J Endocrinol Reprod 3 (1) : 1-10 (1999)
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ASSOCIATION OF ENHANCED EPIDIDYMAL β-GALACTOSIDASE ACTIVITY WITH BREEDING SEASON IN MALE GOATS

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(Received 9th October 1998)

SUMMARY

The impact of environmental cues on breeding in male goats has been investigated. Rainfall, temperature, humidity and day length characteristic of the monsoon season were found to be important factors that influenced breeding. An abattoir survey revealed a good linear correlation between the weight of testis and epididymis sampled from goats during the rainy months of July, August and September. The activity of β-galactosidase, en enzyme that participates in post-translation modification of glycoproteins leading to sperm maturation in the epididymis, was also elevated during the monsoon months. This phenomenon is visualized as one among several physiological adjustments that goats make in response to environmental signals for successful mating.

Key words: Environmental cues; Epididymis; β-Galactosidase; Seasonal breeding.

INTRODUCTION

Seasonal regulation of breeding in mammals is a complex process and involves at one level or another a proper interaction of appropriate ecological and physiological principles (1,2). The precise timing of breeding is made possible year after year by a diverse array of environmental signals that are perceived. This leads to neuroendocrine adjustments which ultimately triggers breeding at the appropriate time (3,4).

In the case of goat, the reproductive behavior is governed by two types of factors categorized "ultimate" and "proximate". The pathways by which both these factors influence breeding have been extensively reviewed (5). It is generally recognized that ultimate factors enable the animal to carryout the coarse adjustments as far as reproduction is concerned while proximate factors do the fine-tuning. The fact however remains that the goats belonging to the

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same species but inhabiting tropical and temperate zones breed at different times in the year. Obviously, the type of vegetation and the climatic conditions prevalent in these zones have a profound role to play in the timing of events leading to reproduction. Since goat has been chosen as our animal model for the present studies, we have attempted to locate their breeding season at Roorkee and identify the environmental factors that control breeding in males.

Just as in other mammals, in goats too, it is expected that sperm maturation is an important prelude of successful mating. Glycosylation and deglycosylation form part of the mechanism that leads to extensive remodeling of sperm surface proteins in the epididymis (6,7). β-Galactosidase is one among several enzymes believed to participate in this process (8-10). In this paper, we present data to show that an elevation in epididymal β-galactosidase activity in goats is associated with its breeding season.

MATERIALS AND METHODS

Location : The present studies were carried out at Roorkee, which is located at 29.52° N and 77.53° E. The exact daily information regarding rainfall, relative humidity, temperature and the time of sunrise and sunset were collected from the local meterological station. Based on climatic conditions prevalent, five seasons were distinguished annually namely, winter (December, January, February), spring (March, April), summer (May, June), monsoon (July, August, September) and autumn (October, November).

Animals : The present investigations were conducted on locally available sexually mature domestic male goats (*Capra indica*) of all age groups reared at the local breeding station under natural environmental conditions. The animals were group housed together, outdoors, under roofed shelter and were fed on seasonally available plants and grass. The animals were also allowed to graze in the open during the daytime and water was made available at all times.

Collection of tissue : Male reproductive tissues were collected through the period of investigations as and when animals were slaughtered for meat consumption. The tissues were brought to the laboratory in ice within one hour of slaughter. The testis and epididymis were cleared of adhering adipose and connective tissues and weighed. While the testis was discarded, the epididymis was saved for biochemical studies. The epididymis was divided into twelve serial segments as previously described (11) and the activity of β-galactosidase in each segment determined.

Enzyme assay : β-Galactosidase activity was determined according to the original procedure of Conchie *et al.* (12) with slight modifications. The enzyme was extracted in 0.1 M sodium acetate buffer, pH 4.8 and assayed using p-nitrophenyl β-D-galactopyranoside as substrate. Protein was measured by the method of Lowry *et al* (13). Specific activity of β-galactosidase is expressed as units where 1 unit refers to the quantity of enzyme capable of liberating 1 μ mole of p-nitrophenol min^{-1} mg $protein^{-1}$.

Statistical analysis : Regression analysis of data on the weight of tissues obtained at different seasons of the year was done employing user friendly software (Lotus 123) on a personal computer. Student's *t*-test was used to compare the data on enzyme activity.

