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Outlook for development of high-throughput cryopreservation for small-bodied biomedical model fishes ☆

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ABSTRACT

With the development of genomic research technologies, comparative genome studies among vertebrate species are becoming commonplace for human biomedical research. Fish offer unlimited versatility for biomedical research. Extensive studies are done using these fish models, yielding tens of thousands of specific strains and lines, and the number is increasing every day. Thus, high-throughput sperm cryopreservation is urgently needed to preserve these genetic resources. Although high-throughput processing has been widely applied for sperm cryopreservation in livestock for decades, application in biomedical model fishes is still in the concept-development stage because of the limited sample volumes and the biological characteristics of fish sperm. High-throughput processing in livestock was developed based on advances made in the laboratory and was scaled up for increased processing speed, capability for mass production, and uniformity and quality assurance. Cryopreserved germplasm combined with high-throughput processing constitutes an independent industry encompassing animal breeding, preservation of genetic diversity, and medical research. Currently, there is no specifically engineered system available for high-throughput of cryopreserved germplasm for aquatic species. This review is to discuss the concepts and needs for high-throughput technology for model fishes, propose approaches for technical development, and overview future directions of this approach.

1. Introduction

High-throughput processing has been widely applied for sperm cryopreservation in livestock for decades (Pickett and Berndtson, 1974). The technology was developed based on advances made in the laboratory and was scaled up for increased processing speed, capability for mass production, and quality assurance. Cryopreserved germplasm combined with high-throughput processing constitutes an independent industry encompassing animal breeding, preservation of genetic diversity, and medical research. High-throughput includes not only efficient large-scale production, but also encompasses uniformity and quality assurance of products, and standardized and streamlined procedures.

Fish sperm cryopreservation first began in the laboratory in the 1950s (Blaxter, 1953), which was about the same time as for humans and livestock species. However, it has remained at a research scale for aquatic species although there have been several hundred species reported for sperm cryopreservation (Tiersch and Mazik, 2000; Tiersch and Green, 2011). In the past 10 years, equipment and processes in place for livestock have been tested for feasibility of use with fish (e.g., Haffray et al., 2008) or directly for fishes by using the protocols developed for dairy bulls (Lang et al., 2003; Roppolo, 2000), and shellfish such as Pacific oysters *Crassostrea gigas* (Dong et al., 2005; Dong et al., 2007). In addition, extensive large-scale protocols have been developed for zebrafish *Danio rerio* sperm cryopreservation (Draper and Moens, 2009).

Recently, a survey of fish culturists revealed a high demand for the type of genetic improvements that can be provided by cryopreservation in aquaculture (Boever, 2006). High-throughput protocols for sperm cryopreservation of blue catfish *Ictalurus furcatus* were initiated with automatic processing equipment developed for livestock and biomedical applications (Hu et al., 2011). After evaluation of factors such as sperm concentration, cryoprotectant concentration, and cooling rates, an optimized protocol was developed for high-throughput processing. With this protocol, post-thaw sperm from 10 individual males yielded uniform results in terms of post-thaw motility and fertility (Hu et al., 2011). This is desirable for high-throughput technology because it can provide products with consistent and predictable output. In addition, protocols compatible with the automated filling and sealing equipment used for livestock were developed for sperm cryopreservation with zebrafish (Yang et al., 2007a), Eastern oyster *Crassostrea virginica* (Yang et al. unpublished data) and Atlantic salmon *Salmo salar* (Hu et al. unpublished data).

Overall, the application of high-throughput processing for aquatic species sperm cryopreservation is just beginning. There is no

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specifically engineered system available for large-scale production of cryopreserved germplasm for aquatic species. With growing recognition of the importance of germplasm and genetic resources for aquaculture, breeding programs, conservation programs, and archiving of biomedical fish lines, automatic processing for sperm cryopreservation for aquatic species comes with high expectations.

