



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

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**COMPARISON EFFECTS OF GONADOTROPIN-RELEASING HORMONE
ANALOGUE (BUSERELIN) ADMINISTRATION AT DIFFERENT DOSES WITH
OVAPRIM ON OVULATION AND SPAWNING INDUCTION IN *RUTILUS
FRISIIKUTUM***

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ABSTRACT

Rutilus frisiikutum (Caspian Kutum) is mentioned as one of the most valuable fish in Caspian Sea. From March to May this species migrates to shallow river waters for spawning. Overfishing, water pollution increasing and degradation of river beds are mentioned as threatened factors by the Iranian Fisheries Organization. The aim of present study was to evaluate the effects of gonadotropin-releasing hormone (GnRH) analogue (Buserelin) at different doses with Ovaprim on final oocyte maturation, ovulation and spawning in Caspian Kutum. So 45 Adult females were selected randomly and injected once with Buserelin Acetate (BUS) and divided into 5

groups (n=9). Group 1: (BUS) 5 $\mu\text{g kg}^{-1}$ BW, Group 2: (BUS) 10 $\mu\text{g kg}^{-1}$ BW, Group 3: (BUS) 20 $\mu\text{g kg}^{-1}$ BW, Group 4: Ovaprim, 0.5 ml kg^{-1} BW (positive Control), Group 5: Intact (negative Control). Our results showed that the highest mean value in ovulation success, ovulation index, fertilization success, relative fecundity and the number of eggs belonging to the Ovaprim treatment. On the other hand, spawning was more successful in BUS 5 $\mu\text{g kg}^{-1}$ (67%) treatment than the other doses. Therefore, it could be concluded that BUS 5 $\mu\text{g kg}^{-1}$ as an optimum dose induced ovulation and spawning in Kutum, so BUS 5 $\mu\text{g kg}^{-1}$ as well as Ovaprim (sGnRHa combined with domperidone) is preferred for ovulation and spawning induction in this species.

Keywords: Caspian Kutum; GnRHa; Ovaprim; ovulation success; Final oocyte maturation
INTRODUCTION

Ovulation and spawning in reared fish can be induced by gonadotropin-releasing hormone (GnRH), its super active analogues alone or in combination with a dopamine antagonist [1], that has been proved as an effective and reliable method in ovulation and spawning of several freshwater fish [2], [3]. Hormonal manipulations to induce final oocyte maturation (FOM), ovulation and spawning are used as a useful managerial tool to elevate the efficiency of egg production and fertility [4], [5]. GnRH as a decapeptide trigger acts on pituitary gland which secretes gonadotropins. As the matter of the fact GnRH can develop reproductive activity by stimulating production [6]. Gonadotropins induce biosynthesis of steroids activation (mainly estradiol/progesterone in females) and the other non-steroidal substances (i.e. activin, follistatin, inhibin) modulation in fish species [7].

Hormonal therapies to induce FOM, ovulation and spawning have been applied in fish culture. GnRH analogues (GnRHAs) stimulate reproductive functions by directly or indirectly stimulating of hormones synthesis and releasing being involved in oocyte maturation, ovulation and spawning [8]. GnRHAs remain in circulation for a longer time and also represent increased binding affinity to the pituitary GnRH receptors. their function are 30–100 times more effective than the native GnRHs in inducing Gonadotropin(GTH) II releasing [1].

Principally in freshwater fish, dopamine antagonist have mostly been used in combination with GnRH agonists in order to hormonal manipulations. In these species, dopamine inhibits GTH II releasing [9]. In aquaculture industry hormonal induction of ovulation and spawning for some species i.e.

tench *Tinca tinca* are well-established using a low dose of GnRHa [10]. The level of plasma GnRHa after a single injection are enhanced for a few hours to a few days, dependent on the particular GnRHa, primary dose, fish species and water temperature [11]. Caspian Kutum *Rutilus frisii kutum*, one of the most valuable commercial and endemic fish in the Caspian Sea, is also considered as anadromous species migrating to shallow river waters for spawning from March to May [12].

The aim of present study was to investigate the effect of GnRHa (Buserelin) administration at different doses on ovulation and spawning induction in Caspian Kutum under artificial conditions by measuring reproductive parameters.

MATERIALS AND METHODS

Fish stocks and maintenance

Shirud River inlets to the Caspian Sea was a place that gravid females *Rutilus frisii kutum* (N=45) were captured and acclimatized in tanks (April 2014). Afterwards they were weighed (922.54 ± 105.5 g) and separated based on their treatment groups.

Chemicals

Buserelin Acetate (Pyr-His-Trp-Ser-Tyr-D-Ser-(But)-Leu-Arg-Pro ethylamide) GnRH analogue was purchased from Sanofi-Aventis Deutschland GmbH D-65926 Frankfurt am

Main, Germany company. Ovaprim (D-Ala⁶, Pro⁹-Net)-sGnRH+Domperidone (Syndel Laboratories, India) was purchased from Virbac Animal Health India company.

