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Seasonal variations in the lipid composition of ram (*Ovis aries*) testis

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Abstract. The seasonal variations in the various categories of lipids have been investigated by dividing the year into five seasons, i.e. spring, summer, rains, autumn and winter. The data have been analysed by using student's *t*-test. Non-significant seasonal variations ($p < 0{\cdot}05$) are found when all the seasons are compared for total lipids, non-polar lipids, glycerides and unidentified lipids and the ratio of phospholipids and non-polar lipids. All the various lipid components and their ratios vary non-significantly ($p < 0{\cdot}05$) in summer vs autumn, summer vs winter and autumn vs winter. Some lipid components vary significantly at different levels when seasons are compared. The significance of the results has been discussed.

Keywords. Seasonal changes; testis; lipids; biochemistry.

1. Introduction

Some observations have been made on lipid composition and its metabolism in the ram testis (Johnson et al 1967a, b; Scott and Setchell 1968; Neill 1974; Neill and Masters 1975). To the best of our information, no attempt has been made to determine the biochemical seasonal changes in the lipids of any mammalian testis. The present studies report the seasonal variations in various categories of lipids in the ram testis throughout the year.

2. Materials and methods

2.1. *Material*

Silica gel G used was from E. Merck (Darmstadt, Germany). The standard lipids were obtained from biochemical unit, V.P. Chest Institute, Delhi or Sigma chemicals, St. Louis, Missouri, USA. All the solvents and other chemicals were of AR grade.

2.2. *Material collection, extraction and separation of lipids*

The testicular material was taken immediately after slaughtering the rams (*Ovis aries*) in the slaughter house. The sexual maturity of the testis was confirmed by the presence of all stages of spermatogenesis in the material taken from each testis fixed in Zenker-formol, dehydrated in ethanol, sectioned and stained with haematoxylin-eosin. The year was divided into following five seasons and in each season five animals were used :

(i) Spring (mid February to end of March).
(ii) Summer (mid May to end of June).
(iii) Rains (last three weeks of July to first two weeks of August).
(iv) Autumn (mid September to end of October).
(v) Winter (mid December to end of January).

The lipids were extracted according to Folch *et al* (1957). The lipids from 5 g of each testis were extracted with 100 ml chloroform : methanol (2: 1) solvent. The crude total lipids were purified by using 5 ml of 0·9% NaCl per 100 ml of lipid extract. For thin layer chromatographic (TLC) separation of lipids with silica gel G, petroleum ether : diethyl ether : acetic acid (80 : 20 : 1, v/v/v) (Mangold 1969) and chloroform : methanol : 7 N ammonia (65 : 25 : 5, v/v/v) (Rouser *et al* 1969) were the solvent systems used for neutral and polar lipids respectively.

2.3. *Identification of individual lipid components*

The spots for both polar and non-polar lipids were first detected by placing the dried chromatograms in sealed chambers saturated with iodine vapours (Sims and Larose 1962). Iodine stained spots were marked and iodine from the plates to be subsequently used was removed by slight heating. These identifications were further confirmed by spraying the dried chromatograms with H_2SO_4 (Heftmann *et al* 1966). The Rf values of the unknown spots were compared with the standard reference lipids run under similar conditions. Modified spray reagent for phospholipids (Voskovsky and Kotetsky 1968) was used for identifying phospholipids. Choline containing lipids, amino-lipids and glycolipids were identified with Dragendroff's reagent (Mangold 1961), ninhydrin (Dittner and Lester 1964) and α-naphthol (Kates 1972) respectively. Phosphotungstic acid (Randerath 1964), spray sequence for glycerides (Clark 1961) and spray sequence for free fatty acids (Dudzinski 1967) were used to identify cholesterol/esters, glycerides and free fatty acids respectively.

2.4. *Quantitative analysis*

The total lipids were determined gravimetrically. Total phosphorus estimated after Ames and Dubin (1960, as cited in Ames 1966) was multiplied by 25 to work out the amount of phospholipids. Cholesterol was estimated according to the method of Cook (1958). The free cholesterol was precipitated with 1% digitonin (in 80% ethanol) and esterified cholesterol was estimated as cholesterol. The free cholesterol was calculated by substracting esterified cholesterol from total cholesterol. Free fatty acids and glycerides were estimated according to Chakraborty *et al* (1969) and Van Handel and Zilversmit (1957) respectively. The

amount of total non-polar lipids was calculated by substracting the amount of phospholipids from that of total lipids.