2. The needs for high-throughput sperm cryopreservation of biomedical model fishes

2.1. The needs for biomedical model fishes

With the development of genomic research technologies, species-specific genetic and molecular mechanisms can be identified, and comparative genome studies among vertebrate species are becoming commonplace for human biomedical research. Fishes, as the largest class of vertebrates, offer unlimited versatility for biomedical research. With extensive studies using these fish models, tens of thousands of specific strains and lines have been created, discovered, and cataloged, and are currently housed worldwide as live animals in resource centers, such as the Zebrafish International Resource Center (University of Oregon, Eugene, OR, USA) which holds around 1080 inbred, transgenic, knockout and mutant strains. However, with the increasing accumulation of new strains every day, it is becoming more difficult to maintain these valuable genetic resources as live animals. Large-scale sperm cryopreservation is urgently needed to preserve these genetic resources. Sperm banking of these fishes can allow the creation, maintenance, and transport of the associated genetic information more easily and safely, and can represent a readily transferable form of bankable wealth with the ability to accumulate, archive, and catalog germplasm.

2.2. High-throughput processing for sperm cryopreservation

Germplasm banking for biomedical fishes must deal with the large number of strains awaiting preservation and quality assurance of the banked products to guarantee recovery of specific strains. Therefore, high-throughput approaches need to be developed to accomplish this task. This process includes biological and cryobiological principles, equipment and facility development, process control for sample handling, inventory and databasing, quality control and assessment, standardization and establishment of industrial practices, and institution of biosecurity systems — more than simply freezing of sperm samples. In addition, high-throughput processing needs to be scalable to the needs of individual laboratories and a central pathway needs to be established to accommodate current and future levels and methods of application. Simultaneously, these activities should be funneled into a standardized approach that can utilize industrial methods supported by commercial vendors of specialized equipment, supplies, and reagents, and industrial-level service providers for cryopreservation, storage, and quality control.

2.3. Quality assurance and product uniformity

Assessment of gamete quality is not well developed for aquatic species in general, and is made more complicated in small fishes due to the miniscule volumes of sperm available and the high value of the few eggs available (Yang and Tiersch, 2009).Currently assessment of sperm motility is performed subjectively by microscopy, or objectively by computer-assisted sperm analysis (CASA), and is the most common method used for the assessment of sperm samples from aquatic species. The correlation between motility and fertilization varies among species and studies (e.g., Cabrita et al., 2009), indicating that more effective methods for prediction of fertilization and cryopreservation success are required. In the mid-1990s, CASA systems developed for mammals were first applied to fish. Since then 56 peer-reviewed research articles have been published addressing this topic (Yang and Tiersch, 2011a). The bulk of this research addresses the feasibility of CASA application; however, as yet no standardized methodology exists for aquatic species. This is important because there are a number of fundamental differences between the sperm of mammals and aquatic species including cell size, motility activation mechanisms, and swimming speed and duration. Moreover, few of these publications address thawed sperm and most utilize fresh sperm collected by stripping.

Flow cytometry is another powerful technique to evaluate sperm quality, and has been used in a range of aquatic species (Daly and Tiersch, 2011). This technique enables rapid screening of large numbers of cells to detect structural and functional changes thereby providing an objective means to assess quality. However, there is considerable variability in flow cytometric assays among studies on aquatic species, making the direct comparison of results problematic. For example, one of the most commonly tested parameters is plasma membrane integrity, using the SYBR14/propidium iodide (PI) assay. This assay is used qualitatively to assess cryopreservation protocols (e.g., Cabrita et al., 2005; Paniagua-Chavez et al., 2006) or sperm toxicity (e.g., Favret and Lynn, 2010; Segovia et al., 2000). Discrepancies include variation in sperm concentration, dye concentration, collection parameters (e.g., population gating), fluorescence compensation, and temporal components such as the time between collection or thawing and assessment, duration of treatments, and time between staining and flow cytometric assessment. In addition, the measurement and control of sperm concentration is usually not recognized, nor considered when assessing sperm quality. As such, flow cytometry results are typically reported as percentage data with no indication of the actual number of competent cells within a sample. A standardized approach to sperm quality assessment for aquatic species is urgently required for research and high-throughput application (Leibo, 2000).