RESULTS

The seasonal changes in several environmental parameters collected over one year have been consolidated in fig.1

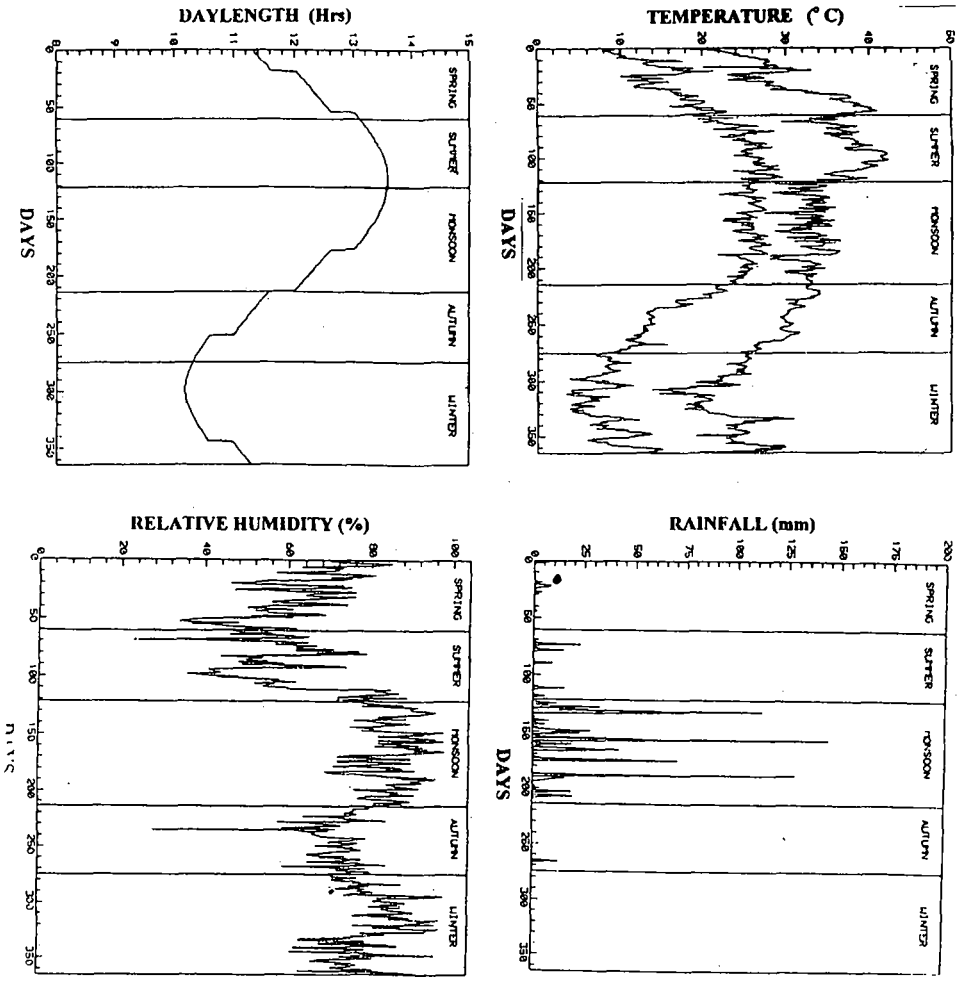

Figure 1 : Daily changes in temperature, rainfall, day length and relative humidity experienced at various seasons in Roorkee

The total rainfall experienced season-wise was : spring (35.3mm), summer (89.2mm), monsoon (933.5mm), autumn (14.8mm) and winter (154.8mm). Thus, as expected the heaviest rainfall was recorded during the monsoon months.

The range of minimum and maximum temperature observed were : spring (15.52 ± 0.40; 30.69 ± 0.74), summer (25.33 ± 0.30; 37.80 ± 0.34), monsoon (25.44 ± 0.14; 32.90 ± 0.23), autumn (15.00 ± 0.50;30.29 ± 0.30) and winter (8.15 ± 0.28; 23.04 ± 0.34). From the figures it is obvious that the temperatures are relatively more stable and also in a range comfortable to animals during the entire monsoon season than at any other season.

