

Effect of Passive Immunization with Specific Anti-FSH on Testicular Lipids and Some Lipogenic Enzymes of Adult Bonnet Monkeys

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ABSTRACT

The effect of anti-follicular stimulating hormone (FSH) on testicular lipid and the specific activity of testicular enzymes of the isocitrate dehydrogenase (ICDH), pyruvate/malate dehydrogenase (MDH) and malic enzyme involved in lipogenesis were studied in mature bonnet monkeys, *Macaca radiata*. Immunization of monkey with anti-FSH for 24 days did not produce any significant changes in the body weight, organ weight and pituitary weight. Testicular isocitrate dehydrogenase (ICDH) and malic enzyme activities were decreased significantly but MDH activity was stimulated by anti-FSH treatment. Testicular total lipid, phospholipid and cholesterol, were not altered significantly by the Anti-FSH treatment. Increased level of free cholesterol was also observed after FSH treatment. Among glyceride glycerol sub classes, triacyl glycerol showed a significant increase. Among testicular phospholipid classes, phosphatidyl inositol was markedly decreased by anti-FSH immunization. Data on serum hormonal profile, shows that there were no alteration in serum testosterone, prolactin (PRL) and luteinizing hormones (LH) but FSH was significantly decreased. The present study reveals that immunization with anti-FSH has significant effect on different class Of testicular lipids and pyruvate malate enzymes cycle. *Iran. Biomed. J. 2: 39-44, 1998.*

Keywords: Anti-FSH, lipogenic enzymes, passive immunization.

INTRODUCTION

It is well established that the pituitary gonadotropins are involved in the process of spermatogenesis and function of male accessory sex organ [1, 2], FSH is essential for normal initiation of the spermatogenic process and maintaining the normal fertilizing capacity of adult male bonnet monkeys [2]. Westhoff reported that FSH-specific antiserum contained antibodies that recognized amino acid sequence 37-55 of FSH [3]. Short term passive immunization against FSH specifically affected spermatogenesis without impeding the endocrine function of the testis in monkeys [3-5]. Long term active immunization of bonnet monkey with ovine FSH also resulted in continued testicular dysfunction leading to acute oligospermia or azoospermia [6]. It was also reported total absence of 4c and s phase cells in adult monkeys immunized with FSH, suggesting spermatogenic block at the premeiotic DNA synthetic phase and formation of primary

spermatocytes and proliferation of spermatogonia (from A1 to type B spermatogonia).

The high level lipids found in normal testis suggests the important role of lipid in metabolic activities of testis [7]. Lipids associated with nongerminal cells in testis [8] plays a vital role in spermatogenic and steroidogenic process. Phospholipids form the major portion of total lipids in testis and it is well associated with sperm maturation process [9] and androgen secretion [10]. About 50% of the neutral lipids in rat testis is triacyl glycerol and other half is mainly cholesterol [11]. Cholesterol is the base of steroidogenesis in normal rat testis. Free cholesterol predominates the esterified cholesterol as they are the direct source for steroid synthesis [12].

The passive and active immunization against FSH is a possible method for male contraception [13]. Most of the available information in this field is related to the study of sperm count, spermatogenesis and serum androgen profiles. In the present study,

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due to their significance as nutritive and structural component of the testis, various classes of neutral and phospholipids were investigated in the monkeys treated with anti-FSH. Apart from these, few important enzymes closely associated with lipogenesis NADP⁺-isocitrate dehydrogenase (Ec 1.1.1.42), malate dehydrogenase (Ec 1.1.1.37) and malic enzyme (Ec 1.1.1.40) Were also considered. Serum FSH, LH, prolactin and testosterone were assayed.

MATERIALS AND METHODS

Animals: Healthy mature male bonnet monkeys *Macaca radaita* weighed 7- 10 kg were fed with standard monkey pellet diet (Hindustan lever, India) along with daily supplement of fresh fruits and vegetable. Water was made available *ad libitum*. These animals were housed in a well ventilated temperature controlled animal house with a light schedule of 14 hr light and 10 darkness, The monkeys were acclimatized to the animal house and were isolated from female monkeys two months before and throughout the study. A total of 10 monkeys divided into two groups of five monkeys each were used.

