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Embryology

EFFECTS OF QUANTITY AND QUALITY OF CARP SPERM ON EGG FERTILISATION SUCCESS

WPŁYW ILOŚCI I JAKOŚCI NASIENIA KARPIA NA STOPIEŃ ZAPŁODNIENIA IKRY

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During the artificial spawning, the carp (*Cyprinus carpio* L.) eggs were fertilised with sperm added in various volumetric and quantitative proportions and assessed in terms of spermatozoa motility. The spermatozoa/egg ratio was based on spermatozoa densities in each sample. The analysis of the fertilisation success served to verify conditions crucial to the artificial fertilisation of carp. The results obtained show the percentage of fertilised eggs to increase with increasing number of spermatozoa per 1 egg, provided the spermatozoa are performing the progressive movement.

INTRODUCTION

Reproduction of fish under artificial conditions makes it possible to test the quality of eggs (Brzuska and Bieniarz, 1977) and of sperm Ginzburg, 1968; Tomasik, 1973; Winnicki and Tomasik, 1976) prior to fertilisation. So far, sperm quality vs. net result of fertilisation was tested in trout (Goryczko and Tomasik, 1975), while the results of carp egg fertilisation were considered only in terms of the sperm to eggs ratio, the sperm (its texture, amount of water) being evaluated macroscopically; in some cases, the

spermatozoa motility was assessed (Dyk and Lucky, 1954; Kossmann, 1973; Stein, 1975). Matlak (1970) reported positive results obtained when using 3–4 cm^3 of sperm per 250–300 g of roe. Wolny (unpubl.) considers a proportion of 200 cm^3 of roe to 13–20 drops of sperm obtained from at least 3 males and mixed to be satisfactory for carp.

It seems necessary to find an objective criterion for the carp sperm quality assessment by means of accurate microscopic methods.

The aim of the present study was to work out such an assessment method and apply it to define how the amount and quality of sperm influences the fertilisation success.

MATERIALS AND METHODS

Artificial spawning of carp was conducted in 1981 at the Laboratory of Fish Biology and Aquatic Environment hatchery, Zator. The available materials were used; the roe was obtained from 21 females of the following strains: Hungarian (8), Yugoslav (10), and Starzewo (3), while the sperm was collected from 42 males of the following strains: Hungarian (22), Yugoslav (6), Zator (12), and Starzewo (2).

Before spawning, the females were injected with the carp hypophysis homogenate: 0.5 mg/kg body weight and 2.0 mg/kg body weight at the first and second injection, respectively, the second one being administered 10 h after the first.

Some females yielded oocytes for the vital sexual maturity determination prior to the injections (as in Brzuska and Bieniarz, 1977). The males were not injected with the hypophysis hormone.

Prior to fertilisation the sperm was evaluated in terms of spermatozoa viability and density. The viability was measured as the duration of various movement phases (in 0.01 min.) and percentage of spermatozoa performing various movements: progressive, circling, and oscillatory. The evaluation involved estimating the percentage of spermatozoa at a given phase of movement and resulted from observing a few fields of view at about 200X magnification. The following point score scale was used:

a) 100% of spermatozoa in progressive movement — 3 points
   100% of spermatozoa in circling movement — 2
   80% of spermatozoa in progressive movement — 1
   80% of spermatozoa in circling movement — 1
b) duration of progressive movement: 0.30–0.35 min. — 4
   0.36–0.40 min. — 3
   0.41–0.46 min. — 2
   0.46–0.50 min. — 1

The total result in the sum of scores from a) and b).

