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Temporal Variation in Reproductive Physiology of Small Indian Mongoose Male, *Herpestes javanicus*, Inhabiting Potohar Plateau, Pakistan

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Abstract.- Small Indian mongoose *(Herpestes javanicus)*, a small carnivore, has a crucial role in the food web, especially in agro- ecosystems where it serves as biological control agent for rodents, snakes, and some insects. In Pakistan, the scientific studies on reproductive biology of this species, vital for its management perspective, are scanty. We investigated male reproductive pattern of this species in Potohar Plateau by estimating concentrations of its male reproductive hormones and studying cellular changes inside the testes. The concentrations of testosterone, FSH (*follicle-stimulating hormone*) and LH (*luteinizing hormone*) were measured by using ELISA kits, while cellular changes inside the testes were studied by using histological procedures. Testosterone concentrations were found elevated from January till April and in August. The FSH levels were also elevated in January, February and March while those of LH were higher in February, March and April. Sperm count was higher in April, June and September, while diameter of seminiferous tubules was found greater in April and August. The study concludes that small Indian mongoose male breeds twice a year with its breeding season ranging from February to April, and July to September (6 months).

Key words: Small Indian mongoose, testosterone, FSH, LH.

INTRODUCTION

The small Indian mongoose (*Herpestes javanicus*) occurs naturally in the southern and south eastern regions of Asia (Wozencraft, 2005); it has a native range from Pakistan and northern India to southern China and the Malay Peninsula. It is also found on Hainan Island and Java. In the west, it extends to southern Iran (Corbet and Hill, 1992), south western Afghanistan (Hassinger, 1968), and along the shore of Persian Gulf it extends up to Kuwait and Iraq (Harrison, 1968).

Pakistan hosts two species of mongooses *viz.*, *Herpestes javanicus*, commonly called as small Indian mongoose and *Herpestes edwardsii*, the large Indian mongoose (Roberts, 1997).The small Indian mongoose occurs in southern Sindh, extending throughout Tharparkar, Thatta and Dadu districts, Bahawalpur division in southern Punjab, Gujranwala, Kasur, Lahore, Jhelum and Sialkot districts, and in the Potohar region, it occurs in Salt range, Jhelum, Rawalpindi and Attock districts. It is adapted to inhabit the village bordering areas and towns (Roberts, 1997).

The small Indian mongoose prefers living near human habitations; generally it is found in agriculture lands, coastal lands, deserts, natural and planted forests, range and grasslands, riparian zones, disturbed scrub, shrub lands, urban areas and wetlands (Nellis, 1989). It is well adapted to better wooded regions of the Indus plains which have a slightly higher rainfall, and thus it shows the distribution of typical Oriental faunal zone species (Roberts, 1997).

The small Indian mongoose is sexually dimorphic whereby its body length varies from 50−67 cm, males being larger in size (650g in body weight) compared to the females (430g) on average. Average age of male at sexual maturity is approximately 122 days and males after getting mature, continuously produce spermatozoa for the whole life of the individual. Earlier published literature shows that in their introduced range on various islands, mongoose breed twice or three times a year, have no definite breeding season;

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however, breeding mostly occurs during the summer months (Ewer, 1977).

Most of studies on reproductive biology of mongooses globally have been conducted so far on introduced populations on islands where the species is reported to be a seasonal breeder. It breeds mainly in the increasing photoperiod and longest days (Gorman, 2009). Mongoose in Hawaii (Pearson and Baldwin, 1953), Puerto Rico (Pimental, 1955), and St. Croix (Nellis and Everard, 1983) have similar breeding seasons. Occurrence of monthly reproductive activity has been reported for the species from some other parts of the world (Nellis and Everard, 1983; Gorman, 2009). Males exhibit a bimodal pattern with peaks in testicular size occurring in spring and early fall. All adult males are reproductively active throughout summer. Adult males do not exhibit regressed testes in the summer, which follows the active summer pattern in the north (Pearson, 1944).

During the annual reproductive cycle of the large Indian mongoose *Herpestes auropunctatus* introduced to the Fijian island of Viti Levu testis weight has been reported to follow a seasonal cycle with a low from February to July and a peak from September to December, however, spermatogenesis continues throughout the year. The cycle of numbers of spermatozoa stored in the epididymis followed that of testis weight. Leydig cell nuclei showed no seasonal pattern, but weight and histology of the accessory organs of reproduction indicated a seasonal cycle of androgen production, similar in pattern to that of testis weight (Gorman, 2009).

