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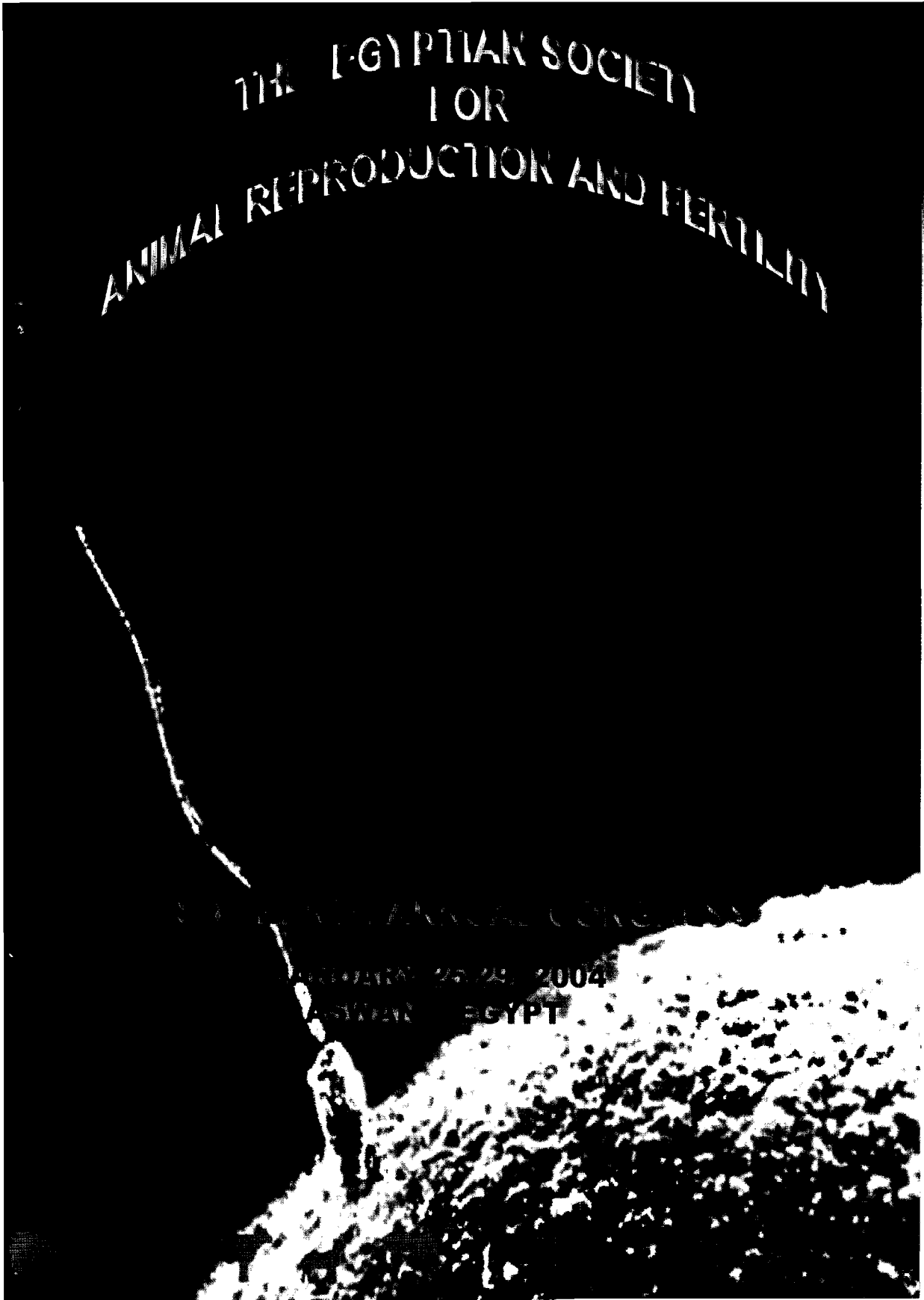

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EFFECT OF SOME TREATMENTS ON MILT CHARACTER AND HISTOLOGICAL STRUCTURE OF THE TESTES IN COMMON CARP

