applied sciences

MDPI

Article

The Effect of Apilarnil Administration on Spermiation and Semen Quality in Sterlet (*Acipenser ruthenus*)

Dragoș Moraru [1,†], Violeta Igna [2,*], Adrian Grozea [1,*], Sandra Antonia Mihailov [1], Ana Codruța Chiș [3] and Silvia Pătruică [1,†]

[1] Faculty of Bioengineering of Animal Resources, University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului no. 119, 300645 Timisoara, Romania; dragos.moraru@usvt.ro (D.M.); sandramihailov@usvt.ro (S.A.M.); silviapatruica@usvt.ro (S.P.)
[2] Faculty of Veterinary Medicine, University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului no. 119, 300645 Timişoara, Romania
[3] Faculty of Agriculture, University of Life Sciences "King Mihai I" from Timișoara, Calea Aradului no. 119, 300645 Timişoara, Romania; codruta_chis@usvt.ro
* Correspondence: violetaigna@usvt.ro (V.I.); adriangrozea@usvt.ro (A.G.)
† These authors contributed equally to this work.

Abstract

The severe decrease in natural sturgeon stocks has led to intensified efforts toward the development of sturgeon aquaculture and the application of reproductive biotechnologies to ensure sustainable production. In cultured male *Acipenser ruthenus* (sterlet), spermiation must be hormonally induced to obtain high-quality semen; however, reducing hormonal dosages while maintaining or improving sperm quality remains a major challenge. In this study, we investigated the effects of reduced doses of carp pituitary extract (CPE) and luteinizing hormone-releasing hormone agonist (LH-RH) combined with Apilarnil supplementation on spermiation and semen quality in *A. ruthenus*. Semen volume, pH, sperm concentration, total sperm output, total motility, and spermatozoa velocity were evaluated. Administering a reduced CPE dose (1.1 mg·kg^{-1}) combined with 1 g of Apilarnil significantly increased semen volume, sperm concentration, and total sperm output, as well as improved sperm kinematic parameters (total motility and velocity) compared with the conventional hormonal protocol. Furthermore, treatments using 50% of the standard LH-RH dose supplemented with 1 g or 2 g of Apilarnil resulted in significantly higher sperm motility and velocity than observed in the control group. These results demonstrate that Apilarnil supplementation allows for a substantial reduction in exogenous hormonal doses while enhancing spermiation efficiency and semen quality in sterlet, supporting the spermiation-induction protocol for sturgeon aquaculture.

Keywords: sturgeon; Apilarnil; spermiation induction; semen quality

Check for updates

Academic Editor: Franco Mutinelli

Received: 11 December 2025
Revised: 11 January 2026
Accepted: 13 January 2026
Published: 15 January 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

The severe decrease in natural sturgeon populations has resulted in their classification as an endangered species. While wild sturgeons still exist, most populations are at serious risk of extinction. The sterlet (*Acipenser ruthenus*) is a key species of sturgeon in aquatic ecosystems. It plays an essential role in maintaining ecological balance and supporting commercial fisheries worldwide. However, its populations are declining due to factors such as habitat degradation, overfishing, and environmental pollutants, which also affect its reproductive success. Numerous studies are aimed at exploring methods to protect and

restore these fish, as sturgeons are among the most endangered species globally. Expanding sturgeon aquaculture and advancing reproductive biotechnologies represent the primary strategies for restocking depleted natural habitats and meeting both consumer demand and industrial needs.

Aquaculture system conditions differ substantially from those experienced by broodfish in natural environments [1]. Artificial rearing settings are generally deficient in spawning triggers, resulting in inadequate physiological responses [2]. Consequently, cultured sturgeon frequently exhibit reproductive impairments, particularly during the final stages of spermatogenesis [3]. These dysfunctions have been counteracted by enhancing reproductive performance via the administration of gonadotropins or gonadotropin-releasing hormone (GnRH), aimed at compensating for pituitary hormone deficiency [4]. Pituitary extracts act directly on the gonads, whereas gonadotropin-releasing hormone is responsible for the release of LH from the pituitary gland, which, subsequently, stimulates steroidogenesis and spermiation [1,5]. Among steroid hormones, androgens, specifically testosterone and 11-ketotestosterone, are central to regulating spermatogenesis and spermiation. Progesterone derivatives, particularly 17α,20β dihydroxy 4-pregnen-3-one (17α,20β-DHP), primarily regulate spermatozoa maturation in sturgeon species [6,7]. Spermatogenesis is the complex process of spermatozoa formation and includes numerous mitoses, meiosis, and cellular morphological transformation [5]. The final phase, spermatozoa maturation, corresponds to the last physiological stage of spermatogenesis and confers their characteristic morphological appearance, the competences for movement, and the fertilization capacity of spermatozoa [8]. In sturgeon, testicular spermatozoa must interact with urine to acquire motility potential [9], a prerequisite for successful fertilization [10], and this suggests urine's crucial role in the final maturation stage.

Under aquaculture conditions, sterlet sturgeon are exposed to exogenous endocrine stimulation to induce spermiation and ensure the production of high-quality semen for reproduction. In sturgeon, intramuscular administrations of GnRH analog [11,12] and sturgeon or carp pituitary extracts (SPE or CPE) have been frequently used to induce spermiation [13,14]. Some studies have demonstrated that semen quality parameters—including semen volume, spermatocrit, spermatozoa concentration, and motility—vary depending on the hormonal treatment employed [15,16]. Despite their effectiveness, the application of hormonal agents in aquaculture raises concerns regarding potential adverse effects on environmental integrity and human health, particularly through endocrine-disrupting mechanisms. Hormonal substances require careful handling to ensure environmental, biological, and food safety, as they may contaminate aquatic ecosystems and induce hormonal imbalances with possible consequences for consumers. Nevertheless, information regarding hormone residues in farmed fish remains limited, largely due to the absence of comprehensive monitoring programs assessing hormone use in aquaculture production systems [17,18].

Apilarnil is a homogenate of male honey bee brood obtained from seven-day-old larvae and honeycomb cell contents [19–21].

The biochemical composition of Apilarnil includes proteins, lipids, fatty acids, carbohydrates, vitamins (A, B, E, and D), and various minerals. Notably, Apilarnil collected during the early phases of larval development contains high levels of sex hormones, such as testosterone, prolactin, progesterone, and estradiol [22]. This product has a variety of benefits, including pharmaceutical, antioxidant [23], energizing, toning, vitalizing, and psychotonic properties [24], which help stimulate the immune system. The results of previous studies have indicated that Apilarnil possesses antioxidant and neuroprotective activities, contributes to fertility enhancement, and displays antiviral properties, while also acting as a source of androgenic hormones [25]. The authors of various studies have reported data suggesting that Apilarnil supplementation can positively influence reproductive param-

eters in humans and other animal species [26]. These parameters can include increased gonadal development, improved sperm and egg quality, and increased reproductive hormone secretion. These effects are attributed to Apilarnil's rich nutritional composition, which includes essential amino acids, vitamins, minerals, and bioactive compounds that are known to support reproductive function in humans and animals. Furthermore, the antioxidant and immunomodulatory properties of Apilarnil may provide protective effects against oxidative stress and pathogens, further promoting reproductive health [27].