The spermatozoa density was measured in the Bürker chamber. Additionally, the total number of spermatozoa in sperm unit volume (1 cm^3) and in the sperm dose used was calculated. The latter served to calculate the spermatozoa/egg ratio. The fertilising

Table 1

Results of Experiment I. Incubation in small battery

Female No.	Male No.	Spermatozoa movement (min.) % moving spermatozoa)			Sperm volume (cm^3)	Roe volume (cm^3)	Sperm/roe volumetric ratio	Spermatozoa no. in. dose($x10^9$)	No of eggs per sample ($x10^3$)	Number of spermatozoa number of eggs ratio	% fertilisation after 24 h	Roe treatment method
		progressive	circling	oscillatory								
W-150 (23.05.81)	W-25	0.38/100	0.20/100	0.52/50	0.01	30	1 : 3000	0.245	24	10208 : 1	11.6	Woynarovich,
					0.1	30	1 : 300	2.450	24	102083 : 1	53.9	Woynarovich,
					1.0	30	1 : 30	24.500	24	1020833 : 1	29.3	1980
					2.0	30	1 : 15	49.000	24	2041666 : 1	52.2	
Control sample in large battery	W-2	5% spermatozoa in progressive movement phase, remaining dead or oscillating			0.01	30	1 : 3000	0.320	24	1333 : 1	4.0	Woynarovich,
					0.1	30	1 : 300	3.200	24	133333 : 1	5.3	Woynarovich,
					1.0	30	1 : 30	32.000	24	1333333 : 1	8.0	1980
					2.0	30	1 : 15	64.000	24	2666666 : 1	24.4	
	W-30	0.45/100	0.20/80	0.40/50	5.5	350	1 : 64	170.500	280	608929 : 1	97.5	Woynarovich, 1962 and 1964
W-136 (28.05.81)	Z-11	0.47/100	0.31/100	0.72/80	0.01	30	1 : 3000	0.255	24	10625 : 1	2.6	Woynarovich,
					0.1	30	1 : 300	2.550	24	106250 : 1	0	Woynarovich,
					1.0	30	1 : 30	25.500	24	1062500 : 1	26.3	1980
					2.0	30	1 : 15	51.000	24	2125000 : 1	12.4	
Control sample in large battery	J-37B	0.43/100	0.27/100	0.35/80	0.01	30	1 : 3000	0.170	24	7083 : 1	0	Woynarovich,
					0.1	30	1 : 300	1.700	24	70833 : 1	3.2	1962 and 1964
					1.0	30	1 : 30	17.000	24	708333 : 1	1.5	
					2.0	30	1 : 15	34.000	24	1416667 : 1	32.2	
	Z-11	0.47/100	0.31/100	0.72/800	2.5	90	1 : 36	63.750	72	885417 : 1	20.8	Woynarovich, 1962 and 1964

capability of a given sperm sample was tested: experiment I involved 0.01–2.0 cm^3 of sperm and 30 cm^3 of roe (Table 1); 1.0–7.0 cm^3 of sperm and 50–350 cm^3 of roe were used in Experiment II, while in Experiment III 90–350 cm^3 of roe were fertilised with 2.5–5.5 cm^3 of sperm. It was assumed that 1000 cm^3 of roe contained about 800,000 eggs.

Following its fertilisation in the Woynarovich solution I (40 g NaCl and 30 g urea in 10 l water), the roe was subsequently rendered nonviscous in solutions I and II (16 g tannin in 10 l water) (Woynarovich, 1962, 1964). Those experiments involving small roe samples were conducted in small (about 0.6 l) Weiss jars; the remaining runs were carried out in a large incubation battery (7 l jars).

RESULTS

Experiment I. The roe was collected from 2 Hungarian females. As stated in Table I, 5% of sperm collected from the Hungarian male W-2 showed progressive movements, the remaining males yielding sperm of a very good motility: 100% of spermatozoa in progressive and 80–100% in circling movements. The volumetric sperm/roe ratio in

