EFFECT OF PREGNANT MARE'S SERUM GONADOTROPIN (PMSG) ON TESTICULAR FUNCTION IN THE IMMATURE BUFFALO BULL (Bubalus bubalis)

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Summary

Responsiveness of the testis to pregnant mare's serum gonadotropin (PMSG) was studied in immature Nili-Ravi buffalo bulls. Four month old bull calves weighing between 66 to 100 kg raised under uniform condition, were treated with 1000 IU PMSG or vehicle, daily, for six days. PMSG induced an increase in the size of the testis, enlargement of the seminiferous tubules and activation of the spermatogonia. The number of differentiated Leydig cells in the testis of gonadotropin treated animals increased considerably over that of the control testes. A significant increase in plasma testosterone concentrations was observed 24 h following the first injection of PMSG and the levels continued to increase until day 6. In vehicle treated animals plasma testosterone levels remained more or less at pretreatment values. The data suggest that buffalo bull testis is functionally responsive to gonadotropin at an early stage of prepubertal development.
(Key Words: PMSG, Testis, Spermatogenesis, Testosterone, Buffalo)

Introduction

Sufficient information has accumulated in recent years about the hormonal regulation of gonadal function and endocrine changes associated with the onset of puberty in the male cattle (Amann, 1983).

One of the approaches used to assess the role of endocrine stimuli in the maturation of the pituitary-testis axis has been by studying the effects of exogenous administration of GnRH or gonadotropins during different phases of sexual development (Golter et al., 1973; Sundby and Farhat, 1978; Sundby and Torjensen, 1978). An increase in LH secretion in response to GnRH administration has been reported in bull calves as early as 2 months of age (Sundby and Velle, 1980). However, testicular androgenesis appears to remain refractory to treatment with GnRH or LH until the age of six months (Mongkonpunya et al., 1975; Chantaraprateep and Thibier, 1979; Lacroix and Pelletier, 1979a; McCarthy et al., 1979).

The age related changes in the peripheral LH and testosterone levels in the male buffalo have been the subject of some reports in the past years (Dwaraknath et al., 1981; Agarwal et al., 1983; Sharma et al., 1984; Hemeida et al., 1985). Findings of a recent investigation have suggested that the testicular quiescence in the Nili-Ravi buffalo bull extends from birth to 6-7 m of age (Ahmad et al., 1989). Nonetheless, our understanding of the endocrine regulation of puberty in this animal remains highly inadequate. The present investigation was carried out to evaluate the responsiveness of the immature buffalo bull testis to chronic stimulation with pregnant mare's serum gonadotropin (PMSG).

Materials and Methods

Six Nili-Ravi buffalo bull calves ranging in age from 13-17 weeks were used in this study. The animals were raised from birth at the animal farms of the National Agricultural Research Centre, Islamabad. The calves were housed collectively and were subjected to identical feeding and managemental regimes. Green fodder and tap water were available to animals ad libitum. The experiments were conducted in the month of December.

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The body weight and the testicular size in animals used in the present study, were comparable to those previously described for this age in the Nili Ravi buffalo bull (Ahmad et al., 1984) and for some other buffalo breeds (Bongso et al., 1984; Sharma et al., 1984). Body weight and scrotal circumference were recorded prior to the initiation of the experiment and at the end of the experiment. The scrotal circumference was measured with a flexible measuring tape around the greatest diameter of the scrotum. Before measurement, the testes were firmly pushed into the scrotum.

Three of the six animals selected randomly, were treated with 1000 IU (im) PMSG (Antex Leo, Ballerup, Denmark) daily, for 6 days. The remaining three animals which served as the controls were treated with vehicle (1 ml normal saline). The injections were given between 09:00 and 10:00 h. Daily blood samples were obtained from the jugular vein in a heparinized syringe, immediately before the injection. The blood was centrifuged at 3000 rpm for 10 minutes and plasma obtained was stored at −15°C until it was used for hormone determination.

On the day following the last injection, the animals were anaesthetized with Rompun (2-[2, 6-xylidino]-5, 6-dihydro-4H-1, 3-thiazine-hydrochloride; Bayer, Leverkusen, FRG; 23 mg/100 kg BW) and the testis of the right side was removed, weighed and a portion of the testicular tissue fixed in Bouin's fluid for histological examination. Serial paraffin sections were cut at 6 μm and stained with Harris's haematoxylin. Cytometric observations were made with an ocular micrometer. Values for seminiferous tubules diameter were obtained by averaging 20 measurements of tubules for each animal.

Plasma testosterone levels were determined by radioimmunoassay (RIA) as described previously (Nieschlag and Loriaux, 1972) and validated for buffalo plasma (Ahmad et al., 1989). Aliquots of 500 μl plasma were extracted with 5 ml diethyl ether. Recovery of labelled and unlabelled testosterone added to plasma was > 90%. All determinations were made in duplicate. The sensitivity of testosterone RIA in terms of lowest dose of the linearized standard curve was 16 pg/tube. The intra and interassay coefficients of variation were 4.5% and 11%, respectively. The results of RIA were calculated according to the procedure described by Rodbard and Lewald (1970). The treatment effects were analyzed using Student's t test (Steel and Torrie, 1960).

