toward increasing their sperm production, and suggest that more selenium was available to sustain the process of spermatogenesis (MARIN-GUZMAN *et al.*, 2000b). In our study, boars fed OH-SeMet at 0.3 mg/kg produced + 3.26 semen doses for intracervical AI (3×10^9 sperm) or even + 6.52 units for post-cervical AI (1.5×10^9 sperm) per ejaculate, when compared to males fed sodium selenite at the same level. It is undoubtedly a critical gain for the dissemination of superior genes and possibly for the profitability of AI-centers. Our results are in according to López *et al.* (2010) and Martins *et al.* (2014, 2018) that published similar findings to the boar sperm concentration and total sperm count, respectively, when tested the Se-yeast form. On the other hand, others studies did not demonstrate any difference in semen quantity parameters (SPEIGHT *et al.*, 2012; LOVERCAMP *et al.*, 2013; PETRUJKIĆ *et al.*, 2014).

Considering the role of selenium on sperm maturation in the epididymis (MARIN-GUZMAN *et al.*, 1997; 2000a), and on the survival of these cells after ejaculation as protective selenoproteins in seminal plasma (KOZIOROWSKA-GILUN *et al.*, 2011; MICHAELIS *et al.*, 2014), the quality of raw and stored semen in a liquid state (17 °C) at 72 h was also evaluated

in the current study. In this way, our results demonstrated that the different selenium sources or levels of organic selenium used in the diet of boars did not affect semen quality under these two conditions, similar to observed by other authors evaluating Se-yeast, who did not find consistent differences when no stressful challenge was included in the study (LOVERCAMP *et al.*, 2013; MARTINS *et al.*, 2014, 2015). The expected beneficial effects from organic selenium were only seen when it was performed successive semen collection of the animals, establish long-term liquid storage for the sperm, or some *in vitro* tests as sperm thermal resistance and hypoosmotic swelling (SPEIGHT *et al.*, 2012; PETRUJKIĆ *et al.*, 2014). So, we believe that under our good conditions, without significant causes of stress for boars and sperm, regardless of the source offered, as long as it meets the animal's minimum levels, the semen quality is met. From another perspective, however, at the same time our results did not find differences for semen quality, the current study allows to suggest that sperm production increased by the inclusion of OH-SeMet in the diet of boars was represented by normal morphologically cells and good quality semen by the tests performed in raw and stored samples.

In our study, some points observed in sperm morphology analysis should be discussed. Firstly, the high prevalence of tail defects, predominantly marked by folded tails, is registered in raw semen samples. There were mean values exceeding 20% in some weeks of evaluation. Likewise, a high percentage of sperm presenting swollen acrosome was detected in samples of liquid-stored semen AI-doses (72 h at 17 ºC), more than 18% in some evaluation points, reaching 23% in one of them. These observations were similar for all the treatments, and consequentely dropped the percentage of normal sperm in both moments of semen evaluation. In raw semen, it is very likely that some failure may have occurred in the preparation of the samples for analysis, once sperm with folded tails were observed in lower amounts, and considered normal, for evaluation after 72 h of storage. Regarding stored semen, swollen acrosomes seem to be the result of some of the post-processing steps of the AI-doses, once such alterations were little or were not observed in the morphological analysis of raw semen. Herein, one of the possible causes could be the effect of vibration emissions during the long transport of semen AI-doses via highways to the laboratory where they were evaluated, a path of at least 500 km. Some authors have demonstrated that the transport of semen AI-doses can lead to damages to sperm structures, as acrosome (SCHULZE *et al.*, 2018). The researchers report that both speed and road surface significantly influence the produced vibrations (HAFEMEISTER *et al.*, 2022).

In addition, most of the variables evaluated in this study showed a time effect, regardless of the selenium source or organic selenium level used in the diet of boars. Some of these

variables maintained a pattern over the evaluation points. First, selenium bioavailability in both blood and seminal plasma was increased significantly with each evaluation, proving that selenium supplementation affects these measurements and it is a time-dependent factor. In addition, our results showed that total and progressive sperm motility, as well as sperm membrane integrity, were significantly improved over the weeks in liquid-stored semen AI-doses (at 72 h). Herein, it is interesting to comment that the percentage of sperm with intact plasma and acrosome membranes was more than double in the last two weeks of evaluation compared to the first ones. For us, it is clear that regardless of the dietary treatment offered to the boars, their selenium demand was met and the semen quality was not only maintained, but also improved as a result of selenium supplementation when considered these sperm analyses.