The percentage relative humidity was also another factor that varied during the five seasons: spring (62.37 ± 1.76), summer (59.80 ± 1.79), monsoon (85.49 ± 0.71), autumn (72.18 ± 0.76) and winter (80.21 ± 0.93). Most days during monsoon months showed uniformly high relative humidity, which was rather steady and consistent during the entire season.

The daily variation in the photoperiod was computed from the time of sunrise and sunset. Day length was observed to increase until the third week of June after which there was a decline. The long photoperiod of summer days just prior to the monsoon months may be an important environment cue that triggers breeding in goats at Roorkee.

Scatter plots of testicular and epididymal weights monitored at five distinct seasons are presented in fig 2. Regression analysis show a very good correlation between the two parameters in the monsoon months ($Y = 6.60 X + 5.077$, $r = 0.934$) followed by spring ($Y = 8.59 X + 9.178$, $r = 0.724$). On the other hand, poor correlation was observed during summer ($Y = 5.00 X + 11.31$, $r = 0.244$), winter ($Y = 3.43 X + 28.71$, $r = 0.497$) and autumn ($Y = 3.12 X + 27.50$, $r = 0.538$).

Specific activity of β-galactosidase in the epididymal segments, measured at different seasons, is presented in Table 1. A careful glance of the data brings the following to light. (1) The activity of β-galactosidase varies considerably among the twelve segments of the epididymis. (2) The activity of β-galactosidase in each of the twelve segments changes with season. (3) The onset of monsoon season brings about a spurt in β-galactosidase activity in all segments of the epididymis.

DISCUSSION

Since goats maintained under natural conditions have been used as the animal model for the present study, one could expect several environmental cues to serve as signals for seasonal reproduction. In this context, rainfall is an important factor that governs breeding. Although no direct evidence can be provided to pinpoint its precise role in the regulation of reproduction, rainfall perhaps acts as a stimulant, by promising surplus food. In addition, since goats are herbivorous, it is possible that certain plant compounds particularly those secondary

