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The comparison of sperm motility and density in four different goldfish (*Carassius auratus*) types

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SUMMARY

Different goldfish types play an important role both in ornamental fish farming and science. Considering its historical background, the goldfish is a suitable model animal for the study of artificial selection as well as for developmental biological studies. Sperm motility and cell density is an important parameter in determining sperm quality. The aim of our study was to examine the effects of different goldfish types on the sperm quality. Several sperm motility parameters (progressive motility (pMOT, %), straight line velocity (VSL, µm s^{-1}), curvilinear velocity (VCL, µm s^{-1}), linearity (LIN, %), amplitude lateral Head Displacement (ALH, µm), Beat Cross Frequency (BCF, Hz)) of four different goldfish types (Common goldfish-"wild type" N=5, Shubunkin N=4, Black Moor N=4, Oranda N=5) was compared during 60 hours (at 12-hour intervals) at refrigerated storage (4 °C). The variability of sperm density was also investigated in all types. A similar cell concentration was determined in the four goldfish variants (Common goldfish $2.01*10^{10} \pm 3.46*10^{9}$; Shubunkin $1.71*10^{10} \pm 3.25*10^{9}$; Black Moor $1.66*10^{10} \pm 3.02*10^{9}$; Oranda $1.56*10^{10} \pm 5.83*10^{9}$). Statistically significant difference between the 4 goldfish types in the motility parameters and cell density was not noted. However, a decreasing tendency in Black Moor sperm motility parameters (pMOT, VCL and VSL) was observable, as well as a reduced spermatozoa density in Oranda was also recorded. Our results can contribute to the improvement of the common hatchery propagation of goldfish. Future studies can add more evidence of the possible effects of artificial selection on the reproduction in different goldfish types.

Keywords: *goldfish types; artificial selection; sperm quality*

INTRODUCTION

Goldfish (*Carassius auratus auratus*) is widespread throughout the world as one of the most famous cultured fish. Since their domestication started, the variability of different goldfish types was strongly influenced by spontaneous mutations and human preferences. Thus, goldfish are the most prominently domesticated fish in the world. (Komiyama et al., 2009). Different goldfish types play an important role both in ornamental fish farming and science (Ahmadmoradi et al., 2012). Considering its historical background, the goldfish is a suitable model animal for the study of artificial selection as well as for developmental biological studies (Li et al., 2019).

The refrigerated storage of semen is a simple and inexpensive procedure that can facilitate the management and reproduction programs in aquaculture. The method allows to store semen for *in vitro* reproduction, quality assessment, the support of hatchery propagation, the selective (*in vitro*) breeding, disease diagnosis and advanced molecular studies (Contreras et al., 2019). Refrigerated storage is essential for the shipping of samples and the establishment of cryopreservation programs and germplasm banks for all species (Glenn et al., 2011; Contreras et al., 2019).

Sperm motility and cell density are an important parameter in determining sperm quality. Both parameters can directly affect the fertilization rate in fish (Fauvel et al., 2010). The sperm quality assessment of male broodstock during cold storage is important from the view of the reproductive ability in cultured fish (Contreras et al., 2019). In the previous study of Bernáth et al. (2017) significant difference was observed in case of the chilled and post-thaw stored sperm in three goldfish variants. According to their concept, the variation among different types observed in their experiments can be caused by an extensive selection program. The aim of our study was to examine the effects of short-term storage on the sperm quality of different goldfish types. Furthermore, the possible effects of the artificial selection on the sperm quality in the case of the four goldfish types were also investigated.

MATERIALS AND METHODS

Broodstock management and sampling

The males were kept in a recirculating aquaculture system at 22 °C. The spermiation was hormonally induced using 2 mg bodyweight kg^{-1} of carp pituitary 24 hours prior to sampling. Fish were anesthetized with 2-phenoxyethanol (99%, 0.4 mL L^{-1}) (Saad and Billard, 1987) prior to sampling. Semen was collected by abdominal massage using a 2 mL syringe.