Some research indicates the androgenic effect of Apilarnil and focuses on the source of this effect. It has been suggested that specific bioactive substances present in the lipid fraction, along with endogenous hormones within Apilarnil, may account for its androgenic activity [21]. Recent experimental evidence demonstrated that Apilarnil exerts a protective effect against bisphenol A (BPA)-induced testicular toxicity in adult male albino Wistar rats. BPA administration was found to decrease the serum levels of pituitary gonadotropins, testosterone, and glutathione (GSH), as well as to reduce total sperm count, motility, and vitality. Furthermore, BPA administration increased sperm abnormalities, elevated malondialdehyde (MDA) concentrations, and suppressed expression of proliferating cell nuclear antigen (PCNA). Treatment with Apilarnil significantly mitigated these adverse effects, as evidenced by restored serum testosterone concentrations, normalization of FSH and LH levels, improved antioxidant status reflected by GSH and MDA values, and enhanced sperm parameters, including count, motility, morphology, and PCNA expression [25]. Support for the androgenic activity of Apilarnil has also been reported in avian models. In a study involving seventeen-day-old chicks, dietary supplementation with Apilarnil (4 g/day) over a 21-day period resulted in a significant increase in comb size compared with control animals, serving as an indicator of enhanced secondary sexual characteristics [28].

Furthermore, positive reproductive outcomes have been observed in livestock. Breeding bulls receiving 120 g/day of drone larvae homogenate encapsulated as a dietary bio-additive exhibited notable improvements in semen production and quality. Specifically, semen volume increased by 25.2%, spermatozoa concentration by 13.6%, total motility by 80.4%, and progressive motility by 92.8%. In addition, the proportion of viable sperm cells in fresh semen rose by 26.1%, while the percentage of live morphologically normal spermatozoa increased by 43% in fresh semen and by 92.49% in frozen samples [29].

In veterinary and animal-production settings, Apilarnil or drone brood homogenate is mainly used as a nutritional, immunomodulatory, or reproductive support supplement. In animal studies, oral delivery represents the most frequently employed route of administration, achieved either by incorporating the substance into feed [29,30] or drinking water or through direct oral application using a syringe, drench, or oral paste [28,31].

More recently, Lenický et al. (2024) [32] investigated the effects of drone brood on stallion spermatozoa during short-term in vitro incubation, with the aim of evaluating its potential incorporation into equine semen extenders. Their findings provided the first evidence of a positive, dose-dependent influence of fixed drone brood on spermatozoa survival under in vitro conditions. Although parenteral administration is not standard practice for Apilarnil or drone brood homogenate, Bolatovna et al. (2015) [33] used parenteral injection of alcohol extract of drone brood homogenate in boars to improve reproductive qualities. An increase in production and quality of boar semen has been reported, and no adverse reactions were observed during this study.

To date, there are no studies in which the authors provide results on the effects of Apilarnil administration on sturgeon reproduction. This perspective is further supported by findings from a recent review examining the application of bee-derived products in aquaculture [34]. Among the 51 studies analyzed, approximately 96% addressed various beekeeping-derived products, such as propolis, bee pollen, honey, and royal jelly, while

less than 4% considered drone brood. Although experimental data regarding drone brood use in aquaculture remain unavailable, its high content of proteins, essential amino acids, minerals, and steroid hormones indicates promising potential for nutritional applications in fish. In addition, nutraceutical research has demonstrated that lyophilized drone brood, when combined with calcium derived from eggshells, enhances the in vitro bioaccessibility of testosterone, estradiol, calcium, and phenolic compounds [34].

Accordingly, the objective of the present study was to investigate the effects of reducing the conventional hormonal dosages applied for spermiation induction in sterlet (*Acipenser ruthenus*) and to evaluate the impact of Apilarnil supplementation on selected semen quality parameters.

2. Materials and Methods

This study was conducted during the natural spawning season (March) of sterlets (*Acipenser ruthenus*) from the fish farm of the University of Life Sciences "King Mihai I", Timișoara (ULST). The experiment included forty-five clinically healthy male sterlet sturgeons, aged four years and weighing between 0.87 and 1.65 kg.

Two experimental protocols (EPs) were established to induce spermiation: (1) the intramuscular administration of carp pituitary extract—CPE (Rideg & Rideg Fish Farm, Ltd., Homokmégy, Hungary), either with or without the addition of pure Apilarnil (Apicris Natura, Iasi, Romania)—EP I and (2) the intramuscular administration of Luteinizing hormone-releasing hormone agonists LH-RH (Sigma-Aldrich Co., St. Louis, MO, USA), either with or without the addition of pure Apilarnil—EP II.

The experiments were carried out in two locations: the ULST aquaculture facility and the Reproduction Laboratory of the University's Research Laboratory Complex.

2.1. Experimental Stages in Aquaculture Facility

The activities carried out at the university aquaculture facility were conducted according to a protocol developed and refined at our facility over recent years. Four months prior to hormonal stimulation, forty-five male sterlets were selected from the main tanks based on phenotypic evaluation. Sex determination was performed by gonadal biopsy using the instruments and techniques described by Chebanov and Galich [35]. The selected males were subsequently transferred to secondary tanks for overwintering, where they were maintained at water temperatures below 7 °C until two weeks before the optimal breeding period. Thereafter, the water temperature was gradually increased by 1 °C·day^{-1} until reaching 14 °C. The fish were maintained at this temperature for three weeks, after which the reproductive stimulation procedures were initiated.

The selected specimens were individually tagged at the caudal peduncle, weighed, hormonally stimulated according to the predefined spermiation induction protocols, and distributed into small, dedicated experimental tanks.

Semen collection was performed 24 h after the second administration of carp pituitary extract and/or Apilarnil in experimental protocol I (EP I) and between 30 and 37 h after the first administration of LH-RH in experimental protocol II (EP II). Semen was collected by direct aspiration from the spermiduct using a sterile 3 mL plastic Pasteur pipette, a method chosen to minimize the risk of sample contamination, while gentle external abdominal massage in the cranio-caudal direction was additionally applied before and during collection to facilitate semen release [36]. Immediately after collection, the semen was transferred from the pipette into sterile 12 mL centrifuge tubes with volumetric graduations, each tube being labeled with the identification code of the donor male, and the pipette was reinserted as needed into the spermiduct to ensure complete collection of all semen released by each individual.

Experimental protocol I (EP I) for the spermiation induction based on carp pituitary extract (CPE) administration included 25 male sterlets, divided into five groups (C I, E I.1, E I.2, E I.3, and E I.4), differentiated by the hormonal protocol (Table 1).

Table 1. Spermiation induction by carp pituitary extract—experimental protocol I (EP I).

			First Administration		Second Administration	
Nr. Crt.	**Experimental Group**	**Body Weight (kg)**	**Carp Pituitary Extract (mg kg^{-1})**	**Apilarnil (g fish^{-1})**	**Carp Pituitary Extract (mg kg^{-1})**	**Apilarnil (g fish^{-1})**
1.	C I	1.14 ± 0.05	0.2	-	1.8	-
2.	E I.1	1.28 ± 0.17	0.2	-	0.9	1
3.	E I.2	1.24 ± 0.26	0.2	-	0.9	-
4.	E I.3	1.23 ± 0.12	0.2	1	-	1
5.	E I.4	1.33 ± 0.23	0.2	1	-	-

The control group (C I) was treated with carp pituitary extract (2 mg × kg^{-1}), administered as follows: 0.2 mg × kg^{-1} bw (10% of the total dose) on the first day of administration and 1.8 mg × kg^{-1} bw (90% of the total dose) 12 h after the first administration. Semen collection was performed 24 h after the second administration.

The experimental group I.1 (E I.1) was treated with carp pituitary extract 0.2 mg kg^{-1} bw (10% of the total dose of the control group) on the first day of administration and 0.9 mg CPE × kg^{-1} bw (45% of the total dose of the control group) plus 1 g pure Apilarnil, 12 h after the first administration. Semen collection was performed 24 h after the last administration.