Group I: This group served as control and was administered with 1 ml of 0.9% saline as vehicle intramuscularly every alternate day at 10.00 AM for 24 days.

Group II: This group was administered with 1 ml of 1:10 dilution of anti-FSH (used antiserum to human FSH, WHO reagent programme), intramuscularly every 48 hr at 10.00 AM for 24 days. The highly specific human anti-FSH was used to neutralize the FSH in bonnet monkeys as there is a good cross reaction between antisera of human and monkey FSH [14].

Blood was collected through femoral vein on day 0,7,15, and 24 of study. Twenty four hours after the last injection, all the monkeys were sedated by a single intraperitoneal injection of sodium pentobarbitone (30 mg/kg body weight). Testes were dissected out, perfused with physiological saline and cleaned thoroughly from the blood clots.

Methods: Total lipids were extracted [15] and estimated colorimetrically [16]. Cholesterol was determined by the method of Hanel and Dam [17]. The colorimetric procedure given by Van Handel and Zilversmith [18] was used for the estimation of glyceride glycerol. Phospholipid was quantified by

the method of Marinetti [19]. NADP-isocitrate dehydrogenase was assayed by the method of Bernt and Bergmeyer [20]. For the determination of the activity of malate dehydrogenase and malic enzyme the Ochoa's method [21, 22] were used. Protein was assayed by the method of Lowry [23].

The single solvent system [24] was used to separate the fractions of neutral lipid. Phospholipid fractions were separated [25] and was eluted by the method of Skipski [26]. The glyceride, cholesterol were eluted by adding 5 ml of chloroform to the tubes, centrifuged at 5000 xg for 10 minutes and the supernatant were taken in a new set of clean tube and estimated by the method described earlier. Free fatty acid concentration was determined by the method of Regouw [27].

Serum FSH and LH were assayed by RIA using materials obtained from National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Baltimore, Maryland, USA [28]. Testosterone was assayed using materials obtained from Diagnostics Corporation, USA. The data obtained were statistically analyzed using the Student's "t" test [29].

RESULTS

Body, testicular and pituitary weights were unaltered under anti-FSH treatment for 24 days. NADP⁺-isocitrate dehydrogenase ($p < 0.01$) and malic enzyme ($p < 0.05$) activities were significantly inhibited by anti-FSH treatment. However the activity of malate dehydrogenase was significantly increased ($p < 0.01$) (Fig. 1). The testicular total lipids, total phospholipid and total cholesterol were not altered markedly by the administration of anti-FSH. However, testicular total glyceride glycerol were elevated appreciably ($p < 0.05$) (Fig. 2). Administration of anti-FSH significantly increased, the testicular triacyl glycerol ($p < 0.05$) and free cholesterol ($p < 0.001$) (Fig. 3). Testicular free fatty acid, mono and diacyl glycerol and phospholipid fraction were unaltered except phosphatidyl inositol, which was markedly decreased ($p < 0.001$) (Fig. 4).

Serum FSH recorded a significant decrease ($p < 0.05$) after 24 days of treatment. However on 7th and 15th days there was no significant changes in FSH. Anti-FSH administration for 24 days did not bring about any significant changes in serum LH, prolactin and testosterone between 0,7,15, and 24 days of immunization (Fig. 5).

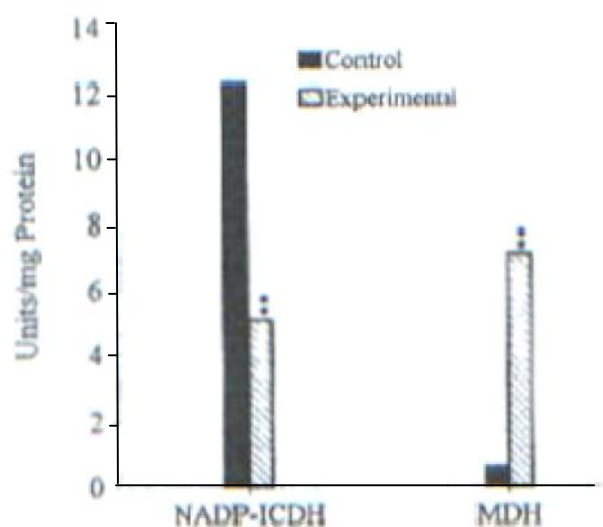


Fig. 1. Effect of anti-FSH on the testicular pyruvate malate cycle enzymes in bonnet monkeys. ** $P < 0.01$ control vs. experimental.

