تقييم الإباضة والخصوبة عند إناث الكارب العام *Cyprinus carpio* المعاملة بموجهة الغدد التناسلية المشيمائية البشرية (HCG) خلال موسم التكاثر

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ملخص:

يعتبر هذا البحث الأول من نوعه الذي يجرى في سوريا ويختبر الحقن الهرموني باستخدام موجهة الغدد التناسلية المشيمائية البشرية (HCG) في إناث الكارب العام وبالتالي تقييم هذه العملية في تحفيز الإباضة والخصوبة. تم حقن إناث أسماك الكارب العام خلال موسم التكاثر بالتراكيز التالية من الـ HCG (250 و500 و750 و1000 وحدة دولية/كغ). وقد بلغ معدل الإباضة 100% لدى جميع الإناث المحفزة بموجهة الغدد التناسلية المشيمائية البشرية (HCG). وسجلت أقل فترة تأخير لحدوث الإباضة 26 ساعة عند التركيز 750 وحدة دولية/كغ. وبلغ أقصى وزن للمبيض، وأكبر خصوبة مطلقة، وأكبر قطر للبويضة عند التركيز 750 وحدة دولية/كغ (1200غ و606000 بويضة و1.50مم تباعاً على التوالي). ولم تسجل فروق معنوية بين التراكيز 500 و750 و1000 وحدة دولية/كغ بالنسبة لوزن المبيض وقطر البويضة ($P < 0.05$)، أما بالنسبة للخصوبة المطلقة فلم تسجل فروق معنوية بين التراكيز 500 و750 وحدة دولية/كغ ($P < 0.05$). لقد أظهرت نتائج استخدام الـ HCG كفاءة في إحداث الإباضة وزيادة الخصوبة لدى إناث الكارب العام المحفزة مقارنة بتلك غير المحفزة هرمونياً.

كلمات مفتاحية: *Cyprinus carpio*، HCG، الإباضة، الخصوبة.

Evaluation of Ovulation and Fecundity of Common Carp Females (*Cyprinus carpio*) Treated with Human Chorionic Gonadotropin (HCG) during The Breeding Season

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Abstract:

This research is the first of its kind to be conducted in Syria and tests hormonal injections using human chorionic gonadotropin (HCG) in Common Carp females, thus assessing this process in the stimulation of ovulation and fecundity. Common Carp females were injected during the breeding season with the following concentrations of HCG (250, 500, 750 and 1000 IU/kg). The ovulation rate was 100% for all females stimulated by HCG. The lowest latency time for ovulation was 26 hours at a concentration of 750 IU/kg. The maximum ovary weight, the largest absolute fecundity, and largest oocyte diameter at concentration 750 IU/kg were 1200 g, 606000 oocytes, and 1.50 mm, respectively. No significant differences between concentrations 500, 750, and 1000 IU/kg for ovary weight and oocyte diameter ($P < 0.05$), and for absolute fecundity, no significant differences between concentrations 500 and 750 IU/kg ($P < 0.05$). The results of using HCG showed efficiency in inducing the ovulation and increasing fecundity in stimulated Common Carp females compared to those of non-hormonal stimulated.

Key Words: *Cyprinus carpio*, HCG, Ovulation, Fecundity.

Introduction:

The culture of the Common Carp (*Cyprinus carpio*) has seen an increase in production and has gained importance in Syria's water farming sector. This is because it contains many advantages (high growth rate, low production cost, cultured under several systems, extreme resistance to diseases and stress) [8].

The process of artificial fertilization is a complementary process to the natural maturity of fish, as hormonal injection stimulates the completion of the development of oocytes inside the ovary and helps to stimulate the ovulation and hence access sexual products. The control of final oocyte maturation and ovulation in females has become a very important practical issue in aquaculture for many reasons, including improving fertility, synchronized ovulation time in a most spawning populations, and increased the rate of fertilization and hatching rate [5; 6]. The use of the human chorionic gonadotropin (HCG) is a simple and convenient method for the bio-stimulatory of fish, for easy preparation and storage does not need to inject anti-dopamine as a companion [1], and mimics the gonadotropin hormone (GtH) that is synthesized and released by the pituitary gland of fish.

Human chorionic gonadotropin (HCG) works much faster by directly inducing the gonads to induce the synthesis and release of sexual steroidal hormones, which in turn play a key role in the final oocyte maturation (FOM) [8].

