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Sperm maturation process in fishes (review)

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Prohlášení

Prohlašuji, že jsem autorem této kvalifikační práce a že jsem ji vypracoval pouze s použitím pramenů a literatury uvedených v seznamu použitých zdrojů.

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Podpis

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Zásady pro vypracování

Understanding the basic features of spermatozoon biology is vital for the development of artificial reproduction technologies in fishes required for aquaculture and species conservation measures. Nowadays, the specific properties of fish spermatozoa in terms of their motility activation and fertilizing ability are actively studied. At the same time, sperm maturation, as a process that develops the potential for motility and fertilization in morphologically fully developed spermatozoa, was studied just in a few fish species. This process is complicated for the study because of the diverse anatomical structure of the urogenital system in different fish taxa. This bachelor thesis aims to review current knowledge in the field of sperm maturation in relation to taxa-specific features of urogenital structure in fishes.

As fishes are a group of animals consisting of several classes, the review in its first part should consider the taxonomy of fishes concerning their taxa-specific structure of the urogenital system. In the second part, the examples of sperm maturation processes in groups of Actinopterygli (e.g., sturgeons and salmonids) and Elasmobranchii (e.g., sharks and stingrays) will be described. Finally, in the third part of the review, examples of the application of knowledge about sperm maturation for fisheries practice will be presented. The review will be elaborated based on an intensive literature search available in the USB library.

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Contents

1. Introduction

Understanding the biology of fish spermatozoa is necessary for any development or advancements in artificial reproduction technologies. Artificial reproduction is required mainly for fisheries practice but also progressively more for species conservation measures. Nowadays, many specific properties of fish spermatozoa and their biology have been researched and are being actively studied, such as their activation of motility, fertilizing abilities, or their structure. On the other hand, not as much is known about the sperm maturation process. During this process, morphologically fully developed spermatozoa acquire the potential for motility activation, via external activating factors. Although this process is necessary for fish egg fertilization, it has been studied in only a few fish species.

Sperm maturation process is a complicated topic for the study as it is highly impacted by the diverse anatomical structures of the urogenital system, which is very taxa-specific among fishes. This process is also closely related to spermatogenesis, its hormonal regulation, and sperm structure and sperm motility regulation. That is why all these aspects will be considered together in this review to understand better how knowledge of sperm maturation will be helpful for the management of fish reproduction in aquaculture and biodiversity conservation measures.

2. Aims

This thesis aims to review the current knowledge in the field of sperm maturation in different fish taxa in relation to:

1) general process of spermatogenesis,

2) sperm structure and motility activation by external factors,

3) taxa-specific features of the urogenital system,

4) hormonal regulation of spermatogenesis and sperm maturation,

5) application for reproduction in practice.

3. General description of spermatogenesis

3.1. Main phases of spermatogenesis in relation to testis structure

Spermatogenesis is a highly organized and coordinated process taking place in the testes, during which spermatozoa develop. It is the male version of gametogenesis, just as in females it is oogenesis. A small number of diploid germ cells, spermatogonia, produce many haploid spermatozoa with a recombined genome.

Two morphologically and physiologically different types of testes can be distinguished, a tubular type (see figure 1A), as seen in the guppy *Poecillia reticulata*. In this type the spermatozoa are stored in a large cavity in the centre of the testis and form clusters called spermatozeugmas. From the cavity, tubules emerge outwards and at their blind end, the so-called apex, spermatogonia concentrate. Below those spermatogonia, type B spermatogonia can be found organized into the so-called cysts, which are progressing towards the centre of the testicular cavity during spermatogenesis. A more advanced lobular type of testes (see figure 1B) can be found for instance in trout. In this type, there is connective tissue extending from the testicular capsule and forming irregular tubes, "lobes".
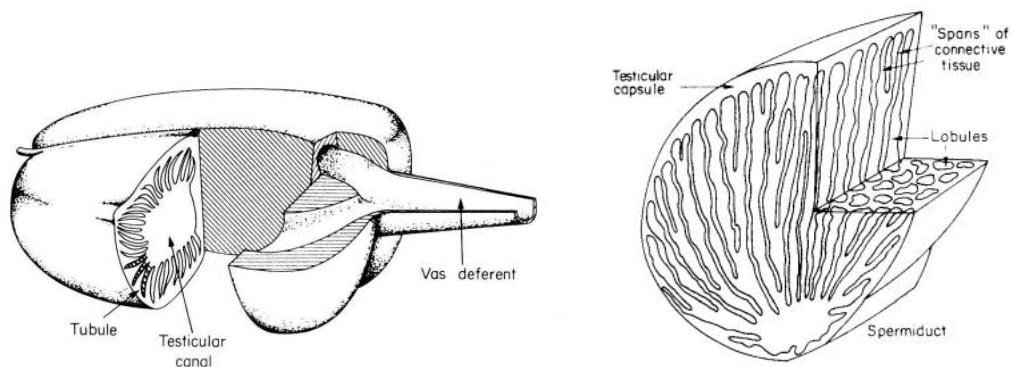
A) Tubular type (guppy)   B) Lobular type (trout)

Figure 1: Schematic structures of the testis of the guppy (A) and trout (B) (Billard, 1986)

The apex of these tubes is very close to the testicular capsule, with the tubes concurring towards a sperm collection system. Type A spermatogonia are found all over the lobule, opposing to the tubular type. As spermatogenesis progresses, the cysts, in which spermatogonia are found just like in the tubular type, move towards the centre of the

lobule only slightly. The spermatozoa are first released into the lobule lumen, and then from there, they reach the efferent and deferent ducts. (Billard, 1986)

Spermatogonia develop in close and continuous contact with somatic elements of the testis, without which they could not survive. Among these elements, Sertoli cells have a crucial role. They form the cysts' walls, providing structural support for the developing germ cells. These cysts are just an organization of a germinal compartment within the testis. Initially, in the earliest stage of spermatogenesis, this cyst consists of a single primary spermatogonium vestured by a few Sertoli cells. The Sertoli cells are in contact with a basement membrane, which makes a border between the germinal compartment and the interstitial compartment of the testis (Schulz et al., 2010). As spermatogonia proliferate, their number inside the cyst rapidly increases. Apart from this purpose, Sertoli cells also secrete a fluid that creates a tubular lumen. Additionally, they get rid of dead germ cells and residual sperm via phagocytosis and regulate spermatogenesis and gonadal steroid hormone production (Cosson, 2019).

Spermatogenesis starts with a single spermatogonium, an undifferentiated male germ cell, that undergoes several mitotic cell cycles. Each generation of spermatogonia slightly and gradually morphologically differentiates from the previous one. The number of cell cycles is dependent on the species of fish. For instance, in the zebrafish, nine mitotic cell cycles have been observed (Leal et al., 2009) and in the Japanese eel, ten cycles have been distinguished (Miura et al., 1991a). When these mitotic cell cycles end, type B spermatogonia emerge, then they enter the last mitosis and develop into a primary spermatocyte. During this mitosis, two daughter cells are created, one remains a spermatogonium and the other differentiates into a primary spermatocyte. This one primary spermatocyte then replicates DNA and makes two secondary spermatocytes via meiosis. At that point, another meiosis takes place. Secondary spermatocytes are very short-lived (1.1-1.7 days). Since they initiate the second meiotic division without synthesizing DNA, the number of chromosomes stays the same, and they develop into four spermatids. These spermatids are the first haploid, and thus, genetically unique cells in this process. Lastly, the spermatids differentiate into spermatozoa by an extensive remodelling process called spermiogenesis. At the beginning of spermiogenesis, spermatids are circular and have a nucleus, Golgi apparatus, centriole, and mitochondria (Kim, 2016). During spermiogenesis, the DNA becomes highly condensed, a head forms, midpiece develops, and a distal centriole forms into an axoneme. And in fishes where it

does, this is when the acrosome develops. Since two meioses took place in the process, four haploid spermatids are created from every single diploid type B spermatogonium.

