Evaluation of The Cryopreservation Technology of Poultry Sperm: A Review Study

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Abstract

With the advancement of the poultry industry, the basic need for the use of cryopreservation technology of poultry sperms has emerged, given that it is the basic technology that preserves the genetic resources of different breeds and establishes genetic banks that in turn contribute to the establishment of different strains and lines. The technology of cryopreservation of sperm has encountered many considerations and obstacles, as the impressions of this technique are divided into three topics, the first impression believes that this technique is largely unsuccessful, while the second suggests a great potential in the preservation process. The third impression believes that cryopreservation is an encouraging and promising operation shortly. Similar to the cryopreservation of sperm in mammals, two methods were used in poultry: the slow and rapid freezing (vitrification). In both types, similar results were obtained where the fertility rates of the sperm did not exceed 40%. Due to the morphological and physiological differences between poultry and mammals’ sperms, three cryoprotectants have been widely used in poultry cryopreservation: dimethyl sulfoxide, dimethylacetamide and glycerol, glycerol is the most widely used due to its molecular properties that contribute to maintaining the highest survival rate and fertility after freezing (high permeability and low cytotoxity). The main obstacle facing this technique remains in how to treat the remaining quantities of the aforementioned cryoprotectants, which lead to a decrease in the fertility capacity after freezing and during artificial insemination. The numerous protocols used, whether in slow or rapid freezing, greatly affected fertility rates, as both the equilibrium and freezing stages played a decisive role in obtaining the highest possible rates of fertility, vitality and survivability of the sperm after thawing. It is concluded from the current review that the cryopreservation technology of poultry sperm is still in a non-advanced stage and needs many new methods to contribute to raising the fertility capacity, vitality and survivability rates after freezing.

Key words: cryopreservation, cryoprotectants, poultry, fertility, spermatozoa

1. Introduction

The industry of poultry has recently witnessed important developments in various production destinations based on scientific trends and disciplines, for example, but not limited to, factors affecting egg storage [1], fodder industry that would raise the production level of birds [2] breeding and genetics improvements [3], and cryopreservation technology of spermatozoa [4].

“Sperm cryopreservation ” is one of the main backbones of assisted reproductive technology (ART). This technology has become a basic alternate in the production of fresh embryos and raising the reproductive efficiency of farm animals. The breeding plans involved many measures to ensure the achievement of the goals, whether it be commercial or research level. In the mammalian cell sector, an intense integration emerged between the freezing of gametes (sperm and oocytes). This integration reached acceptable levels of performance and production, while the issue remained restricted to freezing sperm concerning to the poultry sector, due to the physiological and morphological nature of the eggs, which is totally different on the conditions of oocytes production in mammals, attention was therefore focused on studying the factors and indicators that play a fundamental role in increasing the reproductive efficiency of birds where the most important of which are periods of storage of eggs [1] and the freezing protocols at relatively high temperatures compared to oocytes conservation at mammals. Interest has increased in recent times in poultry semen preservation technology for several reasons, the most important of which is ex- situ conservation, reintroduction projects or to enlarge the genetic pool of a fragmented population, the selection of individuals of genetic importance and the exchange of samples between distant areas [5]. Significant progresses have been achieved in cattle sperm cryopreservation operations, as these processes have been automated, standardized and developed, and this has been based on a commercial aspect,
while progress in this aspect remained less among small ruminants (sheep, goat and pig) and poultry [6]. The studies of Wishart [7] declared that the fertility rates resulted of cryopreserved poultry sperm are lower than those in the domestic mammalian species, where cryopreserved rooster semen retains <2% of the fertilizing ability of fresh semen.

