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Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species

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Abstract

Induction of ovulation in fish by GnRH analogues is overshadowing the pituitary-treatment that has been used for decades. The aim of our research was to develop a GnRH analogue-containing preparation (ovopel) which can be applied in a similar manner to acetone-dried pituitary. Ovopel was tested on four cyprinid species, the common, the silver and the grass carp and the tench.
We compared the effects of ovopel treatment to those of pituitary-treatment in relation to the ratio of ovulated to non-ovulated females. In the case of the common, silver and grass carp, the resolving dose of ovopel was preceded by a priming dose of pituitary or ovopel. The tench received only one dose of ovopel or pituitary. In the common, silver and grass carp, the ovopel treatment (when the priming dose was ovopel) resulted in high rate of responding females. Ovopel induced high rate of ovulation in the tench as well.

Key words: GnRHa-containing preparation, pituitary-treatment, ovulation ratio

1. Introduction

For many years fish farmers and scientists have been using hormone preparations for the artificial propagation of carp and other fish species. In practice, acetone-dried pituitary is the most commonly used agent to induce ovulation (i.e. hypophysation technique). This small organ, which contains the active hormone (gonadotropin), is collected from mixed populations of marketable carp in temperate climate (usually 3-year-old fish).

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The pituitaries (weighing 2–3 mg each) are marketed in different forms. In the simplest form, the freshly dissected and dehydrated glands are stored whole at room temperature until required (Donaldson, Hunter 1983). The disadvantage of this technique is that the whole pituitaries differ in size and in gonadotropin content even when taken from fish of similar weight. Also, gonadotropin quality in the carp pituitary varies among seasons, all of which makes it difficult to obtain an accurate dose (Yaron *et al.* 1984, Yaron, Levavi-Zermonsky 1986).

The problem of dosage can be solved by grinding a large number of acetone-dried glands into a homogeneous powder, and then standardising it with a bioassay of a small aliquot (Donaldson, Hunter 1983). However, this process is complicated, and is not popular with traditional farmers, who prefer to determine dosage by counting whole pituitary glands.

Additional forms of the hormone include partially to fully purified gonadotropins prepared from pituitary glands (Donaldson, Hunter 1983). However, these preparations are also poorly suited to direct application in fish culture as they are packaged in given weights. Once the preparation has been dissolved in water or saline, the active ingredient is only active for a few hours. Therefore breeders have to adapt their breeding project around the packaged amounts.

Apart from these difficulties, it has become increasingly difficult to purchase pituitary glands. In recent years, following the political and economic changes in Eastern Europe, many big fish factories have closed or been divided up. This has reduced the supply of glands from the region; world supply has been reduced, and the market price has soared.

A recent development in the technology of induced breeding is the stimulation of endogenous gonadotropin release from the pituitary of the treated fish by the use of a synthetic analogue of gonadotropin releasing hormone (GnRH) (Anon. 1977, Zohar 1991). However, the injection of GnRH analogues alone is generally infective in inducing ovulation in cyprinid fish species where there is a strong dopamine inhibitory tone on gonadotropin secretion, such as in goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*) (Peter *et al.* 1986), silver carp (*Hypophthalmichthys molitrix*) and grass carp (*Ctenopharyngodon idella*) (Peter *et al.* 1988). To facilitate the gonadotropin releasing activity of the GnRH analogue, it is combined with a dopamine receptor antagonist (Chang, Peter 1983, Peter *et al.* 1988).

Since certain analogues are inexpensive and effective, this method is gaining acceptance throughout the world (Horváth *et al.* 1986, Crim *et al.* 1987, Peter *et al.* 1988, Peter *et al.* 1993), overshadowing the hypophysation techniques that has been used for decades. However, some fish breeders refuse to adopt this new method because application techniques differ from hypophysation. These differences include: 1) the relatively small dose required for successful ovulation, 2) how the dosage for the brood stock is calculated, 3) how the administered agent is prepared and 4) the occasional combination of GnRH analogue-treatment with a dopamine receptor antagonist injection.

The aim of our research was to abolish the differences above, by developing a GnRH analogue-containing preparation (ovopel) and to have the GnRH analogue treatment accepted by traditional fish breeders.

2. Materials and methods

In this study we compared the effects of ovopel treatment to those of hypophysation (control) in relation to the ratio of ovulated to non-ovulated females. Since testing of ovopel requiring expensive brood stock was performed on the large scale, we could not set up the experiments precisely in every respect (e.g. large experimental groups of females from the same stock, treatment of females of different groups at the same time). However, these are

just the wide range trial and the testing on different species that provided an opportunity for us to reach a conclusion whether ovopel can be applied in practice.

Seven Hungarian hatcheries were involved in the experiments and ovopel was tested on four cyprinid species, the common carp (*Cyprinus carpio*), the silver carp (*Hypophthalmichthys molitrix*), the grass carp (*Ctenopharyngodon idella*) and the tench (*Tinca tinca*). The water temperature was maintained at 21–23° C during the experiments.

Ovopel contains a mammalian GnRH analogue, D–Ala^6, Pro^{9}NEt–mGnRH and a water soluble dopamine receptor antagonist, metoclopramide. The concentrations of D–Ala^6, Pro^{9}NEt–mGnRH and metoclopramide are 18–20 μg/pellet and 8–10 mg/pellet, respectively. The average weight of one pellet is 25 mg.