3. Approaches to achieve high-throughput processing

3.1. Automated systems

High-throughput can be achieved in different ways, and adoption of automated systems is one of the most efficient methods. An automated system named MAPI (CryoBioSystem Inc. Paris, France) for loading, sealing, labeling and reading of straws has been developed for mammalian high throughput. Adaptation of this equipment has been evaluated for aquatic species to enable commercial-scale use of cryopreserved sperm (e.g., Hu et al., 2011). Briefly, upon mixing with cryoprotectant, sperm samples are placed on the MAPI system, and filling, sealing and labeling of plastic straws are controlled by a proprietary computer program (SIDE, CryoBioSystem, Inc.). Samples are drawn into 0.5-mL (CBS) plastic straws by vacuum, and are continuously transferred to the sealing platform where both ends are closed by use of 158 °C heat clamps. The straws are labeled with alphanumeric information and bar-coding on the identification jacket with an ink printer (A400, Domino, IL, USA) before transfer for label verification and quality control evaluation. At full operation the system can routinely process as many as 14 straws per min. Controlled-rate freezing follows using standardized procedures (Hu and Tiersch, 2011). Similar to the MAPI, there are other types of automated systems available for sample loading and sealing of 0.25-mL and 0.5-mL French straws at a production scale from companies such as IMV (Paris, France).

3.2. Standardization and streamlining of procedures for sperm cryopreservation

In addition to the use of automated systems, high-throughput requires arrangement of the sequence of procedures and balancing of

the inputs and outputs between connected steps. For any sequence developed, the production resource utility and processing costs (time and money) can be evaluated. Based on the procedures of sperm cryopreservation for major biomedical model fishes such as zebrafish (Yang et al., 2007a), Japanese ricefish (medaka *Oryzias latipes*) (Yang et al., 2010), and *Xiphophorus* fishes (Yang et al., 2007b; 2009), the following 5 major steps can be identified in a basic sequence: sample collection, sample processing, freezing and sorting, storage, and utilization. To meet high-throughput expectations, the time and cost at each step need to be reduced to increase the efficiency of the production cycle.

In contrast to the large-sized food fishes and mammals, biomedical research model fishes are characterized by small body sizes (<5 cm), and thus have limited sperm available from each fish (1–2 μL) (Tiersch, 2001; Yang and Tiersch, 2009). This constrains the application of automated processing equipment to these fishes, especially for samples from single males.

4. Development of high-throughput pathways for biomedical model fishes

To develop high-throughput processing for sperm cryopreservation of biomedical model fishes, a straightforward approach would include: 1) optimization of each step in the process individually and in relation to other steps, 2) assembly and streamlining of these steps after multiple iterations into a pathway, and 3) standardization of the pathway for application to improve overall efficiency of processing, sample usage (e.g., quantifying all steps on a per-sperm basis), post-thaw quality, and fertilization success (Fig. 1) (Tiersch, 2011).

4.1. Optimization of each step in the cryopreservation process

4.1.1. Sample collection — improvement of broodstock conditioning to increase sperm quantity and quality

Baseline protocols compatible with automated processing for sperm cryopreservation and artificial insemination have been developed for zebrafish (Yang et al., 2007a), medaka (Yang et al., 2010), and *Xiphophorus* (Yang et al., 2007b; Yang et al., 2009) by focusing on evaluation of cryoprotectants and cooling rates in 0.25-mL plastic straws which are two major critical cryobiological factors during cryopreservation. In these studies, male-to-male variation of post-thaw motility and fertility was observed, and higher post-thaw motility and fertility were found to be correlated with body condition