Reproductive status of males is more difficult to assess as compared to females which are relatively easily identifiable for their reproductive stage through study of their vaginal smears, and pregnancy status. However, in males, the possible assessment is based on sperm counts, estimation of hormones (testosterone) and the testicular size during breeding and non-breeding seasons. Thus the knowledge of seasonal reproduction in mongoose is based upon examination of male and female reproductive tracts and changes in their organ weights during breeding and non-breeding seasons. In Pakistan, formal scientific studies on reproductive pattern of the small Indian mongoose are lacking although a few published studies have focused its ecological aspects (Siddiqui *et al.,* 2004; Mahmood *et al*., 2011). The current study is aimed to investigate the reproductive pattern of small Indian mongoose male by estimation of its reproductive hormones and studying the structural changes in its testes during breeding and non-breeding seasons.

MATERIALS AND METHODS

Study area

The current study was conducted in the Potohar Plateau (32°33 and 34°36 N and longitudes 71° 89 and 73° 37 E), that embraces four districts; Rawalpindi, Attock, Chakwal and Jhelum, and some areas of the Federal Capital Islamabad . The Plateau stretches across an approximate area of 2.2 million hectares (Bhutta, 1999); its elevation ranges from 305 m to 610 m. The mean temperature varies from 45ºC in summer and below freezing point in winter (Encyclopedia, 2010). Climatically, there are five seasons in the Plateau, winter (which lasts from December till February), spring (from March till end of April), summer (from May till end of June), monsoon (from July-September) and autumn (from October to November). The climate of the Plateau ranges from semi-arid to sub-humid, sub- tropical continental. Pattern of rainfall is bimodal, with two maxima in late summer and winter-spring periods. The average annual rainfall ranges from 300 mm to 500 mm, and about 60-70 percent of it is received during monsoon rainy season from mid- June to mid-September (Shafiq *et al.*,2005).

Study design

Small Indian mongoose males were live trapped from the field on monthly basis from July 2012 to June 2013, using specially designed mesh traps that were set in the evening around 16:00 hours, using poultry intestines as “bait” and checked next morning at around 10:00 h. The traps were covered with grasses and leaves to minimize the neophobia in mongoose. Females that were captured in the traps were released back to the same habitat, and only male specimens were used for data collection. The trapped males were brought to the laboratory, euthanized by inhalation of chloroform, and sacrificed to collect blood and testicular tissue

samples. Blood was drawn directly by cardiac puncture into EDTA containing tubes, centrifuged at 3500 rpm (revolution per minute) for 15 minutes to obtain plasma which was stored at -20°C till final analysis for estimating hormones (Mahmood *et. al.*, 2011). Testes tissue samples obtained were weighed, and fixed in 10% formalin and subsequently processed for standard histological procedures. All animal capture and handling was carried out in accordance with the guidelines of the Ethics Committee of the Department of Wildlife Management, PMAS-AAUR 2007.

Hormonal estimation

Plasma hormonal concentrations were estimated by using commercially available ELISA kits each for testosterone, FSH and LH. The optical density in each case was read at 450 nm with a microtiter within 15 minutes of the final color change. The concentration was calculated for each set of reference standard solutions, control solutions and plasma samples for each hormone.

Histology

The testicular tissue samples of small Indian mongoose were fixed in 10% formalin solution (pH 7.2) for about 18-24 hours and processed through standard histological procedure (Mahmood *et al.*, 2011). Firstly, these were dehydrated in alcohol grades (50%, 70%, 80%, 90%, 100%) and embedded in paraffin wax that was pre-warmed to 59-61°C in a wax dispenser (TBS, Triangle Biomedical Sciences, Durham, NC USA). The embedded tissue blocks were cut into fine sections of 5-7 µm thickness by a rotary microtome (Shandon Finesse). Paraffin containing tissue sections were immediately transferred to water bath (Boekel scientific) at 37°C to stretch. Sections were taken on clean glass slides and left for 2-3 hours on a slide warmer at 37°C. Tissues were deparaffinized in xylene with two changes each for 5 minutes. These were hydrated in descending series of alcohol (100%, 90%, 80% 70% and 50%) for one minute and then stained with 1% Harris Hematoxylin stain for 5 minutes, washed in flowing tap water for 1-3 minutes and then in acid alcohol for a few seconds, washed again in tap water for one minute and then stained with 1% eosin for two minutes, then again dehydrated in ascending series of alcohol (50%, 70%, 80%, 90% and 100%) each for 5 minutes and finally mounted in DPX mounting medium (BDH) and observed under light microscope (Olympus BX50, Japan).