The injectable solution was prepared as follows: Ovopel was applied in a similar manner to acetone-dried pituitary. The necessary amount of ovopel was calculated on the basis of the weight of the spawner. The pellets were pulverized in a mortar and dissolved in a vehicle of 0.7% NaCl. The dissolved material was taken up in a syringe and injected intraperitoneally at a dose of 0.5 cm^3 kg^{-1} fish body weight.

Hormonal treatment:

Induced propagation of cyprinids is more effective if the hormone is administered in two doses (Tamás, Horváth 1984). The first dose (priming dose) is given to bring the eggs into the pre-ovulation stage. Then the second dose (resolving dose) is given to induce ovulation. The interval between the two doses is approximately 12 hours. In the treatment groups of common carp, silver carp and grass carp, one pellet kg^{-1} body weight of resolving dose of ovopel was preceded by a priming dose of pituitary (0.2–0.3 mg kg^{-1} body weight) or ovopel (1/5 pellet kg^{-1} body weight). In the control groups, females received 0.2–0.3 mg kg^{-1} body weight pituitary as a priming dose and 3–4 mg kg^{-1} body weight pituitary as a resolving dose. The tench received only one dose of ovopel (one pellet kg^{-1} body weight) or pituitary (3–4 mg kg^{-1} body weight).

Statistical analysis:

The difference among group means of spawning ratios was tested with the Student's t–test. Fisher's test was used to compare the standard deviations ($P < 0.05$).

3. Results

In common carp, silver carp and grass carp, the mean ovulation ratio was similar between pituitary-treated fish and ovopel treated fish primed with ovopel. However, when ovopel treatment was preceded by a priming dose of pituitary, the mean of ovulation ratio was significantly lower compared to that of pituitary treated control group (Tables I–III.). The tench injected with ovopel also spawned successfully (Table IV).

4. Discussion

Injection of the combination of dopamine antagonist drugs and GnRH analogues has proven to be highly effective means of inducing ovulation of cultured cyprinid fish. The main advantages of this technique include the reduced

Table I. Percentages of ovulated common carp females obtained by using carp pituitary (control) and ovopel. The resolving dose of ovopel was preceded by a priming dose of carp pituitary or ovopel. The asterisk indicates significant ($P < 0.05$) difference (t-test) of the mean compared to the control. Data from 160 females of 19 experimental groups were analyzed. SD – standard deviation

	Control	Ovopel-treated	
		Pituitary--primed	Ovopel--primed
	100	67	83
	85	50	100
	100	60	80
	94	33	60
	72	50	
	75	67	
	82	60	
	50		
n of groups	8	7	4
mean	82.25	55.29*	80.75
SD	16.79	12.05	16.4

Table II. Percentages of ovulated silver carp females obtained by using carp pituitary (control) and ovopel. The resolving dose of ovopel was preceded by a priming dose of carp pituitary or ovopel. The asterisk indicates significant ($P < 0.05$) difference (t-test) of the mean compared to the control. Data from 91 females of 12 experimental groups were analyzed. SD – standard deviation

	Control	Ovopel-treated	
		Pituitary--primed	Ovopel--primed
	93	38.5	100
	90	90	75
	74	0	83
	100	100	100
n of groups	4	4	4
mean	89.25	54.63*	89.50
SD	11.00	44.52	12.56

Table III. Percentages of ovulated grass carp females obtained by using carp pituitary (control) and ovopel. The resolving dose of ovopel was preceded by a priming dose of carp pituitary or ovopel. The asterisk indicates significant ($P < 0.05$) difference (t-test) of the mean compared to the control. Data from 71 females of 10 experimental groups were analyzed. SD – standard deviation

	Control	Ovopel-treated	
		Pituitary--primed	Ovopel--primed
	100	82	100
	73	50	89
	100	67	100
	100	70	100
n of groups	4	4	4
mean	93.25	67.25*	97.25
SD	13.50	13.20	5.50

Table IV. Percentages of ovulated tench females obtained by using carp pituitary (control) and ovopel. Data from 21 females of 5 experimental groups were analyzed. SD – standard deviation

	Control	Ovopel--primed
	100	100
	40	83
n of groups	2	2
mean	70.00	91.50
SD	42.43	12.02

cost of the synthetic drugs, the high rate of ovulation, the completeness of ovulation and the fertilizable eggs and viable embryos.

By developing our GnRH analogue-containing preparation, ovopel, we would like to contribute to the substitution of the use of GnRH analogues for the more expensive pituitary hormone preparations. On the large scale we examined the effects of ovopel on ovulation in cyprinid species with economic importance.

In common carp, silver carp and grass carp ovopel treatment (when the priming dose was ovopel) resulted in high rate of responding females. Interestingly, ovulation occurred successfully in groups where the inducing agent was the same (ovopel or pituitary) for priming and resolving dose. Ovopel induced high rate of ovulation in the tench as well.

In the experiments of Drori *et al.* (1994) only one injection with 10 μg kg^{-1} body weight superactive salmon GnRH analogue (D–Arg^6, Pro^9–NEt–sGnRH) combined with 20 mg kg^{-1} body weight metoclopramide resulted in high ovulation ratio in the common carp. By substituting salmon GnRH analogue for the presently used mammalian GnRH analogue and inreasing the metoclopramide concentration up to 20 mg/pellet in our preparation, one injection of ovopel might be enough to cause ovulation in fish.

According to our research, ovopel could be suitable to have wide application for induced ovulation of cyprinid fish in the future.

5. References

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