The experimental group I.2 (E I.2) was treated with carp pituitary extract at 0.2 mg kg^{-1} bw (10% of the total dose of the control group) on the first day of administration and 0.9 mg CPE kg^{-1} bw (45% of the total dose of the control group), 12 h after the first administration. Semen collection was performed 24 h after the last administration.

The experimental group I.3 (E I.3) was treated with carp pituitary extract at 0.2 mg kg^{-1} bw (10% of the total dose of the control group) plus 1 g Apilarnil pure on the first day of administration. Twelve hours after the first dose, another dose of 1 g of pure Apillarnil was administered. Semen collection was performed 24 h after the second dose of Apilarnil.

The experimental group I.4 (E I.4) was treated with carp pituitary extract at 0.2 mg kg^{-1} bw (10% of the total dose of the control group) and 1 g pure Apilarnil. After 24 h of this administration, semen was collected.

Experimental protocol II (EP II) for the spermiation induction by Luteinizing hormone-releasing hormone agonists (LH-RH) administration included 20 male sterlets, divided into four groups (C II, E II.1, E II.2, and E II.3), differentiated by the hormonal protocol (Table 2).

Table 2. Spermiation induction by luteinizing hormone-releasing hormone agonists LH-RH—experimental protocol II (EP II).

			First Administration		Second Administration	
Nr.Crt.	**Experimental Group**	**Body Weight (kg)**	**LH-RH (µg kg^{-1})**	**Apilarnil (g fish^{-1})**	**LH-RH (µg kg^{-1})**	**Apilarnil (g fish^{-1})**
1.	C II	1.14 ± 0.04	20	-	-	-
2.	E II.1	1.18 ± 0.13	10	1	-	1
3.	E II.2	1.23 ± 0.24	10	1	-	-
4.	E II.3	1.31 ± 0.26	10	-	-	-

The control group (C II) was treated with 20 µg·kg^{-1} LH-RH. Semen collection was performed 30–37 h after administration.

The experimental group II.1 (E II.1) received 50% of the control group LH-RH dose (10 μg/kg) plus 1 g pure Apilarnil. Semen collection was performed 30–37 h after administration.

The experimental group II.2 (E II.2) was treated with 10 $\mu g \cdot kg^{-1}$ LH-RH (50% of the total dose of the control group C II) on the first day of administration, plus 1 g pure Apilarnil. Twelve hours after the first dose, another dose of 1 g of pure Apilarnil was administered. Semen collection was performed between 30 and 36 h after the first dose of LH-RH.

The experimental group II.3 (E II.3) received 50% of the control group LH-RH dose (10 $\mu g\ kg^{-1}$) without the addition of Apilarnil. Semen collection was performed 30–37 h after LH-RH administration.

The reduced hormonal dosages applied in this study were selected based on previously reported effective dose ranges for sterlet and on preliminary observations conducted at the ULST aquaculture facility. Standard protocols for spermiation induction in sterlet commonly use carp pituitary extract at doses between 2 and 6 $mg \cdot kg^{-1}$ body weight and LH-RH agonists at doses around 20 $\mu g \cdot kg^{-1}$. In order to minimize exogenous hormonal input and potential environmental and physiological impacts while maintaining spermiation efficiency, we tested reduced hormone doses corresponding to approximately 50% of the standard protocols.

All hormonal treatments and Apilarnil administrations were performed via intramuscular injection in the dorsal musculature of fish, lateral to the dorsal fin and above the lateral line, avoiding the vertebral column and major blood vessels. For administration of the hormonal products and Apilarnil, the fish was gently removed from the water and placed on a wet, padded surface in lateral recumbency, and the injection area was wiped with sterile saline solution to remove excess mucus. Parenteral administration was performed using sterile syringes and fine needles. Hormonal compound dosages were individually calculated based on body weight, whereas Apilarnil was administered at a fixed dose per fish in accordance with the experimental protocol. In cases where two administrations were required, the second injection was given 12 h after the first one.

The doses of Apilarnil used in this study (1 g and 2 g per fish) were selected on the basis of preliminary, unpublished experiments. In these initial tests, a dose of 0.5 g per fish showed no positive effects on spermiation or semen quality relative to controls. In contrast, administration of doses higher than 2 g Apilarnil per fish was associated with local adverse reactions, including erythema at the injection site, and issues relating to the volume and consistency of the product, as well as other transient local reactions. Consequently, these higher doses were excluded from this study's protocol, in consideration of animal welfare, procedural safety, and experimental reproducibility.

2.2. Laboratory Stage

The second experimental stage, carried out within the "Horia Cernescu" Research Laboratory Complex" of the University of Life Sciences "King Mihai I", Timișoara, consisted of evaluating the semen volume, pH, concentration, sperm count, spermatozoa motility, and velocity.

2.2.1. Measurement of Semen Volume and pH

Semen was collected in sterile centrifuge tubes of 12 mL capacity, printed with volumetric graduations. Immediately after the samples arrived at the laboratory, the containers of semen samples were placed vertically on a flat surface to allow the seminal fluid to drain from the inner walls of the containers and to stabilize the top surface of the fluid. Semen volume was read directly from the graduations. The pH of semen was measured with the inoLab pH 720 pH meter (Xylem Analytics, Weilheim, Germany) at ambient laboratory

temperatures between 23 and 24 °C. Two determinations were carried out on each of the semen samples.

2.2.2. Evaluation of Kinematic Parameters

In advance of sperm motility analysis, each semen sample was checked by microscopic examination (Olympus BX51, Olympus, Tokyo, Japan) of a 5 µL drop of semen for possible contamination with pool water. Semen samples with a motile spermatozoa proportion exceeding 2% were considered contaminated and were excluded from further evaluation. Evaluation of kinematic parameters included the analysis of spermatozoa total motility (TM%) and velocity. Both motility parameters were measured after activation. Spermatozoa were activated by mixing 2 µL of semen with 50 µL of fish tank water, and motility parameters were assessed within a maximum of 10 s after activation. Ten microlitres of each sample were placed on a slide and examined microscopically. First, the ×10 objective was used, followed by the ×20 or ×40 objectives.

Total motility was visually assessed in multiple fields using bright-field microscopy (Olympus BX51 microscope), at 200× magnification. These fields were recorded and re-evaluated using a digital color camera (ColorView II Olympus, Tokyo, Japan) and cell analysis software (Cell F Imaging Software for Life Science Microscopy Olympus, Soft Imaging Solution, Munster, Germany). The percentage of motile spermatozoa was estimated for each semen sample.

The velocity of motile spermatozoa was evaluated using the modified Person's scale [35], which enables assessment based on observed movement patterns. A score of 5 was assigned to samples in which all analyzed spermatozoa exhibited rapid movement, whereas a score of 4 was given when rapid movement predominated. A score of 3 characterized samples in which slow movement was dominant, although a mixture of rapidly motile and immotile spermatozoa was also present. Finally, a score of 2 defined samples in which movement was very slow and oscillatory, with a high proportion of immotile spermatozoa, reaching up to 75%.