Buserelin Acetate (BUS) 5 $\mu\text{g kg}^{-1}$ BW, (BUS) 10 $\mu\text{g kg}^{-1}$ BW, (BUS) 20 $\mu\text{g kg}^{-1}$ BW, Ovaprim (sGnRHa 20 $\mu\text{g ml}^{-1}$ combined with Domperidone 10 mg ml^{-1}) 0.5 ml kg^{-1} BW were injected intramuscularly (IM).

Experiments

The acclimatized females Caspian Kutum were divided into 5 groups. Group 1: Intact (negative Control), Group 2: Ovaprim (positive Control), Group 3: BUS 5 $\mu\text{g kg}^{-1}$, Group 4: BUS 10 $\mu\text{g kg}^{-1}$, Group 5: BUS 20 $\mu\text{g kg}^{-1}$, (n=9). Intramuscular injection (IM) were performed by penetration of the dorsal muscles at the level of dorsal fin [13].

Fish were placed in well-aerated tanks separately with recirculated water after injection. 10 hours after injection they were checked for ovulation by hand-stripping and this was checked hourly. When ovulation occurred, the stream of eggs is directed into a clean, dry bowl. Eggs are usually fertilized with fresh milt. The fresh milt is spread over the eggs and thoroughly mixed by hand. Ovulation success determination ([number of ovulated females/number of injected ones] $\times 100\%$) and ovulation index (OI) (weight of

stripped egg mass/[weight of stripped egg mass + remnant ovaries] $\times 100\%$) was used for assessment of ovulation (2). 24 hours after fertilization when the eggs were at gastrulation stage, fertilization success was assessed by dissecting microscope [14].

Abdominal cavity is a place in Caspian Kutum that total ovulated eggs are placed in which are accessible and released spontaneously. Accordingly relative fecundity was the other index calculating by (total number of eggs in each group/total weight of females) for collected eggs in each treatment at the post spawning period [15]. The percentage of eggweight to body weight was also calculated as follows:

(Weight of stripped eggs mass in each treatment/total weight of females) $\times 100\%$.

The mean time between injection and ovulation was considered as latency period [16].

Statistical analysis

Data are presented as a mean \pm standard error of the mean (S.E.M). Body weight, total length, ovulation success, ovulation index, fertilization success, the number of eggs, relative fecundity and the percentage of eggweight to body weight were determined by one-way analysis of variance (ANOVA, $P < 0.05$) with Tukey's HSD post-test.

RESULTS

Our result showed that no fish ovulated in intact group (negative Control); on the other hand all fish ovulated (100%) in Ovaprim group (positive Control). The highest ovulation success (67%) was observed in BUS 5 $\mu\text{g kg}^{-1}$ group and the lowest one (33%) was obtained in BUS 20 μg group ($P < 0.05$). Although, ovulation success (67%) in BUS 10 $\mu\text{g kg}^{-1}$ treatment was similar to BUS 5 μg group ($P > 0.05$) and a lower latency period in BUS 5 μg group (18 h) than the BUS 10 μg group (19 h), so BUS 5 μg was selected as the optimum dose for female Caspian Kutum.

The ovulation index in Ovaprim was 84%, while it was 71% in BUS 10 μg as a lowest mean value, therefore Ovaprim treatment in comparison with other groups were significantly differed ($P < 0.05$). There is no significant difference ($P > 0.05$) in fertilization success among all treatments that was in the range of 88-93% (Table 1). The number of eggs in Ovaprim group was 67842 (the most mean value) and in BUS 10 μg group was the lowest mean value, 23330; there was no significant difference between BUS 5 μg and BUS 10 μg treatments ($P > 0.05$), but significant difference was observed in BUS 20 μg ($P < 0.05$) and Ovaprim ($P < 0.01$).

The lowest and the highest average value of relative fecundity were 30 eggs g^{-1} in BUS

10 µg and 57 eggs g⁻¹ in Ovaprim groups, respectively. Also there was no significant difference between BUS 5 µg and BUS 10 µg (P>0.05), but significant difference was obtained in BUS 20 µg (P<0.05) and Ovaprim (P<0.01).

The percentage of egg weight to body weight was in the range of 11-17% in all treatments.

In BUS 5 µg and BUS 10 µg groups were in the lowest range and in Ovaprim had the highest mean value. The latency period was in the range of 13-19 h in all treatments, thus significant difference was not observed among different treatments (P>0.05).