Table 1. Effect of Anti-FSH on testicular total lipid, glyceride glycerol, cholesterol and phospholipid in mature monkeys. Each value is mean \pm sem of five animals/observations. Each value is expressed as mg per gram tissue. * $P < 0.05$ control vs. experimental.

Total	Control	Experimental
Lipid	220.10 ± 11.58	187.60 ± 38.50
Glyceride Glycerol	4.38 ± 0.28	9.64 ± 1.76
Cholesterol	3.73 ± 0.15	3.52 ± 0.24
Phospholipid	204.50 ± 31.20	159.20 ± 34.00

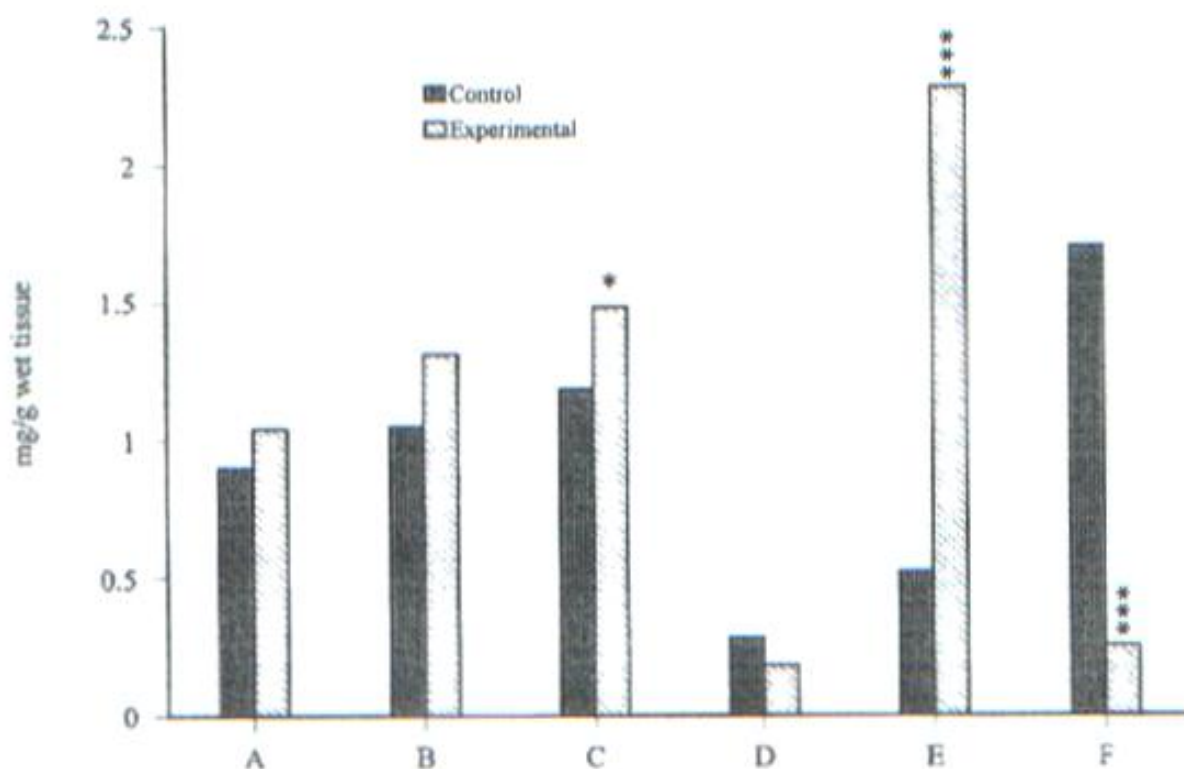


Fig. 2. Effect of anti-FSH on testicular neutral lipid in mature bonnet monkeys. A: monoacyl glycerol, B: diacyl glycerol, C: triacyl glycerol, D: free fatty acid, E: free cholesterol, F: esterified cholesterol. Each value is mean \pm SEM of 5 estimations. * $P < 0.05$, *** $P < 0.001$ control VS experimental.