This research is the first of its kind to be conducted in Syria and tests hormonal injections using human chorionic gonadotropin (HCG) in Common Carp females, thus assessing this process in the stimulation of ovulation and fecundity.

Materials and methods:

The research was conducted at the Faculty of Veterinary Medicine at the University of Hama and the Production and Research Center in the Al-Sin area of the General Authority of Fisheries and Aquaculture from 15.04.2021 to 01.09.2021.

1. Thirty individuals of Common Carp females were selected from the production unit of the General Fisheries and Aquaculture Authority's Al-Sin area, with healthy, disease-free, sexually mature during the natural breeding season of this species, and placed in a

pond (7 m length, 3 m width, 1.5 m depth) after exposure to a saline solution (3%) to ensure that they were protected from any pathogen.

2. Determine the temperature of water (22-26 °C), Dissolved Oxygen (O_2= 8.6 mg/l), pH (7.5).

3. The fish were divided into five groups, including the control group, each group was branded a particular colour for identification, and the following symbols were given (C; G_1; G_2; G_3; G_4) so that each group included six fish. And took their total weights (kg).

4. The concentrations required from the human chorionic gonadotropin (HCG) with brand-name (HuCoG) were attended by the production of a company (Bharat Serums and Vaccines Limited, India) containing the packaging (5000 IU) to stimulate females to ovulation, as follows: The first group (G_1) was given a concentration of 250 IU/kg, the second (G_2): 500 IU/kg, the third (G_3): 750 IU/kg, and the fourth (G_4): 1000 IU/kg. The control group (C) was left without hormonal treatment.

5. Fish injected at only one dose, in the dorsal muscle below the dorsal fin and above the lateral line. After being anaesthetized by a bath of clove oil at 80 ppm/litre of water [9].

6. After injection and recovery, the fish were returned to the pond prepared for the experiment and the temperature was as fixed as possible (22-26°C). It was placed under observation after 10 hours of injection at an hourly rate to monitor the response of the fish to hormonal treatment.

7. When watching the large bulge in the abdominal area of the females, we have resorted to the light pressure on the abdomen with clutching to obtain the oocytes and confirm their formation, with the latency time for ovulation being finely determined (the period from injection until the onset of ovulation is estimated to be hourly), and the ovulation rate [Ovulation rate = (number of females with ovulation/number of females with hormonal treatment) * 100] [11], the ovary weighed after dissection of the fish, absolute fecundity was recorded (number of oocytes in 1 g / ovary weight), the oocyte diameter (mm) was recorded [took 1 g of stripping oocytes and count them under the microscope lens and

measured one micron in diameter at (40X) and subsequently converted to millimetres].

8. Tissue samples of 1 mm were taken from three regions (anterior, middle and posterior) of female ovaries to accurately distinguish the development of oocytes and determine the true degree of maturity they reached as a result of the previous hormonal injection, where they were fixed in formalin (10%), performed the required tissue passages and drafted according to a protocol in force in the pathological anatomy laboratory (Lab private).

9. Statistical analysis was carried out with the assistance of Excel and SPSS (2021): (One-Way ANOVA; Tukey multi comparisons method, $P< 0.05$).

Results and Discussion:

The latency time to ovulation was set at 26 hours after injection in G_3 and ovulation was followed at the remaining concentrations from 32 hours at G_4 to 38 hours in G_2 and 44 hours for (G_1; C) (**Table 1**).

Table 1. Latency time for ovulation of Common Carp.

Group	Latency time (hours)
Control (C)	44
G_1	44
G_2	38
G_3	26
G_4	32

At all concentrations, the female's ovulation rate was 100%. The average of ovary weight after hormonal stimulation ranged from 586.7 to 1131.7 g (**Table 2**), with a clear significant difference in favour of groups G_2, G_3 and G_4 compared to group G_1 and C, with ovary weights of 1054.7, 1131.7 and 1128.3 g, respectively (One-Way ANOVA; Tukey multi comparisons method, $P < 0.05$) (**Figure 1**), while no significant difference was observed between C and G_1, with ovary weights of 605.7 and 586.7 g, respectively (Tukey, $P > 0.05$), as well as no significant difference between G_2, G_3 and G_4 (Tukey, $P > 0.05$) (Tukey, $P > 0.05$) (**Figure 1**).

The absolute fecundity of hormonally stimulated females ranged from an average rate of 245456 to 571492 oocytes (**Table 2**), with a clear significant difference in favour of G_2 and G_3 over the rest of

Table 2. Results of Common Carp females treated by HCG.

