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Effect of some growth factors on *Cyprinus carpio* oocytes maturation (*in vitro* study)

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ABSTRACT

In fish, prior to ovulation, the process that includes oocyte maturation is essential for successful and guaranteed fertilization. In the current study, *Cyprinus carpio* L. oocytes were exposed to three different types of recombinant growth factors: Insulin-like growth factor (IGF), Fibroblast growth factor (FGF), and Transforming growth factor (TGF). A sample of 5 ng /ml of the three upper- mentioned growth factors was utilized on common carp follicles for 3 different periods of time (24,48, and 72 hours). The current study was conducted from December 2019 till January 2020 at the college of veterinary medicine. The results recorded a significant effect ($P<0.01$) between the polarization index (PI) and the different times of incubation. Additionally, an interaction between the treatments and incubation time, using F test, was spotted. Using the Tukey test for multiple comparisons, a highly significant difference ($P<0.01$) between various treatments was noted, except for the comparable relationship between the control and G1.

INTRODUCTION

Cyprinus carpio L., one of the most significant fish species in fish farming, is common and widespread in ponds, lakes, and rivers in central Asia and Europe (**Bakos &Gorda, 2001**).

Fish oogenesis is classified into three stages: previtellogenesis, vitellogenesis, and oocyte maturation. Studies have been conducted dwelling with the two latter stages in addition to the controlling involvement of the hypothalamopituitary- gonadal axis (**Nagahama, *et al.* ,1995**).

Oocyte maturation in teleost (like other vertebrates) occures before ovulation including germinal vesicle migration and breakdown, chromosome condensation and formation of the first polar body **(Patiño & Sullivan, 2002; Nagahama & Yamashita, 2008).**

Many growth factors such as IGF as well as members of the transforming growth factor-b (TGFb) superfamily are remarkable in regulating follicle maturation. IGFs have been determined influencing and considerable for ovarian development even in fish that have similar genera. Actions of IGF-I, leading to oocyte maturation, appear to differ species including various fishes **(Grigorescu *et al.*, 1994; Kagawa *et al.*, 1994; Duan, 1997; Negatu *et al.*, 1998; Poretsky *et al.*, 1999; Mukherjee *et al.*, 2006; Weber & Sullivan, 2000; Weber, *et al.* 2007).**

Mostly, limited data have been conducted on superfamily members and their inhibitors, with a slight attention to the effect of some growth factors on specific species acting upon regulating follicle maturation in fish **(Ge, 2005).**

Watanabe *et al.* (1998) reported that FGF has an outstanding impact on initiating the oocyte development in follicle cell, therefore FGF has been frequently applied in developing the ovary of *Oryzias latipes*. Thus, *Cyprinus carpio,* a considerable local freshwater species in Iraq, recruitable for basic and applied research and discussion, was selected as a model for the present experiment. To specify the effect of the three different growth factors, namely, IGF, FGF, and TGFb on common carp oocyte maturation *in vitro,* was targeted in the current study.

MATERIALS AND METHODS

Specimen collection: The mature fish *(Cyprinus carpio* L.*)* was collected from the fish culturing station/ marine science center in Basrah University. The fish (with weight 2. 75 kg and length 167 cm) was brought to the laboratory alive and then sacrificed (figure 1), the experiment was carried through December 2019 to January 2020.

Laboratory work: The ovaries of sacrificed mature fish were placed in ice-cold basic salt solution (BSS) **(Zuberi *et al.*, 2011b).** The ovaries were reduced and speared to pieces by using fine forceps. To check the oocytes in the follicle of central germinal vesicle (CGV), a sample of the ovarian follicles was treated with clearing fixative solution **(Zhoa & Wright, 1985).**

***in vitro* study:** The experiment of the present study was carried out under sterilized conditions including apparatus, tools, solutions and culture medium in a laminar flow site. The tools were sterilized in an oven or an autoclave. The experiment was achieved in central researches unit in college of veterinary medicine**.** The culture technique for *in vitro* incubation of the ovarian follicles followed the methods of **Upadhyaya and Haider, (1986)** and **Zuberi *et al.* (2002, 2011).** The culture medium (BSS) (with anti-bacterial and antifungal) was divided into four groups: the control group with just BSS, and BSS with 10 ng/ml for each of TGF-β1 for G1, FGF for G2 and IGF-1 for G3 (Us-Biological- USA).

The experiment design: The oocytes were cultured in 24 well- tissue culture plate. the wells were divided into 4 groups where each group contained 6 wells. The three groups were specialized for the three types of growth factors, while the fourth was for the control. After all the wells were filled with oocytes, the four types of media were added to the wells. The plate was closed tightly and covered well by a Parafilm, the date and number were fixed, and the incubation was maintained at 22 ± 0.2°C in a humidified temperature-controlled incubator for 24, 48, and 72 h for each.The plates were duplicated in every stage.

Experimental tests: The test of viability for oocytes was adopted by using trypan blue stain(Pharmacia Fine –Sweden) in samples of cultured oocytes at various time periods. According to this test, the dead oocytes would absorb the stain, whereas the viable and vital oocytes remain clear. The Oculomicrometer was adapted to measure the radius and diameter of the oocytes, and then, examined under an inverted microscope to detect the position of germinal vesicle (GV) by treating them with egg clearing solution, the above steps were achieved in of 0, 24, 48 and 72 h**.(Bancroft & Gamble, 2008)** (Fig. 2). Moreover, the value of polarization index (PI) was calculated according to **Hajirezaee *et al.* (2010) :**

$$PI = \frac{\text{Distance of germinal vesicle from animal pole}}{\text{Distance of germinal vesicle from animal pole} \_ \text{Distance of germinal vesicle from vegetal pole}} \times 100$$

Statistical analysis: Percentage position of germinal vesicle breakdown was calculated and expressed as mean ± S.E. The percentage (%) positions of germinal vesicle, followed by treatment with types of growth factors and incubation periods were analyzed by appropriate ANOVA models using SPSS for Windows (Software version 23).