The production of spermatozoa in the testes is cyclic. It is either continuous throughout the year, as in the guppy, where the production of spermatozoa is permanent, or not continuous, as in trout (see figure 2), tench *Tinca tinca*, pike *Esox lucius* and roach *Rutilus rutilus*, where two distinct cycles success each other. In this case, one cycle's spermatozoa are all eliminated before the next cycle begins (Billard, 1986). In the case of carp *Cyprinus carpio,* spermatogenesis is discontinuous, but unlike in trout, the two cycles overlap.
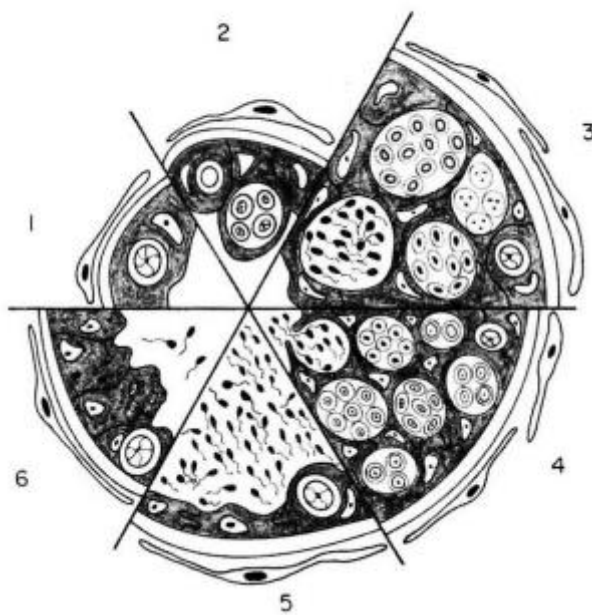
Figure 2: Spermatogenetic cycle in a rainbow trout *Oncorhynchus mykiss* lobule; schematic illustration (Billard, 1986)

1) Inactive phase of spermatogenesis; 2) primary spermatogonium multiplication 3) active spermatogenesis; lobular size increases 4) active spermatogenesis; release of early spermatozoa; 5) spermiation; 6) spermatozoa are eliminated by release or resorption

The period within which spermatogenesis is active depends on the given species and varies greatly even within the same family, e.g., *Cyprinidae*. These periods follow different patterns, for instance, in carp, trout and pike it occurs in summer, in tench spermatogenesis happens entirely in spring and in roach it persists from autumn to spring, although it is interrupted in winter as a consequence of low temperatures.

Spermatogenetic production is surprisingly stable among fish species, averaging around 100,000 spermatozoa per day per gram of testes. This makes weighing testes a

popular method of estimation of spermatozoa yield, however, there are concerns about spermatozoa yield after spermiation, as the percentage of spermatozoa that successfully undergo spermiation can range anywhere from 3% to almost 100% (Billard, 1986).

Spermatozoa already have a structurally complete flagellum (Cosson, 2019). with all its elements – axoneme and plasma membrane surrounding it. During spermatogenesis, energy must be accumulated for later use during sperm motility. However, bioenergetic pathways involved into spermatogenesis is a purely studied area in contrast to sperm motility ones. Simultaneously, decreased spermatozoon cytoplasmic volume during spermatogenesis and the presence of mitochondria suggest the vital role of mitochondrial respiration in sperm bioenergetics. The respiration rate in inactive spermatozoa before motility activation must be fast enough to maintain the proper ATP levels before spermiation. This respiration rate is sufficient for supporting basic sperm metabolism (Dzyuba et al., 2017).

The creation of one male gamete is less resource-draining compared to one female gamete, as eggs are rich in reserves and much larger, however, this is countered by the total number of gametes created (Schulz et al., 2010).

Concluding this part of the review, it should be remarked that spermatogenesis in fishes is a taxon-specific process in terms of it's localization in the testis, the number of cell divisions in the cysts, duration of the whole process and subcellular structures in which spermatogenesis takes place. The later structures (spermatocysts and spermatozeugmas) can be considered important participants in the sperm maturation process, which is the current review's main object.

3.2. Sperm maturation as the final stage of spermatogenesis

Sperm maturation is the last physiological stage of spermatogenesis and more specifically spermiogenesis. During sperm maturation, immature morphologically fully developed spermatozoa get the ability to respond to motility-activating factors. This enables them to respond to motility-activating factors and hence acquire motility and be able to fertilize eggs. This process involves physiological changes; however, no morphological alterations occur. Upon contact with an appropriate environment, mature spermatozoa gain motility immediately. Conversely, under the same external conditions, immature spermatozoa are incapable of becoming motile, showing the necessity of sperm maturation process for fish reproduction (Schulz and Miura, 2002).

Sperm maturation is associated with the process of spermiation, which is determined as the breakage of contact between Sertoli cells by which spermatozoa are released from spermatocysts into the lumen of efferent ducts. In a broader meaning, it indicates that mature spermatozoa have been released into the sperm ducts and are ready to be discharged or stripped. However, in this case, the term spermiation is not entirely correct and could be called the production of milt instead (Baynes and Scott, 1985).

As sperm maturation is a process which varies highly between taxa, examples in some fishes will follow below, and the necessary conditions for maturation of spermatozoa will be described.

3.3. Sperm maturation in different fish taxa

In this thesis, the vertebrate classification introduced in 2016 (Nelson et al., 2016), in which classes of Myxini, Petromyzontidae, Chondrichthyes and some representatives of Osteichthyes class are considered as fishes will be used. Actinopterygii, also called ray-finned fishes, is a subclass of class Osteichthyes with the most species, comprising about 96% of all fish and over 50% of all vertebrates. This subclass encompasses the infraclasses Cladistia (bichirs), Chondrostei (sturgeons and paddlefishes), Holostei (gars, bowfins, and relatives) and subdivision Teleostei, where the majority of fish fall in. Since teleosts are the most widely used fishes in aquaculture, most research and experiments on sperm maturation have been conducted on them. However, there has been some research on Chondrosteans and other fishes.

3.3.1. Sperm maturation in Osteichthyes (the case of Actinopterygii)

Sperm maturation of Teleost fish has been studied on a few different species and suggests different narratives in different species.

Generally, in teleosts, sperm maturation takes place directly in the testes after the completion of sperm formation, and mature sperm capable of activation of motility can be collected via stripping or by the extraction of testes and the usage of testicular spermatozoa.

