REPRODUCTIVE PHYSIOLOGY OF THE STALLION

IV. SEASONAL CHANGES IN THE TESTOSTERONE CONCENTRATION OF PERIPHERAL PLASMA

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The normal equine breeding season begins in the spring and extends through mid-summer. It is characterized by marked increases in sperm output and seminal volume, as well as behavioural changes manifested in decreased reaction time and the number of mounts per ejaculation (Pickett & Voss, 1972). The production of androgenic steroids by the equine testis has been investigated both *in vivo* (Lindner, 1961) and *in vitro* (Savard & Goldzieher, 1960; Bedrak & Samuels, 1969; Oh & Tamaoki, 1970) but the testosterone level in peripheral plasma has not been reported (Lindner, 1961) and seasonal influences upon testicular function have received little attention. The object of this study was to establish the influence of season on the concentration of testosterone in the peripheral plasma of the stallion.

Two ejaculates were collected from five mature Quarter Horse stallions each Tuesday. Blood was withdrawn from the jugular vein of each animal at approximately 10.00 hours on the Wednesday closest to the middle of the month and on the Wednesdays immediately preceding and following for a 13-month period from May 1969 to May 1970. Thus each stallion was bled three times per month for 13 consecutive months.

Each sample of blood (30 to 40 ml) was collected into a 50-ml heparinized plastic centrifuge tube and immediately centrifuged. Then 5 to 6 ml of plasma was removed, placed in 9-ml screw-cap vials and stored at -30°C . Testosterone was determined by radioimmunoassay as described by Ismail, Niswender & Midgely (1972) but modified as follows: an antibody to testosterone- 11β -bovine serum albumin with a greater specificity than that of Ismail *et al.* (1972) was used for radioimmunoassay in conjunction with a testosterone- 11α -tyrosine methyl ester as the radioiodinated antigen. Plasma (1 ml) was extracted twice with 5 ml benzene (1 : 2). The dried extract was dissolved in 1 ml phosphate-buffered saline (pH 7.0) containing 0.1% gelatin and subsequently assayed at two dose levels. Recovery was monitored in each sample by addition of 10,000 d/min (48 pg) of $[1,2-^3\text{H}]$ testosterone before extraction. Testosterone levels obtained before and after thin-layer chromatography (Payne & Mason, 1968) of extracts of horse plasma samples were not different, indicating that only testosterone was quantified. The intra-assay coefficient of variation of

duplicate samples was 5.4%. When the same samples were assayed in seven different radioimmunoassays, the coefficient of variation was 11.4%. Exogenous testosterone in amounts ranging from 0.2 to 20 ng could be quantitatively recovered from the serum of males and cycling and pregnant females.

The significance of treatment effects was determined by analysis of variance, and differences among specific treatment means were further evaluated by the Newman-Keuls sequential range test. Changes in plasma testosterone with time were examined by orthogonal contrasts to test for significant first and second order responses (Steel & Torrie, 1960).

The monthly means for the plasma testosterone concentrations of the five stallions are presented in Text-fig. 1. The highest mean level of testosterone

TEXT-FIG. 1. Concentration (mean $\pm$ S.E.) of testosterone in the peripheral plasma of mature stallions over a 13-month period.

was 3.2 ng/ml in May 1969. Plasma testosterone levels fell below 1.5 ng/ml during October, December and January, and were significantly lower ($P < 0.05$) than in May 1969 but did not differ significantly from levels during the remaining months. Testosterone levels in November were as high as those obtained in the middle of the mating season. The lack of statistically significant differences in levels of testosterone between most months of the breeding season and those of the non-breeding season, as well as the apparent increase in testosterone levels during November, may reflect inadequate sampling frequency. Pulsatile releases, resulting in approximately tenfold changes in the plasma testosterone levels of individual subjects within a few hours, were recently described in bulls (Katongole, Naftolin & Short, 1971) and rats (Bartke, Steele, Musto & Caldwell, 1973). These fluctuations in the secretory rate of testosterone were unrelated to time of day. A four and a half times difference in the testosterone concentration of two plasma samples was obtained from one stallion during a single month, and similar two- to threefold differences were not uncommon among the other stallions. Thus, pulsatile secretion of testosterone may also occur in stallions.

Despite the wide fluctuations within and among stallions, testosterone levels changed in a significant ($P < 0.01$) quadratic fashion over the 13-month period, being significantly greater during the breeding season than the non-breeding season. This seasonal pattern of testosterone secretion may reflect alterations in the release of pituitary gonadotrophins, the responsiveness of the testis to gonadotrophin stimulation, or a combination of these.

Significant ($P < 0.01$) differences among stallions and stallion $\times$ month interactions were observed. The mean plasma testosterone level averaged across months was 1.9 ng/ml, and ranged from 1.68 to 2.08 ng/ml for individual stallions. Differences among stallions and stallion $\times$ month interactions may also reflect pulsatile release of testosterone since the stallion with the lowest plasma testosterone level also had the highest level for an individual plasma sample (5.65 ng/ml). While stallion $\times$ month interactions were highly significant ($P < 0.01$), all stallions exhibited a seasonal pattern of testosterone secretion.

The greatest sperm output in the first and second ejaculates from these stallions occurred during July (Pickett & Voss, 1972), 2 months after the seasonal peak in plasma testosterone levels, and was approximately twice as great as sperm output observed during the winter months. The combined duration of spermatogenesis and extragonadal transit in the stallion is approximately 60 days (Gebauer, Pickett & Swierstra, 1974a, b; Swierstra, Gebauer & Pickett, 1974). Since testosterone stimulates mammalian spermatogenesis (Steinberger, 1971), it is reasonable to assume that elevated testosterone levels during May and June were at least partly responsible for the high sperm output observed in July.

An interval of approximately 60 days is inherent between the imposition of stimuli affecting spermatogenesis and the appearance of maximal response measured as sperm output. Sexual behaviour and accessory sex gland activity should respond more quickly to alterations in the endocrine system. As anticipated, the time required for each stallion to respond to sexual stimulation (reaction time) declined at least elevenfold, and the number of mounts required per ejaculation decreased more than 50% during May 1969 and remained low throughout the breeding season as compared to the winter months (Pickett & Voss, 1972). The accessory sex glands also exhibited seasonal variation in secretory activity, as indicated by the approximately twofold greater volume of first ejaculates during May, June and July of 1969 compared to the months of the non-breeding season.

Secretion of testosterone in the stallion has been shown to be clearly influenced by season. Taken with the seminal and behavioural characteristics (Pickett & Voss, 1972), this finding is consistent with the hypothesis that seasonal variations in libido and the secretory and gametogenic activity of the stallion reproductive tract are mediated, at least in part, by the pattern of testosterone secretion.

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