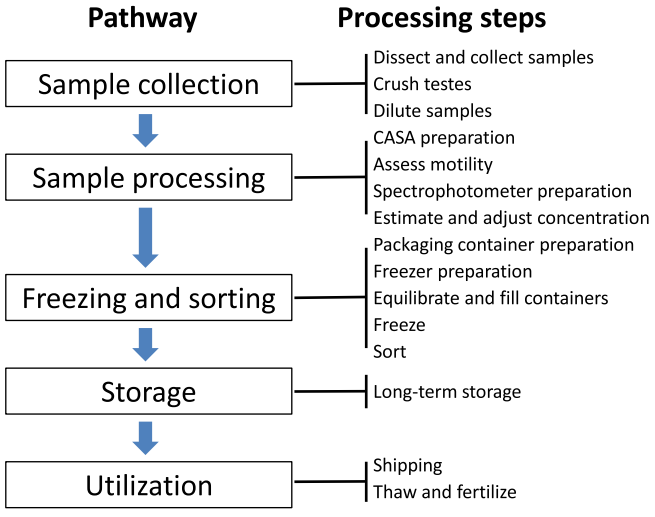

Fig. 1. Schematic representation of a cryopreservation pathway suitable for biomedical model fishes. In its simplest form, the pathway comprises 15 standardized, integrated processing steps from sample collection through thawing and use for fertilization.

factor in zebrafish (Yang et al., 2007a). Based on this knowledge, improvement of broodstock conditioning can be a way to increase gamete quality and quantity and to minimize variability leading to increased efficiency of *in vitro* fertilization with cryopreserved sperm.

Many factors can influence optimal reproductive condition, including age, maturity, feed quality and quantity, temperature, gender ratios, water quality, light cycles, and stocking density (Yang et al., 2010). Consequently, experiments to test all of these factors individually or in combination would be complex. The established standardized culture conditions for model fishes can be used as a baseline for testing such as by manipulating culture temperature or light cycles, and increasing the quantity and quality of feeding to improve gamete quantity and quality in relation to cryopreservation.

4.1.2. Sample collection and processing

Sample processing includes collection, suspension of sperm in extender, concentration adjustment, and quality assessment. The first two steps can currently only be improved in terms of high-throughput with technical training and increasing the number of personnel involved. Concentration determination is an important factor to be standardized during cryopreservation and in-vitro fertilization. However, the small sample size from biomedical model fishes limits standardization because cell concentration determination by methods such as hemocytometer requires a 10-μL sample and about 10 min for counting. Recently, an approach that uses a microspectrophotometer to determine sperm concentration was developed (Tan et al., 2010), which requires less time (sec per sample), and minimal sample volume (1–2 μL of diluted sperm). Equations that relate concentration and absorbance have been established for samples collected by stripping, and by crushing of dissected testis of zebrafish, medaka, and *Xiphophorus*, and the accuracy of this relationship was verified (Tan et al., 2010). Quality assessment of fresh sperm will be discussed below.

4.1.3. Sample packaging

Packaging of samples can influence the cooling rate during freezing, sample storage efficiency after freezing, sample identification, and biosecurity. Currently, several different kinds of containers have been used for biomedical model fishes such as plastic cryovials of different volumes, glass capillaries, and plastic straws (Yang and Tiersch, 2009). The different materials and shapes of these containers result in different heat transfer properties during freezing and thawing. Even for the same style of container, differences can exist with products from different manufacturers, which can result in variability of cooling or thawing rates. Therefore, it is necessary to standardize the packaging method to ensure that protocols will be repeatable especially in different laboratories. Also, the choice of packaging container should consider the potential for high-throughput processing, permanent labeling, reliable sealing, efficient storage, shipping, and inventory.

For research model fishes, the small volumes of sperm available limit the choices for sample packaging. In zebrafish and medaka, glass capillary tubes or cryovials were first used in sperm cryopreservation (Aoki et al., 1997; Draper et al., 2004; Draper and Moens, 2009; Harvey et al., 1982; Krone and Wittbrodt, 1997; Morris et al., 2003). Later, to standardize protocols with potential for automation at high throughput, French straws were chosen for sperm packaging with the smallest commercially available volume (0.25 mL)(Yang et al., 2007a; 2010), and were also used in biomedical *Xiphophorus* fishes (Huang et al., 2004a,b; Yang et al., 2006; Yang et al., 2007b; 2009). The use of French straws has the following advantages: potential for use with automated filling and sealing equipment, permanent labeling, complete sealing, and efficient storage. However, the cotton end of French straw usually absorbs and wastes some sample (10–15 μL for the 0.25-mL straws) although newer straws are available to avoid this loss. Recently, cryotubes (0.5-mL and 1.0-mL) have become commercially available for cell banking with compatibility for automated

handling of liquid in 96-well plates and labeling with two-dimensional laser etching. It is possible that systems such as these could be used for sperm cryopreservation for model fishes, although more investigation is needed.