		Control	Group			
		C	G_1	G_2	G_3	G_4
No. Fish		6	6	6	6	6
Total Weight (kg)	**min**	3.1	3.5	3.4	3.5	3.5
	max	4.4	4.6	4.6	4.4	4.3
	mean	3.8±0.6	4.1±0.6	4±0.6	4±0.5	4±0.4
Ovary Weight (g)	**min**	592	550	1004	1050	1100
	max	625	610	1150	1200	1150
	mean	605.7 ±17.21	586.7 ±32.15	1054.7 ±82.62	1131.7 ±75.88	1128.3 ±25.66
Absolute Fecundity (Oocyte)	**min**	222987	230115	528355	530250	407916
	max	235417	255218	605188	606000	426458
	mean	228135 ±6484	245456 ±13449	555019 ±43477	571492 ±38321	418423 ±9515
Oocyte Diameter (mm)	**min**	1.17	1.44	1.47	1.48	1.48
	max	1.18	1.45		1.50	1.49
	mean	1.17±0.01	1.45±0.01	1.47	1.49±0.01	1.48±0.01

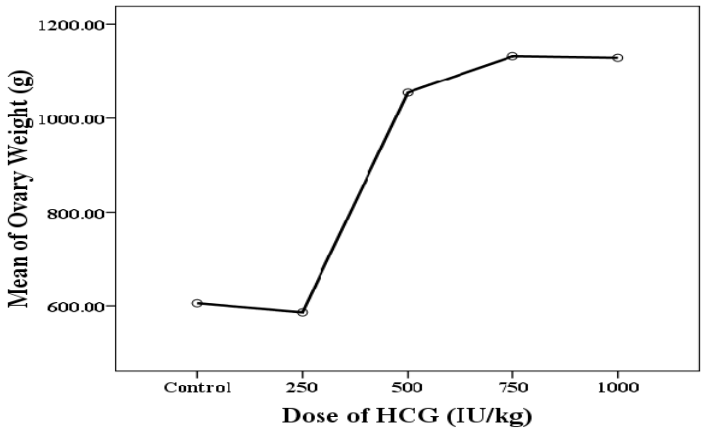


Figure 1. Ovary weight of Common Carp after induction by HCG.

both groups and control, with the absolute fecundity of 555019 and 571492 oocytes, respectively (Tukey, P< 0.05) (**Figure 2**), while the absolute fecundity at G_4 reached 418423 oocytes, and no significant difference was observed between the control (C) and G_1, where the absolute fecundity reached 228135 and 245456 oocytes, respectively (Tukey P > 0.05) (**Figure 2**).

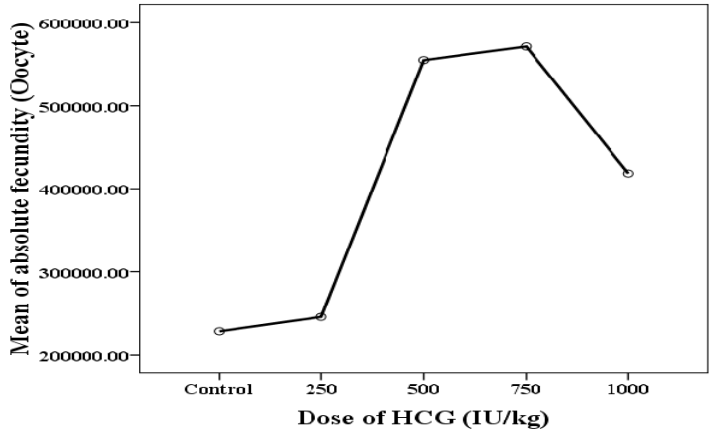


Figure 2. Absolute fecundity of Common Carp after induction by HCG.

The average of oocyte diameter recovered after the ovulation was 1.45 to 1.49 mm (**Table 2**), where it was recorded in groups G_2, G_3 and G_4; 1.47, 1.49 and 1.48 mm, respectively, without achieving a significant difference between them (Tukey P> 0.05) (**Figure 3**) but with a preference for G_1 and C (Tukey P< 0.05). While the oocyte of the control group (C) was 1.17 mm in diameter and G_1 1.45 mm in diameter (**Table 2**).