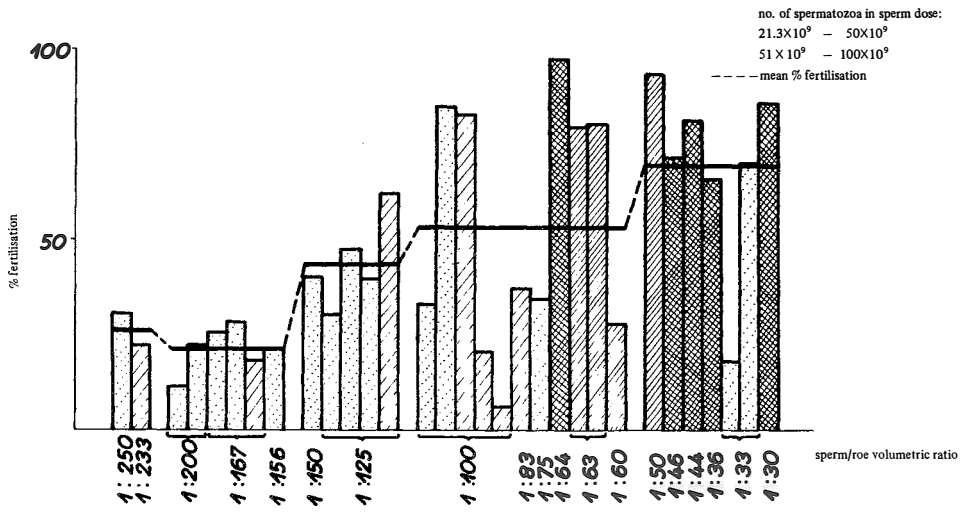

Fig. 1. Fertilisation success vs. sperm/roe volumetric ratio; experiment II.

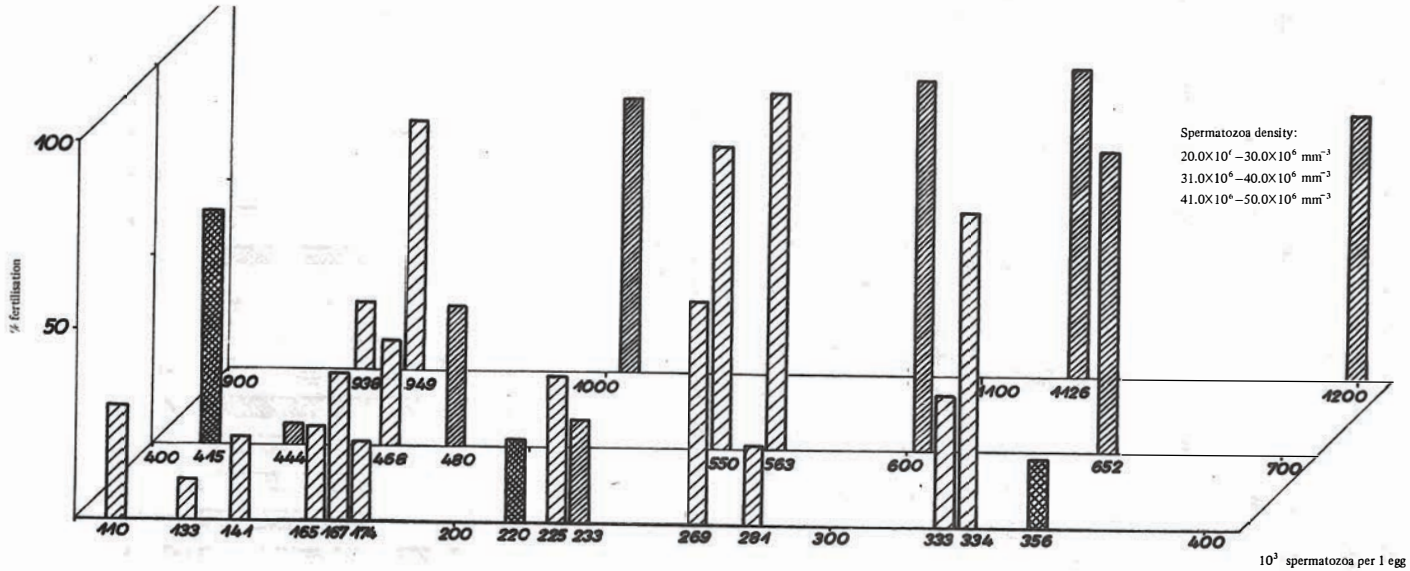

Fig. 2. Fertilisation success vs. spermatozoa number per 1 egg; experiment II.