Figure (1) common carp *(Cyprinus carpio* L.*)*

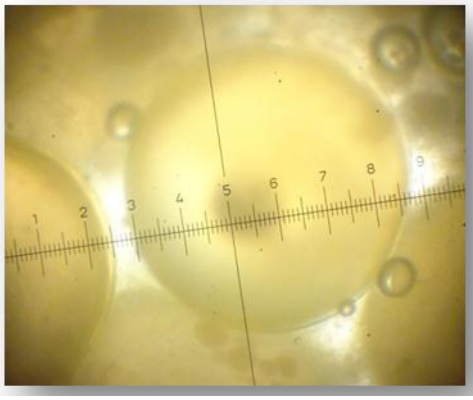

Figure (2) oocyte with oculomicrometer scale

RESULTS

Treatment of the ovarian follicles with different type of growth factors with 10 ng\ml revealed high significant effect (P<0.01) between the values of PI of all groups and different time of incubation. A substantially significant (P<0.01) result in the interaction between the treatments and incubation time was recorded using F test. By using Tukey test for multiple comparisons considering different treatments, the result was high significant (P<0.01) for all treatments with an exception of the comparable relation between the control and G1.

Figure (3) shows the averages of polarization index (PI) of the common carp oocytes nucleus treated *in vitro* for the three groups including the control in different incubation time. The highest value for PI recorded 91.16 in G1 with a duration of 72 hours , while the lowest value was in the 72 hours G2 which recorded 40.11.The average of oocytes diameters varied from one group to the other, the lowest average was in all groups in 0 hour marking 1041µ± 0.2703, while the highest average recorded in G1 (72 h.) was 1346 ± 0.0749 (Table 1).

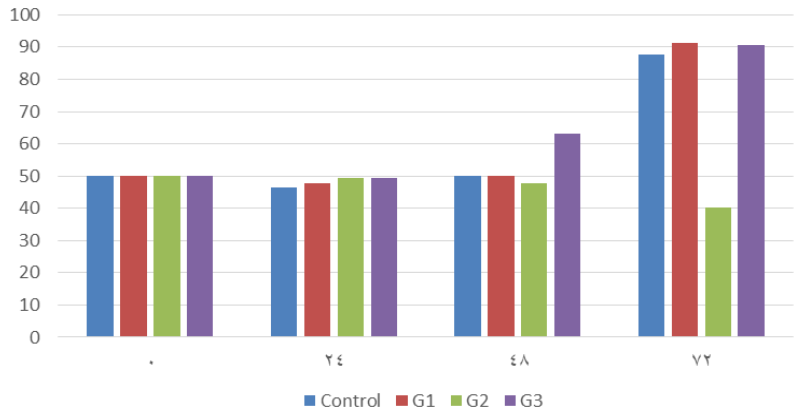

Figure 3: The averages of polarization index (PI) of the common carp oocytes nucleus in three groups plus control in different incubation time.

Table 1: the average of oocytes diameters(µ) in control and treatments groups in different times.

Time(day) / Group	0	24	48	72
Control	1041± 0.2703	977± 0.0851	1202± 0.0844	1225± 0.10682
G1	1041± 0.2703	1273± 0.1214	1136± 0.1576	1346± 0.0749
G2	1041± 0.2703	1175± 0.0935	1175± 0.1517	1171± 0.0892
G3	1041± 0.2703	1128± 0.1351	1260± 0.1797	1225± 0.1039

DISCUSSION

The current study showed that *Cyprinus carpio* (common carp) oocytes responded to IGF,FGF and TGF , an observation that correlates with the studies of **Weber and Sullivan (2000)** , **Mojazi Amiri *et al.* (2001), and Zuberi *et al.* (2011a)** who noticed a similar response, to similar and some other growth factors, in other hormones, during the examination of other species *in vitro*.The average of diameter of fish oocytes increased in the last two incubation time (48,72 h.) compared with 24 h. Therefore, the average of polarization index (PI) ranged between 96.61 - 40.11.

Zuberi *et al*. (2011b) mentioned that *in vitro* the exposure of ovarian follicles for the species *Barilius vagra* to human chorionic gonadotropin (hCG) affected both the the position of germinal vesicle and the maturation process of oocytes as well. This, in turn, coincides with the outcomings of the present experiment that spotlighted the response depending on time and prolonged incubation period, which led to the decrease of central position germinal vesicle and an increase of GVBD.

The results of this study showed that G3(IGF) recorded the highest PI value, a finding that agrees with **Negatu *et al.* (1998),** then followed by G1 (TGF) and finally G2 (FGF).The treatment of ovarian fragments containing oocytes in intact follicles with rhIGF-I performed an increase in concentrations of estradiol-17b and maturation-inducing steroid (MIS) 17,20b,21-trihydoxy-4-pregnen-3-one (20b-S) in the culture medium, and a decrease in testosterone levels**(Weber & Sullivan, 2000),**

Furthermore, **Schmitz (2003)** reported that IGFI effect on LH synthesis an outcome of a specific increase of the transcription level of the LH β subunit.

This study revealed high significant effect (P<0.01) between the values of PI of G1, G2, G3 and the different incubation period, by the use of F test. In addition,it is worth- mentionined that Iinsulin-like growth factor I (IGFI) might play a good role as a link between growth and puberty **(Huang *et al.*, 1998; Huang *et al*.,1999).**

CONCLUSION

All the growth factors had different effects on oocytes maturation with different results.

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