In salmonids, maturation has been shown to be mainly under the control of cAMP (cyclic adenosine monophosphate) and the pH of the water fish swim in during migration for reproductive reasons. Research conducted by Morisawa and Morisawa (1988) on

rainbow trout and chum salmon *Oncorhynchus keta* has demonstrated that the spermatozoa of these fishes, albeit having no potential for motility in the testes despite being otherwise mature, acquire that potential in the main sperm duct, vasa deferentia after passage through the efferent ducts (vasa efferentia). This can be attributed to the higher pH value (approximately 8.0) and a higher concentration of HCO^{3-} in the sperm duct, as opposed to the testes. Since the testicular sperm of rainbow trout and chum salmon that has been collected in this study gained motility after an hour of incubation in artificial seminal plasma containing HCO_3^- and a higher pH level, these circumstances have been identified as the deciding factors. The higher pH level results in an increase in cAMP levels inside spermatozoa. This increased cAMP level in turn allows the acquisition of motility. The hormonal regulation of seminal fluid pH, an ability fish naturally have, appears to be a crucial component of sperm maturation, as demonstrated by various studies. E.g., a study by Morisawa et al. (1993) compared the motility of testicular spermatozoa of chum salmon caught in the sea to the motility of sperm duct spermatozoa of chum salmon caught in the river wherein it migrated. These findings indicate that during the migration as spermatozoa naturally move from the testis to the sperm duct, their motility increases significantly due to the factors stated above.

In eel species sperm maturation is influenced by multiple factors; seminal plasma K^+, pH, HCO_3^- concentration and Na^+ concentration. Similar to rainbow trout and chum salmon, in the Japanese eel there has been no sperm maturation of testicular sperm that has not been in contact with the sperm duct. However, it has been demonstrated that incubation in a solution with specific HCO_3^- concentrations and/or high pH (Miura et al., 1995) has been able to induce motility, increasing with the HCO_3^- concentration used, naturally only up to some concentration ceiling. Interestingly, when a high enough pH (8.4 – 8.7; higher than seminal pH) was introduced, testicular spermatozoa have been able to get their motility induced even though a HCO_3^--free artificial seminal plasma has been used. This demonstrates that one factor, if high enough, can mitigate the need for the other. K^+ concentration has been deemed to be an important factor in eels since experiments conducted on both Japanese eel *Anguilla japonica* and European eel *Anguilla anguilla* showed that removal of K^+ decreased motility rapidly and it could be reversed again with the addition of said ions.

In carp, a study carried out by Redondo-Müller et al. (1991) suggests that the main factor responsible for maturation is an ionic equilibration across the sperm membrane. This has been examined by a dilution of the carp semen into 200 mM KCl medium and

showed semen to preserve its potential motility and in sperm of low initial motility to even regenerate it gradually, demonstrating K^+ dependence for conservational and regenerative capabilities, with the same applying to Na^+.

Chondrostei, sturgeons and paddlefish, have very specific demands for sperm maturation, regarding their unique urogenital system. Spermatozoa undergo final maturation under conditions that are vastly different from those in the testes. Both urine and seminal fluid in the Wolffian ducts possess different properties than sperm has in the testes; they have lower concentrations of ions Na^+, K^+ and Ca^{2+}, but also a higher pH and lower osmolality and protein content (Dzyuba et al., 2014a). Lately, it has been found that the sensitivity of spermatozoa to Ca^{2+} can fluctuate during sperm maturation. An experiment performed by Bondarenko et al. (2017) on the sterlet has revealed that in order to induce the motility of testicular spermatozoa, the activation medium had to be two to three times higher in Ca^{2+} concentrations compared to the spermatozoa in Wolffian ducts.

Previously, it was thought that mixing chondrostean sperm with urine is to be avoided and that it ought to be a contaminating factor. However, experiments carried out by Dzyuba et al. (2014a) on the sterlet show, that not only is the contact between urine and sperm of chondrostean fishes not dangerous but that it is unavoidable, as it is even a necessary maturation step. This conclusion was reached since sperm collected from Wolffian ducts during the study on sterlet was able to become motile, but spermatozoa collected directly from testes, prior to contact with urine, were not able to become motile even after being exposed to an activating medium. Furthermore, it was observed that motility of testicular sperm has been achieved after in vitro incubation in urine or even just in seminal fluid from the Wolffian ducts (Dzyuba et al., 2014b)

In summary, sperm maturation of osteichthyans is diverse, with most notable differences between the groups of 1) eels and salmonids; where sperm maturation takes place in the specific parts of sperm ducts and pH and specific ionic compositions are important, and 2) chondrosteans, where contact of sperm with urine is necessary for maturation and 3) other teleosts, where sperm matures in the testes and testicular spermatozoa can be used. Another different approach to sperm maturation can be observed in Chondrichthyes.

3.3.2.Sperm maturation in Chondrichthyes (the case of Elasmobranchii)

In elasmobranchs, the whole process of sperm maturation occurs in the genital ducts. This has been concluded since in the Banded houndshark *Triakis scillium*, sperm was immotile in the testes but motile in the vesicula seminalis (Minamikawa and Morisawa, 1996). A lot of attention regarding this topic has been drawn to the epididymis (see figure 3), as its biosynthetic, absorptive and resorptive activities have been deemed to be implicated in the spermatozoa maturation process (McClusky, 2015).

It should be noted that there are similarities between the Elasmobranch's reproductive organ anatomy in males to mammalian, bird, and reptilian reproductive anatomy, as in these groups with internal fertilization, sperm undergoes maturation during the passage through the ductus deferens. The epididymis is very long and convoluted in the Elasmobranchs, giving sperm plenty of space for maturation.
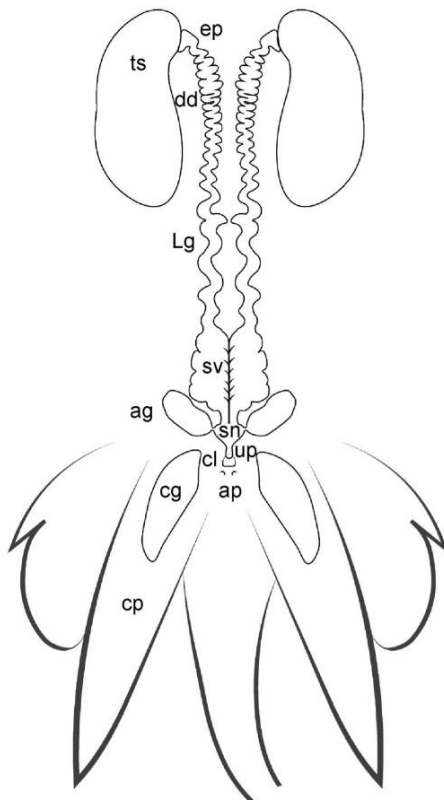

Figure 3: Male reproductive organ anatomy of Chondrichthyes (García-Salinas et al., 2021)

Ag = alkaline gland; ap = abdominal pores; cg = clasper gland; cl = cloaca; cp = clasper; dd = ductus deferens; ep = epididymis; Lg = Leydig gland; sn = sinus; sv = seminal vesicle; ts = testes; up = urogenital papilla

The secretory activities of the epididymal epithelium in vertebrates is important for the spermatozoa maturation since that epithelial activity is partly responsible for the production of the luminal fluid, a fluid highly distinctive from the seminal plasma (Cornwall, 2008). Lately, similar narrative has been suggested in some Elasmobranch; in the Magdalena river stingray *Potamotrygon magdalenae*, the epididymis showed epithelial changes correlated to the observed macroscopic external regions. This is likely related to different functions in the sperm maturation process, however, this has not been described in other Elasmobranchs, where the epithelium mostly remains stable during sperm maturation, as in the ray *Himantura signifer* (Del Mar Pedreros-Sierra and Ramírez-Pinilla, 2014).