Experiment I ranged from 1:15 to 1:3000. The spermatozoa density ranged from 17.0 x 10^6/mm^3 to 32.0 x 10^6/mm^3, which – when converted to the number of spermatozoa per dose – gave a range of 0.17 x 10^9 to 170.5 x 10^9 (Table 1). A 30 cm^3 roe sample was calculated to contain about 24 x 10^3 eggs, the controls containing 72 x 10^3 and 280 x 10^3 eggs. The per cent fertilisation ranged from 0.0 to 97.5% /♀ W–150 ♂ W–30 control sample. Table 1 shows the poorest results to have been obtained from the W-2 and Z-11 males, 4.0–24.4% and 20,8% fertilisation, respectively, the highest per cent fertilisation (11.6–53.9%) being obtained for the female W–150 roe and male W–25 sperm. Better fertilisation results were obtained when using sperm of a very good and good motility.

Experiment II. Fig. 1 illustrates the relationship between the fertilisation success and sperm/roe volumetric ratio, while Fig. 2 shows the per cent fertilisation as related to the number of spermatozoa/number of eggs ratio in the same experiment. The fertilisation success ranged from 6.3% to 97.5% and increased with increasing volume of sperm per roe unit volume (Fig. 1). Additionally, Fig. 1 shows the range of total numbers of spermatozoa in various sperm doses (21.3 x 10^9–216.15 x 10^9). The highest per cent fertilisation was obtained in the roe samples mixed with sperm doses containing 100 x 10^9–200 x 10^9 spermatozoa. Fig. 2 reveals a growing trend in the per cent fertilisation at a higher spermatozoa number per 1 egg. Additionally, Fig. 2 presents the spermatozoa densities (20.0 x 10^3–50.0 x 10^3/mm^3) in the samples.

Experiment III. This experiment includes following the effects of spermatozoa motility in sperm portions used. The range of sperm/roe volumetric ratio was 1:36 to 1:100 (Fig. 3). Based on the total number of spermatozoa (17.5 x 10^9–41.0 x 10^9/cm^3), numbers of spermatozoa in various doses were calculated to amount from 57.0 x 10^9 to 196.9 x 10^9.

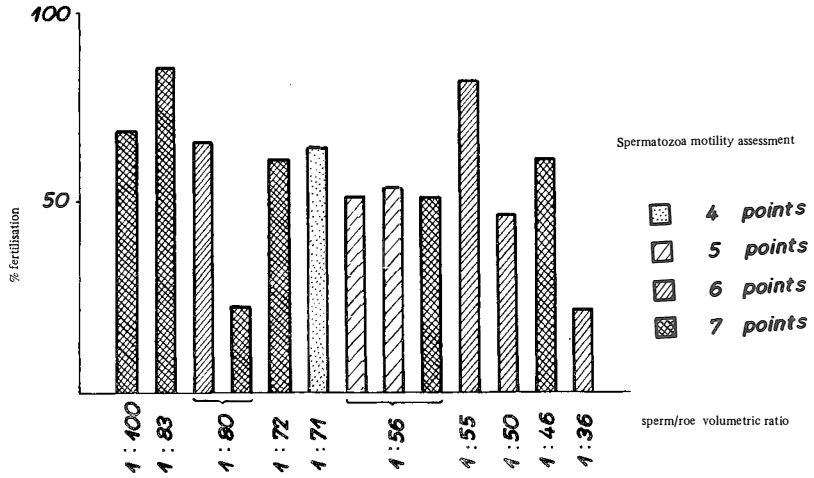

Fig. 3. Fertilization success as evidenced by experiment III; spermatozoa motility.

The per cent fertilisation in this experiment was found to range from 20.8% to 85.2%. As shown in Fig. 3, the motility of spermatozoa bears a considerable effect on egg fertilisation. The maximum motility score obtained was 7. The score was in a reverse proportion to the duration of phase I (the progressive movement) and in direct proportion to the number of spermatozoa performing this type of movement. A certain increase in the per cent fertilisation was found at a higher score, even at a higher i.e., less favourable sperm/roe volumetric ratio (e.g., 1:100, 1:83).