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The present study was conducted upon a total number of 25 male Common carp. Those males were divided into 4 groups; the 1st (n= 5) was treated with 1.5mg/kg Bw pituitary extract, the 2nd (2=5) was treated with 75Mg/kg B.W. LH. RH, the 3rd (n=5) was treated with 10 Mg/Kg B.W. LH. RH and 5 mg /kg BW Dopamine artagonists and the 4th (n=10) received no treatment. Milt samples were collected from all males anaesthetized with Thiopental sodium and MS 222 during the breeding season. Milt volume, percentage of motile and viable spermatzoa and sperm cell count were recorded. The histological structure of the testes was also observed. The ultra structure of spermatozoa and tests were also investigated. It was found that the injection of pituitary extract improved the milt volume and percentage of motile and viable spermatozoa, followed by those males treated with GnRH. The injection of GnRH and Dopamine antagonists improved the milt volume and live sperm percentage and reduced the percentage of sperm motility. Meanwhile, the sperm cell concentration did not vary among all groups. Regarding the effect of season, the milt volume and live sperm percentage increased significantly and sperm motility and sperm cell concentration non significantly increased during spring than autumn. The microscopic picture of the testes in addition to the ultra structure of spermatozoa and testes were discussed.

INTRODUCTION

The common carp usually becomes sexually mature during the first year in both tropical and subtropical regions **(Woynarovich and Harvath, 1980).** They reported also that most of common carp spawn during spring and its spermatozoa are motionless in the testis but become motile when come in contact with water. Moreover, the spermatogenesis is usually completed by the end of summer and spawning occurs during the spring of the following year **(Billard *et al.*, 1992).**

The common carp spermatozoa is one of the most primitive among Teleost fish **(Billard, 1986).** Invasive endocrine technique has been used to enhance sperm production in common carp **(Saad and Billard, 1987).** Moreover, the spermatozoa

of warm water fishes moves actively by their filamentous tail for 0.5-1.0 minute **(Woynarovich and Harvath, 1980).**

The present investigation was conducted to evaluate the effect of treatment with pituitary extract, GnRH or GnRH and Dopamine antagonists on milt characters and histological structure of the testes in common carp. The ultrastructure of spermatozoa and testes were also observed.

MATERIAL AND METHODS

The present work was carried out on a total number of 25 broostock male common carp during the breeding seasons early in spring and late in autumn (1998-2002) at El-Abassa Fish Hatchery, Sharkia Governorate. Those fish fed twice daily with diet at an approximate of 3% body weight (B.W.) according to **Gissi *et al.* (1991).**

Based on the type of treatment, those males were divided into 4 groups: the 1st (n = 5) was treated with pituitary gland extract (1.5 mg/kg B.W.) according to **Woynarovich and Harvath (1980),** the 2nd (n = 5) was treated with single dose of LH.RH, the 3rd (n = 5) was treated with single dose of LH.RH (10 mg / kg B.W.) and single dose of Dopamine antagonists (5 mg/kg B.W) according to **Line and Peter (1996)** and the 4th (n = 10) received no treatment and used as control group. All males were anaesthetized either by using Thiopental sodium (25 mg/L) for 10-13 minutes **(Shaheen *et al.*, 1996)** or MS222 (100 mg) at pH 8 **(Lahnsteiner *et al.*, 1997).** A safe technique for repeated anaesthesia was used without side effects **(Gutierrez and Herrera, 1995).**

Milt collection and evaluation :

Males of common carp were subjected to natural cycle of temperature and photoperiod **(Line and Peter, 1996).** The ripe males were stripped during the spawning period **(Woynarovich and Harvath, 1980 and Yao and Crim, 1995).** The ventral surface of male fish was carefully dried with clean towel, the bladder was cleared by gentle abdominal pressure and the genital papilla was dried to avoid contamination with excreta slime and urine, then the milt was collected in a dry graduated tube and used for evaluation after the first drop of it was wiped away. Milt volume (ml), percentage of sperm motility and livability, in addition to the sperm cell count (x 10^{12} / ml) were recorded.