Sperm motility assessment

Sperm motility of four different goldfish types (Common goldfish- "wild type" *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5) was compared during 60 hours (at 12-hour intervals) chilled (refrigerated storage, 4 °C). In our experiments, progressive motility (pMOT, %), straight line velocity (VSL, µm s^{-1})

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curvilinear velocity (VCL, μm s^{-1}), linearity (LIN, %), amplitude lateral Head Displacement (ALH, μm), Beat Cross Frequency (BCF, Hz) in the 4 various types were analyzed using a CASA (Computer-assisted Sperm Analysis; Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA) system. Sperm cells were activated in a modified activating solution for cyprinids (45 mM NaCl, 5 mM KCl, 30 mM Tris, pH 8.0 ± 0.2, Saad et al., 1988) in a mixture with 0.01 g mL^{-1} bovine serum albumin (BSA). Measurements were carried out at least in duplicates (at least 300 cells per activation).

Sperm concentration

Individual samples were diluted in a salt-based extender (200 mM KCl, 30 mM Tris, pH 8 ± 0.2, Kollár et al., 2013) at a ratio of 1:999. Diluted sperm was loaded into a Bürker-type haemocytometer (Marienfield Superior, Paul Marienfeld GmBH & CO. KG, Lauda-Königshofen, Germany). Cells were counted using the open-source software Image J 1.48v (Image J for Windows, National Institutes of Health, USA).

Statistical analysis

Data obtained from motility and cell density measurements were analyzed using GraphPad Prism 8.0.1. for Windows (GraphPad Software, La Jolla, California, USA). Differences between groups were analyzed using Kruskal-Wallis followed by Dunn's post hoc tests at the significance level of $p<0.05$.

RESULTS

Based on the examined CASA parameters, significant difference was not observed between the 4 variants, as well as significant decrease was not recorded during 60 hours (hr) of storage in neither of them (*Figure 1–6*).

Figure 1: **The pMOT of the four goldfish types measured during 60 hours chilled storage. Columns represent mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**

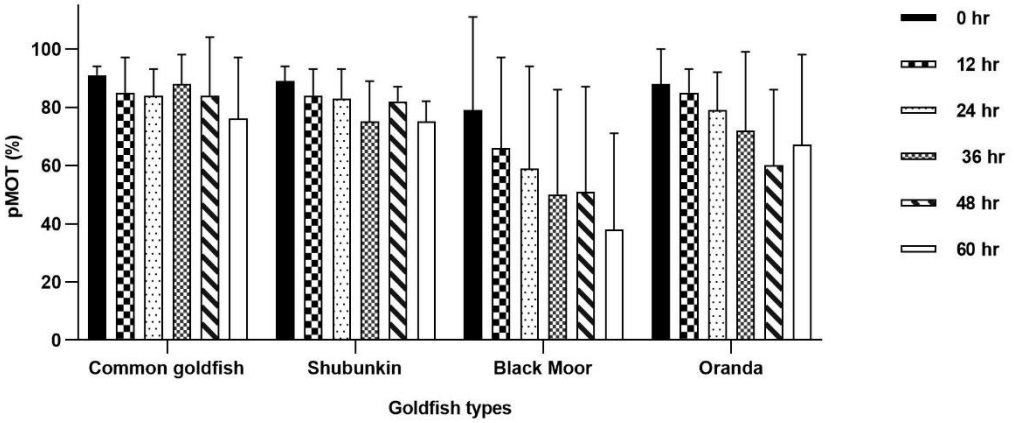

Figure 2: **The VSL of the four goldfish types measured during 60 hours chilled storage. Columns represent mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**

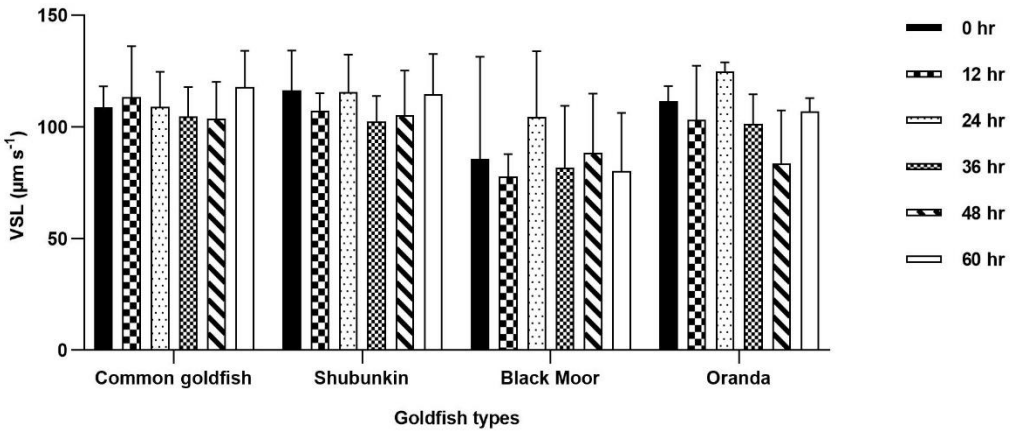

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Figure 3: **The VCL of the four goldfish types measured during 60 hours chilled storage. Columns represent mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**