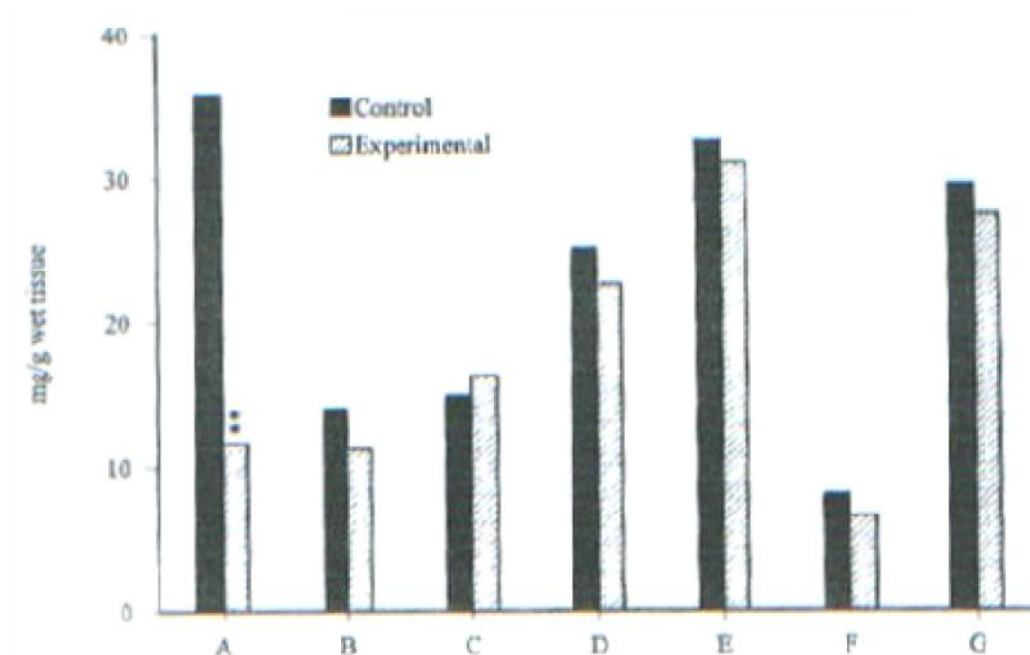


Fig. 3. Effect of anti-FSH on testicular phospholipid fraction in mature bonnet monkeys. A: phosphatidyl inositol, B: phosphatidyl serine, C: sphingomyelin, D: phosphatidyl choline, E: phosphatidyl ethanol amine, F: cardiolipin and G: phosphatidic acid. Each value is mean \pm SEM of 5 animals/observation. ** $P < 0.05$ control VS experimental.

DISCUSSION

Spermatogenesis in man and other mammals are under the influence of LH and FSH [1]. FSH acts on Sertoli cells to facilitate spermatogenesis [30] and maturation of late stages (stages 16-19) of spermatid [1]. These specific effects of FSH has been considered as the site of interference for possible male contraceptive [3, 13].

The study on serum hormones suggest a selective suppression of FSH unaffected steroidogenic function of testis as there was no significant alteration in testosterone, LH and prolactin. This may prove the specificity and cross reaction of anti-FSH used in the present investigation. Unaltered testicular and pituitary weight reveals that passive immunization for short period of 24 days may not alter the structural component of the testis. Early report on rats also revealed unaltered weight of testis after passive immunization with FSH [31]. However, immunization for longer duration of 90 to 210 days decreased the weight of testes in rats [32] and different species of monkeys, *macaca fasciata* [5] *Macac mulata* [33] and *macaca radiata* [34]. Probably, only longer duration of anti-FSH treatment alone may alter the spermatogenic function and testicular weights.

