Universal biotechnological medium for sperm dilution during poultry artificial insemination

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Abstract. The aim of the research was to develop a universal biotechnological environment for diluting sperm during artificial insemination of birds. The creation of the medium was carried out in 4 stages. The first stage is the selection of environmental components of their influence on the survival of sperm cells in vitro. During the second one, the combination of components and their effect on sperm of different species of birds was studied. Subsequently, the effect of the complex of combinations of ingredients on the preservation of sperm motility was determined upon dilution of the ejaculates of roosters, turkeys, cesars, drakes, husaks during in vitro storage. Ejaculates of males of the studied species in quality close to the average species value were used. During the experiments, the ratio of chemical components was determined, which ensured the optimal value of the concentration of hydrogen ions when the medium was diluted in distilled water. The permissible pH of the medium has been established; it must be neutral or slightly alkaline. The pH was adjusted by injecting sodium acetate. The created medium is stored in a dry state for up to 2 months at a temperature of + 6–8 °C in hermetically sealed containers. Sperm diluted with this medium contributes to maintaining the high fertilizing ability of rooster sperm for up to 24 hours, for turkeys and cesaries for 7 hours, for waterfowl for 3-4 hours.

1. Introduction
One of the fundamental elements that determine the efficiency of reproduction of birds when using artificial insemination, is the dilution of sperm. This is because storing undiluted bird sperm at room temperature for 20 and 60 minutes reduces its fertilizing capacity by almost 10% in the first and 50% in the second case, and storage at + 30 °C for half an hour generally leads to zero fertilization of eggs. Storage of ejaculates at a temperature of 2–4 °C for 3 hours during insemination reduces the fertility of chicken eggs by 25%. Fresh turkey sperm loses their fertilizing ability even faster [1-3].

In order to extend the fertilizing ability of sperm in artificial insemination in the early 20th century, I.I. Ivanov [4] proposed to use dilution of sperm with artificial media. In artificial insemination of animals, many different liquids were tested as diluent media for sperm. Some of them are still successfully used in animal husbandry to this day. However, due to differences in mammalian sperm, these media are not effective for birds [5].

In the middle of the last century basing on a detailed study of the effect of various chemicals on the viability of bird sperm, Russian and foreign scientists created and received the ubiquitous medium developed in VNITIP (FRC "VNITIP" RAS) A3, A5, A7, C2, VNITIP 97, 2016 [6,7], in NIIGRZh
(VNIIGRZh branch of the Federal Research Center “VIZh” named after L K Ernst) [8,9], as well as foreign thinners of Lake and others [1,10]. Some of the diluents listed above are used in artificial insemination of poultry to this day.

Considering that in the last decade new knowledge in the field of physiology, biochemistry and other related sciences related to the reproduction of poultry was obtained, large-scale studies were conducted at the FRC “VNITIP” of the Russian Academy of Sciences to create biotechnological media to dilute sperm of various types of poultry. The composition of the media used in artificial insemination of chickens, turkeys, guinea fowls, ducks, geese, differed slightly [6,11,12].

In connection with the foregoing, in order to unify diluent media to make it cheaper, through the application of a powder production technology, the goal was set to create a universal biotechnological environment for diluting sperm during artificial insemination of all species of birds.

2. Materials and methods
Development of a universal biotechnological environment for diluting sperm during artificial insemination of birds was carried out in 4 stages.

The first stage was devoted to the selection of components and the study of their effect on the survival of rooster sperm, at the second stage - the combination of components and their effect on the semen of other bird species. The components used in previously created environments were taken as a basis. Subsequently, the effect of the complex of combinations of ingredients on the preservation of sperm motility was determined by diluting the ejaculates of roosters, drakes, czars, turkeys, husks during in vitro storage. In studies, male ejaculates were used in quality close to the average values for each bird species (table 1).