2.5. *Statistical analysis*

The data were analysed using Student's *t*-test (Snedecor 1959).

3. Results and discussion

The data for the amounts of various lipid components and the ratios of certain components are given in table 1. Significance of comparisons between various seasons for different lipid constituents is shown in table 2. Total lipids, non-polar lipids, glycerides and unidentified lipid components show non-significant ($p < 0{\cdot}05$) seasonal variations (table 2). The ratio of phospholipids and non-polar lipids also varies non-significantly ($p < 0{\cdot}05$). All the various lipid components and their ratios show non-significant variations ($p < 0{\cdot}05$) in summer vs autumn, summer vs winter and autumn vs winter (table 2). When different seasons are compared, some lipid components show significant variations (table 2).

The main seasonal variations in different categories of lipids are found in rains vs autumn or rains vs winter or rains vs summer; however, slight differences in these variations in three sets of seasons do exist (table 2). The exact physiological significance of such seasonal variations cannot be specified. But to account for the seasonal variations in the semen quality under similar environmental factors, these seasonal variations in the lipid components may be correlated with the physiological functioning of testis. Shukla and Bhattacharya (1952) have reported that on the whole, the quality of semen (number of spermatozoa, and quality and quantity of seminal plasma) in ram improves during the cooler months of the year and becomes worst with high humidity. It is well established that qualitative and quantitative production of semen is dependent upon the functioning of testis which is achieved by the regulation of spermatogenesis and the activity of male accessory sex organs by the male steroid hormones (primarily testosterone). So it is suggested that the testicular activity is best during cooler months (autumn and winter) and worst during rains. This suggestion is further supported by Katongale *et al* (1974) who reported the higher level of testosterone in ram during cooler months, when the level of esterified cholesterol is minimum (table 1) and significantly different ($p < 0{\cdot}01$) when compared with that in rains (table 2). The amount of esterified cholesterol is maximum in rains (table 1) and it varies significantly when compared with that in other seasons (table 2). Based on these points, a negative correlation is suggested between esterified cholesterol and steroidogenesis in the testis.

Among lipids, the spermatozoa mainly contain phospholipids (Komarek *et al* 1964, 1965a, b; Scott *et al* 1967). So if the alterations of the lipids in other testicular cells are not considered, the direct correlation between the number of spermatozoa and amount of phospholipids in the testis may be suggested. Based on the above discussion, on the whole, it is suggested that among lipids, the ratio of phospholipids and esterified cholesterol may be used as the criterion for the physiological functioning of testis, i.e. for qualitative and quantitative

Table 1. The amount (mg/g fresh testis) of various lipid components and their ratios during various seasons of the year in the ram testis (Mean ± SEM).

Lipid components	Spring	Summer	Rains	Autumn	Winter
Total lipids	27·22 ± 2·84	25·77 ± 2·64	24·45 ± 2·78	25·77 ± 2·92	24·85 ± 2·44
Phospholipids (PL)	16·64 ± 1·29	12·42 ± 1·10	13·68 ± 1·43	13·13 ± 1·47	12·40 ± 1·13
Non-polar lipids (NL)	10·58 ± 1·19	13·35 ± 1·34	10·77 ± 1·51	12·64 ± 1·53	12·45 ± 1·45
PL : NL	1·57 ± 0·23	0·94 ± 0·16.	1·27 ± 0·20	1·04 ± 0·15	1·10 ± 0·12
Total cholesterol (TC)	2·32 ± 0·14	2·83 ± 0·20	2·17 ± 0·15	2·74 ± 0·18	2·77 ± 0·20
Esterified cholesterol (EC)	0·47 ± 0·06	0·46 ± 0·07	0·67 ± 0·05	0·37 ± 0·04	0·40 ± 0·05
Free cholesterol (FC)	1·85 ± 0·10	2·36 ± 0·15	1·50 ± 0·12	2·37 ± 0·73	2·37 ± 0·30
FC :EC	3·92 ± 0·35	5·78 ± 0·48	2·23 ± 0·28	6·54 ± 0·55	5·98 ± 0·52
Free fatty acids	1·22 ± 0·02	1·03 ± 0·03	1·01 ± 0·03	1·02 ± 0·02	1·03 ± 0·02
Glycerides	5·01 ± 1·22	7·22 ± 1·14	5·44 ± 1·33	6·58 ± 1·02	6·35 ± 1·14
Unidentified	2·13 ± 0·52	2·27 ± 0·43	2·15 ± 0·34	2·30 ± 0·42	2·23 ± 0·53
PL : TC	7·17 ± 0·63	4·39 ± 0·38	6·30 ± 0·54	4·79 ± 0·41	4·49 ± 0·42
PL : BC	35·40 ± 3·72	26·96 ± 2·93	20·42 ± 2·88	36·01 ± 3·87	31·82 ± 3·15
PL : FC	8·99 ± 0·95	5·24 ± 0·48	9·12 ± 0·88	5·54 ± 0·45	5·42 ± 0·39