2.2.3. Determination of Sperm Concentration and Sperm Count

The semen concentration refers to the number of spermatozoa per mL of semen. The sperm count is the total number of spermatozoa upon semen collection [37] and is calculated by multiplying the concentration by the volume of semen [38]. Sperm concentration was determined using a Bürker-Türk hemocytometer (BRAND GMBH + CO. KG, Wertheim, Germany). The raw semen was diluted with pool water (ratio 1:2000) and mixed for a few seconds to homogenize. A coverslip was applied on top of the hemocytometer's chambers, and a drop of 10 µL diluted semen sample was placed into the groove of the cell counting chamber. This procedure was repeated to fill the two etched grids of the hemocytometer chamber. Capillary action ensured a uniform distribution of the suspension within the chamber. A heated plate (Hamilton Thorne Bioscience, Beverly, MA, USA) was applied to the coverslip surface corresponding to the counting chamber, which was maintained for 3 s, with the aim of stopping the movement of spermatozoa through thermal shock, thus facilitating their counting. The hemocytometer was placed on a microscope stage (Olympus BX51, Olympus, Tokyo, Japan) and analyzed with a 40× objective, in bright field. Spermatozoa from five middle squares of the grid were counted, as follows: 4 middle squares located on the diagonal and 1 middle square, placed anywhere on the grid. The same procedure was applied to both grids of the hemocytometer. The average of the two values was calculated. The concentration of the semen sample was calculated, applying the following formula: Concentration = Average of counted sperm × Dilution factor × Multiplication factor [39].

2.2.4. Statistical Analysis

Descriptive data analysis was performed using IBM SPSS Statistics 26, and statistical significance tests were performed using the Mann–Whitney U test within the same program. To control for the risk of type I errors associated with multiple testing, *p*-values were adjusted using the Benjamini–Hochberg method.

3. Results

3.1. Measurement of Semen Volume and pH

The volume of semen collected from male sterlet stimulated for the spermiation induction with carp pituitary extract (CPE) ranged between 1.52 ± 0.65 mL and 33.5 ± 1.17 mL (Table 3). The greatest semen volume (33.5 ± 1.17 mL) was collected from experimental group E I.1 using the spermiation induction protocol based on carp pituitary extract, with the second dose reduced to half and supplemented with 1 g pure Apilarnil. The volume of sperm collected indicates that reducing the hormonal dose by half at the second administration slightly increases the average semen volume from 28.32 ± 10.49 mL (C I group) to 31.40 ± 6.11 mL (E I.2 group). Halving the dose of the hormone associated with the addition of Apilarnil improved the volume of semen collected (33.5 ± 1.17 mL/E I.1 group), indicating the favorable effect of this product on semen production.

Table 3. Semen parameters: pH, volume, concentration, and total number of spermatozoa per collection, after spermiation induction in sterlet (*Acipenser ruthenus*) using carp pituitary extract (CPE).

Experimental Group	Semen pH	Semen Volume (mL)	Semen Concentration (Sperm × 10^9/mL)	Total Sperm Number (Sperm × 10^9/collection)
C I	6.76 ± 0.14 ^{a}	28.32 ± 10.49 ^{a}	4.33 ± 2.47 ^{a}	107.98 ± 39.63 ^{a}
E I.1	7.09 ± 0.35 ^{ab}	33.5 ± 1.17 ^{a}	3.14 ± 0.28 ^{a}	105 ± 13.24 ^{a}
E I.2	6.84 ± 0.10 ^{a}	31.40 ± 6.11 ^{a}	2.57 ± 0.63 ^{a}	80.31 ± 23.93 ^{a}
E I.3	7.62 ± 0.35 ^{b}	3.60 ± 3.41 ^{b}	1.96 ± 1.38 ^{a}	10.67 ± 16.88 ^{b}
E I.4	6.22 ± 0.33 ^{a}	1.52 ± 0.65 ^{b}	1.42 ± 1.82 ^{a}	3.00 ± 4.82 ^{b}

In all experimental groups, the data were expressed as means ± SD. The same index letter indicates non-significant differences ($p > 0.05$) between experimental variants. For the considered parameters, pairwise comparisons were performed using the Mann–Whitney U test, with Benjamini–Hochberg correction.

Reducing the carp pituitary extract dose to 10% of that administered to the control group resulted in a significant decrease in the volume of semen collected from the fish, even when a single dose of 1 g of Apilarnil or two doses were administered: 1.52 ± 0.65 mL (E I.4) and 3.60 ± 3.41 mL (E I.3).

The values of the semen volume collected from sterlet males stimulated for the spermiation induction with LH-RH, in the four experimental variants, are presented in Table 4. The highest semen volume was reported in the C II control group (16.62 mL).

Table 4. Semen parameters: pH, volume, concentration, and total number of spermatozoa per collection, after spermiation induction in sterlet (*Acipenser ruthenus*) using luteinizing hormone-releasing hormone agonists (LH-RH).

Experimental Group	Semen pH	Semen Volume (mL)	Semen Concentration (Sperm × 10^9/mL)	Total Sperm Number (Sperm × 10^9/collection)
C II	7.04 ± 0.62 ^{ab}	16.62 ± 10.53 ^{a}	1.71 ± 0.80 ^{ab}	32.51 ± 31.27 ^{a}
E II.1	7.17 ± 0.37 ^{a}	4.88 ± 4.04 ^{b}	2.30± 0.71 ^{a}	12.00 ± 13.35 ^{a}
E II.2	7.04 ± 0.5 ^{ab}	5.20 ± 7.04 ^{ab}	1.73 ± 0.01 ^{ab}	10.91 ± 12.92 ^{a}
E II.3	6.50 ± 0.16 ^{b}	13.81 ± 9.00 ^{ab}	1.15 ± 0.50 ^{b}	15.82 ± 22.36 ^{a}

In all experimental groups, the data were expressed as means ± SD. The same index letter indicates non-significant differences ($p > 0.05$) between experimental variants.

Reducing the dose of LH-RH by half resulted in a slight decrease in sperm volume (13.81 ± 9.00/E II.3 group), but statistical analysis did not indicate a significant difference between the two groups ($p > 0.05$). A significant decrease in sperm volume was observed in groups that were stimulated with a reduced dose of LH-RH associated with the addition of Apilarnil, either in a single dose (E II.2 group) or two doses (E II.1 group).

As shown in Tables 3 and 4, the mean semen pH values measured approximately 60 min after collection ranged from 6.50 to 7.62 across the nine experimental variants included in the study.

3.2. Determination of Sperm Concentration and Sperm Count

In the experimental group that was administered carp pituitary extract (CPE) for spermiation induction, the highest sperm production, revealed by the sperm concentration ($4.33 \pm 2.47 \times 10^9$ spermatozoa/mL) and number of sperm per collection ($107.98 \pm 39.63 \times 10^9$), was reported in control group C I (Table 3). Reducing the dose of CPE to 0.9 mg/kg at the second administration caused a decrease in both sperm concentration and the total number of spermatozoa per collection. Statistical comparisons indicated no significant differences between the control group and groups E I.1 and E I.2. Higher quantitative sperm production was recorded when the reduction in CPE dose was accompanied by the addition of Apilarnil: $105 \pm 13.24 \times 10^9$ spermatozoa in E I.1 versus $80.31 \pm 23.93 \times 10^9$ spermatozoa in E I.2. These findings may reflect the favorable impact of Apilarnil on maturation and spermiation.

3.3. Evaluation of Sperm Motility

No significant differences in total motility and velocity after comparisons between study groups using the Mann–Whitney U test with Benjamini–Hochberg correction were observed among the five experimental groups that were treated with carp pituitary extract—CPE (Table 5 and Figure 1).

Table 5. Sperm kinematic parameters (total motility and velocity) after spermiation induction in sterlet (*Acipenser ruthenus*) using carp pituitary extract (CPE).

	Experimental Group	Total Motility (%)	Velocity (Score)
1.	C I	86.20 ± 7.89 [a]	4.00 ± 1.00 [a]
2.	E I.1	90.20 ± 2.05 [a]	4.80 ± 0.45 [a]
3.	E I.2	73.80 ± 15.01 [a]	3.00 ± 0.71 [a]
4.	E I.3	84.80 ± 9.23 [a]	4.20 ± 0.45 [a]
5.	E I.4	88.00 ± 4.06 [a]	3.60 ± 0.89 [a]

In all experimental groups, the data are expressed as means ± SD. Different superscript letters indicate statistically significant differences between experimental groups (Mann–Whitney U test, $p < 0.05$).