DISCUSSION

The principal objective of the three experiments performed was to compare the volumes and densities of sperm used with corresponding volumes of roe and to look for a possible relationship between the fertilisation success and the number of spermatozoa/number of eggs ratio.

Kiselev (1957) related the success of fertilisation to the sperm volume: the percentage of fertilised eggs increased from 0.5 to 44.4% when 1.0 cm^3 of sperm was used for 5×10^3 eggs (about 6 cm^3 of roe). The results contained in table 1 fail to reveal any unequivocal relationship between the sperm volume used and per cent fertilisation. However, a tendency for an increasing fertilisation success with increasing sperm volume per roe unit volume is supported by the results of Experiment II (Fig. 1). The results of all three experiments allow to suppose that the optimal sperm/roe volumetric ratio is within the range of 1:100–1:50 in spite of a positive result (53.9% fertilisation) being also possible at 1:300 ratio (Table 1).

On the other hand, a relationship between the per cent fertilisation and number of spermatozoa per 1 egg is evident. The number of spermatozoa in a sperm dose is determined by the spermatozoa density (Wolny, 1974); e.g., 2 cm^3 of sperm obtained from the male W–2 contained 64×10^9 spermatozoa, i.e., almost twice as many as in 2 cm^3 of sperm of the male J–37B (34×10^9). Furthermore, the higher the number of spermatozoa in a sperm dose, the higher fertilisation success as shown in Table 1 comparing the data for all the males.

The males used in Experiment II showed a range of total spermatozoa number in their sperm, which resulted in a range of spermatozoa number in doses used for fertilisation ($21.3 \times 10^9 - 216.15 \times 10^9$); a mean per cent fertilisation (Fig. 1) exceeds 50% when the spermatozoa number in a dose is $100 \times 10^9 - 200 \times 10^9$. Fig. 2 confirms the fact of increasing fertilisation success with increasing spermatozoa number/egg number ratio. On the other hand, the spermatozoa densities in Experiment II shown in Fig. 2 do not seem to be an unequivocal measure with which to assess the sperm quality unless they are compared to number of eggs. So far, effects of sperm concentration on fertilisation success have been assessed. Moczarski (1976) found a statistically significant correlation between per cent fertilisation of eggs from a single female and spermatozoa density. This single fact of the correlation occurred under the following conditions: amount of roe to

be fertilised: 3 cm^3; amount of sperm used: 0.4 cm^3; spermatozoa density: 31 x 10^3–160 x 10^3/mm^3. According to Mussielus (1951), the higher sperm concentration, the better viability of the offspring and the higher mean body weight of fry. That author reports results of an experiment showing a mean of 60.13% fertilisation of roe fertilised with sperm of 26.9 x 10^6 spermatozoa/mm^3, while a mean of 37.94% fertilisation was obtained when using sperm containing 18.5 x 10^6 spermatozoa/mm^3.

It seems that the number of spermatozoa per a defined number of eggs can be an indicator of sperm utility provided the spermatozoa viability is considered as well. For example, the success of fertilisation was relatively high with the sperm from males W–25 and W–30 (Table 1) with only 50% of the spermatozoa performing the oscillatory movement and 100% moving progressively. The remaining males produced a lower per cent fertilisation, which might have resulted from a differential spermatozoa motility; e.g., the male W–2 yielded sperm with only 50% of spermatozoa in the progressive movement, the fertilisation success being, however, also the result of a high number of spermatozoa in a dose. Experiment III supports to some extent the results of Experiment I in terms of the spermatozoa motility effect on fertilisation success. The relationship is not unambiguous, but as can be seen in Fig. 3, samples of sperm with high scores (6 and 7) gave higher per cent fertilisation in most cases than those with lower scores.

When analysing the results of all three experiments one can conclude that in order to obtain a satisfactory fertilisation success there should be 300,000 spermatozoa per 1 egg provided 100% of them perform the progressive movement lasting from 0.3 to 0.4 min., the movement being probably very important in fertilisation (Żuromska, 1981).