Table 1 shows the data obtained on the basis of a synthesis of published works, as well as the results of their own research.

| Bird species | Volume of ejaculate, sm³ | Sperm concentration, billion/sm³ | Sperm motility, scorexx)
|---------------|--------------------------|----------------------------------|-----------------
| Roosters      | 1.2                      | 0.2                              | 5.0             | 2.0             | 2.5             | 9 – 10         |
| Turkeys       | 1.0                      | 0.1                              | 10              | 0.3             | 7.0             | 6 – 9          |
| Gosak         | 1.3                      | 0.2                              | 1.3             | 0.2             | 6.0             | 6 – 8          |
| Drakes        | 0.5                      | 0.05                             | 8.0             | 1.5             | 3.0             | 8 – 9          |
| Caesar        | 0.2                      | 0.01                             | 7.0             | 1.5             | 4.0             | 6 – 10         |

xx) Minimum values apply to males used for artificial insemination.

As it can be seen from table 1, gooses have the largest volume of ejaculate. The average sperm volume exceeds more than three times the cesar and is significantly higher than that of the males of other species. The concentration of sperm is the highest among turkeys, and the lowest - among goose dogs. The level of sperm production in various bird species is on average 0.3–1.4 billion in each ejaculate. The highest sperm have turkeys, and the lowest - gander. Different sperm concentrations affect their motility.

For studies we used sperm with average concentration and motility. The sperm concentration was evaluated by centrifuging. The motility of sperm was assessed visually under a microscope at a magnification of 200 times on a 10-point scale. Diluted sperm was stored at a temperature of + 5-6 ° C in closed bottles in a domestic refrigerator. The main factor taken into account when creating a universal biotechnological environment was the assessment of sperm motility during dilution and storage in vitro.

3. Research results and their discussion
When choosing the components of the created medium, we proceeded from the peculiarities of the in vitro reaction of sperm of various bird species to certain chemical elements.
Salt was selected as the main substance, dissociating when dissolved into positively charged Na ions and negatively charged ions of the acid residue, resistant to further dissociation. Thus, the quantitative ratio of anions and cations created a buffer system necessary to maintain a positive charge on the sperm, the presence of which did not allow sperm to stick together and ensure their forward movement. In addition, the presence of electrolytes and non-electrolytes contributed to the maintenance of the required parameters of osmotic pressure, and sodium ions had a positive impact on the preservation of mobility, survival and fertilizing ability of sperm due to the preservation of enzymes contained in sperm cells.

To provide energy for the movement of sperm, sugar was introduced into the medium, which, in addition to the energy source, is involved in maintaining the optimal osmotic pressure in the diluent and serves as the antipode for Na ions, which is an electrolyte.

8 chemical compounds were tested in 17 combinations. They were used in various ratios: sucrose, glucose, tartaric and acetic acids, salts - K, Na tartrate, potassium phosphate, sodium carbonate.

Options were selected to ensure the motility of sperm in ejaculates from 8 to 9 points, diluted in environments with a pH in the range from 6.95 to 8.15. The number of components in these environments was from 3 to 6.

As further studies have shown, despite the insignificant difference in the survival of sperm in freshly diluted ejaculates at pH close in value in different combinations, the fertilizing ability of sperm during insemination was different and ranged from 50 to 92%.

As a result of laboratory experiments, the ratio of components was found, providing the optimal value of the concentration of hydrogen ions when the medium was diluted in distilled water. To maintain the pH of the diluent, sodium acetate was added to the medium in an amount determined in laboratory experiments. In artificial insemination, it is necessary to maintain a neutral or weakly alkaline medium in diluted sperm. This environment contributes to the neutralization of lactic acid, carbon dioxide and carbon dioxide emitted by sperm during movement.

In further studies, 2 media with the best fertility of eggs during artificial insemination of poultry were used. In two experiments, the pH change in these media was studied at 1, 5, 24, and 48 hours after dilution with distilled water and stored in a domestic refrigerator at a temperature of +5–6 °C. These environments differ in a different set and a different quantitative ratio of components (table 2). Therefore, as it follows from the data in the table, the pH changes in used media during storage to the alkaline side in proportion to the amount of CO₂ released by motile sperm.