Results

Body Weight and Testicular Size

The mean body weight and scrotal circumference of PMSG treated calves did not differ significantly from animals treated with vehicle, at the end of treatment period (table 1). Furthermore, an increase in mean testicular weight observed in gonadotropin treated animals over that of control (6.6 ± 1.4 vs 4.6 ± 0.8 g) was shown to be statistically insignificant ($p > 0.05$).

Testicular Morphology

The mean values for seminiferous tubule diameter in control and PMSG treated calves (38.81 ± 2.62 and 53.17 ± 1.48 μm, respectively) were significantly different from each other ($p < 0.05$). The histological appearance of control testes was fairly uniform in all the three animals (figure 1).

TABLE 1. SCROTAL CIRCUMFERENCE, TESTICULAR WEIGHT AND SEMINIFFROUS TUBULE DIAMETER IN PREPUBERTAL BUFFALO CALVES TREATED WITH PMSG

Treatment	Age (weeks)	Body weight (kg)	Scrotal circumference (cm)	Testicular weight (g)	Seminiferous tubule diameter (μm)
Vehicle[a]	16.3 ± 0.3	82.7 ± 5.8	11.0 ± 0.3	4.6 ± 0.8	38.8 ± 2.6
PMSG[b]	15.3 ± 0.8	84.0 ± 13.6	11.0 ± 0.8	6.6 ± 1.4	53.1 ± 1.4[c]

Values are Mean ± SEM (n = 3)

[a]Normal saline (1.0 ml) administered (im) daily for 6 days

[b]1000 IU in 1.0 ml saline administered (im) daily for 6 days

[c]Significantly different from the vehicle treated group ($p < 0.05$; Studnet's t test)

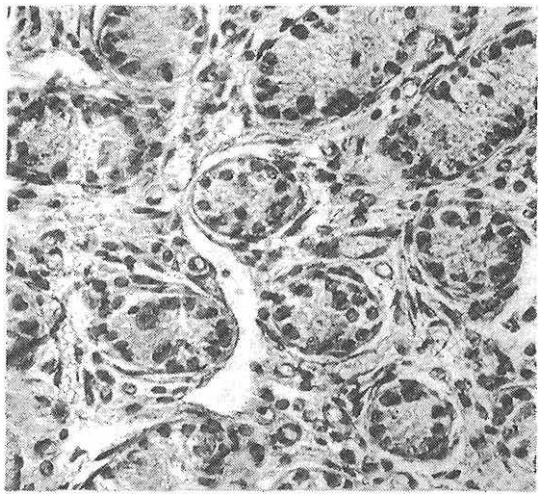

Figure 1. Section of testis from saline treated buffalo calf showing early spermatogonia and pre-Sertoli cells near the basement membrane. (× 400)

The tubules were solid cords filled with granular cytoplasm. The seminiferous epithelium contained early spermatogonia and pre-Sertoli cells situated close to the basement membrane. The intertubular compartment was mainly composed of undifferentiated mesenchymal cells and fine stromal tissue. However, a few differentiated Leydig cells could also be recognized. Gonadotropin treatment induced not only an increase in the size of the seminiferous tubules but also a hyperplasia and hypertrophy of the tubular tissue (figure 2). In the treated testes, the number of spermatogonia had increased in number and some of these cells were seen to have migrated towards the centre of the tubule. These cells generally possessed a larger and more vesicular nucleus compared to those of germ cells which were situated near the basement membrane. A few of the larger cells could be identified as differentiating spermatogonia or early spermatocytes. Furthermore, there was a marked increase in the amount of the intratubular cytoplasm. In the PMSG stimulated testis, the interstitial cells were differentiated into Leydig cells and were characterized by spherical nuclei and eosinophilic cytoplasm.

Plasma Testosterone

Testosterone profile of buffalo bull calves before and after treatment with 1000 IU PMSG or saline daily for a period of 6 days is shown in figure 3. Plasma testosterone levels were determined at days −1, 0, 1, 2, 4, 6 and 7 relative to the initiation of treatment at day 0. Testosterone

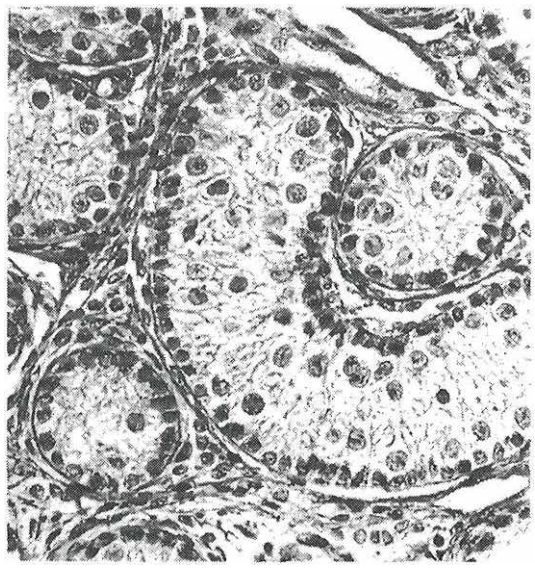

Figure 2. Section of testis from PMSG treated buffalo calf showing hypertrophy of seminiferous tubule accompanied by an increase in the number of pre-Sertoli cells. (× 400)