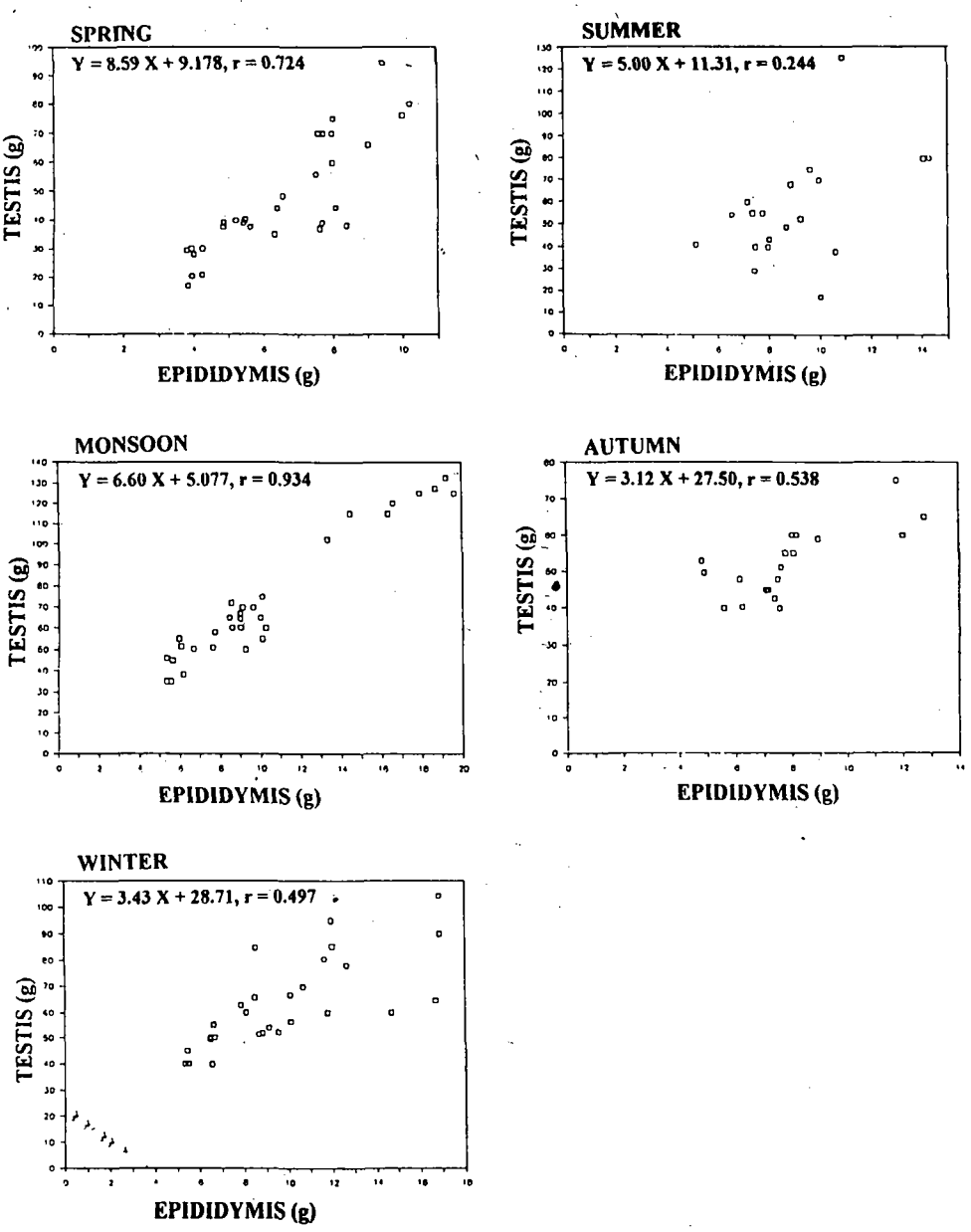

Figure 2 : Regression analysis of the data on goat epididymal and testicular weight obtained during the five distinct seasons at Roorkee

Table 1: Seasonal variation in the specific activity of β-galactosidase (μ-moles p-nitrophenol liberated, min^{-1} mg protein $^{-1}$) along twelve epididymal segments of goat

Epididymal segments	Spring (n = 32)	Summer (n = 32)	Monsoon (n = 36)	Antumn (n = 28)	Winter (n = 36)
1.	8.97 ± 2.01	14.17 ± 3.60	17.59 ± 2.96 *	8.81 ± 2.33	10.76 ± 2.15
2.	23.54 ± 3.59	28.67 ± 3.73	36.60 ± 4.72 *	19.64 ± 3.84	21.80 ± 3.60
3.	28.07 ± 3.86	32.41 ± 4.12	38.51 ± 4.75 *	22.99 ± 3.22	22.08 ± 3.87
4.	28.07 ± 3.66	39.51 ± 4.09	50.60 ± 5.92 *	28.16 ± 3.70	34.04 ± 3.82
5.	26.08 ± 4.19	43.82 ± 5.13	50.41 ± 5.63 *	30.59 ± 3.91	32.90 ± 4.34
6.	22.47 ± 3.67	42.58 ± 4.22 *	44.30 ± 5.57 *	25.46 ± 3.16	29.65 ± 3.23
7.	19.36 ± 2.82	34.91 ± 3.65	41.70 ± 4.17 *	23.08 ± 2.63	25.11 ± 3.91
8.	17.97 ± 2.84	16.13 ± 3.48	31.72 ± 3.06 *	17.61 ± 2.36	19.75 ± 2.83
9.	16.32 ± 2.17	29.10 ± 2.93 *	32.68 ± 3.14 *	18.91 ± 2.49	17.73 ± 2.50
10.	21.02 ± 2.84	43.98 ± 4.11 *	47.10 ± 5.06 *	23.35 ± 3.17	26.27 ± 3.76
11.	24.80 ± 3.99	45.05 ± 4.39 *	47.60 ± 5.06 *	26.02 ± 3.63	30.88 ± 3.47
12.	10.05 ± 1.76	11.99 ± 1.93	13.08 ± 1.44 *	6.61 ± 1.62	7.46 ± 1.14

Each value represents mean ± SEM. All comparisons are made against values obtained in winter (the non-breeding season). *$p<0.01$ Student's *t*-test.

metabolites produced during sprouting may act as dietary signals for the onset of breeding. In fact some plant compounds that influence reproduction in seasonal breeders under natural conditions have even been identified (14, 15). Thus, it is likely that rainfall influence breeding indirectly through the food chain.