The average velocity score shows a decrease when the second dose of CPE was halved in the E I.2 group and completely removed in the E I.4 group. However, sperm velocity was increased in group E I.1, which received Apilarnil at a dose of 1 g $fish^{-1}$ concomitantly with the halving of CPE at the second administration, compared to group E I.2, in which the reduction in CPE was not counterbalanced by the supplementation with Apilarnil. The increase in sperm velocity was also exhibited in group E I.3, which received a single dose of CPE at 0.2 mg × kg^{-1} plus 1 g × $fish^{-1}$ Apilarnil, followed by the same dose of Apilarnil, twelve hours later (Table 5).

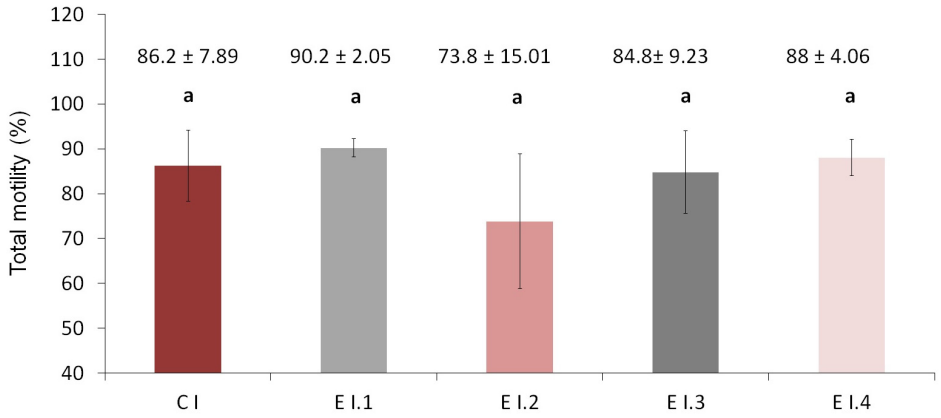


Figure 1. Percentage of total sperm motility in sterlet (*Acipenser ruthenus)* following spermiation induction using carp pituitary extract (CPE). Data are expressed as mean ± SD. The same index letter indicates non-significant differences (Mann–Whitney U test, $p > 0.05$) between experimental variants.

Halving the dose of luteinizing hormone-releasing hormone agonists (LH-RHs) and the administration of 10 μg × kg^{-1} led to a decrease in total sperm motility (38.20 ± 7.50%) in experimental group E II.3 compared to the control group C II (Table 6 and Figure 2).

Table 6. Sperm kinematic parameters (total motility and velocity) after spermiation induction in sterlet (*Acipenser ruthenus*) using luteinizing hormone-releasing hormone agonists (LH-RHs).

	Experimental Group	Total Motility (%)	Velocity (Score)
1.	C II	67.4 ± 13.46 ^{a}	3.00 ± 1.00 ^{a}
2.	E II.1	89.60 ± 5.77 ^{be}	4.20 ± 0.45 ^{a}
3.	E II.2	90.60 ± 1.14 ^{ce}	4.60 ± 0.89 ^{a}
4.	E II.3	38.20 ± 7.50 ^{d}	3.75 ± 1.02 ^{a}

In all experimental groups, the data were expressed as means ± SD. The same index letter indicates non-significant differences ($p > 0.05$) between experimental variants.

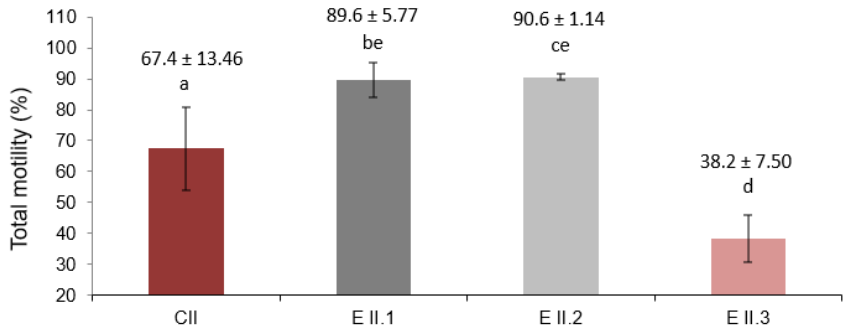


Figure 2. Percentage of total motility of sterlet sperm samples from the four groups belonging to the experimental protocol treated with luteinizing hormone-releasing hormone agonists (LH-RHs). Groups with different letter indexes are statistically significantly different (Mann–Whitney U test, $p < 0.05$).

Significantly higher values of total motility were observed in group E II.2, which received 50% of the LH-RH dose administered to group C II, plus 1 g of pure Apilarnil. Similarly, high values were observed in group E II.1, which received the same dose as group E II.2, plus an additional 1 g of pure Apilarnil twelve hours after the initial Apilarnil dose.

No differences in velocity score after comparisons between study groups using the Mann–Whitney U test with Benjamini–Hochberg correction were observed among the four experimental groups that were treated with LH-RH (Table 6 and Figure 2).

4. Discussion

In aquaculture, efficient utilization of males largely relies on the acquisition of adequate amounts of high-quality spermatozoa, either for immediate fertilization or for subsequent storage and use [8]. Male reproductive performance is commonly characterized by semen volume and spermatozoa quantity. Both total semen volume and sperm output vary considerably among species as well as among individuals within the same species [40].

Because sturgeons rarely undergo natural spawning under captive conditions, hormonal stimulation is routinely applied to promote sperm maturation and induce spermiation [15]. The endocrine regulation of spermiation involves modulation of the hypothalamic–pituitary–gonadal axis, with pituitary gonadotropins and gonadal steroid hormone synthesis playing central roles in this process [41]. Through hormonal induction, it is possible to obtain substantial semen volumes—ranging from several milliliters to several hundred milliliters—characterized by semen concentrations of approximately 0.02–10 $\times$ 10^9 spermatozoa mL^{-1} and high sperm quality, including velocities of 90–180 $\mu m\ s^{-1}$ and motility values reaching up to 100%. These parameters are strongly species-specific and depend on the hormonal protocols applied [41].

Sperm production can be stimulated by injections of various gonadotropic agents, including acetone-dried pituitary extracts from carp or sturgeon, as well as gonadotropin-releasing hormone (GnRH) analogs administered in combination with dopamine receptor antagonists. In sterlet, spermiation is commonly induced using carp pituitary extract (CPE) at doses of 4 mg kg^{-1} [1,42] or 6 mg kg^{-1} [42]. In the present study, a reduced CPE dose of 2 mg kg^{-1} was administered to the control group, corresponding to the standard protocol routinely applied at the ULST Fish Farming Facility. For the experimental groups, we reduced the CPE dose and administered it with or without the addition of 1 or 2 g Apilarnil per fish. Halving the CPE dose (1.1 mg kg^{-1}) associated with the addition of Apilarnil resulted in sperm quantitative parameter values (volume, concentration, and total sperm number per collection) similar to those obtained with the administration of 2 mg kg^{-1} CPE. The favorable effects of Apilarnil could be linked to the sexual steroids contained in this product. Thus, the influence of sex steroids on the sterlet sperm maturation was reported. Elevated circulating levels of the steroid hormones: testosterone, 11-ketotestosterone, and 17,20β-progesterone following hormonal stimulation of spermatogenesis before the breeding season, led to in vitro maturation of sterlet spermatozoa. Increases in sperm volume and percentage motility of spermatozoa collected from the Wolffian duct were recorded [9]. Sex steroid hormones also exert both positive and negative feedback on the brain–pituitary axis, thus regulating pituitary gonadotropin (GTH) release. A major positive action of the steroids is to enhance pituitary responsiveness to GnRH, probably by stimulating GnRH-R. A major negative action of these steroid hormones is exerted through the dopaminergic system, increasing dopamine (DA) turnover and thus enhancing the DA inhibitory tone over GTH secretion. Although sex steroid profiles have been extensively characterized in most sturgeon species, limited information is available regarding the feedback effects of these hormones on gonadotropin-releasing hormone (GnRH) and gonadotropin (GTH) secretion. Pavlick and Moberg (1997) [43] reported a significant increase in pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels following the implantation of exogenous testosterone in white sturgeon. These findings indicate that the pituitary gland of adult sturgeon responds to positive testosterone feedback [43]. Reducing the dose of carp pituitary extract at 10% of the dose administered to the control group, by excluding the second administration, was associated with a significant decrease in the volume of semen collected from the fish, concentration, and total sperm number, even when a single dose of 1 g of Apilarnil or two doses were administered.