The technique of poultry spermatozoa cryopreservation has achieved many successes; however, these achievements remain linked to many variables. Perhaps the most important of those variables are those that are related to fertility [8]. Fulton [9] declared that much of the unique poultry genetics were established and maintained. These unique genetics are being lost due to increasing costs in maintaining them and to decreasing interest. Shaffner et al [10] noted that chickens were one of the first species to have sperm cryopreserved and offspring produced using artificial insemination of frozen sperm. Whereas Polge et al. [11] achieved a great success by cryopreserving rooster sperm in 1949 using glycerol as a cryoprotectant agent. On the other hand, many studies have shown that the process of cryopreservation of poultry sperm is not successful in general based on several considerations, the most important of which are the biological and morphological composition of poultry sperm, the poor fertility rates after thawing and decrease in sperm counts which are not sufficiently reliable [12,13,7]). Here, in this review, the importance of cryopreservation technology in poultry was presented, the most important impressions, the general situation, methods of cryopreservation, types of cryoprotectants used and the most important considerations that must be taken into account during the application of the technique.

2. Cryopreservation technologies

As in the case of cryopreservation of mammalian gametes, the cryopreservation process of poultry sperm went through two basic stages: slow freezing and vitrification. A close correlation between the cryoprotectants and the freezing method was observed through the previous methods. The slow freezing method maintains the viability of gametes at very low temperatures. The freezing chamber reduces the temperature of the in a stepwise manner during the cryopreservation process [14]. In poultry, this method was applied according to programmed cooling rates (1-10°C/min) to reach the target temperature (between-70 and -196°C), as well as programmed thawing rates were applied (50-70°C/min). The degree between -5°C and -15°C was considered one of the most important critical time intervals in this method, in which the semen turns from a liquid state to solid state [15]. The slow freezing method is characterized by high equipment costs, higher incidence of intracellular ice formation, low concentration of cryoprotectants and less toxic. Vitrification method depends basically on the use of high concentrations of cryoprotectants and the very fast freezing rates that may reach 1 minute in liquid nitrogen at -196°C [16]. Vitrification led to a historical change in the principles of cryobiology. The method has many advantages compared to the slow freezing method, as this method reduces the formation of ice crystals, which provides a significant increase in the cooling rate [17]. High fertility rates were obtained using the vitrification method in some studies [18,19] On the other hand, Phuong et al. [20] obtained close fertility rates (20%) in both methods of cryopreservation. Both technologies have been applied in other species such as turkeys, guinea fowl, ducks, ganders and some wild species; however, the fertility rates are often less successful than chicken with wide intra-species variability. Sperm surviving after freezing-thawing is usually 40–50% of the initial population. Damage during this process affects both fertilizing ability and its duration in poultry species; however, the effect of cryopreservation on the sperm ability to undergo the acrosome reaction, the initial event of fertilization, is still in question in birds [21]. With the development of cryopreservation, methods have developed a variety of successful semen preservation protocols that have proven effective in fertilization trials. It is based on freeze protection by amides [22,23,24,25,26,27,28]. The protocols have a wide variety of styles, such as electron microscope grids [29], open pulled straw (OPS) [30], closed pulled straw (CPS) [31], cryotops [32], cryoloops [33] and minimum drop size [34].

3. Cryoprotectants

Perhaps the most popular cryoprotectants used in the scope of cryopreservation of bird sperm are dimethyl sulfoxide (DMSO), dimethylacetamide (DMA), Dimethylformamide (DMF) and glycerol (G). These cryoprotectants have been used in both cryopreservation techniques (slow freezing and vitrification) across various protocols and extenders. Among the cryoprotectant, glycerol has been widely included in the freezing protocols to preserve sperm from all livestock species [35,36]. Perhaps glycerol exhibits contraceptive effects to
rooster spermatozoa when inseminated into the hen reproductive tract (1% v: v) [37]. Molecularly, the previous cryoprotectants are characterized by high permeability inside the cell and low toxicity compared to other cryoprotectants. DMA is commonly used in cryopreservation protocols because it does not require multiple methods when dissolving and it gave high fertility rates [18,38]. Table 1 displays the results of some studies on the fertility potential of some poultry species through the use of different cryoprotectants that have been added to different types of extenders. It is noticed from the previous table that the values of fertility ranged between 2.1% and 41.6% [39] with the exception of one study, in which rates were 83.78%.