4.1.4. Sample filling, labeling, and sealing

In a manner similar to that use for manual filling of straws, automated systems apply a vacuum at the cotton end of straws and fill using suction. The accuracy of needles or nozzles that insert into the straw is critical to ensure reliable sealing after sample loading. To avoid cross contamination, the tube and nozzle can be replaced. In bull semen freezing, straws can be printed beforehand or labeled individually during or after sealing. This produces feasibility to control the process and avoid waste. Nevertheless, in either case, close estimation of straw numbers will aid the efficiency and scheduling of production. There is no established coding system for aquatic germplasm. Unlike the dairy industry, the variety of species and protocols encompassed by aquatic species would challenge the format applied by the dairy and beef industries (www.naab-css.org). If a universal code for aquatic germplasm is to be developed, it should provide comprehensive procedure descriptions. As one of the most important biosecurity steps, straw sealing finalizes the packaging. The powder and gel sealing used in the past poses potential risk because of physical weakness. The heat sealing used for CBS straws and ultrasound sealing for French straws can solve this problem. Filling, labeling and sealing are interactive aspects in straw quality assurance. Many common problems such as empty or partially filled containers, untraceable straws, contamination of inventory, and sample explosion during thawing all relate to these steps.

4.1.5. Freezing

The cooling process can be accomplished by different methods. For model fishes, these include placement on dry ice, suspension in liquid nitrogen vapor, and controlled cooling with a programmable freezer. The first two methods are inexpensive and do not need expensive equipment, and thus can be used in field situations. However, to produce accurate and repeatable cooling rates, programmable freezers need to be used, especially for sperm that are sensitive to small variations in cooling rate during freezing. For example, for medaka sperm, a change of 5 °C/min in cooling rate yielded a significant change in post-thaw sperm motility (Yang et al., 2010). Therefore, strict control of cooling rate can assure quality and uniformity of cryopreserved sperm.

4.1.6. Sample sorting for storage

Holding of frozen samples in vapor-phase (−120 °C) or liquid-phase nitrogen (−196 °C) in a storage Dewar is a standard method for cryogenic storage of samples. During frozen storage, the important considerations are sample identification, potential contamination, and ease of sample inventory. The use of French straws for packaging offers the advantages of permanent alphanumeric and barcode labeling by printer, and complete sealing which prevents transfer of materials (e.g., sperm cells or bacteria) among samples stored in the same Dewar (Morris, 2005). When storage is in the liquid phase of nitrogen, another important consideration is floating of frozen samples, especially when small volumes are packaged in lightweight containers with a large air space.

Sample sorting into bulk containers for long-term storage is often needed after freezing. This process should be done under liquid nitrogen, and can be time consuming if the labeling of samples is not easily discernable. If samples can be frozen in the storage containers without affecting the cooling process, the sorting step could be skipped or minimized, and the whole process could be accelerated.

4.1.7. Sample thawing and fertilization

Improvement of reproduction and *in vitro* fertilization can increase offspring production, minimize variability, and provide efficient use of cryopreserved samples. The process of *in vitro* fertilization needs to be standardized and quantified in terms of the sperm number used for specific numbers of eggs (e.g. sperm-to-egg ratio), the associated volumes and concentrations, gamete holding time, and activation of the gametes for fertilization. In addition, age, body weight, body length, or culture condition of fish need to be quantified and the correlations need to be identified with the success of fertilization. Optimization of these conditions will greatly improve the efficiency of fertilization and offspring production.