Micrometry and sperm counts

The histological sections of testes were observed under light microscope, and interpreted on the basis of diameter of seminiferous tubules both during breeding and non-breeding seasons, as well as their epithelial cell heights from initial, middle and terminal segments using stage and ocular micrometers. For measuring diameter of seminiferous tubules, five tubules per slide were selected and their diameter measured while epithelial cell height of three tubules per slide was measured.

Sperm concentration or sperm count was done using ocular grid technique. For sperm count, sperms in three cells per slide were counted. Sperm count in each seminiferous tubule of the histological sections of testes was made by using an ocular grid placed in the eye-piece of the microscope. It was fixed on observation of slide on the targeted seminiferous tubule. First, the total number of ocular grids filled with seminiferous tubules in each row and number of squares in each row (that contained the seminiferous tubule) were counted. Secondly, the total number of ocular grids filled with sperms in each row was counted.

The frequency of occurrence of sperms in each seminiferous tubule of the testicular section was calculated by following and Alipayo *et al*. (1992) by using the formula;

$$\text{Frequency of occurrence} = A/B \times 100$$

where, 'A', represents No. of rows (ocular grid) filled with complete seminiferous tubules; B, No. of rows (ocular grid) filled with sperms on the sample slide.

Statistical analysis

Results obtained were statistically analysed using students paired *t*-test to compare concentrations of testosterone, FSH and LH between breeding and non-breeding seasons, and

histological changes in the testicular sections viz., sperm count, and the diameter of seminiferous tubules.

RESULTS

Body measurements

Fifteen males were live trapped, the monthly capture rate was one individual per month, however, during November 2012 and April 2013, two males each were captured, their data were pooled and their hormonal concentrations were taken as mean of two samples during these months. Body measurements including gross weight, total body length and testes weight of small Indian mongoose males were recorded in the laboratory before being sacrificed (Table I). Body weight of individuals ranged between 800g and 1129g in most cases (n= 12) except for three individuals which were juveniles. Average body length of the specimens was between 53.34 cm to 76.2 cm (n=15).

Testes weight

Weight of the testis of small Indian mongoose males ranged from 3g to 7g (Table II), with maximum testes weight recorded during April and May 2013 (7 and 6 g, respectively). Average testicular weight during breeding season also showed no significant difference from that in non-breeding season (t = 0.237; p = 0.82) (Table III).

Hormonal concentrations

Testosterone concentrations were found elevated (Table II) during the months of January (16.2 ng/ml), February (16.1 ng/ml) and March 2013 (16.0 ng/ml), then started to decline in April (14.3 ng/ml) and May 2013 (9.64 ng/ml), however, the levels were found again elevated in August (14.4 ng/ml). The average testosterone levels during breeding season were slightly elevated (13.49±1.25 ng/ml) compared to (11.32±1.35 ng/ml) that during the non-breeding season. However, Student's paired *t-test* showed non- significant difference between the two values (t = 0.89; p = 0.41; df = 5) (Table III).

The FSH levels were also elevated during January (1.40 mIU/ml), February (1.41 mIU/ml) and March 2013 (1.42 mIU/ml). LH levels showed increase from February 2013 up to April 2013 (11.45 mIU/ml, 17.9 mIU/ml, and 24.6 mIU/ml respectively), then declined but again elevated during September (Table II). However, average FSH concentration during breeding season (1.23±0.06 mIU/ml) was not different from non-breeding season (1.11±0.12mIU/ml) with no significant difference between the two seasons (t = 0.848; p = 0.416; df = 5).