At the same time, the presence of sperm maturation process in elasmobranchs, occurring along the sperm transit through the reproductive tract (more precisely epididymis) was strongly confirmed by Dzyuba et al (2019b).

Interestingly, on the Banded houndshark, it has also been shown that the motility of its spermatozoa has been quite stable under the conditions of pH levels ranging from 5-9, suggesting that the seminal plasma pH may not be as important a factor as in some other fishes (Minamikawa and Morisawa, 1996).

Concluding this chapter, it should be summarised that sperm maturation itself is a final stage of spermatogenesis, which exists in evolutionary distant fish taxa. This process impacts the success of fertilization via physiological processes responsible for the preparation of spermatozoa for motility. Because of the remarkable diversity of fish reproductive performances, their spermatozoa vary greatly among fish taxa and this variability will be shortly described below.

4. Remark of spermatozoa diversity in fishes

Sperm exhibit extraordinary morphological diversity and are among the most variable of all known cell types (Pitnick et al., 2009).

Spermatozoa structure in fish is of interest for studies mainly because specific morphological differences among sperm may reflect differences in functional capabilities and phylogeny (Psenicka et al., 2007). One of the differences between fishes' spermatozoa structure is the presence or absence of the acrosome, an organelle containing degradative enzymes for the sperm to get into the ovum. Currently, there is no clear conclusion whether the sperm maturation process is associated with acrosome

modifications. In Actinopterigian fishes spermatozoa are qualified as "simple sperm", as the flagellum structure lacks additional columns on the sides of the axoneme, as can be found in mammalian sperm or Chondrichthyes spermatozoa (Jamieson, 1991).

In the first studies based on the structure, mode of fertilization, and cellular organization, spermatozoa were classified as either basal or derived. Basal spermatozoa are usually found in animals with external fertilization. They are characterized by having, among other things, an acrosome, and a spherical nucleus.

Most Teleostei fishes have retained the original method of insemination by emission of gametes into the water and therefore were previously considered to be of the basal type. Most Teleostei spermatozoa have also retained the primitive spherical or subspherical head found in basal sperm, however, most have lost their acrosome. Their middle piece is short and usually contains about 4 or 5 mitochondria at the base of the nucleus, which would also indicate the spermatozoa of Teleostei to be basal, as that is characteristic of this type. However, later, the studies of Mattei (1991) have shown that this issue is not so straightforward and the diversity in the shape of fish spermatozoa is immense.

The acrosome has been lost in the Neopterygii subclass, in which Teleostei fish fall in along with Holostei. That is one of the only common traits regarding this topic among Neopterygii. Given the purpose of the acrosome, these fish had to substitute it by presenting a micropyle in the egg. That is a structure in the egg envelope that gives spermatozoa access to the oocyte, allowing it to pass through the otherwise impenetrable chorion.

Teleosts are a very diverse group of fishes with over 26,000 species being currently described, so it's no wonder there's a high diversity of sperm structure among them.

In both chondrosteans and chondrichthyans, the acrosome is present. All species of Chondrichthyes have an internal mode of fertilization, unlike in Osteichthyes, where only about 2% of species use this strategy. Species with external fertilization exhibit a simpler sperm organization in comparison to internally fertilizing species. Interestingly, in sharks with a lot of post-copulatory sexual selection, sperm has been shown to increase in flagellum length (Rowley et al., 2019), showing the variability and adaptability of sperm cells.

The sperm of different fish taxa differs mainly by size, the number of mitochondria in the midpiece, the presence or absence of an acrosome and the flagellum structure. The flagellum's shape and structure can affect spermatozoa's ability to move through water or

the female reproductive tract and thus its fertilizing capability. Remarkable differences can be observed between spermatozoa of internally and externally fertilizing fishes. Spermatozoa of internally fertilizing species are more adapted to survive longer and compete with spermatozoa from other males inside female reproductive tract than ones in species with external fertilization.

When spermatozoa are fully morphologically developed and undergo maturation, at the very end of spermatogenesis after spermiation, they are able to become motile under the right circumstances.

5. Activation of motility

Since most fish species undergo external fertilization and given the aquatic environment, the activation of sperm motility is typically initiated through water exposure in most species and spermatozoa are generally immotile inside the testes and sperm ducts, as their motility is suppressed by iso-osmolality and specific ionic content (Dzyuba et al., 2019a).

However, there are some exceptions, e.g., some species of Cottidae such as the sperm of freshwater sculpin *Cottus hangiongensis,* which can already be motile in the sperm duct (Koya et al., 1993).

In fish species where sperm activation occurs in an external environment, two modes of activation are commonly distinguished. An ionic mode, where motility is triggered by external ions, or an osmotic mode of activation, where the motility of spermatozoa gets activated by an osmotic shock. Fish with an osmotic mode of activation have specific requirements for water conditions regarding their osmolality, as freshwater fish require a hypoosmotic shock in the water environment to initiate sperm motility. This also relates to issues with urine activation, since urine is hypoosmotic to seminal fluid in fish. On the other hand, marine fish have the opposite requirements, as the osmolality of seawater is around 1000 mOsm*kg^{-1} while the fish body is around 300 mOsm*kg^{-1}, therefore their spermatozoa gain motility under hyperosmotic conditions. The ionic mode of activation is typically found in salmonids and sturgeons, where sperm is activated by the efflux of intracellular K$^+$, which is reached since the concentration of K+ ions is much lower in freshwater. The optimal osmolality for sperm motility in freshwater fish varies between species, and ranges from sturgeons with 0 to 120 mOsm*kg^{-1}, through carp with 150 – 200 mOsm*kg^{-1} to Salmonidae with up to 300 mOsm*kg^{-1}.

Motility can be activated even via some other signalling molecules, such as CO_2 in some cases (Inaba et al., 2003), but it's rather rare, so it mostly relates to the osmolality and the ionic fluxes in some cases. Some fish species need extracellular calcium in order to activate sperm motility. This has been shown in the Tilapia *Oreochromis niloticus*, some herrings and some salmonid species (Yanagimachi and Kanoh 1953; Baynes et al., 1981). Tilapia is a specific case regarding Ca^{2+}, as since it is a euryhaline species, it can reproduce in both freshwater and marine environments. In freshwater-acclimated tilapia, their spermatozoa don't need external Ca^{2+} for activation, yet in fish acclimated to marine conditions, Ca^{2+} is required (Linhart et al., 1999).

In chondrichthyans, sperm is already mature in the male reproductive tract, however, in some species does not gain its motility until contact with the female's uterus fluid. This has been concluded from study of shark, *Triakis scyllia* since when immotile spermatozoa have been experimentally diluted into electrolyte solutions with the same concentrations as the uterus fluid, they became motile (Minamikawa and Morisawa, 1996). However, in other chondrichthyan species, ocellate river stingray *Potamotrygon motoro* spermatozoa are motile in seminal fluid (Dzyuba et al., 2019a).

As it is clear, that motility activation requires spermatozoa to be fully mature, and since sperm maturation is greatly related to the urogenital system structure of fishes, a review of it will now follow.

