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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 impending 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 Al 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 1.1.137) 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-FSHβ (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 antiFSH. 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).

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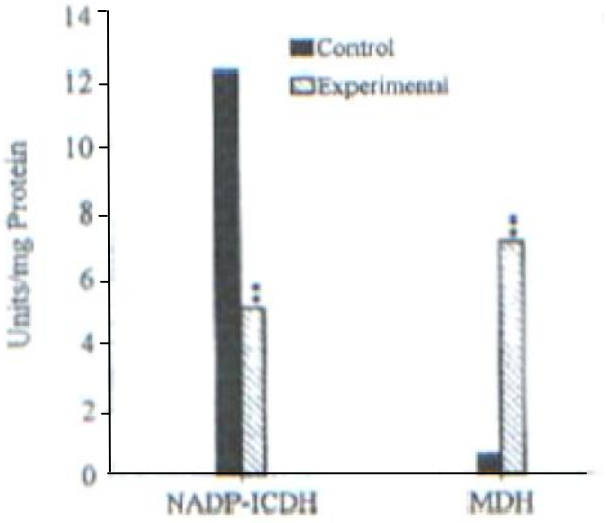


Fig. l. Effect of anti-FSH on the testicular pyruvatemalate cycle enzymes in bonnet monkeys. ** P<0.01 control vs. experimental.

Table 1. Effect of Anti-FSH on testicular total lipid, glyceride glycerol, cholesterol and phospholipid in mature monkeys Each value is mean ± 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

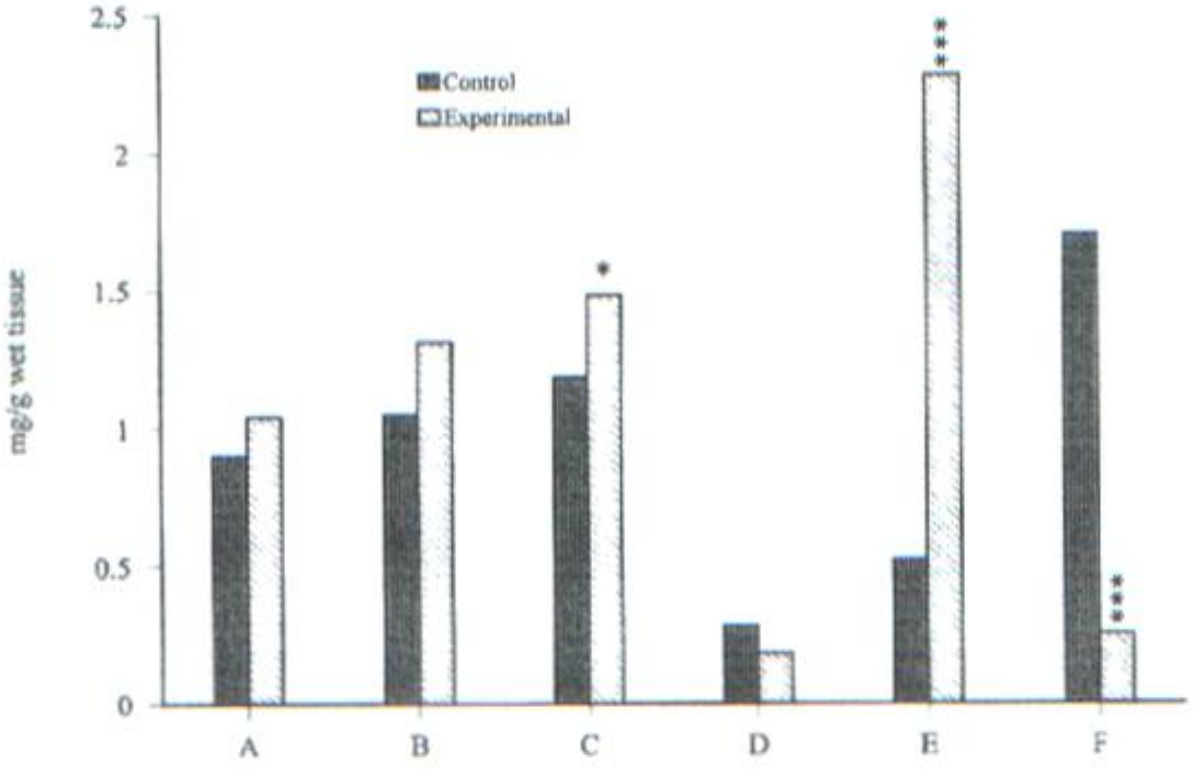


Fig. 2. Effect of anti-FSH on testicular neutral lipid in mature bonnet monkeys. A: monoacyl glycerol, B: diacyl glycerol, C: triacyl glycerol, D: free fatty acid, E: free cholestrol, F: esterified cholestrol. Each value is mean ± SEM of 5 estimations. **P<0.05, ***P<0.001* control VS experimental.