Histological examination of the testes:

The testes were separated after killing the male, then cut it into several small pieces (1 mm thick) and allowed to be fixed in alcoholic Bouin's solution for

6 days. Paraffin block embedded and sections of pieces were performed using standard histological techniques **(Martoja and Martoja, 1967).** Sections of 7 M thick were mounted on slides and stained using H & E stain, then examined under light microscope.

Ultra-structure examination of the spermatozoa and testicles:

It was done under electron microscope according to the technique reported by **Bancroft and Stevens (1982).**

Statistical analysis:

Data obtained were statistically analyzed using the **Statistical Analysis System (SAS, 1987).**

RESULTS

Data of the current investigation are recorded in Tables 1, 2 and 3. The microscopic picture of common carp testicles are depicted in Figures 1, 2 and 3. The ultrastructures of common carp spermatozoa are illustrated in Figures 4, 5, 6 and 7 and those of the testicles are shown in Figure 8.

Table 1. Effect of treatment by pituitary gland extract, GnRH and GnRH + DOPA antagonists on milt characters of Common carp (M ±SE).

Milt characters	Control group (n = 10)	Treated groups		
		Pituitary (n = 5)	Gn.RH (n = 5)	GnRH + DOPA antagonist (n = 5)
Milt volume (ml)	0.70 ± 0.06^{d}	5.60 ± 0.40^{a}	4.00 ± 0.32^{b}	3.40 ± 0.24^{c}
Sperm motility (%)	77.00 ± 2.13	81.00 ± 3.32	80.00 ± 2.74	75.00 ± 2.24
Live sperm (%)	94.70 ± 0.79^{b}	97.60 ± 0.51^{a}	96.40 ± 1.08^{ab}	93.80 ± 2.01^{ab}
Sperm cell Conc. (x10^{12}/ml)	2.51 ± 0 .10	2.46 ± 0.16	2.30 ± 0.07	2.46 ± 0.16

Means with different superscripts in each category are significantly different ($P < 0.05$).

Table 2. Effect of season on body weight and milt characters of Common carp (M ± SE).

Characters	Spring	Autumn
Body weight (Kg)	4.40 ± 0.87^{a}	3.50 ± 0.22^{a}
Milt volume (ml)	5.60 ± 0.40^{a}	1.80 ± 0.25^{b}
Sperm motility (%)	80.00 ± 3.16^{a}	76.00 ± 2.91^{a}
Live sperm (%)	97.60 ± 0.51^{a}	93.80 ± 0.58^{b}
Sperm cell conc. ($x10^{12}$/ml)	2.46 ± 0.81^{a}	2.30 ± 0.53^{a}

Means with different superscripts in each category are significantly different ($P < 0.05$).

Table 3. Correlation coefficient among body weight and milt characters of Common carp.

Characters	Melt volume	Motility (%)	Live sperm (%)	Sperm count ($x10^{12}$/ml)
Body weight (Kg)	0.019	0.213	0.277	0.090
Milt volume (ml)	-	0.175	0.325	0.149
Sperm motility (%)	-	-	0.004	0.337
Live sperm (%)	-	-	-	0.142

DISCUSSION

There was a significant increase in milt volume of common carp treated by pituitary gland extract, GnRH, GnRH + dopamine antagonists than the control one (Table 1). This results came in accordance with the previous studies conducted by **Oliveira *et al.* (1991) and Christ *et al.* (1996)** in common carp fish, **Suquet *et al.* (1992)** in *Rainbow trout* **Mylonas et al. (1995)** in *American shad.* In contrast

Fig. 1 Cross section in testes of C carp before treatment showing narrow seminiferous tubules, little number of spermatozoa and thin tunica albuginia (H &E, X 100)

Fig. 2 Cross section in testis of C carp showing great number of spermatozoa, spermatids and all the developmental stages inside the lobules (H&E, X 100)