Table 2. Significance of comparisons between various seasons for different lipid components in ram testis.

	Total lipids	PL	NL	PL: NL	TC	EC	FC	FC:EC	FFA	Glycerides	Unidentified	PL: TC	PL: EC	PL: FC
Spring/ Summer	NS	*	NS	NS	NS	NS	*	NS	**	NS	NS	**	NS	*
Spring/ Rains	NS	NS	NS	NS	NS	*	NS	**	**	NS	NS	NS	*	NS
Spring/ Autumn	NS	NS	NS	NS	NS	NS	*	**	**	NS	NS	*	NS	*
Spring/ Winter	NS	*	NS	NS	NS	NS	NS	*	**	NS	NS	**	NS	*
Summer/ Rains	NS	NS	NS	NS	*	*	**	**	NS	NS	NS	*	NS	**
Summer/ Autumn	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Summer/ Winter	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Rains/ Autumn	NS	NS	NS	NS	*	**	**	**	NS	NS	NS	NS	*	**
Rains/ Winter	NS	NS	NS	NS	*	**	*	**	NS	NS	NS	*	*	**
Autumn/ Winter	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS : Non-significant at 5%; *: Significant at 5%; **: Significant at 1%.

production of semen including the number of spermatozoa. This ratio follows a decreasing sequence in autumn, spring and winter (table 1), but the differences are non-significant (table 2). On the whole, the quality of semen in winter is better than that in spring (Shukla and Bhattacharya 1952) but the percentage of live spermatozoa in semen shows the reverse order; however, the differences are non-significant ($p < 0{\cdot}05$) (Chahal 1977).

The relatively higher levels of different forms of cholesterol in summer (table 1) and their significant variations in comparison to those in rains (table 2) are supported by the high levels of total plasma cholesterol and total plasma corticoids in summer and their significant variations in comparison to those in rains (Chahal 1977). But the percentage of live sperm is minimum in summer (Chahal 1977). The "summer sterility" and corresponding lowered breeding efficiency in ram is also well-known (see Dutt 1960). On the whole also, the semen in summer is better than that in rains only (Shukla and Bhattacharya 1952). The variations of lipid constituents in summer vs rains differ from those in rains vs winter, or rains vs autumn only in the fact that in these seasons, the ratio of phospholipids and esterified cholesterol varies non-significantly ($p < 0{\cdot}05$) (table 2). As the lipids play a very important role in the physiology of testis (Johnson 1970; Coniglio 1977), it may be said that among lipids, the ratio of phospholipids and esterified cholesterol may be responsible for the differences in the semen qualityl of summer as compared to that in winter and autumn. So it further supports the suggestion that among lipids, the ratio of phospholipids and esterified cholesterol may be used as the criterion for physiological functioning of testis. When spring, autumn and winter are compared with rains for the ratio of phospholipids and esterified cholesterol, all the seasons show similar significant variations ($p < 0{\cdot}05$). Therefore it may be suggested that testicular activity is almost similar in spring, autumn and winter. Further, it decreases from summer to rains, because spring, autumn and winter show non-significant variations when compared with summer (table 2). So the testicular activity may decrease in the order of autumn or spring or winter, summer and rains. The quality of semen on the whole follows a decreasing sequence in winter, spring, summer and rains (Shukla and Bhattacharya 1952).

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