The data on enzymes associated with lipogenesis

suggest an impaired lipogenesis in testes of bonnet monkeys. Malic enzyme which acts on malate, utilizing NADP as a cofactor to generate NADPH and NADP-isocitrate dehydrogenase also produces NADPH by the conversion of isocitrate into α -ketoglutarate [12] were decreased in anti-FSH treated monkeys immunized against FSH. Pentose phosphate pathway which is the other major source of NADPH [12] also found to be affected in these monkeys (unpublished data). Therefore, data on the enzymes associated with lipogenesis may suggest impaired lipogenesis in the testes of bonnet monkeys due to anti-FSH treatment. However, a differential response by anti-FSH was evident in the testes of monkeys, as malate dehydrogenase activity was stimulated. This may probably shunt more oxaloacetate to facilitate gluconeogenesis as there is inhibition of lipogenesis.

The data on lipid reveal an increase in the ratio of free cholesterol:esterified cholesterol which may suggest increased mobilization of cholesterol which is not fully utilized due to decreased FSH availability. This view is supported by the study of Gambal and Ackerman [10] who reported increased free cholesterol without any alteration in esterified cholesterol in rats treated with anti-FSH. Selective suppression of FSH may affect the utilization of glyceride and this may be the reason for the

Table 2. Effect of Anti-FSH on serum FSH, LH, Prolactin and testosterone in mature bonnet monkeys. * $P < 0.05$ control vs. experimental day 0, vs. experimental 24 day.

Hormone	Animal	Days			
		0	7	15	24
FSH MIU/ml	Control	5.23 ± 0.19	5.26 ± 0.08	5.28 ± 0.16	5.03 ± 0.24
FSH MIU/ml	Experimental	5.10 ± 0.53	3.85 ± 0.83	3.50 ± 0.75	2.80 ± 0.69*
LH	Control	5.24 ± 0.44	5.55 ± 0.76	5.37 ± 0.41	8.20 ± 1.00
LH	Experimental	4.11 ± 0.92	3.48 ± 0.84	3.31 ± 1.22	5.36 ± 1.44
Prolactin (ng/ml)	Control	4.10 ± 0.35	4.40 ± 0.43	4.30 ± 0.18	4.65 ± 0.21
Prolactin	Experimental	3.84 ± 0.19	3.46 ± 0.20	3.82 ± 0.28	4.08 ± 0.40
Testosterone	Control	6.50 ± 0.29	6.32 ± 0.16	5.91 ± 0.21	5.90 ± 0.17
Testosterone	Experimental	6.26 ± 0.20	6.12 ± 0.26	5.86 ± 0.27	6.02 ± 0.32

observed accumulation of the same in the testis. Glyceride are the main source of energy for germ cells [35]. Accumulation of glyceride may suggest distribution of spermatogenesis. During active spermatogenic process reduction in testicular glyceride was reported [11]. Therefore, the observed accumulation of glyceride may indicate disruption of spermatogenic process. The increase in total glyceride seems to be mainly due to increased concentration of triacyl glycerol as there was no significant change in mono diacyl glyceride. Unlike glyceride, obvious alteration in total phospholipids and various classes of phospholipids were not evident, except phosphatidyl inositol. In normal testis with active spermatogenic process glyceride are comparatively low and phospholipids are high [8]. Increased concentration of phospholipid is being attributed to large number of germ cells which contain membrane phospholipids [12]. However, the ratio of glyceride: phospholipid was elevated due to anti FSH treatment, suggesting the possibility of distribution in spermatogenesis. Phosphatidyl inositol is an integral part of plasma membrane and may be under the stimulatory effect of tropic hormone and facilitate the action of tropic hormone at the level of target cells [36]. The decreased concentration of phosphatidyl inositol in monkeys treated with anti FSH may suggest a specific stimulatory effect of FSH on this phospholipid class.

In general, the present study reveals that immunosuppression of FSH for even a shorter period may have an adverse effect on testicular biochemistry, particularly lipids. It is also interesting to note that in the present investigation in spite of decreased FSH, due to anti-FSH treatment, testosterone, LH and prolactin were not found to be affected significantly.

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