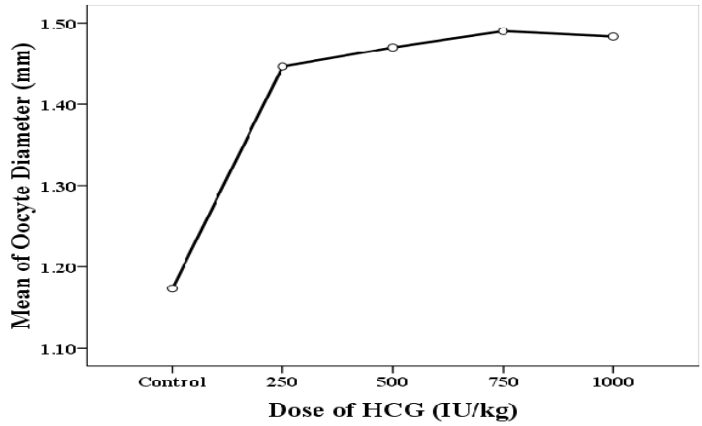
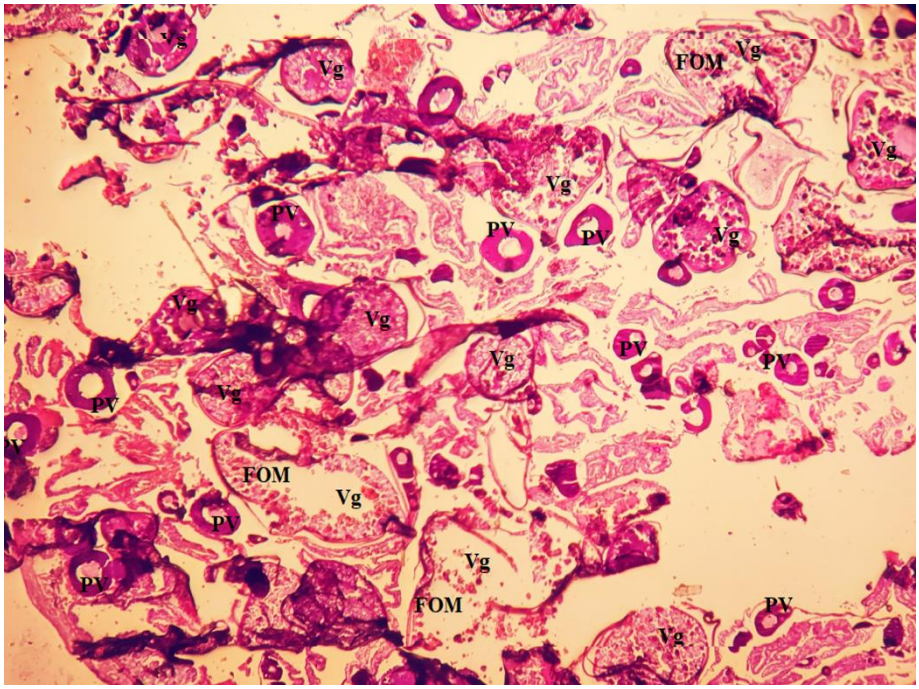


Figure 3. Oocyte diameter of Common Carp after induction by HCG.

Histological sections of female ovaries stimulated by human chorionic gonadotropin (HCG) showed a clear differentiation of mature oocytes formed in the vitellogenic growth stage, and very few oocytes in the previtellogenic growth stage [12] (**Figure 4**).
In the current study, it was found that the human chorionic gonadotropin (HCG) succeeded in accelerating ovulation in Common Carp (*Cyprinus carpio*), the lowest latency time for ovulation was recorded at the average value 26 hours after injection of 750 IU of HCG/kg compared to all other concentrations. The results in the current study did not correspond to those obtained by Assal and Salihe [3] when using the dose of 1000 IU of HCG/kg, which showed a latency time for ovulation of 15 hours compared to our result of 32 hours, while Faraj *et al*. [7] recorded 16 hours at the