Table 1. Fertility of poultry semen reported after freezing in different extenders containing different cryoprotectants in different concentrations [39].

| Breed   | Extender                        | Cryoprotectants         | Fertility (%) | References |
|---------|---------------------------------|-------------------------|---------------|------------|
| Rooster | Modified Beltsville poultry semen extender (BPSE) | Dimethyl sulfoxide (DMSO, 4%, v/v) | 38.2 ± 2.5   | [40]       |
| Rooster | Modified extender                | DMSO, (4%, v/v)         | 41.6 ± 0.0    | [41]       |
| Rooster | Lake and Ravie medium            | Dimethylacetamide (DMA, 6%, v/v) | 10.8 ± 0.0    | [42]       |
| Rooster | Lake and Ravie medium            | DMA (6%, v/v)           | 33.6 ± 13.4   | [43]       |
| Rooster | Beltsville extender              | Glycerol (2%, v/v)      | 29.2 ± 2.8    | [44]       |
| Rooster | Lake and Ravie medium            | Glycerol (%11, v/v)     | 2.1 ± 0.0     | [42]       |
| Rooster | Lake and Ravie medium            | Glycerol (8%, v/v)      | 31.3 ± 0.0    | [45]       |
| Gander  | EK diluent                       | Dimethylformamide (DMF, 6%, v/v) | 83.78 ± 9.5   | [46]       |
| Turkey  | Lake diluent                     | Glycerol (11%, v/v)     | 8.6 ± 2.1     | [47]       |
|         | Lake diluent                     | Glycerol (8%, v/v)      | 10.7 ± 2.3    |             |

4. The main considerations and basic dilemmas in the sperm cryopreservation process

There is no doubt that the cryopreservation of poultry sperms has paralleled those of mammals. In general, there are some considerations that should have been taken into account while performing the cryopreservation process, which can be summarized as follows:

1. The unique nature of poultry spermatozoa: poultry spermatozoa have unique spermatological characteristics that are different to a great extent from mammalian spermatozoa, this difference makes the process of cryopreservation difficult and sensitive. The head of the sperm in poultry generally narrow and contains a small amount of cytoplasm, the tail of the sperm is very long compared to the sperm of mammals, this makes the motility of the sperm in the cryoprotectants fluids very impeded and will lead to damage in ultrastructure of sperm cells, especially the mitochondria and midpiece [48,49]. Perhaps the fluidity membrane in the poultry sperm is closely related to the cholesterol/phospholipid’s ratio, as the high content of phospholipids reduces the effectiveness of cryopreservation and fertility after freezing [50]. During cryopreservation, the bis-allylic methylene group of plasma membrane phospholipids is severely damaged due to an increase in reactive oxidising species (ROS) production which in turn leads to lipid peroxidation [51].

2. The amount of maintenance of the lowest possible percentage of respiratory oxidative stress in sperm: unlike oocytes, sperms are characterized by permanent movement and high-level metabolic activity (high respiratory rate) during a very short time, so most of the vital characteristics of sperms deteriorate and fall to standard levels both in optimum temperatures (in vivo) or in conditions of in vitro freezing [52].
By comparing the semen of poultry and mammals concerning size and concentration, it is noted that the concentration of spermatozoa is very high in poultry (6 to 10 \times 10^9 sperm/mL) compared to mammals (1 to 2 \times 10^6 sperm/mL; bull), while the size of the ejaculate (0.4-1.5 ml) is less than that of mammals (4-7 ml), and therefore the high concentration of sperm count in poultry will be negatively affected by excessive dilution, especially after freezing and thawing \[53\]. Besides, the ATP loss is severe after the processes of freezing and thawing, noting that this loss varies from one strain to another and from one species to another (Table 2; \[52\])

Table 2. Adenosine triphosphate concentrations (pmol/10^9 sperm cells) in fresh and frozen/thawed rooster spermatozoa \[52\].