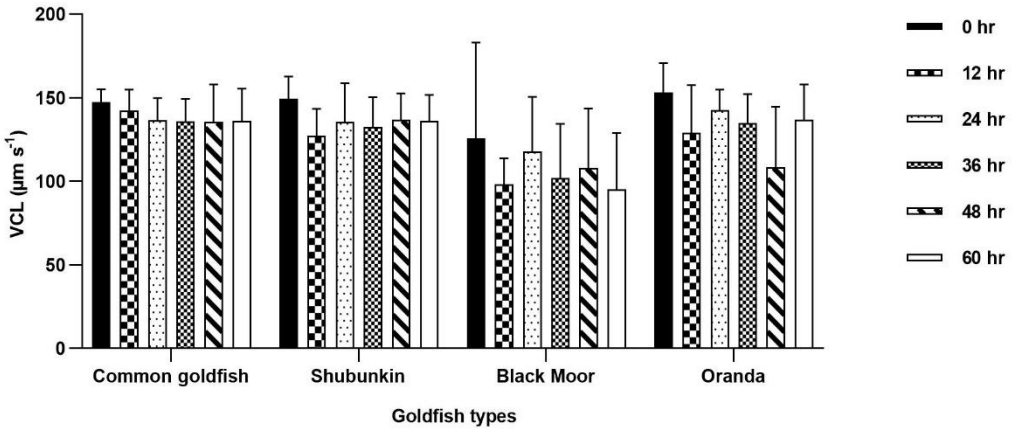

Figure 4: **The LIN of the four goldfish types measured during 60 hours chilled storage. Columns represent mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**

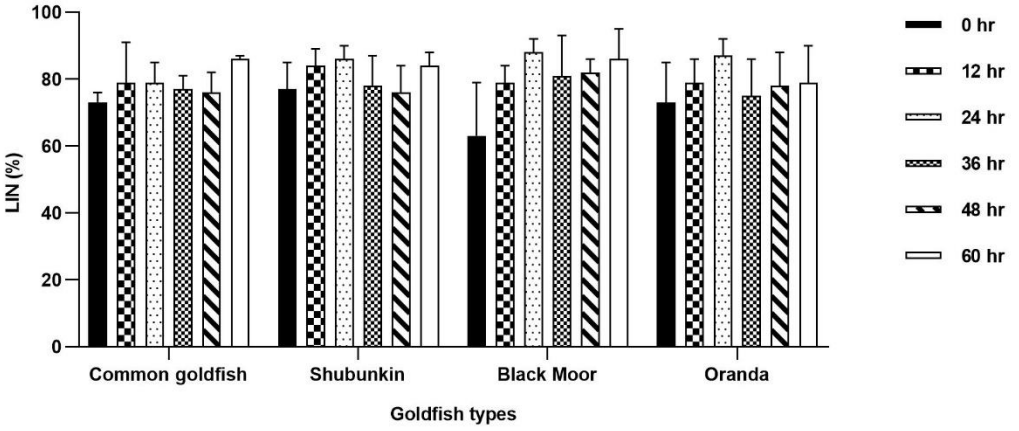

Figure 5: **The ALH of the four goldfish types measured during 60 hours chilled storage. Columns represent mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**