Vg
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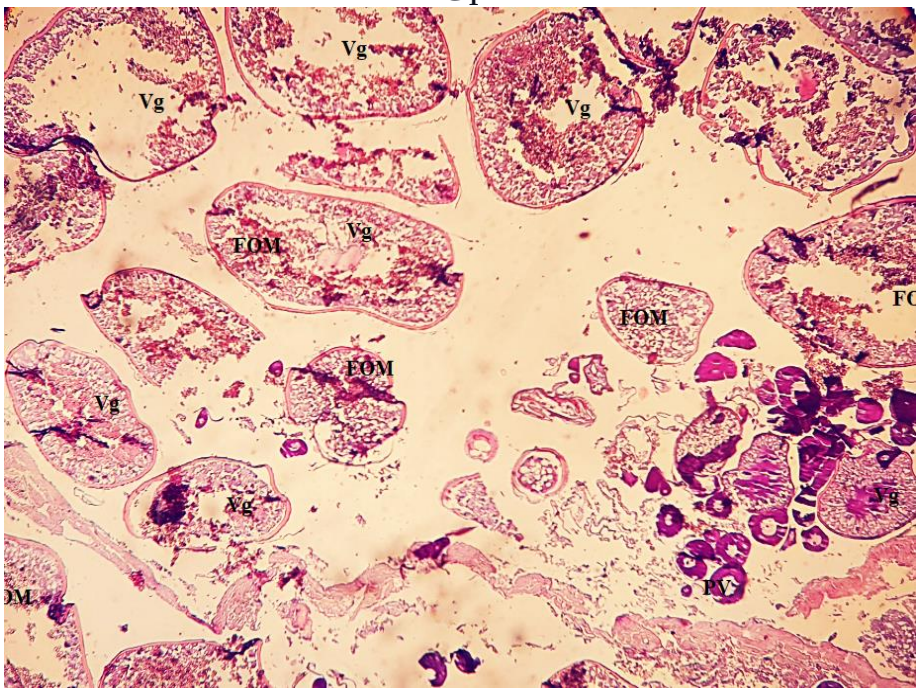
G_1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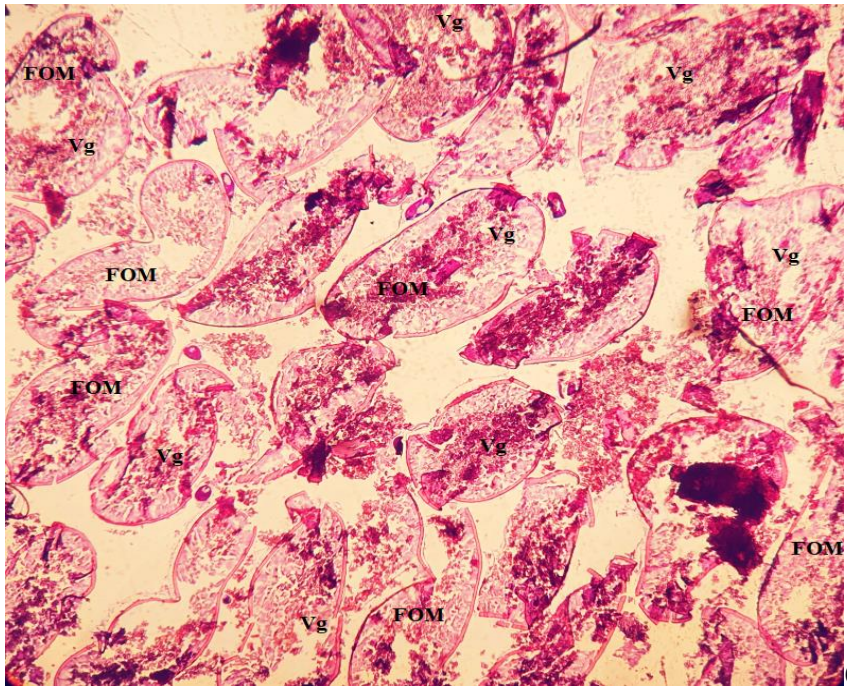
G_2