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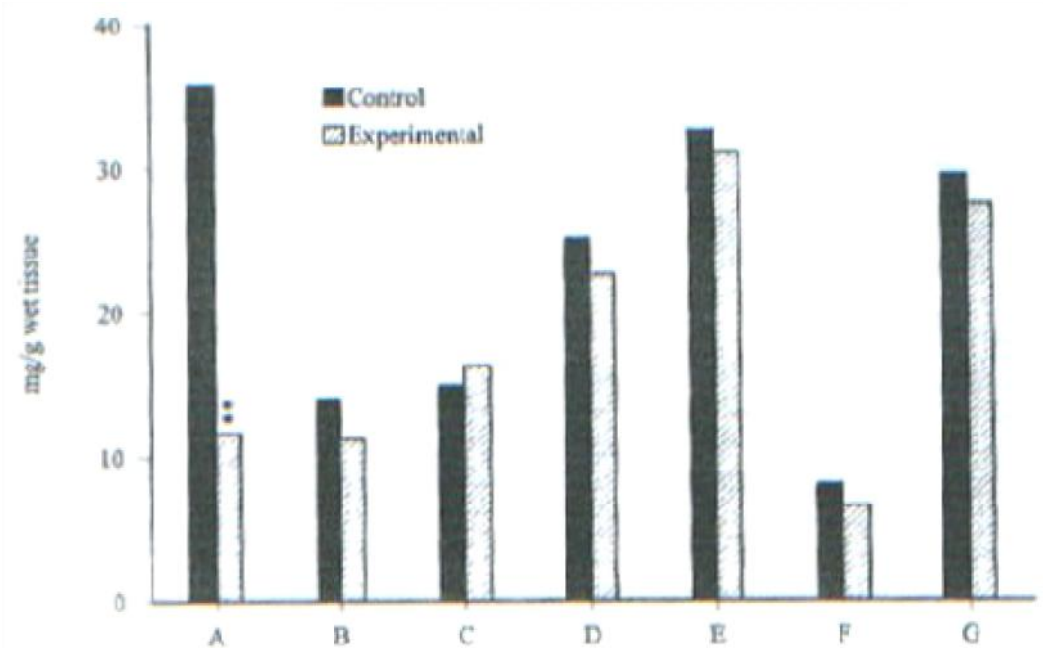


Fig. 3. Effect of anti-FSH on testicular phospholipid fraction in mature bonnet monkeys. A: phosphatidyl inositol, **B:** phosphatidyl serine, C: sphingomyelin, D: phosphatidyl choline, E: phosphatidyl ethanol amine, F: cardiolipin and G: phosphatidic acid. Each value is mean ± SEM of 5 animals/observation. ***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 fasiculta* [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-ketoglutrate [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 availabil ity. 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

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Table 2. Effcet 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.

		Days			
Hormone	Animal	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 o 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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REFERENCES

1. Steinberger, E. (1971) Hormonal control of mammalian spermatogenesis. *Physiol. Rev. 51: 1-22.*
2. Eshkol, A. and Lunenfeld, B. (1975) The immunization with hormone in reproduction research. pp 5881, North Holland/American Elsevier, Amsterdam. Westhoff, W.E Slootstra. J.W. Kuperus, D. Flinterman j. F Schaaper, W.M.M. Oonk, H.B., and Meloen, R.H. (1996) Detection of epitopes on FSH and FSH-antiserum-induced suppression of bioactivity of FSH and LH. *J. of Reprod Immunol. 30: 133-149.*
3. Nieschlag, E. and Wicking, E.J. (1982) Immunological neutralization of FSH as an approach to male fertility control. *Int.J. Androl. Suppl. 5:1-26.*
4. Madhwa Raj, H.G. Murthy, G.S.R.C. Siaram, M.R. and Talbert, L.M. (1982) Control of spermatogenesis in primates. Effect of active immunization against FSH in the monkey. *Int. J. Androl. Suppl. 3: 2733.*
5. Moudgal, R. N. and Rajan, D.R. (1985) An immunological approach to study gonadotropin action at the cellular and molecular level. *Bio-Sciences Report 5: 943-955.*
6. Johnson, A.D. (1970) Testicular lipids. In: *The testis,* Vol.2 (Johnson, A.D. Gomes, W.R. and Van Denmark, N.L, eds.) pp. 193-257, Academic Press, New York.
7. Free, M. J. (1970) carbohydrate metabolism in the testis. In: *The Testis* Vol, 2 (ed. Johnson, A.D., Goomes, W.R. and Van Denmar, N. L.) pp. 125-192, Academic press, New York
8. George, J.C. and Ambadkar, P.M. (1963) Histochemical demonstration of lipid and lipase activity