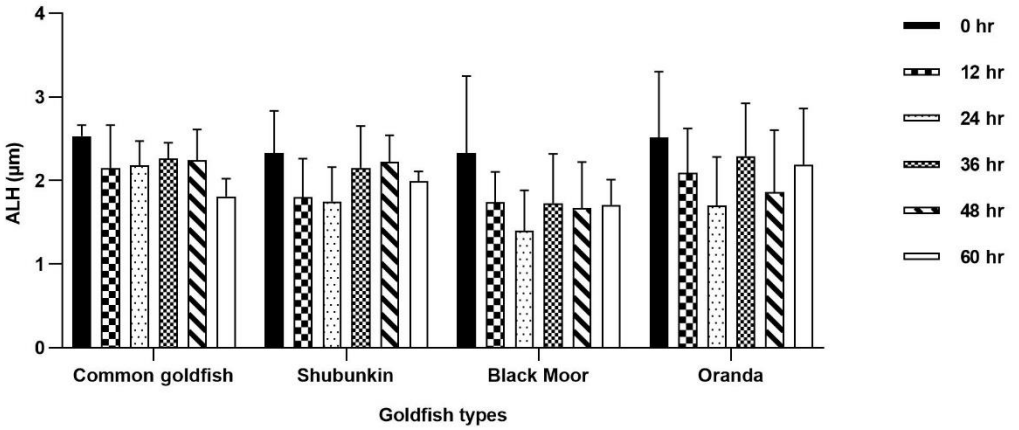

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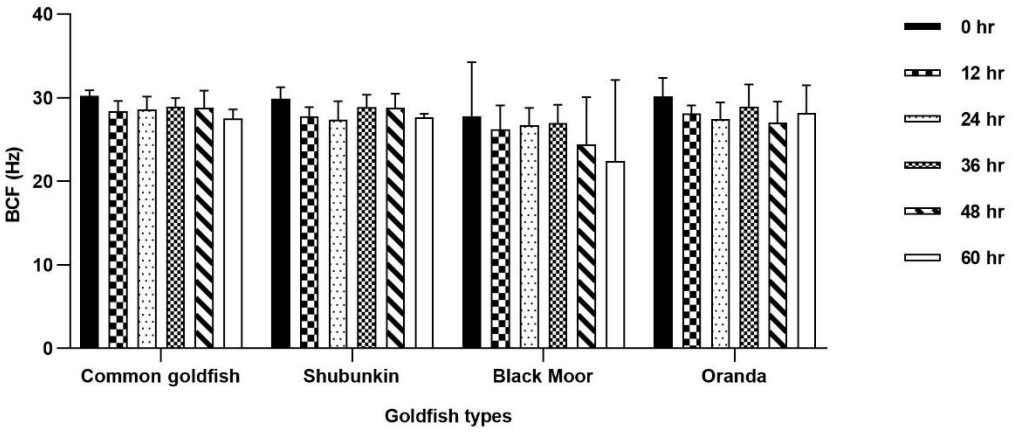
Figure 6: **The BCF of the four goldfish types measured during 60 hours chilled storage. Columns represent mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**



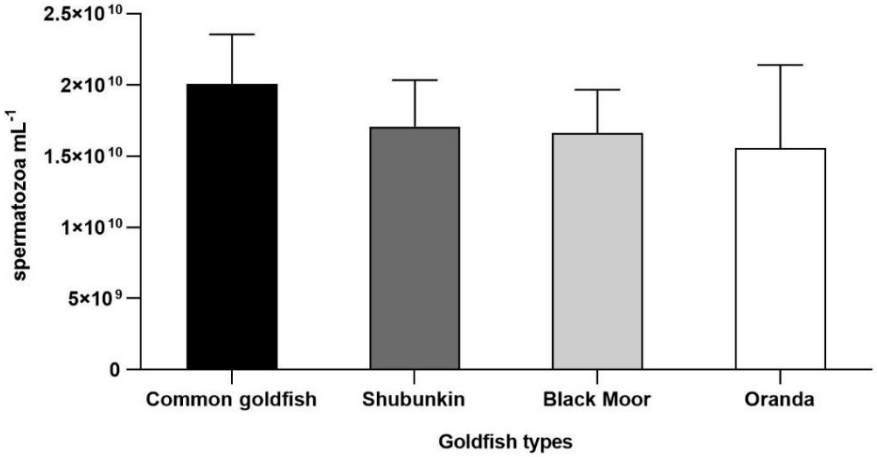
Measured CASA parameters of the four types at 60 hr compared to 0 hr did not decrease significantly. However, a slight reduction was observable in Oranda, as well as a perceptible decreasing tendency in Black Moor (*Table 1*).

Furthermore, a similar cell concentration was determined in the four goldfish variants (Common goldfish $2.01*10^{10} \pm 3.46*10^{9}$; Shubunkin $1.71*10^{10} \pm 3.25*10^{9}$; Black Moor $1.66*10^{10} \pm 3.02*10^{9}$; Oranda $1.56*10^{10} \pm 5.83*10^{9}$) (*Figure 7*).