It is important to mentioned that our study was carried out during the autumn-winter in Brazil, when the seasonality do not seem to be a negative factor on boar semen quality as it occurs in spring-summer season (ZASIADCZYK *et al.*, 2015; PEÑA *et al.*, 2019). Added to that, we believe that the excellent conditions offered by the AI-centers regarding ambient temperature, health and nutrition, did not characterized challenges for the demand for selenium and selenoproteins to be increased, and the beneficial effect of OH-SeMet was maximized. These reflections are supported by the recent description of the vitagene concept in farm animals, a family of genes responsible for their adaptation to stress conditions (SURAI *et al.*, 2019; SURAI; KOCHISH; FISININ, 2021). The authors describe that these specific genes are activated under the most varied causes of stress into the productive system (technological, environmental, nutritional and internal), promoting an increase in the synthesis of protective molecules, such as the case of some selenoproteins.

The excellent results observed for *in vivo* fertility in our study leave no room for doubt about the real benefits of organic selenium for boar reproduction. Semen AI-doses from boars fed OH-SeMet resulted in a greater pregnancy rate in pig farms already was very efficient, even with the use of the inorganic source sodium selenite (99.3 vs. 97%). It is known that improving something that is already very good is not an easy task. In this way, an increase of 2.3% in the pregnancy rate as found by the present study, makes us believe that OH-SeMet contributes in any way to better maintenance of sperm fertilizing ability after semen collection, processing, transport, and storage. In good Brazilian farms with 1000 females and using the average NBA from our study, these gains correspond to approximately 52 more farrows in the year and more than 1200 weaned piglets in the same period (AGRINESS, 2021). Herein, a doubt can naturally appear about how a better reproductive performance observed for boars fed OH-SeMet can be explained if their semen quality was similar to those fed the inorganic source. For us, three

points must be raised and need further investigation.

The first, and very clear for andrologists, is that the tests carried out in this study are far from thoroughly evaluating the sperm function and fertilizing ability. Although routine evaluations are essential to predict not so good semen quality, these tests can be many times insufficient to predict the fertility results in the field (OLIVEIRA *et al.*, 2012; ANDRADE *et al.*, 2017; ANDRADE, 2021), and here is an important reason for the increasingly demand for fertility assays, or others approaching them, made by the major publishing journals in animal reproduction. Indeed, sperm can suffer functional damages during the first 72 h of storage in the liquid state, which cannot be assessed by conventional tests, being necessary other ones to evaluate their response to essential events for trigger fertilization (HENNING *et al.*, 2012), as *in vitro* capacitation and acrosome exocytosis (YESTE *et al.*, 2015; PAVANELI *et al.*, 2019, 2020), or even *in vitro* sperm-oviduct binding assays (HENNING *et al.*, 2019).

Another thing is, which form of selenoprotein can be increased in the seminal plasma of boars fed OH-SeMet, once selenium concentration was greater in these animals? Maybe, a deeper and more complete investigation of this fluid could help us to better understand the link between organic selenium in the diet and better results on the farms. At this point, selenoprotein P, widely known for being the main selenium transporter into the body, has been pointed out also as a potential and stable biomarker of semen quality once present in the men's seminal plasma (MICHAELIS *et al.*, 2014). As our study did not measure this specific selenoprotein in boar seminal plasma, it is not possible to affirm that it was important here. However, recent studies have been demonstrated that among other factors, the chemical forms of supplemental selenium (inorganic or the different organic ones) affect the expression of selenogenome, regulating transcripts of multiple selenoproteins (SURAI; KOCHISH; FISININ, 2021). In broiler chickens, authors found that Selenoprotein P was one of the selenoproteins that had their activity and gene expression upregulated by offering OH-SeMet in the diet, which leads us to think that it can also have happened in our study (ZHAO *et al.*, 2017; SUN *et al.*, 2021).

Lastly, it is important to comment about epigenetics. It is evident that sperm transmit not only the paternal haploid genome to the oocyte, but also DNA methylation profile, DNA-associated proteins, nucleo-protamine distribution pattern, and non-coding RNA established during spermatogenesis. Herein, several studies have been demonstrated that this “information pack” can impact on sperm fertility ability, embryo and placental development, and also on health of the offspring (CARRELL; HAMMOUD, 2010; WANG *et al.*, 2013; CHAMPROUX *et al.*, 2018; GALAN; KRYKBAEVA; RANDO, 2020), while it is constantly changed by environmental exposures and male lifestyle, such as the diet consumed

(SCHAGDARSURENGIN; STEGER, 2016). Although the effects of offering different selenium sources in the diet of boars on their sperm epigenetics landscape is unknown, considering the biological roles of selenium via selenoproteins on epigenetic regulation (SPECKMANN; GRUNE, 2015), it is possible to suggest that boars fed OH-SeMet were more efficient to transfer relevant factors to the sperm survive and function in the female reproductive system, as well as to the quality of the placenta, and then, to the nutritional support to fetus during pregnancy. In fact, females inseminated with semen doses from boars fed OH-SeMet had a reduction of stillborn piglets in their litter when compared to values obtained in the sodium selenite group. However, caution must be exercised when interpreting this result, since many factors influence this variable (parity order, farrowing duration, farrowing assistance), not all of which could be controlled in this study.