4.2. Assembly and streamlining of the steps during cryopreservation — establishment of pooled samples for high-throughput batch production with automated equipment

Due to the limited sample volumes available from most model fishes, increases in sample volume need to come from pooling of samples from different individuals. This would enable processing with a high-throughput approach, minimize male-to-male variation, and provide uniform batches of cryopreserved samples that can receive rigorous quality control and evaluation. More importantly, the pooling of samples can greatly reduce the time needed at certain procedure steps such as motility estimation and sperm concentration determination to achieve the goal of high throughput, and substantially reduce the time and sample volume necessary for quality assessment of fresh and thawed samples (Table 1). It is true that some genetic applications such as targeting induced local lesions in genomes (TILLING) requires cryopreservation of sperm from individual males, but other approaches aimed at line preservation readily lend themselves to sample pooling strategies.

In most studies on model fishes, single males have been used for evaluation of motility and fertility of cryopreserved samples, but it has also been common to pool individual males to produce sufficient sample volume for certain experimental designs (Yang et al., 2007a; Yang et al., 2007b; Yang et al., 2010). To evaluate the efficiency of pooling for germplasm banking, pooled samples and individual samples need to be cryopreserved at the same time for post-thaw comparisons of: 1) relative production from each sample type; 2) motility and velocity estimated by CASA; 3) membrane integrity estimated by flow cytometry, and 4) fertility estimated by in-vitro fertilization. This research should provide solid information for use of pooled samples which will have potential for high-throughput processing. Automated systems for filling and sealing of 0.25-mL straws are available currently (e. g. model MRS 1, IMV Technologies, Maple Grove, MN, USA), and future commercial equipment will hopefully use a similar but smaller straw (e.g., 50–100 μL) designed for aquarium fishes. This offers the possibility for true high-throughput operation of sperm cryopreservation with the necessary quality and reproducibility.

4.3. Standardization of the pathway for application to improve overall efficiency of processing

4.3.1. Establishment of computer-based processing models for the cryopreservation pathway based on quality, efficiency, expense, time, and the number of animals or samples available or needed

An ultimate application goal for high-throughput cryopreservation technology would be to establish “central facilities” accessible to research institutions or other users. That could serve functions similar to dairy improvement centers around the country. To expand the utility of standardized pathways, computer modeling of existing processes can help to build a basic structure. The modeling starts by generating flow charts from protocols. This breaks the process into operations, decisions, records, transportation, and inspirations. Then, the time and cost of each step are added to create an operation sheet, which provides standard operation instructions. Meanwhile, simulation-based modeling can evaluate the process which offers opportunities to adjust and optimize

Table 1
Summary of biological data for males of zebrafish, medaka, and four species of *Xiphophorus* including sperm production per male (Yang and Tiersch, 2011b), and calculation of sperm numbers that would be produced by pooling of samples from 20 males for high-throughput processing. It is assumed that sperm would be adjusted to a concentration of 5×10^7 cells/mL with a loading volume of 100 μL per 0.25-mL French straw, and used for fertilization of 50–100 eggs from one female for zebrafish, or 50–100 eggs from 10 females for Japanese ricefish (medaka), or 1–2 females for *Xiphophorus*. Pooling would allow quality assessment to be performed on the batched material and would greatly reduce waste compared to sampling from individual males.

	Sample size	Body length (cm)	Body mass (g)	Testis mass (mg)	Sperm number per mg testis ($\times10^6$)	Sperm production per male ($\times10^6$)	Number of straws (per male)	Number of straws if pooling 20 males
Zebrafish	45	2.4 ± 0.2	0.295 ± 0.066	3.2 ± 2.0	7.7 ± 2.0	24.6	5	98
Medaka	74	2.6 ± 0.2	0.311 ± 0.052	2.0 ± 0.6	2.0 ± 0.4	4.0	1	16
X. helleri	45	3.2 ± 0.3	0.630 ± 0.168	9.2 ± 5.5	5.4 ± 2.2	49.7	10	199
X. couchianus	66	2.0 ± 0.2	0.184 ± 0.052	3.1 ± 1.0	5.4 ± 2.5	16.7	3	67
X. maculatus	117	2.5 ± 0.3	0.474 ± 0.149	7.1 ± 3.7	5.8 ± 2.8	41.2	8	165
X. variatus	35	2.5 ± 0.2	0.298 ± 0.096	6.4 ± 3.4	2.4 ± 1.3	15.4	3	61

under various constraints. In this way, the process can be tested with real-life situations before decisions are made. Different user groups could design their own central facilities with consideration of specific research, geographic, or economic specifications.