Table 1: **The CASA parameters recorded at 0 hr and 60 hr in the four goldfish types. Mean ± SD (p<0.05). Common goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**

	COMMON GOLDFISH		SHUBUNKIN		BLACK MOOR		ORANDA	
	0 hr	60 hr	0 hr	60 hr	0 hr	60 hr	0 hr	60 hr
PMOT (%)	91 ± 3	76 ± 21	89 ± 5	75 ± 7	79 ± 32	38 ± 33	88 ± 12	67 ± 31
VSL (µM S^{-1})	109 ± 10	118 ± 16	116 ± 18	115 ± 18	86 ± 46	80 ± 26	111 ± 7	107 ± 6
VCL (µM S^{-1})	147 ± 8	136 ± 19	149 ± 13	136 ± 16	126 ± 57	95 ± 34	153 ± 18	137 ± 21
LIN (%)	73 ± 3	86 ± 1	77 ± 8	84 ± 4	63 ± 16	86 ± 9	73 ± 12	79 ± 11
ALH (MM)	2.53 ± 0.13	1.81 ± 0.21	2.33 ± 0.50	1.99 ± 0.19	2.33 ± 0.92	1.71 ± 0.30	2.51 ± 0.79	2.19 ± 0.67
BCF (HZ)	30.23 ± 0.24	27.49 ± 1.12	29.90 ± 1.33	27.63 ± 0.48	27.74 ± 6.53	22.45 ± 9.68	30.16 ± 2.21	28.21 ± 3.27

Figure 7: **The sperm density recorded in the four goldfish types. Columns represent mean ± SD (p<0.05). Common Goldfish *N*=5, Shubunkin *N*=4, Black Moor *N*=4, Oranda *N*=5.**



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DISCUSSION

In aquaculture, semen short-term storage is a technique designed to improve reproductive procedures and retain semen in suitable conditions to maintain its fertilizing capacity (Contreras et al., 2020). In the case of the four investigated goldfish types the chilled storage for 60 hr had no effect on the sperm motility parameters. Contrary, negative effect of the short-term storage was identifying in some different goldfish types in the experiment of Bernáth et al. (2017). The sperm was investigated during 48 hr at 4 °C. A significant decrement in pMOT after 42 (23 ± 2%) and VCL after 36 (94 ± 12 μm s^{-1}) hours was observed in the Oranda type. In Calico type pMOT decreased significantly already after 18 (42 ± 26%) and VCL after 6 (10^5 ± 8 μm s^{-1}) hours. Similar to our results motility parameters of Black Moor sperm did not decrease significantly during 48 hours of storage, although a regressive trend was observable in both studies. According to our results and the former study of Bernáth et al. (2017) selective breeding can affect sperm sensitivity for chilled storage in different goldfish types as well as in different populations.

In the experiment of Glenn et al. (2011) three different extenders (C-F HBSS, NaCl, and $NaHCO_3$) were examined in the case of goldfish. Percent motility of sperm samples in the extenders was assessed at 24-hour intervals. The storage in extenders had a significant effect on goldfish sperm motility. Motility of sperm diluted in C-F HBSS was 57 ± 52%, in NaCl was 46 ± 32% at day 3. Sperm cells failed to fully suspend in solutions of $NaHCO_3$, and motility ceased within 1 day. Furthermore, motility was not observed also in undiluted sperm samples at day 4. No motility occurred in any activated sperm following 6 days. The short-term storage in adequate extender can elongate the motility of the sperm.

Sperm density has been traditionally used for the assessment of milt quality (Zadmajid et al., 2013). In our experiment the lowest cell density was determine in the case of Oranda ($1.56*10^{10}$ ± $5.83*10^9$). Bernáth et al. (2017) diluted sperm samples of three different goldfish types (Black Moor, Oranda and Calico) in a sugar-based extender. Similarly to our results, the lowest cell concentration was measured in the Oranda type however, the value did not differed significantly from the two other types (Bernáth et al., 2017). According to our hyphothesis, in the former study of Bernáth et al. (2017) and in our study the sample size was not sufficient to reveal the clear differences between the investigated goldfish variants.

CONCLUSIONS

In the case of the four investigated goldfish types the chilled storage is applicable for 60 hours. This finding can contribute to the improvement of the common hatchery propagation. Based on the high standard deviation, the sample size was not sufficient for this experiment. A higher number of individuals can help to record the exact differences between the different goldfish types in sperm quality. Future studies can add more evidence of the possible effects of artificial selection on the reproduction in different goldfish types and in different populations.

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