Finally, the present study aimed to identify at which supplementation level OH-SeMet could be more efficient from a reproductive perspective. It is evident that with the rapid genetic advance of boars in AI-centers, it is necessary to have a greater monitoring of the nutrition of these animals, seeking to understand what their real needs are. Under our conditions, this study showed that offering OH-SeMet at 0.6 mg selenium/kg did not impact sperm production, semen quality and reproductive performance of the animals, providing a response very similar to that obtained with the level of 0.3 mg/kg. However, further studies are needed on the use of OH-SeMet in boars under different AI-centers' realities, as well as evaluate its effects in commercial hybrid boars.

8 CONCLUSION

Our results indicate that two scenarios in pig breeding may benefit when OH-SeMet instead of sodium selenite is used as dietary supplementation for boars. Firstly, tends toward increasing boar sperm production and, consequently, the number of semen AI-doses produced per ejaculate. It means more efficient dissemination of superior genes by semen processing centers, which may still be economically interesting for them. On the other hand, increasing the pregnancy rate in AI programs reflects a better use of the sows within the system, positively impacting non-productive days on pig farms. Regarding OH-SeMet recommendations, under the experimental conditions of the present study, its use at 0.6 mg/kg does not imply greater gains that those obtained with 0.3 mg/kg. However, the use of OH-SeMet within the studied range could be considered safe for practical situations, which it is not always possible to have the precision and accuracy of how much selenium each boar is ingesting.

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APPENDIX A – DISTRIBUTION OF THE ANIMALS INTO THE GROUPS

Dietary Treatment											
SS3				OH-SeMet3				OH-SeMet6			
Animal	Months	Score	Breed	Animal	Months	Score	Breed	Animal	Months	Score	Breed
BR4722	15	67.2	DB30	BR4736	10	67.0	DB30	AM5808	31	67.3	DB20
BR4280	11	92.3	DB20	BR8673	9	111.0	DB20	BR3618	15	106.3	DB30
BR9015	8	114.2	DB30	BR4557	19	142.6	DB30	BR4710	14	100.5	DB30
BR2268	24	120.1	DB30	BR4285	11	147.2	DB20	BR8756	9	132.2	DB20
AM9850	24	136.7	DB20	BR4456	19	156.7	DB30	BR3663	16	133.8	DB30
BR4319	9	141.8	DB30	BR4958	17	167.2	DB30	BR9013	8	144.9	DB30
BR4909	12	151.2	DB30	BR4315	10	170.0	DB20	BR3630	17	160.9	DB20
BR4323	9	167.7	DB30	BR3664	17	180.9	DB20	BR10516	9	161.3	DB30
AM7713	23	169.8	DB30	BR4290	11	215.4	DB20	BR4747	9	171.5	DB20
BR4284	11	181.7	DB30	BR2958	20	234.2	DB30	BR8845	9	203.1	DB30
BR8896	9	224.7	DB20	BR10470	10	279.5	DB30	BR4720	14	242.7	DB30
BR4953	18	302.3	DB20	BR3669	16	286.0	DB30	BR3670	16	281.2	DB20
BR4955	18	332.7	DB30	BR2380	28	305.8	DB20	BR9973	8	305.4	DB20
BR8835	9	343.7	DB20	BR4298	13	135.2	DB30	BR4703	13	374.9	DB30
Mean	14.3	181.8		Mean	15.0	185.6		Mean	13.4	184.7	
SD	6.0	87.6		SD	5.4	69.9		SD	6.0	87.3	
NR/G	DB20	5		NR/G	DB20	6		NR/G	DB20	6	
	DB30	9			DB30	8			DB30	8	