6. Diversity of fish male urogenital system structure

The urogenital system of fishes is a complicated subject with very diverse anatomical structures between different taxa, with implications on the sperm maturation process.

The morphology of the urogenital system has evolved during fish speciation.

All fishes can be generally categorized into three distinctive groups, depending on the type of connection, or lack of it, between the reproductive and excretory systems. Most commonly, the genital and urinary ducts are fully separated, found in the Chondrichthyes (sharks, rays, chimaeras) and the Teleostei. The Chondrostei and the Dipnoi have common urogenital ducts for sperm and urine discharge. At last, some fishes have only partially connected ducts (Cyclostomata, Cladistia, Holostei). These groups further subdivide in fishes with a connection between ducts depending on the severity of the connection. In fishes with separated ducts, the external openings of these ducts are either separated and distant, or they combine into a urogenital papilla. These

distinctions may seem insignificant, however, they can and do affect numerous aspects of fish spermatology. (Dzyuba et al., 2019a)

Teleost fishes fall under the category of fish with fully separated reproductive and urinary ducts (see figure 4). Despite the rich diversity of Teleost fish, the structure of the urogenital system stays highly similar among the different species.

In most teleosts, testes are usually rounded and elongated, and their fused sperm ducts open directly to the outside without connecting to urinary tracts (see figure 4).
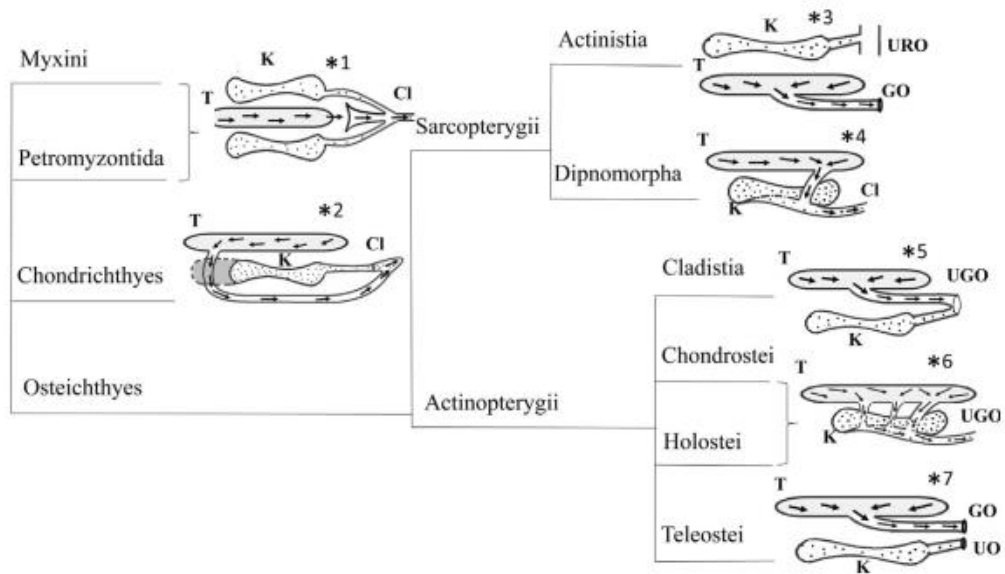

Figure 4: Simplified schematic representation of male urogenital system structure in fishes (Dzyuba et al., 2019a).

Cl = cloaca; GO = genital opening; K = kidney; T = testis; UGO = urogenital opening; UO = urinary opening; URO = uro-rectal opening

There are however some outliers; for instance in salmonids (see figure 5), the degree to which urinary and genital ducts are separated is unique even among all vertebrates (Lombardi, 1998). Salmonids have their testes paired and extending the length of the coelomic cavity. The ducts of each testis fuse into one, forming a common duct. This common duct then opens into a coelomic cavity, wherein the coelomic funnel is located. Furthermore, this coelomic funnel connects the surrounding coelomic cavity to the outside, using a single genital pore, located between the anus and the excretory pore

(Lombardi, 1998). Generally, the distinctions between urogenital systems between different taxa influence where sperm maturation takes place.
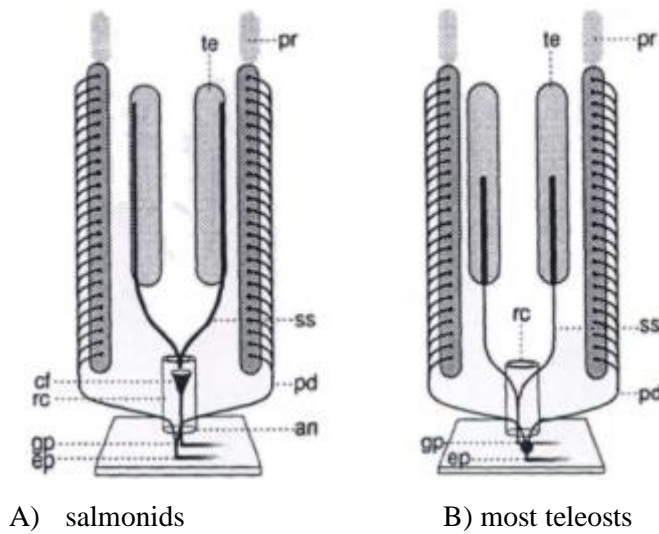

A) salmonids   B) most teleosts

Figure 5: Diagrams showing the integration of male urinary and genital components in teleosts (Lombardi, 1998).

an = anus; cf = coelomic funnel; ep = epoophoron; gp = genital pore; pd = primary urinary duct; pr = pronephric rudiment; rc = rectum; ss = secondary spermiduct; te = testis

The teleost kidney has multiple functions, as it is usually divided into two functionally different parts, with the cranial part being responsible for lymphoid, interrenal, hematopoietic and suprarenal functions and the caudal part mostly for renal function. These two parts can be fused fully or partially, or entirely separate. From the kidneys, two mesonephric ducts emerge and further connect to transfer urine outside. (Dzyuba et al., 2019a)

Regarding the separation of urinary and genital ducts, the role of urine in teleost fishes' sperm biology is almost negligible, especially compared to chondrostean fishes and the Dipnoi reviewed later, only raising concerns about possible contamination of sperm during artificial sperm collection, since urine in freshwater fishes is hypotonic to semen, meaning there is a potential to induce motility of spermatozoa upon contact. In artificial reproduction, this is unfavourable since if motility is achieved too early, the sperm quality is reduced (Alavi and Cosson, 2006). In natural spawning, urine contamination is unlikely.

In fisheries practice, two methods have been introduced to deal with urine contamination; in some species, such as the carp, basic precautions are sufficient enough,

since urine can be manually separated from sperm. In others, such as the tench, species-specific immobilizing media must be used due to the impossibility of manual separation of urine from sperm (Rodina et al., 2004).

As sturgeons are the most notable chondrostean family, out of all the chondrostean fishes, most research about the urogenital system has been conducted on them.