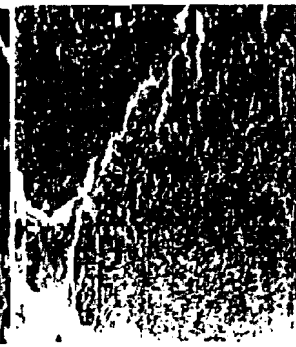

Fig. 3 Cross section in testis of C carp after treatment showing thick tunica albuginia and wide seminiferous tubules (H& E, X 100)

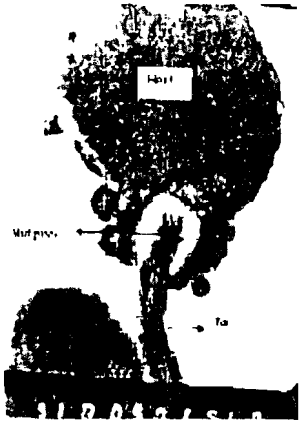

Fig. 4 EM of C carp sperm showing normal structure and there is no acrosomes

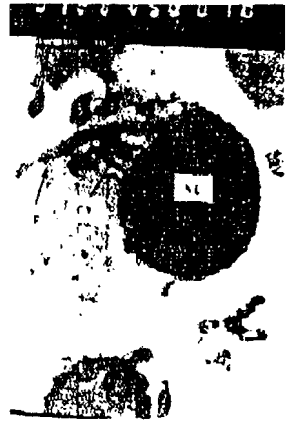

Fig. 5 EM of C carp sperm showing the normal structures, nucleus (UN), basal body (B), cytoplasmic channel (CY) and falgellum (F)

Fig. 6 EM of C carp sperm cross section in tail region showing the flegella which composed from two central microtubules and nine pairs of pripheral microtubule

Fig 7 EM of C carp sperm showing normal sperm structure and abnormal ones with detached tails

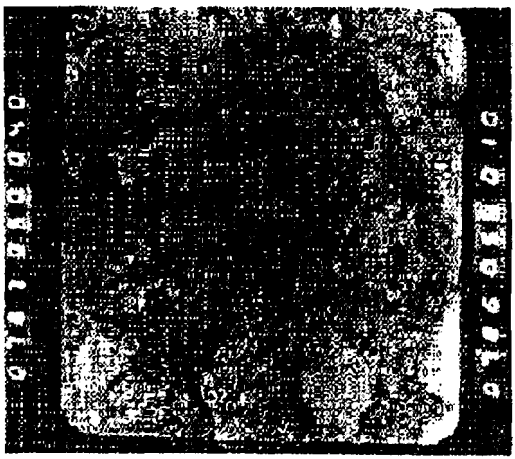

Fig. 8 EM of C carp testicle showing all the developmental stages of spermatogenesis inside the lobules The spermatids are smaller than the spermatocytes

Methven and Crim (1991) recorded that the milt volume of *Atlantic halibut* ranged between 1-60ml This can be explained through neuroendocrine regulation of GTH-II secretion in Teleost mainly under a dual neurohormonal system GTH-II release is stimulated by a gonadotropin-releasing hormone (GnRH) and inhibited by dopamine which act as a gonadotropin release-inhibitory factor **(Peres *et al.*, 2000)**

Table 1 showed a non significant difference in mass activity of milt among males treated by pituitary gland extract, GnRH, GnRH + dopamine antagonists and control This results were similar to those observed previously by **Oliveira *et al.* (1991) and Billarad and Cosson (1992)** in common carp, **Fauvel *et al.* (1999)** in Sea bass *Dicentrarchus Sabrax*, **Lahnsteiner and Patzner (1998)** in Boops *boops*, *Diplodus sargus*, *Mullus barbatus*, and Trachurus *mediterraneus*. But in contrary to those observed by **Mylonas *et al.* (1995)** in *American shad*. Before extension of

milt, the spermatozoa of Common carp were usually motionless but they became active motile after their dilution with distilled water. The induction of motility might be related to the particular environmental conditions including temperature, salinity, pH, season and fish species during the spawning **Lahnsteiner *et al.* (1995).**

The present study revealed a non-significant difference in percentage of live spermatozoa between fish treated by pituitary gland extract, GnRH, GnRH + dopamine antagonists and control. This results came in consistent to those observed by **Oliveira *et al.* (1991)** for common carp.