| Media number | Experiment | Freshly prepared medium | The storage time of the diluent, hours |
|--------------|------------|-------------------------|---------------------------------------|
|              |            |                         | 1  | 5  | 24 | 48 |
| 1            | 1          | 7.1                     | 7.1          | 7.25 | 7.4 | 7.6 |
|              | 2          | 7.1                     | 7.25          | 7.45 | 7.6 | 8.0 |
|              | Average    | 7.1                     | 7.17            | 7.35 | 7.5 | 7.8 |
| 2            | 1          | 8.05                    | 8.05           | 8.15 | 8.2 | 8.25 |
|              | 2          | 8.05                    | 8.15            | 8.35 | 8.5 | 8.65 |
|              | Average    | 8.05                    | 8.1             | 8.25 | 8.35 | 8.45 |

As it is seen from the data of table 2, the exact match of pH in different experiments was not observed, however, differences in pH values are within the measurement error. On average, in the first medium the pH changes by 0.7 units, in the second - by 0.4. The second medium in both experiments is characterised by more stable pH. These experiments indicate a rational selection of components, as a result of which, when dissolved, the chemicals do not react.

Sperm diluted with both media maintained high biological qualities for 6–8 hours. The fertilization of chicken eggs when using environment No. 1 is 94.5–96 %, environment No. 2 – 93.9–95.0 %. This fertilization of eggs after a day of storage of diluted sperm decreased by 3-3.5% and amounted to 91.5 and 92.5%, respectively.
Environments underwent production testing at various poultry farms. When using medium No. 1, fertilization of eggs was 90–93.6%, and that of medium No. 2, 91.1–95.6%.

**Table 3.** Fertilization of eggs in artificial insemination of poultry with sperm diluted with different media.

| Bird species | Environment No. 1 (VNITIP - 2016) | Environment No. 2 (new) |
|--------------|-----------------------------------|------------------------|
|              | storage of diluted sperm, hours    | egg fertilization, % a) | storage of diluted sperm, hours | egg fertilization, % a) |
| Chickens     | 24                                | 91.0                   | 24                                | 94.5                   |
| Guinea Fowl  | 4                                 | 89.0                   | 7                                 | 92.0                   |
| Turkeys      | 4                                 | 87.0                   | 7                                 | 92.0                   |
| Ducks        | 2                                 | 89.0                   | 4                                 | 94.0                   |
| Geese        | 0.5                               | 85.0                   | 3                                 | 91.0                   |

a) Indicators of egg fertilization are given using freshly diluted sperm.

Studies have shown that the newly obtained medium in dry form is stored for 2 months, without losing quality, at a temperature of + 6–8 °C in hermetically sealed containers. Sperm diluted with this medium contributes to maintaining the high fertilizing capacity of rooster sperm for up to 24 hours, turkeys, cesars for 7 hours, waterfowl for 3–4 hours. Increasing the shelf life of diluted sperm is a very important factor when organizing work in conditions of high production of hatching eggs. Medium diluted with distilled water is well preserved in a domestic refrigerator for 24 hours, and at room temperature for 8–10 hours.

Higher fertility of eggs is due to the fact that the new environment contributes to the preservation of the fertilizing ability of sperm, not only in vitro, but also in the oviduct of females. The proposed biotechnological environment allows us to extend the collection of hatching eggs after each insemination and increase the intervals between subsequent insemination by 1–2 days. This reduces the number of insemination and reduces labour costs in obtaining incubation eggs by 10–15%.

**4. Conclusion**

Studies have shown that the newly created medium-diluent of bird sperm in dry form can be stored in hermetically sealed containers, at a temperature of + 6–8 °C, for 2 months without losing quality. A patent of the Russian Federation No. 2637774 has been received for the environment. Sperm diluted with this medium contributes to maintaining the high fertilizing ability of rooster sperm for up to 24 hours, turkeys, cesars for 7 hours, waterfowl for 3–4 hours.

Dilution of sperm by the biotechnological medium (RF patent No. 2637774) helps to reduce the cost of maintaining males by increasing the sex ratio, as well as extending the biological usefulness of sperm “in vitro”. Dilution of sperm provides fertilizing ability in the genital tract of females of all types of poultry by extending the viability of sperm and reducing their enzymatic constant.

The extension of the time needed to preserve the biological usefulness of sperm in vitro is also necessary for the rational organization of labour during artificial insemination in large volumes of hatching eggs.

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