The mean semen pH values measured approximately 60 min after collection ranged from 6.50 to 7.62. Compared with previously published pH values for *A. ruthenus* semen, our results are lower than those reported by Baradaran Noveiri et al. (2019) for bester (female *Huso huso* × male *Acipenser ruthenus*), where the mean sperm pH was 8.09 ± 0.34 (range 7.51–8.68) [44]. This value is also lower than the pH reported for *A. ruthenus* by Psenicka et al. (2008), who found an average of 8.13 ± 0.19 [45]. The pH values obtained in the present study align with the known physiological features of sperm maturation in sturgeons and reflect the natural pH gradients observed between testicular seminal fluid, urine, and Wolffian duct secretions. In sturgeon species, immature spermatozoa produced in the testes are transported through the kidneys toward the Wolffian ducts, which open directly into the external environment [8]. During this process, testicular spermatozoa are mixed with urine, resulting in the dilution of the seminal fluid, reduced osmolality, and lower spermatozoa concentrations compared with those observed in many other teleost species. This interaction with urine is essential, as it enables spermatozoa to acquire motility upon subsequent dilution with water and to achieve fertilization competence [9]. As reported by Dzyuba et al. (2014), the pH of testicular seminal fluid in sturgeon is approximately 6.5, whereas the Wolffian duct seminal fluid exhibits a more alkaline pH of around 8.5, and urine has an intermediate pH of about 8.0 [9].

In the present study, semen pH was measured approximately 60 min after collection by direct aspiration from the spermiduct, with strict precautions taken to avoid contamination. Nevertheless, the final phase of sperm maturation in sturgeons involves the physiological mixing of testicular sperm with urine during passage through the kidneys and Wolffian ducts. Consequently, the pH of the seminal fluid is influenced by the relative contributions of testicular secretions, urine, and ductal fluids at the time of collection, which may vary according to the stage of sperm maturation and spermiation, as well as the interval following hormonal stimulation. The experiments were carried out under controlled aquaculture conditions, and the water used both for maintaining the broodstock and for sperm activation had a near-neutral pH, which may have indirectly influenced the variability of the recorded values. Furthermore, sperm pH was measured approximately 60 min after collection. This time interval may allow for slight shifts in the acid–base balance to occur, as compared to measurements taken immediately after collection in other studies.

Sturgeon spermatozoa remain immotile within the testes and seminal plasma and become motile only after release into the aquatic environment [46]. This inhibition of motility in seminal plasma is largely attributed to elevated potassium (K^+) ion concentrations [47]. Optimal sperm motility in Acipenseridae has been reported at alkaline pH values ranging from 8 to 9, particularly under low dilution conditions (1:50) [48,49].

Ingermann et al. (2002) [50] demonstrated that within the pH range 7.5–8.5, sperm contribute perhaps 5–10% of the buffering capacity in semen, whereas they appear to make no contribution over the pH range of 6.0–7.0. The relatively minor contribution of sperm activation at the higher pH values seems unlikely to be physiologically significant and may simply be a consequence of the presence of sperm proteins. Low semen buffering capacity represents a physiological adaptation allowing the epithelial cells of the reproductive tract to exert control over the capacity for sperm motility by regulating semen pH via bicarbonate secretion. Also, low semen buffer capacity may permit control of the potential for sperm motility via pH regulation mediated via acid/base secretions into the semen.

Sperm motility is influenced by multiple physicochemical factors, including semen pH, ionic composition (K^+, Na^+, and Ca^{2+}), osmotic pressure, and temperature [18]. As a critical functional attribute of male gametes, motility enables spermatozoa to actively migrate toward and penetrate the female gamete [18]. In the present study, treatments

involving 50% of the conventional LH-RH dose supplemented with either 1 g or 2 g of Apilarnil led to significantly enhanced sperm motility and velocity.

The present study has several limitations that should be taken into account when interpreting the findings. Specifically, the physiological and endocrine pathways through which Apilarnil may affect the last step of spermatogenesis, spermiation, and sperm quality were not directly examined, and reproductive hormone concentrations or oxidative stress markers were not measured in either serum or gonadal tissues. In addition, sperm quality assessment was limited to basic functional parameters, without detailed evaluation of sperm morphology, viability, or ultrastructural features. Future research should include mechanistic studies examining the influence of Apilarnil on the hypothalamic–pituitary–gonadal axis and oxidative balance in sturgeons using specific hormonal and biochemical assays. Long-term effects of the combined hormone–Apilarnil treatment on fertilization, hatching rates, larval performance, and offspring health should also be assessed. Moreover, implementing CASA-based sperm motility analysis and standardized activation conditions will improve reproducibility and relevance for sturgeon aquaculture studies.

Considering that aquaculture and advanced reproductive biotechnologies are widely recognized as key tools for preventing the extinction of most sturgeon populations, future research, based on functional and genetic analyses of spermatozoa, would provide valuable diagnostic information for identifying strategies to improve the success of advanced reproductive biotechnologies applied to sturgeon. We believe that future studies should be aimed at further developing these approaches, with a particular focus on elucidating the pathways and mechanisms by which Apilarnil, through its components, acts both at the level of the hypothalamic–pituitary–gonadal axis and at the cellular and molecular level.

5. Conclusions

Through the present study, we revealed the favorable impact of Apilarnil on the spermiation and some spermatic parameters. Reducing the carp pituitary extract (CPE) dose to 1.1 mg kg^{-1}, combined with Apilarnil supplementation, produced sperm quantitative parameters (volume, concentration, and total sperm number per collection) comparable to those achieved with a 2 mg kg^{-1} CPE dose. Treatments using 50% of the standard LH-RH dose supplemented with 1 g or 2 g of Apilarnil resulted in significantly higher sperm motility and velocity. Further investigation is necessary to determine optimal doses of hormones and Apilarnil to reliably induce spermiation and enhance sperm quality in cultured *A. ruthenus* sturgeon.