Vg
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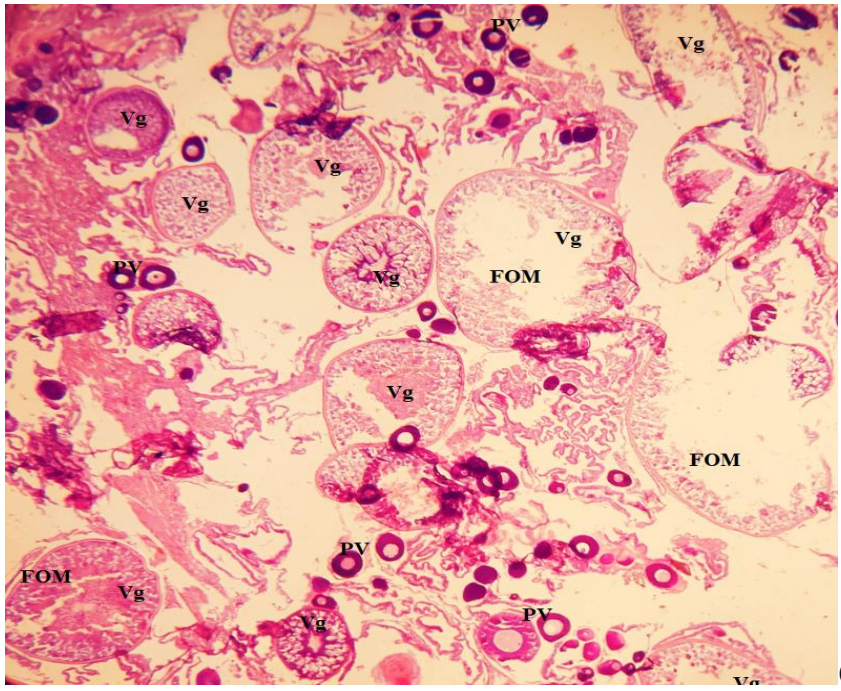
G_3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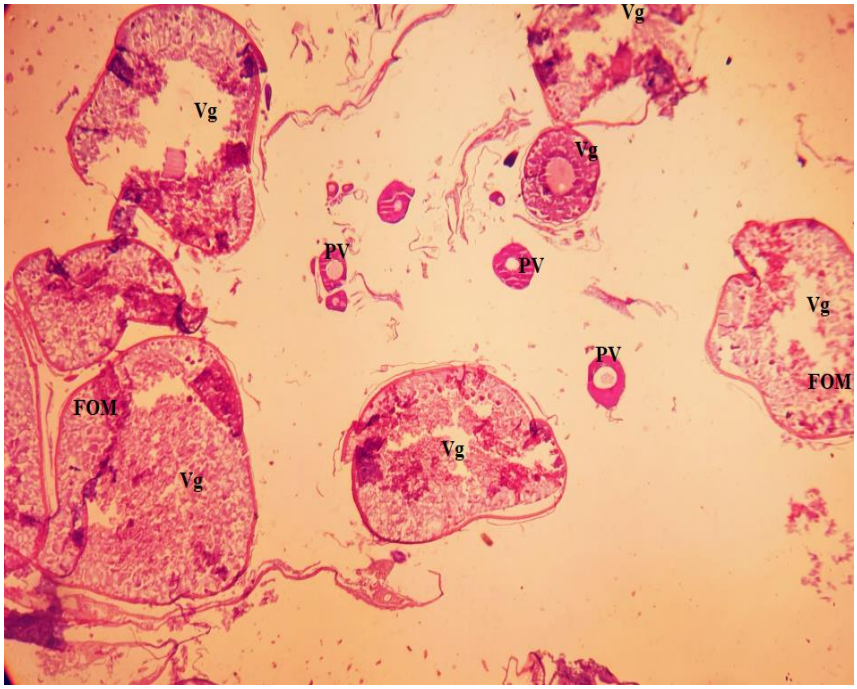
G_4



C

Figure 4. Ovarian tissue sections of the Common Carp induction by HCG show the oocytes in Provitelogenic (PV) growth stage and Vitelogenic (Vg) growth stage of their evolution, especially the Final Oocyte Maturation (FOM). Group: G_1, G_2, G_3, G_4, C. (H&E – 40X)

dose of 1500 IU/kg, and Akar *et al*. [2] recorded 18 hours on the dose of 2000 IU/kg (in two equal doses of 1000 IU/kg).

Our results also showed a very good response to ovulation at a rate of 100% in all females (hormonal treatment) treated hormonally of previous concentrations of HCG, as opposed to what Yeasmin *et al*. [13] found with no effect of 400, 500 and 600 IU of HCG/kg in inducing ovulation.

The higher latency time for ovulation may be due to insufficiency of gonadotropin in plasma, which is necessary for final maturity and ovulation [4; 10] the differences in the latency time may have been due to the type of commercial hormones circulating, the hormone injection doses used, the water temperature, injection time, and fish maturity.

Conclusions:

1. The lowest latency time for ovulation was recorded at an average value 26 hours after injection of 750 IU of HCG/kg.
2. The maximum ovary weight was 1200 g at 750 IU of HCG/kg. No significant difference between 500, 750 and 1000 IU of HCG/kg.
3. The maximum absolute fecundity was 606000 oocytes at 750 IU of HCG/kg. A clear significant difference in favour of 500 and 750 IU of HCG/kg over the rest of both groups and control.
4. The maximum Oocyte diameter was 1.50 mm at 750 IU of HCG/kg. No significant difference between 500, 750 and 1000 IU of HCG/kg.

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