A control sample incubated in a large battery Weiss jars gave much better results (95% fertilisation) than eggs incubated in a small battery, in spite of a smaller number of spermatozoa per 1 egg than in many other cases in Experiment I (Table 1). This difference may have been brought about by lower water temperature in the small battery; the water was not heated, while the temperature is an important factor in fertilisation (Matlak, 1969). Additionally, the roe quality may have influenced the results: the female W–150 roe showed usually a higher per cent fertilisation than the roe obtained from the female W–136.

CONCLUSIONS

1. Fertilisation success tends to increase with increasing sperm/roe volumetric ratio.
2. The optimal sperm/roe volumetric ratio ranges from 1:100 to 1:50.
3. Fertilisation success increases with growing ratio between the number of spermatozoa and number of eggs; satisfactory results are obtained when using at least 300,000 spermatozoa in the progressive movement phase per 1 egg.
4. Spermatozoa motility seems to be a key factor in fertilisation; fertilisation success is in

inverse proportion to the duration of the progressive movement and in direct proportion to the number of spermatozoa performing this type of movement.

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WPŁYW ILOŚCI I JAKOŚCI NASIENIA SAMCÓW KARPIA NA STOPIEŃ ZAPŁODNIENIA IKRY

STRESZCZENIE

Materiał do badań stanowiły tarlaki karpia (*Cyprinus carpio* L.).

Podczas sztucznego tarła ikra była zapładniana w różnych proporcjach objętościowych i ilościowych nasieniem karpia. Ilościowy stosunek plemników do jaj został oparty na obliczeniu koncentracji plemników w każdej z prób. Oceniano również ruchliwość plemników od momentu ich aktywacji w wodzie. Czas trwania poszczególnych typów ruchów plemników mierzony był stoperem. Analiza stopnia zapłodnienia ikry posłużyła do weryfikacji istotnych czynników w procesie zapłodnienia.

Z rezultatów badań wynika, że wraz ze wzrostem stosunku plemników do jaj, próbki zapłodnionej ikry wykazują wzrastający procent zapłodnienia. Zaobserwowano wyraźny wpływ ruchu postępowego plemników na stopień zapłodnienia ikry. Mimo, że jakość spermy uzależniona jest od indywidualnych właściwości samca, co jest zgodne z twierdzeniem wielu autorów, to jednak wydaje się, że ruch postępowy plemników istotnie wpływa na zapłodnienie. Przypuszczalnie plemniki karpia charakteryzują się aktywnością życiową w środowisku wodnym w zakresie czasowym 0,3–0,4 min.

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ВЛИЯНИЕ КОЛИЧЕСТВА И КАЧАСТВА СЕМЕНИ САМЦОВ КАРПА НА СТЕПЕНЬ ОПЛОДОТВОРЕНИЯ ИКРЫ

Р Е З Ю М Е

Материалом для исследований служила маточная рыба карпа (Cyprinus carpio L.). Во время искусственного нереста икру оплодотворяли разными количествами и объёмами семени карпа. Количественное соотношение сперматозоидов к яйцеклеткам получено на основании вычисления концентрации сперматозоидов для каждого образца. Оценивалась также подвижность сперматозоидов с момента активирования их в воде. Продолжительность отдельных фаз движения сперматозоидов измеряли с помощью секундомера. На основании анализа степени оплодотворения икры производилась проверка факторов, существенных для процесса оплодотворения.

На основании полученных результатов установлено, что по мере увеличения количественного соотношения сперматозоидов к яйцеклеткам образцы оплодотворенной икры отличаются повышенным процентом оплодотворения. Наблюдается также отчётливое влияние поступательного движения сперматозоидов на степень оплодотворения икры. Хотя по мнению многих авторов качество спермы является индивидуальным признаком самца, однако по всей вероятности поступательное движение сперматозоидов проявляет существенное влияние на оплодотворение. Сперматозоиды карпа по всей вероятности отличаются способностью к оплодотворению в водной среде в диапазоне времени 0,3 - 0,4 минут (фаза поступательного движения).

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