Author Contributions: All authors contributed to the study conception and design. Conceptualization, original draft preparation, D.M., S.P., A.G. and V.I.; methodology, S.P., A.G. and V.I.; formal analysis, D.M.; statistical analysis, A.C.C. and S.A.M.; review and editing and validation, S.P., A.G. and V.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by funds from the University of Life Sciences "King Mihai I" from Timișoara.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The report of the analyses performed for the samples in the paper can be found at the "Horia Cernescu" Research Laboratory Complex of the University of Life Sciences "King Mihai I", Timișoara.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Podhorec, P.; Knowles, J.; Vysloužil, J.; Boryshpolets, S.; Kubová, K.; Rodina, M.; Kholodnyy, V.; Sotnikov, A.; Gela, D.; Dzyuba, B. Induction of Spermiation in Sterlet *Acipenser ruthenus* by PLGA Microparticle Delivery with Sustained Alarelin Release. *Animals* **2021**, *11*, 3305. [CrossRef] [PubMed]
2. Podhorec, P.; Kouril, J. Induction of Final Oocyte Maturation in Cyprinidae Fish by Hypothalamic Factors: A Review. *Vet. Med.* **2009**, *54*, 97–110. [CrossRef]
3. Alavi, S.M.H.; Hatef, A.; Mylonas, C.C.; Gela, D.; Papadaki, M.; Rodina, M.; Kaspar, V.; Psenicka, M.; Podhorec, P.; Linhart, O. Sperm Characteristics and Androgens in *Acipenser ruthenus* after Induction of Spermiation by Carp Pituitary Extract or GnRHa Implants. *Fish Physiol. Biochem.* **2012**, *38*, 1655–1666. [CrossRef] [PubMed]
4. Yaron, Z.; Bogomolnaya, A.; Drori, S.; Biton, I.; Aizen, J.; Kulikovsky, Z.; Levavi-Sivan, B. Spawning Induction in the Carp: Past Experience and Future Prospects—A Review. *Isr. J. Aquac.-Bamid.* **2009**, *61*, 5–26.
5. Schulz, R.W.; Miura, T. Spermatogenesis and Its Endocrinology. *Fish Physiol. Biochem.* **2002**, *26*, 43–56. [CrossRef]
6. Amiri, B.M.; Maebayashi, A.; Adachi, S.; Yamauchi, K. Testicular Development and Serum Sex Steroid Profiles during the Annual Sexual Cycle of Male Sturgeon Hybrid, the Bester. *J. Fish Biol.* **1996**, *48*, 1039–1050.
7. Barannikova, I.A.; Bayunova, L.V.; Semenkova, T.B. Serum Levels of Testosterone, 11-Ketotestosterone and Oestradiol-17β in Three Species of Sturgeon during Gonadal Development and Final Maturation Induced by Hormonal Treatment. *J. Fish Biol.* **2004**, *64*, 1330–1338. [CrossRef]
8. Dzyuba, B.; Cosson, J.; Dzyuba, V.; Fedorov, P.; Bondarenko, O.; Rodina, M.; Linhart, O.; Shelton, W.L.; Boryshpolets, S. Sperm Maturation in Sturgeon (Actinopterygii, Acipenseriformes): A Review. *Theriogenology* **2017**, *97*, 134–138. [CrossRef]
9. Dzyuba, B.; Cosson, J.; Boryshpolets, S.; Bondarenko, O.; Dzyuba, V.; Prokopchuk, G.; Gazo, I.; Rodina, M.; Linhart, O. In Vitro Sperm Maturation in Sterlet, *Acipenser ruthenus*. *Reprod. Biol.* **2014**, *14*, 160–163. [CrossRef]
10. Dzyuba, B.; Boryshpolets, S.; Cosson, J.; Dzyuba, V.; Fedorov, P.; Saito, T.; Psenicka, M.; Linhart, O.; Rodina, M. Motility and Fertilization Ability of Sterlet *Acipenser ruthenus* Testicular Sperm after Cryopreservation. *Cryobiology* **2014**, *69*, 339–341. [CrossRef]
11. Williot, P.; Brun, R.; Rouault, T.; Pelard, M.; Mercier, D.; Ludwig, A. Artificial Spawning in Cultured Sterlet Sturgeon, *Acipenser ruthenus* L., with Special Emphasis on Hermaphrodites. *Aquaculture* **2005**, *246*, 263–273. [CrossRef]
12. Bayunova, L.; Canario, A.V.; Semenkova, T.; Dyubin, V.; Sverdlova, O.; Trenkler, I.; Barannikova, I. Sex Steroids and Cortisol Levels in the Blood of Stellate Sturgeon (*Acipenser stellatus* Pallas) during Final Maturation Induced by LH-RH Analogue. *J. Appl. Ichthyol.* **2006**, *22*, 334–339. [CrossRef]
13. Linhart, O.; Mims, S.D.; Gomelsky, B.; Hiott, A.E.; Shelton, W.L.; Cosson, J.; Rodina, M.; Gela, D. Spermiation of Paddlefish (*Polyodon spathula*, Acipenseriformes) Stimulated with Injection of LHRH Analogue and Carp Pituitary Powder. *Aquat. Living Resour.* **2001**, *13*, 455–460. [CrossRef]
14. Sieczyński, P.; Glogowski, J.; Cejko, B.I.; Grygoruk, C. Characteristics of Siberian Sturgeon and Sterlet Sperm Motility Parameters Compared Using CASA. *Arch. Pol. Fish* **2012**, *20*, 137–143. [CrossRef]
15. Zohar, Y.; Mylonas, C.C. Endocrine Manipulations of Spawning in Cultured Fish: From Hormones to Genes. *Aquaculture* **2001**, *197*, 99–136. [CrossRef]
16. Mylonas, C.C.; Duncan, N.J.; Asturiano, J.F. Hormonal Manipulations for the Enhancement of Sperm Production in Cultured Fish and Evaluation of Sperm Quality. *Aquaculture* **2017**, *472*, 21–44. [CrossRef]
17. Hoga, C.A.; Almeida, F.A.; Reyes, F.G.R. A Review on the Use of Hormones in Fish Farming: Analytical Methods to Determine Their Residues. *CyTA J. Food* **2018**, *16*, 679–691. [CrossRef]
18. Islam, P.; Hossain, M.I.; Khatun, P.; Masud, R.I.; Tasnim, S.; Anjum, M.; Islam, M.Z.; Nibir, S.S.; Rafiq, K.; Islam, M.A. Steroid Hormones in Fish, Caution for Present and Future: A Review. *Toxicol. Rep.* **2024**, *13*, 101733. [CrossRef]
19. Pătruică, S. *Biotehnologii în Apicultură și Sericicultură*; Editura Eurobit: Timișoara, Romania, 2021; pp. 39–62.
20. Iliești, N.V. *Apilarnil*; Editura Apimondia: București, Romania, 1991.
21. Erdem, B.; Özkök, A. Can Food Supplement Produced from Apilarnil Be an Alternative to Testosterone Replacement Therapy? *Hacet. J. Biol. Chem.* **2017**, *45*, 635–638. [CrossRef]
22. Sidor, E.; Dżugan, M. Drone Brood Homogenate as a Natural Remedy for Treating Health Care Problems: A Scientific and Practical Approach. *Molecules* **2020**, *25*, 5699. [CrossRef]
23. Moraru, D.; Alexa, E.; Cocan, I.; Obiștioiu, D.; Radulov, I.; Simiz, E.; Berbecea, A.; Grozea, A.; Dragomirescu, M.; Vintilă, T.; et al. Chemical Characterization and Antioxidant Activity of Apilarnil, Royal Jelly, and Propolis Collected in Banat Region, Romania. *Appl. Sci.* **2024**, *14*, 1242. [CrossRef]
24. Sidor, E.; Miłek, M.; Zaguła, G.; Bocian, A.; Dżugan, M. Searching for Differences in Chemical Composition and Biological Activity of Crude Drone Brood and Royal Jelly Useful for Their Authentication. *Foods* **2021**, *10*, 2233. [CrossRef]
25. Elashal, M.H.; Abd El-Wahed, A.A.; Mohamed, M.A.; Hamad, R.; Abd Eldaim, M.A.; Khalifa, S.A.M.; Aldahmash, B.; El-Seedi, H.R.; El-Aarag, B. Apilarnil Ameliorates Bisphenol A-Induced Testicular Toxicity in Adult Male Rats via Improving Antioxidant Potency and PCNA Expression. *Reprod. Toxicol.* **2024**, *125*, 108570. [CrossRef]