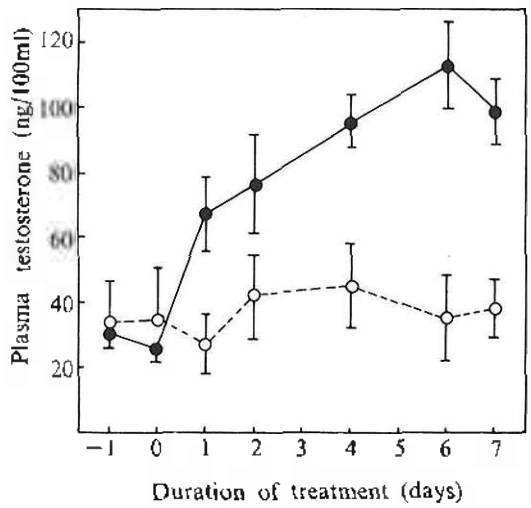

Figure 3. Plasma testosterone profiles in buffalo calves (n = 3) treated with 1000 IU of PMSG (solid line) or vehicle (broken line).

values increased approximately two-fold on day 1 and the levels continued to increase until day 6. At the end of the experiment, the mean plasma testosterone concentrations in the PMSG treated calves were significantly higher than that of control animals and the pretreatment values ($p < 0.05$).

Discussion

The present data indicate that PMSG administered to 4-month old buffalo calves for a period of 6 days induced a stimulation of the testis as reflected by activation of the seminiferous epithelium and Leydig cell function. The mean values for scrotal circumference were comparable in the two groups prior to treatment and increased only slightly during the experimental period. However, a detectable increase in mean testicular weight observed in gonadotropin treated calves over that of control animals at the end of the treatment could be ascribed mainly to the enlargement of the seminiferous tubules under PMSG stimulation.

The histological appearance of the testicular tissue from untreated calves was similar to that described previously in the young buffalo (Goyal and Dhingra, 1973; Dhingra and Goyal, 1975; Deshpande and Janakiraman, 1985) and cattle (Amann, 1983). In control animals, the seminiferous cords contained undifferentiated small cells situated along the basement membrane. The large cells characteristic of the infantile bull testis were virtually absent although an occasional large cell (gonocyte) was present in the centre of the tubule. Goyal and Dhingra (1973) have observed that these large cells persist in 15-16 week old buffalo calves and constitute 17% of the total cell population at this stage of testicular development. PMSG stimulation in the present study resulted in a significant increase in the mean diameter of the seminiferous tubules and activation of the spermatogenic cells although there was no evidence of lumen formation. Interestingly, the size and histology of the gonadotropin stimulated tubules paralleled closely the normal tubular morphology described for older (50-52 weeks of age) buffalo calves (Goyal and Dhingra, 1973).

The gonadotropin induced increase in the amount of the interstitial tissue and a differentiation of the precursor cells into Leydig cells observed in this study, has also been reported for other mammalian species (Chemes et al., 1976; Arslan et al., 1981). In the present study, a significant increase in testosterone concentrations occurred 24 h after the first injection of PMSG. It has previously been shown in this laboratory that plasma testosterone does not show any significant change during the first 8 h following acute PMSG treatment to 3-4 month old calves (Arslan et al., unpublished data), the present data, therefore, suggest that the initial steroidogenic response of the testis to gonadotropin unlike the adult buffalo bull (Arslan et al., 1985), involves a lag period of several hours. These observations in the immature buffalo bull are apparently at variance with the reports that in young cattle the testis does not respond to LH stimulation until 6 months of age (Mongkonpunya et al., 1975; Lacroix and Pelletier, 1979b) although a release of LH in response to GnRH at this stage of prepubertal development has been shown (Haynes et al., 1977). In our study, testosterone response to PMSG stimulation indicates that gonadotropin responsive steroidogenic tissue is present in buffalo calf testis even at 3-4 months of age.

The increase in testosterone levels observed in chronically treated calves could be due to a functional differentiation of the interstitial cells and partly to an increase in the number of Leydig cells as evidenced by testicular histology.

On the basis of this investigation, it is concluded that PMSG brings about a stimulation of the spermatogenic and steroidogenic function of the immature bull testis. Furthermore, it is suggested that the buffalo bull testis becomes functionally responsive to gonadotropin at a period as early as 4 months during the postnatal life.

Acknowledgements

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