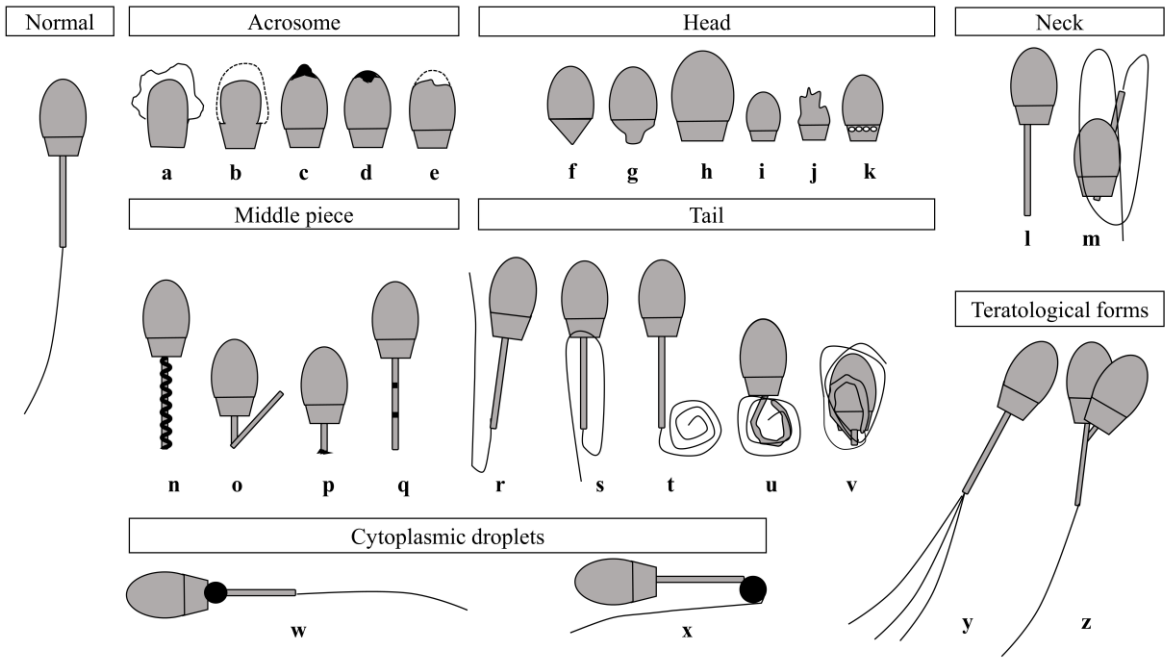
Source: Pavaneli (2022).
Legend: Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine. DB20, Large-White; DB30, Landrace. SD, standard deviation; NR/G, number of breed repetitions per group.

APPENDIX B – BASAL DIET COMPOSITION (AS-FED BASIS)

Item	Amount
Ingredient, %	
Corn	51.497
Soybean meal, 46% CP	26.450
Soybean hulls	10.000
Sugarcane yeast	1.250
Sugar	3.750
Soybean oil	2.450
Dicalcium phosphate, 19.5% P	2.005
Sodium bicarbonate	0.130
Limestone	0.390
Multi-enzymatic complex	0.005
Salt	0.500
Citric acid	0.150
Chromium methionine	0.020
Zinc chelate of glycine hydrate	0.012
Inert	0.300
Vitamin E, 50%	0.030
Vitamin C, 35%	0.100
L-Carnitine	0.002
Vitamin-mineral premix	0.500
L-Valine, 98%	0.080
L-Lysine HCl, 78.8%	0.200
L-Threonine, 99%	0.077
L-Tryptophan, 99%	0.022
DL-Methionine, 99%	0.080
Analyzed composition	
Dry matter, %	91.990
Mineral matter, %	5.620
Crude protein, %	20.490
Crude fiber, %	4.950
Ether extract, %	5.990
Non-nitrogenous extract, %	62.940
P, %	0.600
Ca, %	0.630
Selenium, mg/kg	0.133

Source: DB Company (2019).

APPENDIX C – SPERM MORPHOLOGICAL ABNORMALITIES



Source: Pavaneli (2022); CBRA (2013).
Legend: **a**, swollen; **b**, detached; **c-d**, knobbed; **e**, incomplete; **f**, narrow at the base; **g**, pyriform; **h**, normal giant; **i**, normal small; **j**, abnormal small; **k**, pouch formation; **l**, abaxial tail implantation; **m**, retroabaxial tail implantation; **n**, corkscrew-shaped; **o**, folded; **p**, broken; **q**, segmental aplasia; **r**, folded; **s**, tightly folded; **t**, coiled; **u**, tightly coiled; **v**, coiled around head; **w**, proximal cytoplasmic droplet; **x**, distal cytoplasmic droplet with folded tail; **y**, multiple tails; **z**, double head.