Although substantial rainfall is experienced at two seasons, spring and monsoon, peak sexual activity is distinctly seen in monsoon months. This shows that rainfall may not be the

only signal required for sexual activation of male goats in Roorkee. Reproduction requires tremendous energy inputs and in order to utilize the available energy properly, nature has perhaps chosen a season wherein least energy is spent for thermoregulation. In this respect the extremes of temperature experienced during summer and winter do not favour reproduction. On the other hand, it appears that moderate temperatures combined with high relative humidity makes the monsoon season most suited for sexual performance. In this season, there is little deviation in the daily minimum and maximum temperatures from optimal levels. Thus, a major portion of energy that would otherwise have been utilized in thermoregulation is perhaps diverted for reproduction.

In many species, light acts as a time of the breeding season either by providing the signal that synchronizes an endogenous circannual rhythm or by stimulating the neuroendocrine gonadal axis (16, 17). Among mammals that respond reproductively to photoperiods, two groups can be distinguished: one group that breeds with the onset of long days and the other that breeds with the onset of short days (18, 19). From the data collected in the present study, it appears that goat found in the Roorkee region are "short day breeders". The switch over from the long photoperiods of summer to the relatively short photoperiods experienced during the monsoon seems to stimulate breeding in goats at Roorkee. The existence of a similar mechanism has been reported in goat and ram inhabiting the temperate regions (20, 21).

It may now be reasonable to conclude that regulation of reproduction in male goats is a synchronized cascade of response of seasonal changes in rainfall, temperature, humidity and photoperiod. Information gathered from several animal breeders at Roorkee confirms that maximum sexual activity in goats occur during the rainy months of July, August and September. The annual resumption of reproductive activity in this particular season enables the animal to prepare metabolically for the favourable period of time when the chances of survival of its offspring are maximal.

When the weight of the testis and the corresponding epididymis collected over one year period was analyzed season-wise, an interesting linear correlation emerged, which was particularly impressive during the monsoon season. It is well known that sperm produced in the testis undergo maturation in the proximal epididymis and are stored in a viable form at its distal end until ejaculation (23, 24). Also daily sperm production in the testis and the rate of flow through the rete testis in discontinuous breeders has been reported to vary with the season (5). Obviously, the rate of sperm production in the testis and removal from the male reproductive tract is more balanced in the sexually active male goats during the breeding season than at other seasons.

Measurement of epididymal β-galactosidase has demonstrated that these animals actually anticipate the upcoming season by making the necessary physiological adjustments. β-Galactosidase is a glycosidic enzyme believed to be involved in processing glycoproteins (7, 8). The present studies have shown abundant enzyme activity in segments of goat epididymis which suggest that the enzyme is either synthesized and / or activated in the

epididymis and indeed should be involved in events that take place here. In fact some studies (8-10) have also suggested that epididymal β-galactosidase may be responsible for enhancing fertilizing ability of sperm by altering the carbohydrate composition of sperm surface glycoproteins.

In almost every segment of the epididymis, a conspicuous increase in the activity of β-galactosidase has been observed in the monsoon months and in some segments during the preceding summer season. Since breeding in this animals occurs during the monsoon season, it is highly likely that the enzyme is involved in processing sperm surface glycoproteins and thereby sperm maturation which may be necessary for successful mating. Earlier studies have also suggested that β-glucosidase can be used as an additional index for seasonal reproduction in ram (25). It may be useful to recall that the onset of short photoperiods during the mating season brings about a rise in androgen levels in goat (26-28). Epididymal β-galactosidase being an androgen dependent enzyme (12) is perhaps showing higher activity in response to the increased levels of androgens associated with the mating season during the monsoon months.

ACKNOWLEDGMENT

This work was supported by grants from UGC, India. MG and NS thank the Council for Scientific and Industrial Research, India for the award of Senior Research Fellowships during which time this work was carried out.

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