The pronephroi of the chondrosteans are formed during early embryonic stages. Soon after hatching, pronephroi are fully developed and begin to deteriorate (Dodd, 1983), and are then replaced by opisthonephroi, the definitive kidneys, which develop side by side with pronephroi. Kidneys researched on the sterlet *Acipenser ruthenus* have been shown to take up almost the whole length of the body cavity and be subdivided into three parts: 1) the paired cranial compartment, functioning mainly as a hematopoietic organ; 2) the fused middle part with hematopoietic tissue, intact nephrons and testicular excretory ducts; and 3) the fused caudal part made up by hematopoietic tissue and intact nephrons (Wrobel and Jouma, 2004).

Sturgeons have paired testes with central testicular canals. From these canals, several vasa efferentia extend. The purpose of vasa efferentia is to transport sperm, they make a connection between the central testicular canals and the external lateral kidney canals. Spermatozoa created in the seminiferous tubules in the testes first travel to the testicular canals, then enter the vasa efferentia, from where they are transported to the kidneys, concretely the external and internal kidney canals. There they mix with urine and finally get to the Wolffian ducts. That means that in case of sturgeons, the Wolffian ducts have a dual purpose, as they transport both sperm and urine. There are two Wolffian ducts, which create a urogenital sinus by joining. This sinus opens to the outside via a single urogenital pore (see figure 4). (Dzyuba et al., 2019a)

Interestingly, the part of the kidneys where sperm enters still serves its urinary function and the nephrons there are not distinguishable in any way from the other nephrons, which is unique among fish species (Wrobel and Jouma, 2004).

As fishes are a large group of animals consisting of multiple classes with very different approaches to their urogenital structure, it is important to also mention other fishes apart from Teleostei and Chondrostei, although they are arguably the most relevant in our geographical region.

Myxini is a class of animals with an eel-like body living near the bottom of oceans with a simple kidney structure compared to more complex fishes, they excrete urine into and through two Wolffian ducts leading into a cloaca. Little is known about their

reproductive strategies, however, according to Lombardi (1998), mature spermatozoa are released into the body cavity, from where they travel through a coelomic funnel that further reaches and connects to a urinary duct (Wolffian duct), forming a common urogenital sinus, opening through a urogenital pore directly in the cloaca (see figure 4), meaning their sperm and urine connect only a short distance in front of the cloaca. It has been found that urinary and genital tracts may however not even connect at all and have each their own opening to the cloaca. In Petromyzontidae, the lampreys, mature spermatozoa are released into the body cavity similarly to hagfish, from where they travel through a genital funnel. The urogenital connections are also comparable to those of hagfish, with the difference that lamprey sperm does not travel to the cloaca but rather to the outside directly from the genital funnel (see figure 4) (Johnson et al., 2014).

Due to the character of urogenital structure in these two groups, sperm may potentially contact urine at ejaculation or moments before it, however, any higher importance of the contact between urine and sperm for motility activation is improbable (Dzyuba et al., 2019a).

Chondrichthyes is a large class consisting of two subclasses; Elasmobranchii, where sharks and rays belong, and Holocephali, known as chimaeras.

The internal male reproductive system comprises paired testes and paired reproductive tracts. The testes fill a large portion of the body cavity and are embedded in an epigonal organ located near the cephalic region of the animal. In these tracts diverse sections can be recognized; the proximal long convoluted part called the epididymis, the vas deferens (Wolffian ducts) and the distal widened part of the vas deferens called the seminal vesicle or ampulla, where the sperm is stored (see figure 4) (Walker, 2020). Adjacent to the ductus deferens there is a Leydig's gland, which empties its contents into the ductus as well as onto the epididymis.

Elasmobranchii pronephroi get reduced at the early stages of egg development and are soon replaced by mesonephros. Ultimately, opisthonesphroi are formed and have two different regions with separate functions.

The two different regions of opisthonephros are the anterior part, which is transformed into the epididymis and Leydig's gland, and serves as a maintenance organ for the testis, and the posterior part of opisthonephros, which on the other hand preserves its excretory function, and has a duct independent of the ducts of the anterior region. This duct then has its own urogenital sinus, opening to the cloaca. Due to this separation, male sharks

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and rays have their urinary ducts entirely separated from sperm ducts. (Dzyuba et al., 2019a)

Although Holocephali are a much smaller and thus a less studied subclass of Chondrichthyes, it is generally believed that the character of the urogenital system in these fishes is similar to that of Elasmobranchii, except the fact that Holocephali have no cloaca (García-Salinas et al., 2021).

The involvement of urine in sperm biology in chondrichthyans is very unlikely, not only are the urinary and genital tracts separated, making the contact possible only at the cloaca (or urogenital sinus in Holocephali), but also the spermatozoa are already mature in the male reproductive tract before any contact with urine (Minamikawa and Morisawa, 1996).

Osteichthyes, also called bony fishes comprise the most extant fish species. This class contains two subclasses; all Sarcopterygii and all Actinopterygii fish belong to this group. Especially Actinopterygii is an extremely diverse and aquaculturally important group of vertebrates, however, now other groups of Actinopterygii than Teleostei and Chondrostei will be reviewed. Firstly, Sarcopterygii consists of two classes; Actinistia, comprising coelacanths with only two currently living species *Latimeria chalumnae* and *Latimeria menadoensis*, and Dipnomorpha, with lungfish and species related to them. Since coelacanths are endangered species, not much is known about their male urogenital system, however, according to a study carried out by Dingerkus et al. (1978), two deferent ducts descending from the testes fuse into a common duct with an opening to the outside. Kidneys of male *Latimeria chalumnae* are opisthonephric and fuse together. From these kidneys, two ureters enter into urinary bladders and from there, urethras leave and discharge into the posterior region of the rectum without ever fusing, therefore at least males of *Latimeria chalumnae* have a single conjoint uro-rectal opening, which is completely separated from the genital ducts. It should also be noted that according to Dingerkus et al. (1978), this also means that these coelacanths have no cloaca (see figure 4), however, a study by Locket (1980) has shown the exact opposite; a connection between the urinary and genital ducts and a presence of cloaca.

Due to these ambiguous findings, no clear conclusion can be drawn on the topic of whether urine has an impact on sperm maturation in coelacanths.

In Dipnomorpha, known as lungfishes, pronephroi appear during embryonic stages (Kerr, 1900) and paired mesonephroi develop after the formation of head kidneys, at

relatively later stages in life. Unlike chondrichthyans' connection with the anterior part of the kidneys, in Dipnoi, the kidneys are connected to the testes via efferent ducts in the posterior part (see figure 4). These efferent ducts then join the Wolffian ducts, through which both sperm and urine (from the anterior part of the kidneys) transfer. Wolffian ducts either conjoin into one and enter the cloaca, or enter into the cloaca independently, depending on the species.

Due to the connection of urinary and genital tracts, urine ought to influence sperm maturation in Dipnomorpha similar to sturgeons (Dzyuba et al., 2019a)

The pronephroi of Cladistia, the bichirs, form during embryonic but degenerate early in life to be replaced by opisthonephroi. The kidneys are paired, however, do not fuse. A large urinary sinus is formed by the linkage of ureters (Budgett, 1901). No connection between the genital and urinary systems has been observed, according to Budgett (1901), the urinary sinus narrows down and joins two genital ducts just before the urogenital opening (see figure 4).

Although very little is known about the sperm biology of bichirs, due to there being no connection between urinary and genital ducts, urine implication in the sperm maturation process is improbable, with possible contaminating effects.