The concentrations of LH were found elevated during February, March, April and September with maximum LH levels recorded in April (24.6 mIU/ml). Average LH concentration was found elevated during breeding season (14.29±2.59 mIU/ml) as compared to non-breeding season (9.57±1.31 mIU/ml) but showed no significant difference (t = 1.619; p = 0.13; df = 5) between the two seasons (Table III).

Sperm counts

Maximum frequency of spermatozoa (732) inside the seminiferous tubules (SNT) of the testes was recorded during September 2012, followed by 686 during June 2013 and 671 during April 2013 (Table II, Fig.1). Average frequency of spermatozoa as well as the diameter of seminiferous tubules was also no different between breeding and non-breeding seasons (Table III, Fig. 1).

Diameter of Seminiferous tubules (SNT)

Diameter of seminiferous tubules inside testes started to increase from February 2013 (157 µm), reaching the maximum (203 µm) during April 2013. However, lowest diameter of SNT was recorded in October 2012 (124 µm) (Table II, Fig. 1B).

Breeding season

Based upon the testosterone concentrations obtained during the current one year study period, it is indicated that small Indian mongoose males, in the study area, breeds twice a year; from February to April and from July to September (6 months) while its non-breeding season spreads over May to June, and October to January (6 months).

Table I.- Body measurements of small Indian mongoose male trapped during the study period (July, 2012-June, 2013) from Potohar Plateau.

Sampling months	Sampling Site	Geographical coordinates	Body weight (g)	Body length(cm)
July-2012	AAUR Fields	N=33°39.010 E=073°04.907	805	68.326
Aug-12	AAUR Fields	N=33°39.010 E=073°04.907	123	57.15
Sep-12	Chakora	N=32°34.248 E=072°47.925	941	55.88
Oct-12	Mohal Dina	N=32°59.526 E=073°37.923	959	58.42
Nov-12	Chakri Road	N=33°33.728 E=073°01.085	859	55.88
Nov-12	Dhoke Fateh	N=33°45.264 E=072°21.641	850	53.34
Dec-12	Dhoke Fateh	N=33°45.264 E=072°21.641	987	59.69
Jan-13	Lallkurti	N=33°34.48.2 E=073°03.243	800	60.96
Feb-13	Lallkurti	N=33°34.48.2 E=073°03.243	893.5	57.15
Mar-13	Harley Street	N=33°34.33.5 E=073°02.41.8	479	60.96
Apr-13	Harley Street	N=33°34.33.5 E=073°02.41.8	1000	71.12
Apr-13	Harley Street	N=33°34.33.5 E=073°02.41.8	1005	76.20
May-13	Lalazar	N=33°34.48.2 E=073°03.24.3	975	71.12
June-13	Lalazar	N=33°34.48.2 E=073°03.24.3	166	38.608
June-12	Jubairpur	N=32°53.559 E=072°45.054	1129	76.20

Table II.- Testicular functions (concentrations of testosterone (ng/ml), FSH (mIU/ml), and LH (mIU/ml) and frequency of spermatozoa (per cm^2) and diameter of seminiferous tubules (µm) of the testes of small Indian mongoose male trapped from July 2012 to June 2013.

Trapping month	Testes weight (g)	Testosterone (ng/ml)	FSH (mIU/ml)	LH (mIU/ml)	Frequency of spermatozoa (per cm^2)	Diameter of seminiferous tubules (µm)
July-2012	4	12.2	1.21	9.75	176	150
Aug-2012	3	14.4	1.01	6.95	463	144
Sep-2012	4	7.95	1.14	15.1	732	141
Oct-2012	4	6.30	0.73	10.9	323	124
Nov-2012	4	13.2	0.91	10.7	401	149
Dec-2012	4	11.1	0.89	8.40	472	149
Jan-2013	4	16.2	1.40	5.00	409	165
Feb-2013	3	16.1	1.41	11.45	441	157
Mar-2013	3	16.0	1.42	17.9	357	171
April-2013	7	14.3	1.20	24.6	671	203
May-2013	6	9.64	1.39	7.94	490	191
June-2013	3	11.5	1.36	14.50	686	161

Table III.- A comparison of hormonal concentrations and testicular parameters of small Indian mongoose malesfrom the Potohar Plateau during breeding and non-breeding seasons.