The sperm cell count of common carp treated with pituitary gland extract, GnRH, GnRH + dopamine antagonists and control in this study came in agreement with those obtained by other authors for common carp (**Oliveira *et al.*, 1991; Christ *et al.*, 1996** and **Ravindere *et al.*, 1997**); for *American shad* (**Mylonas *et al.*, 1995; Fauvel *et al.*, 1999**) and for *Atlantic halibut* (**Harald *et al.*, 2001**).

Regarding the effect of season Table 2 revealed a significant increase in the volume and the live sperm percentage on spring in comparing to autumn but there was non-significant difference in sperm motility and sperm count among both seasons. This finding was similar to **Billard *et al.* (1992).** They found also that the spermatogenesis is usually completed by the end of the summer and spawning occurs in the spring of the following year. Moreover, **Saad and Billard** (1987) reported that spermiation given regularly over 9 months after the completion of spermatogenesis in October. **Shimizu (1997)** found that the fish gonads of yearling fish were quite immature in September. He added that during late autumn and winter, a gradual increase in the GSI of both sexes was observed and the spawning period of the yearling fish was from late March to August judging from the presence of milt-producing males and most of fresh water fish spawn during spring.

The Common carp spermatozoa, are characterized by its round head, the nucleus occupies all the head area, the presence of two mitochondrial processes around the mid piece in between appear cytoplasmic channel, basal body, and very short flagellum. One of the most exiting chricteria is the absence of acrosome. In cross of the sperm tail, it was noticed that filaments arranged in (9 + 2). These finding are similar to that observed in other studies upon Common carp spermatozoa (**Billard *et al.*, 1995**). In another study, it was mentioned that the head region is elongated in the spermatozoon of *C. gobio* with absence of acrosome and asymmetrical flagellum which inserts laterally at the nucleus, the mid piece is

approximately cylindrical in shape and it contains five to six large and ovoid mitochondria **(Lahnsteiner *et al.*, 1997).** Moreover, **Lahnsteiner and Patzner (1999)** reported that spermatozoa of *Siganus Rivulatus* is symmetrical in its longitudinal axis, has no acrosom, an almost spherical nucleus, a small mid-piece with six mitochondria, centriols arranged at an acute angle to each other, no nuclear fossa and a flagellum. In Tilapia, **El-Ashram (1997)** describe the spermatozoa consists of a spherical head and long tail, the head was devoid of an apparent acrosom, the head length was 2.38 and 2.56um. **Suquet *et al.* (1998),** noticed in the morphology of *Turbot* spermatozoa that the mature cells contained dense chromatin.

By electronomicrograph of the common carp testicles in the present study, the inner part of lobule filled up with spermatozoa observed in mature sexual lobular type; the lobules were irregular shape in variable size. All developmental stages were observed inside the lobules, at the edge of the lobule and within individual cysts the spermatocytes (I) are slightly bigger than the spermatocytes (II) and have dense and less rounded nuclei. The spermatids even smaller than the spermatocytes II have very dense, rounded nuclei. Spermatozoa are packed in the inner part of the lobules and fill the lobular lumen. On examining cross section of common carp testes by light microscope before treatment semniferous tubules were narrow with a few cellular lining, little numbers of spermatozoa and thin tunbica albuginia, interstitial connective tissue fibers rich with collagen, general aspect of lobule organization. Partial view of lobule section showing the all development stages inside the lobules, at edges of lobules within individual cysts. The spermatocytes I are slightly bigger than the spermatocytes II have very dense rounded nucleus. In the inner part of the lobules spermatozoa are packed and fill the lobular lumen. In other cross section in testes after treatment showing thick tunica albuginia and wide seminiferous tubules. There was an increase in both size and lumen of semniferous tubule in addition to highly proliferation in lyding cells. These findings came in accordance with that mentioned in other studies **(Billard *et al.*, 1992; Oteme *et al.*, 1996; Grier and Taylor, 1998; Brown-Peterson *et al.*, 2002).**

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