| Male | Fresh sperm | Frozen/thawed sperm |
|------|-------------|---------------------|
| 1    | 5,163.2 ± 0.34^a | 65.04 ± 0.02^b     |
| 2    | 3,644.20 ± 0.53^a| 58.97 ± 0.06^b     |
| 3    | 2,141.50 ± 0.45^a| 64.21 ± 0.03^b  |
| 4    | 3,321.70 ± 0.3^a  | 67.03 ± 0.03^b     |
| 5    | 2,958.12 ± 0.67^a | 59.61 ± 0.04^a     |
| 6    | 2,772.34 ± 0.55^a | 50.26 ± 0.03^b     |
| 7    | 1,643.71 ± 0.32^a | 50.49 ± 0.05^b     |
| 8    | 1,434.16 ± 0.67^a | 48.05 ± 0.02^a     |
| 9    | 2,734.49 ± 0.23^a | 52.07 ± 0.04^a     |
| 10   | 3,600.65 ± 0.44^a | 59.01 ± 0.03^a     |
| 11   | 1,842.32 ± 0.62^a | 63.29 ± 0.07^b     |
| 12   | 3,194.77 ± 0.14^a | 60.07 ± 0.05^b     |

^a,bDifferent superscripts indicate significant differences within rows.

3. The amount of preservation of the highest possible rate of vital characteristics in sperm after the cryopreservation process (e.g. viability, motility and normality): it is worth noting that there is a wide dispersion in the rates obtained after cryopreservation, as each school is advising to follow its own best method in these processes. In references, the percentages generally ranged between 30% to 40% for various vital characteristics \[43\].

4. Cytotoxicity of the cryoprotectants and their chemical behaviour in the sperm cell: cryoprotectants have a toxic effect, the severity of which varies from one type to another, this effect will lead to a decrease in the various vital and morphological characteristics of the sperm in addition to the fertility capacity. The membrane permeability of gametes (sperm and oocytes) to water is determined basically by the composition of membrane phospholipid, the water and ion channel proteins and cytoskeletal elements, but the cryoprotectants change this permeability \[54\]. Glycerol causes oxidative stress and a contraceptive effect \[13,55\]. DMSO has low toxicity compared with glycerol to the spermatic cells \[56\]).

5. The disposition of cryoprotectants after thawing: perhaps the presence of cryoprotectants in the extender is one of the most important issues that warrant research due to the cytotoxicity and the damage they cause to the female genital tract in addition to the low fertility rates. In order to reduce the cytotoxicity effects of the cryoprotectants, several studies recommend some steps to be taken (specifically for glycerol): a) gradient centrifugation; b) dialysis; and c) step-wise dilutions with centrifugation \[57, 58,59\]. The previous steps have been widely applied to the level of poultry sperm, and have given encouraging results in high fertility rates and vital characteristics but they need more work and development in laboratories \[60,59,35\].

6. Post -thawing stage: post -thawing fertilizing ability is one of the factors that play an important role in the various vital characteristics of the sperm because it depends on the time factor (e.g. 5,10 and 15 minutes) and temperature (e.g. 5, 10, 15 °C), as the duration of thawing varies from one protocol to another and the type of the extender. It is known that post-thawing fertilizing ability of chicken semen varies among breeds or lines \[52,8,61,62\], this stage is limited to some strains and lines where specific genotypes can require the modification and adjustment for differences in cryopreservation suitability of poultry semen \[24,25,47,63\].

**Conclusion**

The following is concluded from the present review:
1. Both methods of slow freezing and vitrification were applied in preserving poultry sperm with a relative improvement in fertility rates in the vitrification method.
2. The fertility rates and vital indicators remained relatively low despite the wide variety of cryoprotectants used.
3. Among the cryoprotectants, dimethylacetamide is promising and a strong competitor to glycerol.
4. The poultry sperm-cryopreservation technique is still not achieving its desired success.

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