Parameters	Breeding season	Non-breeding season	t-value	Significance level (p-value)
Testosterone (ng/ml)	13.49 ±1.25	11.32 ± 1.35	0.890	0.41
FSH(mIU/ml)	1.23 ± 0.06	1.11 ± 0.12	0.848	0.41
LH (mIU/ml)	14.29 ± 2.59	9.57 ± 1.31	1.619	0.13
Testes weight	4.33± 0.55	4.16 ± 0.40	0.237	0.82
Frequency of spermatozoa	473 ± 83.48	463.5 ± 50.6	0.086	0.93
Diameter of SNT	161±9.46	152.5 ±9.17	0.631	0.55

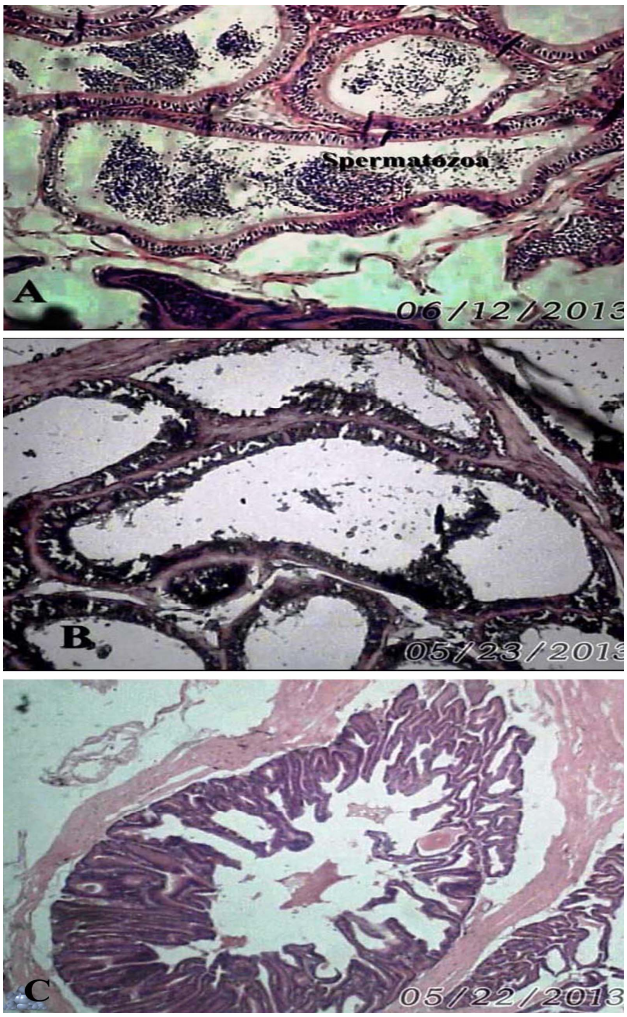

Fig. 1. Histological structure of the testes of small Indian mongoose showing; **A)** concentration of spermatozoa inside seminiferous tubules during breeding season, **B)** concentration of spermatozoa during non-breeding season, and **C)** increased seminiferous tubular diameter during breeding season. Magnification: 10x; Stain: haematoxylin & eosin.

DISCUSSION

Small Indian mongoose is an important component of biodiversity performing its role in the environment especially in agro- ecosystems. It serves as an important biological control agent for rodents and snakes. A few published studies about this species in Pakistan have been focused on its morphological characteristics, distribution and food habits (Siddiqui *et al.*, 2004; Mahmood *et al.*, 2011). However, data about its breeding pattern and the profile of its reproductive physiology under local conditions of the environment are scarce; the only literature reference about the breeding of this species from the sub-continent is that of Roberts (1997), quoted Powell (1913) who kept a tame female in the eastern part of the Punjab (unspecified location in pre-partition India) which paired with a wild male on 11 July and produced a litter of three after a gestation period of just six weeks. The current study aimed to investigate the breeding pattern of small Indian mongoose male in Potohar Plateau from July 2012 to June 2013.

The breeding pattern of different wildlife species may vary in different regions of the world under local conditions. Some studies on small Indian mongoose have been conducted in islands where this species was introduced and reported to be a seasonal breeder (Pearson and Baldwin, 1953; Pimental, 1955; Gorman, 2009; Nellis and Everard, 1983). A small number of females may breed in any month (Nellis and Everard, 1983), fertile males occur in all months, however, the onset of ovulation in females is seasonal and probably in response to increasing photoperiod (Gorman, 2009).