APPENDIX D – DISTRIBUTION OF FEMALES (N) BY PARITY WITHIN THE DIETARY TREATMENTS PROVIDED FOR BOARS

Parity	Pregnancy Rate			Litter Size		
	SS3 (*n* = 301)	OH-SeMet3 (*n* = 427)	OH-SeMet6 (*n* = 403)	SS3 (*n* = 286)	OH-SeMet3 (*n* = 415)	OH-SeMet6 (*n* = 394)
0	107	147	139	102	144	135
1	58	87	84	55	84	82
2	45	69	72	43	69	71
3	41	60	50	39	55	49
4	26	41	40	25	40	39
5	24	23	18	22	23	18

Source: Pavaneli (2022).
Dietary Treatments: SS3, 0.3 mg selenium/kg as sodium selenite; OH-SeMet3, 0.3 mg selenium/kg as hydroxy-selenomethionine; OH-SeMet6, 0.6 mg selenium/kg as hydroxy-selenomethionine.

APPENDIX E – SELENIUM CONCENTRATION IN DIFFERENT FEED SAMPLES

Feed Sample	Selenium (mg/kg)
Pre-experimental phase[1]	0.226
Experimental phase[2]	
W1	0.353
W2	0.061
W3	0.048
W4	0.068
W5	0.104
W6	0.079
W7	0.129
W8	0.079
W9	0.148
W10	0.177
W11	No sample
W12	0.101
W13	0.251
W14	0.042
Pool[3]	0.133

Source: Pavaneli (2022).
[1]Complete diet (selenium-supplemented).
[2]Basal diet (no selenium supplementation).
[3]Mixture of samples from the 14 weeks of study.
Legend: W, week.

APPENDIX F – ANALYSES OF FEED SAMPLES COLLECTED BEFORE AND DURING THE EXPERIMENT

	% In the diet							
Feed Sample	DM	MM	CP	CF	EE	NFE	P	Ca
Pre-experimental phase[1]	91.20	4.67	21.21	5.35	5.5	63.25	0.49	0.43
Experimental phase[2]	91.99	5.62	20.49	4.95	5.99	62.94	0.60	0.63

Source: Pavaneli (2022).
[1]Complete diet (selenium-supplemented), just one sample.
[2]Basal diet (no selenium supplementation), pool of samples from the 14 weeks of study.
Legend: Ca, calcium; CF, crude fiber; CP, crude protein; DM, dry matter; EE, ether extract; MM, mineral matter; NFE, nitrogen-free extract; P, phosphorus.

APPENDIX G – PUBLISHED SCIENTIFIC PAPER

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Reproduction

REPRODUCTION

Hydroxy-selenomethionine as an organic source of selenium in the diet improves boar reproductive performance in artificial insemination programs

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Abstract

This study aimed to compare different selenium (Se) sources in the diet on boar's semen quality and fertility. For this, 28 boars aged 8 to 28 mo were fed with the following dietary treatments for 95 d: 0.3 mg Se/kg as sodium selenite (**SS**; $n = 14$) and 0.3 mg Se/kg as hydroxy-selenomethionine (**OH-SeMet**; $n = 14$). During this period, two experiments were carried out. In experiment 1, the semen of all boars was evaluated every 2 wk. Raw semen was initially evaluated for the processing of seminal doses, which were stored at 17 °C for 72 h, followed by sperm quality assessments. Furthermore, Se concentration and glutathione peroxidase (**GPx**) activity were measured in the seminal plasma. In experiment 2, 728 females were inseminated weekly with seminal doses from boars of the different experimental groups to further assess in vivo fertility and litter characteristics. Results demonstrated that boars fed OH-SeMet had more Se in their seminal plasma ($P < 0.05$), showing the greater bioavailability of the organic source in the male reproductive system. Moreover, boars fed OH-SeMet tended ($P < 0.10$) toward a higher total sperm count in the ejaculate (66.60 vs. 56.57 × 10^9 sperm) and the number of seminal doses (22.11 vs. 18.86; 3 × 10^9 sperm/dose) when compared with those fed SS. No effect of the dietary treatments was observed on GPx activity in seminal plasma ($P > 0.05$) as well as on raw and stored semen quality ($P > 0.05$). Under in vivo conditions, seminal doses from boars fed OH-SeMet tended ($P < 0.10$) toward a higher pregnancy rate at weeks 3, 5, and 8, and also resulted in a higher ($P < 0.05$) percentage of pregnant females in the overall period (99.30 vs. 97.00). In conclusion, the replacement of SS with OH-SeMet in boars' diet can improve sperm production and results in better reproductive performance for them, bringing greater productivity and profitability to artificial insemination centers and commercial pig farms.

Key words: bioavailability, boar spermatozoa, dietary selenium, fertility, liquid-stored semen

Source: Pavaneli (2022).
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