26. Suleiman, J.B.; Bakar, A.B.A.; Mohamed, M. Review on Bee Products as Potential Protective and Therapeutic Agents in Male Reproductive Impairment. *Molecules* **2021**, *26*, 3421. [CrossRef]
27. Nainu, F.; Masyita, A.; Bahar, M.A.; Raihan, M.; Prova, S.R.; Mitra, S.; Emran, T.B.; Simal-Gandara, J. Pharmaceutical Prospects of Bee Products: Special Focus on Anticancer, Antibacterial, Antiviral, and Antiparasitic Properties. *Antibiotics* **2021**, *10*, 822. [CrossRef] [PubMed]
28. Yücel, B.; Açıkgöz, Z.; Bayraktar, H.; Seremet, Ç. The Effects of Apilarnil (Drone Bee Larvae) Administration on Growth Performance and Secondary Sex Characteristics of Male Broilers. *J. Anim. Vet. Adv.* **2011**, *10*, 2263–2266.
29. Zhumanov, K.; Tadzhiev, K.; Seisenov, B.; Shimelkova, R.; Mustafin, M.; Tileuberdiev, A.; Kali, M. Influence of Drone Homogenate on Sperm Productivity and Semen Quality of Stud Bulls in the Republic of Kazakhstan. *Casp. J. Environ. Sci.* 2025; *in press*.
30. Shoinbayeva, K.B. Biologically Active Preparation and Reproductive Function of Stud Rams. *Asian J. Pharm.* **2017**, *11*, 184–191.
31. Altan, Ö.; Yücel, B.; Açıkgöz, Z.; Şeremet, Ç.; Kösoğlu, M.; Turgan, N.; Özgönül, A.M. Apilarnil Reduces Fear and Advances Sexual Development in Male Broilers but Has No Effect on Growth. *Br. Poult. Sci.* **2013**, *54*, 355–361. [CrossRef] [PubMed]
32. Lenický, M.; Sidor, E.; Dianová, L.; Tirpák, F.; Štefunková, N.; Dżugan, M.; Halo, M., Jr.; Halo, M.; Slanina, T.; Urban, I.; et al. The Effect of Bee Drone Brood on the Motility and Viability of Stallion Spermatozoa—An In Vitro Study. *Vitr. Cell. Dev. Biol. Anim.* **2024**, *60*, 596–608. [CrossRef]
33. Bolatovna, K.; Rustenov, A.; Eleuqalieva, N.; Omirzak, T.; Akhanov, U. Improving Reproductive Qualities of Pigs Using the Drone Brood Homogenate. *Biol. Med.* **2015**, *7*, BM-091-15.
34. Lo Presti, V.; Cavallaro, M.; Di Rosa, A.R. Bee-Derived Products in Aquaculture Nutrition: A Comprehensive Review of Impacts on Fish Performance, Health, and Product Quality. *Animals* **2025**, *15*, 3153. [CrossRef]
35. Chebanov, M.S.; Galich, E.V. *Sturgeon Hatchery Manual*; FAO: Ankara, Turkey, 2013.
36. Igna, V.; Telea, A.; Florea, T.; Popp, R.; Grozea, A. Evaluation of Russian Sturgeon (*Acipenser gueldenstaedtii*) Semen Quality and Semen Cryopreservation. *Animals* **2022**, *12*, 2153. [CrossRef]
37. Hafez, E.S.E. *Reproduction in Farm Animals*, 5th ed.; Lea & Febiger: Philadelphia, PA, USA, 1987.
38. World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th ed.; WHO: Geneva, Switzerland, 2010. Available online: https://iris.who.int/items/04913c19-c92f-44a6-ad69-afd32205fd95 (accessed on 10 December 2025).
39. Chenoweth, P.J.; Lorton, S.P. *Animal Andrology: Theories and Applications*; CABI: Wallingford, UK, 2014.
40. Kowalski, R.K.; Cejko, B.I. Sperm Quality in Fish: Determinants and Affecting Factors. *Theriogenology* **2019**, *135*, 94–108. [CrossRef]
41. Alavi, S.M.H.; Rodina, M.; Gela, D.; Linhart, O. Sperm Biology and Control of Reproduction in Sturgeon: (I) Testicular Development, Sperm Maturation and Seminal Plasma Characteristics. *Rev. Fish Biol. Fish* **2012**, *22*, 695–717. [CrossRef]
42. Piros, B.; Glogowski, J.; Kolman, R.; Rzemieniecki, A.; Domagała, J.; Horváth, Á.; Urbányi, B.; Ciereszko, A. Biochemical Characterization of Siberian Sturgeon (*Acipenser baerii*) and Sterlet (*Acipenser ruthenus*) Milt Plasma and Spermatozoa. *Fish Physiol. Biochem.* **2002**, *26*, 289–295. [CrossRef]
43. Pavlick, R.J., Jr.; Moberg, G.P. Dopaminergic Influence on Gonadotropin Secretion in White Sturgeon (*Acipenser transmontanus*). *Fish Physiol. Biochem.* **1997**, *16*, 35–43. [CrossRef]
44. Baradaran Noveiri, S.; Noori, A.; Bahmani, M.; Yazdani Sadati, M.A.; Akbarzadeh, A. Effects of seminal plasma ionic content, pH and osmolality on spermatozoa motility in bester (Female *Huso huso* × Male *Acipenser ruthenus*) sturgeon. *Iran. J. Fish Sci.* **2019**, *18*, 395–404.
45. Psenicka, M.; Alavi, S.M.H.; Rodina, M.; Cicova, Z.; Gela, D.; Cosson, J.; Nebesarova, J.; Linhart, O. Morphology, Chemical Contents and Physiology of Chondrostean Fish Sperm: A Comparative Study between Siberian Sturgeon (*Acipenser baerii*) and Sterlet (*Acipenser ruthenus*). *J. Appl. Ichthyol.* **2008**, *24*, 371–377. [CrossRef]
46. Islam, M.S.; Akhter, T. Tale of Fish Sperm and Factors Affecting Sperm Motility: A Review. *Adv. Life Sci.* **2011**, *1*, 11–19. [CrossRef]
47. Billard, R. Spermatogenesis and Spermatology of Some Teleost Fish Species. *Reprod. Nutr. Dev.* **1986**, *26*, 877–920. [CrossRef]
48. Toth, G.P.; Ciereszko, A.; Christ, S.A.; Dabrowski, K. Objective Analysis of Sperm Motility in the Lake Sturgeon, *Acipenser fulvescens*: Activation and Inhibition Conditions. *Aquaculture* **1997**, *154*, 337–348. [CrossRef]
49. Alavi, S.M.H.; Cosson, J.; Karami, M.; Amiri, B.M.; Akhoundzadeh, M.A. Spermatozoa Motility in the Persian Sturgeon, *Acipenser persicus*: Effects of pH, Dilution Rate, Ions and Osmolality. *Reproduction* **2004**, *128*, 819–828. [CrossRef] [PubMed]
50. Ingermann, R.L.; Holcomb, M.; Robinson, M.L.; Cloud, J.G. Carbon dioxide and pH affect sperm motility of white sturgeon (*Acipenser transmontanus*). *Exp. Biol.* **2002**, *20*, 2885–2890. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.