in the rat testis. *J Histochem. Cytochem. 12: 587590.*
9. Gambal, D. and Ackerman, R.J. (1967) Hormonal control of rat testicular phospholipids. *Endocrinology 80, 231-239.*
10. Carpenter, M.M. and Oshima, M. (1965) lipid composition of the rat testis. *Federation Proceedings 24: 663.*
11. Mayes, P.A. (1988) Metabolism of glycogen. In: *Harpers Biochemistry.* (Murry, R.K. Grames, D.K. and Rodwell, V.W., eds) pp 165-171, Appleton and Lange, California.
12. Talwar, G.P. (1980) Immunological of contraception pp. 48-58. Camelot Press Ltd. South Great Britannia.
13. Wide, J. and Newton, J. (1971) An immunological cross reaction between monkey gonadotropin preparation and antisera to human gonadotropin. *J Reprod. Fertil. 27: 103-106.*
14. Folch, I. Lees, M. and Salone Stanley, G. H. (1959) A simple method for the isolation and purification of total lipids from animals tissues. *J. Biol. Chem. 226: 496-509.*
15. Frings, G.S., Frendley, T.W., Dunn, R.T. and Queen, G.A. (1972) Improved determination of total serum lipids by the sulpho-phosphovanillin reaction. *Clin. Chem. 18: 673-674.*
16. Hanel,H.K. and Dam,H. (1955) Determination of small amount of total cholesterol by the Tschugaeff reaction with a note on the determination of Lathosterol. *Acta Chemica Scandinavea 98: 677682.*
17. Van Handel, F. and Zilversmith, D:B. (1959) Micromethod for direct determination of serum triglycerides. *J. Lab. Invest. Clin. Med. 50: 152-157.*
18. Marinetti, G.V. (1962) Chromatographic separation, Identification and analysis of phosphatides. J. *Lipid Res. 3: 1-10.*
19. Bernt, E. and Bergmeyer, H. U. (1974) Isocitrate dehydrogenase (NADP) In : *Methods of enzymatic analysis,* Vol.2 (Bergmeyer, H.U., eds) pp. 624-627, Academic press, New York.
20. Ochoa,S. (1955a) Malic dehydrogenase from pig heart. In: *Methods in Enzymology vol.* 1 (ed. Colowick, S.P. and Kaplan, N.O.) pp. 735-739, Academic Press, New York.
21. Ochoa,S. (1955b) Malic dehydrogenase from pigeon liver and wheat germ. In: *Methods in Enzymology, vol.* 1 (Colowick, S.P. and Kaplan, N.O., eds) pp. 739-742, Academic Press, New York.
22. Lowry, 0. H. Rosebrough, N. J., Farr, A.L. and Randall, R. J. (1951) Protein measurement with the folin phenol reagent. J. *Biol. Orem. 193: 256-257.*
23. Duthie, A. M. and Atherton, H.U.(1970) One stage solvent system and the dimensional thin layer chromatographic separation of milk simple lipid classes on 20x20 cm plates. *J. Chromatogr. 51: 319-321.*
24. Narayanan, V., Riebes, J.A. and Hilf, R. (1985) effect of streptozotocin induces diabetes and insulin on phospholipid content of R3230AC mammary tumor cell. *Cancer Res. 45: 4833-4837.*
25. Skipski, V.P. Peterson, R.F. and Barclay, M (1964) Quantitative analysis of phospholipid by thin layer chromatography. *Biochem. J. 90: 374-378.*
26. Regouw, B.J.N., Comlissen, P.J.H.C., Helder, R.A.P., Spijkers, J.B.P. and Weber, Y.M.M. (1972) Specific determination of free fatty acid in plasma. *Clinical Chimica Acta 31: 187-194.*
27. Frants, A. G., Kleinberg, D.W. and Noel, G.L. (1972) Studies on prolactin in man. *Recent Progress in Hormone Research 28: 527-674.*
28. Ostle, B. (1954) In: *Statistics in Research,* Oxford and IBH publication, New York.
29. James, E.G. and Wilson, J.D. (1985) Disorder of the testes and male reproductive tract. *In:* William's Text Book of Endocrinology 7th ed. (Wilson and Foster, eds) pp. 259-312, W.B. Saunders Company, Tokyo.
30. Dym, M., Madhwa Raj, H.C., Lin, Y.C., Chemes, H.E., Kotite, S.N., Nayfeh, S.N. and French, F.S. (1979) Is FSH required for maintenance of spermatogenesis in adult rats. *J. Reprod. Fertil. Suppl. 26: 175-181.*
31. Davis, R. V., Man, S.J., Laurie, M.S. and Setchell, B.P. (1979) The effect of long term administration of either a crude inhibition preparation or an antiserum to FSH on serum Hormone level. Testicular function and fertility of adult male rats. *J. Reprod. Fertil. Suppl. 26: 183-191.*
32. Wicking, E.J. Usadel K.H. and Nieschalag, E. (1980) The role of follicle stimulating hormone in testicular function of the mature rhesus monkey. *Acta Endocrinologica 94: 117-128.*
33. Murthy, G.S.R.C., Sheela Rani, G.S. Moudgal, N.R. and Prasad, M.A.M. (1979) Effect of passive immunization with specific antiserum to FSH on the fertility of adult male bonnet monkeys (Macaca radita). *J. Reprod. Fertil. Suppl. 26:147-163.*
34. Davis, B.K. (1976) Inhibitor effect of synthetic phospholipid vesicle containing cholesterol on the fertilizing ability of rabbit spermatozoa. *Proc. Soc. Exp. Biol. Med 152: 257-261.*
35. White, A. Handler, P. and Smith, E.L. (1973) In: *Principles of Biochemistry,* pp 452-603, Kokgakusho Ltd., Singapore.

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