Body weight of the mongoose changes with the reproductive cycle. Male mongoose attains peak weight in February, before the onset of breeding, and then rapidly declines in weight. Males regain weight and body fat immediately after the mating period; they were significantly heavier in November than in August (Nie *et al.*, 1975). In the current study maximum body weight of the male animals was recorded in April (approximately 1000g), May (975g) and June (1129g). Field observation of the pups was made in the months of June and July and then again during September and October. Considering that the gestation period of female mongoose is about 49-51 days (in this study), the field observations of the pups indicate that the mating period of the species in the study region could be in March and April and then again during June and July, which is also the onset of monsoon season in the study region. According to Roberts (1997), who quoting Powel (1913) reported

gestation period of six weeks (42 days) in this species, also showed that breeding of small Indian mongoose is not confined to any particular season, however, most records of breeding in the wild seem to be around the end of summer, coinciding with the monsoon season when insect life is abundant and amphibians and reptiles are also available.

Breeding of mongoose has been reported to be seasonal, falling mainly during the period of increasing photoperiodicity and the longest days (Gorman, 2009). Mongoose in Hawaii (Pearson and Baldwin, 1953), Puerto Rico (Pimental, 1955), and St. Croix (Nellis and Everard, 1983) had similar breeding seasons. Monthly trends of reproductive activity occurring in small Indian mongoose already reported from some other parts of the world show that males exhibit a bimodal pattern with peaks in testicular size occurring in spring and early fall. All adult males are reproductively active throughout summer. Adult males do not exhibit regressed testes in summer, which follows the active summer pattern in the north (Pearson, 1944). According to Gorman (2009) the breeding season of large Indian mongoose in Fijian Islands is quite different occurring from August to February and non-breeding season from March to July. Soares and Hoffmann (1982) have reported that testes weights followed a seasonal cycle, as the testes weights were significantly higher during breeding season compared to the inactive season. In Pakistan spring season starts in March, however, the testicular weights were found elevated during April (7g) and May (6g) whereas testes weight during the fall (September and October) remained only up to 4g.

Body weight measurements did not change significantly ($p>0.05$) between breeding and non-breeding seasons in the study area. These results are somewhat similar to some earlier published records (Soares and Hoffmann, 1982; Gorman, 2009) that body weights of small Indian mongoose did not change significantly as a function of season. In addition, the body length showed non-significant ($p=0.144$) difference between active and inactive seasons. Similar findings were reported by Gorman (2009) that the small Indian mongoose body length did not vary seasonally. However, testes weights of the small Indian mongoose captured from Potohar Plateau showed individual variations among male mongooses in different months of breeding and non-breeding seasons, but still those differences were statistically non significant ($p = 0.82$).

Results of the hormonal estimations show that testosterone, FSH and LH concentrations in plasma samples were variable among individuals and during different months of the study period, and although the mean concentration of testosterone, FSH and LH were found higher during the breeding season compared to the non-breeding season but in neither case were the differences statistically significant ($p >0.05$). Soares and Hoffmann (1982) showed that serum Androgen, FSH and LH of the small Indian mongoose in Hawaii were significantly higher during the active season (breeding) compared to the inactive non-breeding season.

The changes within the seminiferous tubules including tubular diameter, and frequency of occurrence of spermatozoa inside the seminiferous tubules, inside the testes of small Indian mongoose during the current study have also shown variation among the individuals during different study months. The frequency of occurrence of spermatozoa in the seminiferous tubules was found slightly higher in active season (473) compared to the inactive season (463), however, the results were non-significantly different. Similarly, average diameter of seminiferous tubules during breeding and non-breeding season (161±9.46 μm) was non-significant. In contrast Gorman (2009) had reported that seminiferous tubules diameter, epithelial cell height and frequency occurrence of spermatozoa in the tubules was significantly high ($P>0.001$) during the active season.

CONCLUSION

The study concludes that the small Indian mongoose male in the Potohar Plateau breeds twice a year and its breeding season continues from February to April and from July to September (6 months). The concentrations of LH, FSH and testosterone, in general increase during March and April indicating the breeding season. The sperm count and diameter of seminiferous tubules and epithelial cell heights are non-significantly different in the active and inactive seasons.

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