Holostei comprises gars and bowfins, in bowfin *Amia calva*, it has been shown that pronephroi are created during embryogeny, are present for a limited time after hatching and are ultimately lost completely in adults (Ballard, 1986), similarly to gars (Long and Ballard, 2001). The kidneys of adult bowfins fuse in the posterior region, with the anterior region containing no nephrons; only consisting of hematopoietic tissue. Their kidneys gradually contain more nephrons the closer to the posterior region. It has been concluded that there is a kidney-testis relation in bowfins (see figure 4); testes are connected to the kidneys by a mesorchium, and the sperm ultimately travels to the urogenital ducts after sequentially passing through the lateral testicular canal, numerous vasa efferentia, the lateral kidney channel and opisthonephroi tubules. The urogenital ducts widen into expansions similar to the urinary bladder, and join, forming an unpaired canal transporting both urine and sperm outside the body (Smet, 1963, cited by Dzyuba et al., 2019a).

In adult gars, the opisthonephroi behave as excretory organs and are separated anteriorly and fused posteriorly. Large, paired testes of gars are connected to

opisthonephroi by a mesorchium containing multiple vasa efferentia. Some of those vasa efferentia join the Wolffian ducts after passing through the Malpighian bodies and urinary tubules. Sperm is then transported outside through them, opening through a small papilla (Pfeiffer, 1933). As there are connections between urinary and genital ducts in gars and bowfins, urine probably doesn't have a contaminating damaging effect, however, due to insufficient information on the topic no conclusions can be drawn about its necessity for sperm maturation.

The structure of the male urogenital system of fishes plays a crucial role in the process of sperm maturation, nevertheless, the regulation of sperm maturation and spermatogenesis alike is in the control of the endocrine system.

7. Hormonal regulation of spermatogenesis and sperm maturation

Hormones serve a critical regulatory role in spermatogenesis. Three steps of spermatogenesis at which reproductive hormones are crucial regulators are: balancing between self-renewal and differentiation of spermatogonia, the transition between type A spermatogonia to rapidly propagating type B spermatogonia and the entry of spermatocytes into meiosis (Schulz et al., 2010). The most important regulatory hormones participating in spermatogenesis are FSH, LH, and sex steroids. Endocrine regulation of spermatogenesis is reached via signalling molecules derived from sources outside germ cells. Sertoli cells are crucial in endocrine communication, as they respond to an endocrine stimulus by changing the release of a growth factor, effectively mediating the hormone's impact on the germ cells. On the other hand, germ cells do not possess such receptors for FSH and sex hormones and can not respond to an endocrine stimulus themselves. When the stimulation via gonadotropins FSH and LH takes place, spermatogonial mitosis changes to rapid proliferation, preparing to later initiate meiosis, contrary to the former slow self-renewal pathway. During this early stage of spermatogenesis, FSH has a major regulatory duty, on the other hand LH is more important in the later stages of spermatogenesis, sperm maturation. For the initiation of meiosis, an indispensable hormone is one of the group of progestins, DHP (dihydroprogesterone). DHP was shown to be present in the testis during early spermatogenesis and exhibited to promote DNA replication of spermatogonia. It is not the only hormone with this function, as 11-Ketotestosterone (11-KT) mentioned below

serves the same purpose, however, a study by Miura et al. (2007) has recently shown that if antibodies of DHP were present (thus preventing DHP functionality), DNA replication of spermatogonia was not initiated despite 11-KT being involved.

Other notable hormones regulating spermatogenesis are the androgens, namely testosterone and 11-KT, and although there has been some discussion about the concrete roles of these androgens in male fish, it is generally thought that they influence some phases of spermatogenesis.

Although estrogens are predominantly female hormones, they are also formed in male fish, and in fact, all male vertebrates. In fish, estrogens have been shown to be important in regulating gene expression in the testis (Pinto et al., 2006). The levels of individual hormones fluctuate throughout the year, for instance, androgens increase gradually during spermatogenesis, peak and then decrease their levels when spermiation occurs.

Sperm maturation and spermiation are also regulated by the endocrine system. The most crucial hormone during sperm maturation is LH, as it initiates an increase in the production of androgens and other hormones. The productions of 11-KT, DHP and 20β-S (17α,20β,21-trihydroxy-4-pregnen-3-one) are all affected. The two latter are from the group of progestins and are greatly expressed in fish gonads in many species. Their levels peak during the spermiation period, and they have been shown to affect several functions of the testes. In Salmonidae and Cyprinidae they have been demonstrated by Ueda et al. (1985) to induce or at least accelerate spermiation, but also to increase milt production (Baynes and Scott, 1985), and stimulate spermatozoa motility (Miura et al., 1992). DHP has been suggested to regulate sperm maturation in some teleosts, e.g., in the Japanese eel (Miura et al., 1991c), as it seems to mediate an increase of seminal plasma pH, hence increasing the cAMP content in sperm and that allows the acquisition of motility as stated earlier. Progestins can also have a more direct effect on sperm motility in some cases, as it has been shown that in Atlantic croaker *Micropogonias undulatus* and seatrout *Salmo trutta trutta*. Progestin 20β-S binds to the sperm plasma membrane and stimulates sperm hypermotility (Tubbs and Thomas, 2008). 11-KT, apart from its function during spermatogenesis as discussed previously, also interferes during spermiation, as its injections have been shown to induce spermiation in goldfish and the biwa trout *Oncorhynchus rhodurus*. Advancement or induction of spermiation has also been achieved in the case of DHP injections when given to some salmonids and cyprinids (Ueda et al., 1985). In summary, androgenic, progestagenic and even estrogenic

hormones are all necessary regulators from the beginning of spermatogenesis all the way to sperm maturation and spermiation.

The endocrine system and its implications on spermatogenesis and the sperm maturation process is a complex topic, knowing how it works can be helpful in fisheries practice or species-conservation measures for the artificial usage of hormones for the desired outcomes.

7.1.   Artificial induction of spermatogenesis and spermiation

Fish spermatogenesis can be accelerated or induced by artificially adjusting hormone levels in the fish system, but these changes are rarely direct, as more often they start a chain of reactions and counter-reactions, finally leading to the desired outcome.

Fish steroid genesis is stimulated by FSH along with LH directly by the activation of a cell type called Leydig cells, which are the primary source of testosterone or androgens in males. Because of this activation role, and since FSH has been found in sexually mature salmonids, in theory, increased FSH signalling may be a way to induce spermatogenesis, by activating Leydig cells and thus eliciting a surge in the secretion of 11-KT. This is a potent endogenous androgenic sex hormone, that is involved in spermatogenesis by pushing Sertoli cells to stimulate spermatogonia before mitosis to complete spermatogenesis. (Nagahama et al., 1994). The same goal can be achieved by adding 11-KT directly to the testicular organ culture system, spermatogenesis was induced from spermatogonial proliferation to spermiogenesis after introducing 11-KT, it has been shown in the case of Japanese eel (Miura et al., 1991b), and in Japanese huchen *Parahucho perryi* (Amer et al., 2001). Another study carried out by Miura et al. (1991a) on Japanese eel, tried using a single injection of human chorionic gonadotropin (HCG) to induce spermatogenesis. These eels previously, before the injection, only had type A and early type B spermatogonia (not yet proliferated), and within just one day Sertoli cells and Leydig cells were notably activated, and after two more days spermatogonia began to proliferate and change into type B spermatogonia. Spermatids and spermatozoa were detected after 18 days.