4.3.2. Standardization of the assessment of sperm quality by use of computer-assisted sperm analysis and flow cytometry

Assessment by CASA is powerful approach for analysis of the characteristics of sperm movement. It is an absolute requirement to have proper parameter settings for collection of the types of data. The default conditions set by the manufacturers of CASA system are based on mammalian sperm which can be continuously motile for days. Fish sperm are usually much smaller than mammalian sperm and swim faster. More importantly, fish sperm can have complicated motility activation modes, and motility for most fishes can last only seconds to minutes. Therefore, the parameter settings need to be modified for different fishes, for sperm collected by different methods such as stripping or dissection of the testis, and for fresh, stored, and thawed conditions. Procedures need to be developed and standardized to ensure accuracy for sample analysis. Furthermore, the profiling of sperm movement can be systematically analyzed together with sperm viability and fertility after thawing to identify correlations, and the identified parameters could be made available as reliable indices for prediction the gamete quality in relation to storage, shipping, and cryopreservation. An integrated methodology with proper settings, standardized protocols, and predictable parameters can provide a powerful platform for systematic analysis of sperm before freezing and after thawing for quality assurance and control.

For flow cytometry, the most commonly used assays for sperm quality are the membrane integrity assay (SYBR14/PI) and mitochondrial membrane potential (e. g. MitoTracker Deep Red). Similar to the situation for CASA, the protocols of these assays for fish sperm need to validated and standardized, and additional assays need to developed and established. In addition, the correlation among measurements and sperm viability and fertility need to be identified through large-scale screening, and thus these assays could be effectively used for prediction of the outcome of cryopreservation, and used for quality assurance and control.

5. Future needs for development of high-throughput cryopreservation in model fishes

For small-sized model fishes, high-throughput cryopreservation is still at the incipient or concept-development stages, and requires a series of activities to be in place for application. These activities have not yet been fully implemented anywhere, and no integrated equipment, devices, or packaging containers are available that are specifically designed for use with the small samples volumes. The large number of research strains held at stock centers or laboratories is continuously growing every day, and high-throughput cryopreservation is needed to preserve these valuable genetic resources. Therefore, the US National Institutes of Health has supported grants for research in this topic, and other proposal solicitations could be forthcoming for related equipment or device development. Currently, the focus is still on technology development and technical problems. As we move forward, the steps involved in development of high throughput could be summarized as follows (Hu and Tiersch, 2011):

1) Development of necessary technical capabilities for high-throughput applications.
2) Establishment of standardized and streamlined procedures for sperm cryopreservation with high-throughput processing and reliable quality control.
3) Specific development of necessary equipment, devices, and facilities for automated handling of small sample volumes.
4) Establishment of quality control protocols and standardization or harmonization of protocols, labeling, terminology, reporting of results, and databases. Development of Best Practices Manuals or other guidelines would assist these efforts.
5) Development of central facilities that have strong operational capabilities, and a sustained effort should be made to develop cooperation with other organizations and facilities. These relationships can include sharing of samples, capabilities, and expertise.
6) Establishment of training programs for procedural efficiency, and education of personnel from different user laboratories.
7) Development of appropriate biosecurity safeguards to control movement in and out of facilities of pathogens and other adverse biological effects.
8) Development of functioning storage repositories, with rules for use and disposal of samples, and with appropriate security and backup for basic services (e.g., electricity, liquid nitrogen, refrigeration capabilities).
9) Implementation of archival-quality labeling of samples and the creation of robust databases capable of handling biological information concerning samples, maintaining correct inventory and identification of sample locations.
10) Integration of sperm repository databases with the existing databases for biological and genetic information of strains or lines, and development of computing capability and information transfer including the ability to interact and exchange information with other databases.
11) Establishment of pricing structures necessary for initial and continued sharing of genetic material and information among researchers.
12) Coordination of regional activities among countries or administration across borders by treaties or other agreements.

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