Table 1: The effect of Buserelin treatments at different doses on ovulation success (%), ovulation index (%), fertilization success (%), relative fecundity (eggs g⁻¹), the number of eggs and eggweight to body weight (%) in Caspian Kutum, *Rutilus frisii kutum*

Treatments	Dosage	Body Weight (g)	Total Length (cm)	Ovulation Success (%)	Ovulation Index (%)	Fertilization Success (%)	Relative Fecundity (eggs g ⁻¹)	The number of eggs	Egg Weight to Body Weight (%)
Intact	—	831.1±84.08	44.2±1.5	—	—	—	—	—	—
Ovaprim	0.5 mg	1341.7±220.4	50.4±2.6	100 ^{c*}	84.3±0.3 ^b	93.1±0.7	57.05±13.2 ^{c*}	67842±25623 ^{c*}	17.9±2.3 ^b
BUS	5 µg kg ⁻¹	806.6±76.88	41±2.08	67 ^a	78 ^a	88	36.59±6.65 ^a	24260±7740 ^a	11.61±1.89 ^a
BUS	10 µg kg ⁻¹	780±12.8	42±2.64	67 ^a	71±1 ^a	90.5±0.5	30.73±0.1 ^a	23330±667 ^a	12.61±0.3 ^a
BUS	20 µg kg ⁻¹	853.3±71.72	44±2.02	33 ^b	75 ^a	89	45.31 ^b	41700 ^b	16.3 ^b

Mean (±SE) values with a different superscript are significantly different; b: (P<0.05), c* : (P<0.01)

DISCUSSION

There is a growing trend to enhance the efficiency of reproductive functions of culture fish like Kutum. GnRH_a and other pharmaceutical agents can be induced physiological reproductive functions [17]. The results of the current study demonstrate that GnRH-I agonist (Buserelin) administration in low dose of 5 µg kg⁻¹ with the lower latency period is a more successful hormonal treatment to induce ovulation and spawning in Kutum than the dose of 10 µg kg⁻¹. In veterinary the most extensive using agonists are the natural decapeptide, Buserelin and Deslorelin [17]. The single

treatment by a small dose of the GnRH-I agonist (Buserelin) in low quantities will induce spawning, after binding to its receptor [18].

Arabacı demonstrated that Buserelin can be also effective for spawning induction in gilthead sea bream [19] and common carp [20]. Podhorec et al. reported similar results in ovulation and spawning induction in females *Tincatinca*, that indicated a low dose of mGnRH_a 1 µg kg⁻¹ was led to 63% ovulation and spawning induction [21]. Another study was also reported that alone GnRH_a treatment enhanced ovulation success in *Tincatinca* [22]. In present study,

BUS 10 $\mu\text{g kg}^{-1}$ administration was also effective and latency period was higher than the BUS 5 $\mu\text{g kg}^{-1}$ treatment, so the lower dose of Buserelin (BUS 5 $\mu\text{g kg}^{-1}$) had higher efficiency with a more appropriate price; consequently this dose is recommended as an optimum dose in female Caspian Kutum. However, [10] demonstrated that almost 70% of females *Tincatinca* ovulated after GnRHa treatment lonely (10 $\mu\text{g kg}^{-1}$)

[15] demonstrated lone GnRHa using acted properly to induce FOM and spawning in the European sea bass. Based on achieved results (100% of successful ovulated fish in Ovaprim treatment and no ovulated fish in Control group) hormonal induction is necessary for Caspian Kutum. In such studies that is also shown about ovulation success (100%) in female Caspian Kutum [23] and African catfish *Clarias gariepinus* [24] by Ovaprim administration. GnRH analogues functions differed in ovulation, latency period and spawning of various fish [25], [26] and it can be attributed to the fish species, amino acid sequence, purity of the GnRH analogue and dose of the GnRH [27].

In present study, OI in ovaprim treatment demonstrated the highest mean value; also the existence of dopamine antagonist (Domperidone) combined with sGnRHa was

necessary for GTH releasing from the pituitary, ovulation and spawning in Kutum. OI in BUS 5 $\mu\text{g kg}^{-1}$ treatment was higher than the other doses. These results indicated that BUS 5 $\mu\text{g kg}^{-1}$ as an optimum dose was capable of inducing significant changes in the ovary and complete ovulation under the hatchery conditions.

Ovaprim treatment showed the highest value for some parameters like as the number of eggs, fertilization success, relative fecundity and egg weight to body weight. Such as these results have been recently searched in African catfish, *Clarias gariepinus* [24]. Because of quietly accessible ovulated eggs being placed in abdominal cavity in Caspian Kutum, total number of eggs and relative fecundity were estimated [15]. The highest average of the number of eggs in Ovaprim treatment showed that Ovaprim led to 100% ovulation.

The results in this investigation clearly showed that Ovaprim (sGnRHa combined with domperidone) in comparison with different doses of Buserelin, had the more effect in FOM, spawning and other factors' induction. Also it can be inferred that Buserelin 5 $\mu\text{g kg}^{-1}$ could improve the effectiveness of artificial reproduction in Caspian Kutum, so it preferred as an

optimum dose for ovulation and spawning induction in this species.

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