Generally, most research regarding induced spawning in fish focuses on female ovulation, since a lot of species of male fish used in aquaculture may produce enough sperm spontaneously (Carral et al., 2003), however, in some species, hand stripping of sperm is not optimal as it may be almost impossible, and then testicular sperm is preferred

for fisheries practice. Knowing whether the sperm is mature in testes, allows us to know if the use of testicular sperm is possible. This can be utilized in pike, catfish, or carp for instance.

In the method of hand stripping, the milt volume may also not be sufficient enough. Generally, spermiation induction increases spermatozoa quality. Artificial induction of spermiation can work on different levels of regulation of the brain-pituitary-gonadal axis.

Stimulation of the brain can be achieved by the usage of 17,20 βP (17,20 β-dihydroxy-4-pregnen-3-one), a progestin naturally involved in the endocrine regulation of spermiation. This progestin is a hormonally derived sex pheromone naturally found in fish and stimulates an increase in serum gonadotropin (GtH) levels and subsequently increases milt production in males in goldfish (Dulka et al., 1992). It has been proven to be effective in improving sperm volume, spermatozoa production and motility in some aquaculturally important species.

Stimulation of the pituitary gland is achieved by the usage of gonadotropin-releasing hormone (GnRH). The upsides are that this stimulation can be more effective than the traditional stimulation at the gonadal level, but also this is not species-specific and raises no pathological concerns. Synthetic advantageous GnRH analogues (GnRHa) have been introduced, mitigating some downsides of GnRH, GnRHa has advantages such as a decrease in biodegradation, or higher affinity to GnRH receptors.

In regard to the stimulation of the testis, hypophysation is the traditional method used to induce spermiation in carp and related species, however, it has its disadvantages, such as a relatively high price, or pathological risks, so there have been attempts to use other methods. One of them is the use of an already mentioned HCG (Human chorionic gonadotropin), which can be highly effective in some species but also have little to no effect and even be harmful in others. In some cases, such as in carp, LHRH (Luteinizing hormone-releasing hormone) and LHRHa, its analogue, have also proven to be very effective in inducing spermiation, as they evoke the release of endogenous pituitary gonadotropin supplies (Weil et al., 1986).

8. Conclusion

The sperm maturation process is a complex process necessary for fish reproduction, as it develops spermatozoa's potential to respond to motility activating factors. Motility is usually activated upon contact with water via an osmotic shock or a specific ionic environment composition.

The sperm maturation process has been described in detail in only a limited number of species, mostly teleosts, and therefore is a field of study with possible further research. This process is greatly related to the urogenital system anatomy, which differs greatly between different fishes and the urogenital system structure directly affects the sperm maturation process by taxa specific manner.

The maturation process of spermatozoa can be affected by various factors, which are species-dependent. The most outstanding examples of the differences in sperm maturation are: 1) in sturgeon, the necessary maturation step is a contact of spermatozoa with urine, possible due to the direct connection between urinary and genital tracts; 2) in eels and trouts, sperm maturation takes place in the specific parts of sperm ducts, due to pH and specific ionic compositions; 3) in chondrichthyans, sperm maturation can be considered as similar to mammals and it is taking place in the specific part of the male reproductive system - the epididymis and the seminal vesicles.

The endocrine system controls the whole process of spermatogenesis, including sperm maturation. This is where all the knowledge about sperm maturation and spermatogenesis and their correlation with the endocrine system is valuable. Hormones can be utilized in fish farming to induce spermatogenesis, sperm maturation or spermiation to enhance results. Utilization of hormones can be executed via the treatment of different parts of the brain-pituitary-testes axis, taking into account species-specific aspects of hormonal regulation of spermatogenesis and sperm maturation. Artificial induction of spermiation has been traditionally carried out by hypophysation, however, nowadays new methods are being developed, be it for economic reasons or others.

In many species of male fish used in aquaculture artificial induction may not be needed, as they may produce enough sperm of sufficient quality spontaneously. In some other species, hand stripping of sperm is not optimal. The milt volume also may not be adequate enough, and artificial induction is then beneficial. One crucial piece of knowledge is whether the sperm is mature in the testes, which allows us to know if the use of testicular sperm is possible. This is popular in fisheries practice in some species,

where there may not be many other options. The knowledge of this also widens our possibilities of using testicular spermatozoa of fishes, where or when no other sources of genetic material are available regarding biodiversity conservation measures.

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10. Abstrakt

Hlavní cíl této práce bylo posouzení aktuálních informací v oboru zrání spermatu (procesu, při kterém nezralé spermie získají potenciál reagovat na faktory podněcující pohyblivost spermií) a také posouzení struktury urogenitálního systému ryb v souvislosti se zráním spermií. Proces zrání spermií se odehrává v různých částech rybího urogenitálního systému, a je velmi rozdílný v závisloti na taxonu dané ryby. Rozlišujeme čtyři hlavní typy ohledně toho, kde a jak zrání probíhá; zrání přímo uvnitř varlat (u ryb skupiny Teleostei, kde jsou močové a pohlavní cesty úplně oddělené), uvnitř specifických částí spermovodů (u úhořů a lososovitých ryb), uvnitř specifických částí samčího pohlavního systému – v epididymisu a semenných váčcích (u příčnoústých) a nebo uvnitř Wolffových vývodů, poté co spermie přijdou do kontaktu s močí (ryby ze skupiny Chondrostei – jeseteři a veslonosi). Faktory které spouštějí tento proces byly také shledány závislými na daném druhu ryby. Jelikož je zrání spermií poslední fází spermatogeneze; procesu při kterém se ve varlatech vytváří spermie, spermatogeneze, její hlavní části, a struktura varlat byly také v práci pokryty. V závěru byla zhodnocena důležitost znalosti procesu zrání spermií pro umělé rozmnožování v praxi.

- Klíčová slova: zrání spermií, pohyblivost spermií, spermatogeneze, spermie, urogenitální systém, spermiace, endokrinní systém, hormonální stimulace, varlata, testikulární sperma, akvakultura, Chondrostei, Teleostei, Chondrichthyes.

11. Abstract

The main aim of this thesis was to review the current knowledge in the field of fish sperm maturation (a process where immature spermatozoa acquire the potential to respond to motility-activating factors) and the urogenital system structure in relation to this maturation. Sperm maturation takes place in different parts of the fish urogenital system, and is very taxa-specific. Four main narratives have been found in regard to the place for sperm maturation. It occurs inside the testes (teleosts with a separation between urinary and genital ducts), in the specific parts of the sperm ducts (eels, salmonids), inside the particular parts of the genital system – epididymis and seminal vesicles (elasmobranchs), and inside the Wolffian ducts after contact of sperm with urine (chondrosteans). The factors stimulating this process were also found to be species-dependent. Since sperm maturation is the last stage of spermatogenesis, the process of creation of sperm cells in the testes, spermatogenesis, its main phases, and testis structure were also discussed. Finally, the importance of knowledge on sperm maturation for artificial fish propagation practice was reviewed.

- Keywords: sperm maturation, sperm motility, spermatogenesis, spermatozoa, urogenital system, spermiation, endocrine system, hormonal stimulation, testes, testicular spermatozoa, aquaculture, Chondrostei, Teleostei, Chondrichthyes