Effect of Induced Hypothyroidism on the Fertility of Male Goats

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ABSTRACT : To study the effect of induced hypothyroidism on fertility status of male Black Bengal goats, 10 adult healthy mature males were divided into control and treated groups.

Hypothyroidism was induced successfully by injecting thiourea subcutaneously initially for 15 days at the rate of 100 mg/kg body weight, followed by 66.7 mg/kg body weight for the subse quent 15 days. This resulted in a decrease ($p < 0.01$) in plasma tri-iodothyronine, thyroxine and testosterone levels, with increasing duration of thiourea treatment. It also adversely affected semen quality, ejaculate volume, sperm concentration, motility and viability. Live percentage declined to 75% for treated and control remained at 90%.

Artificial insemination of female goats with semen of thiourea-treated goats, resulted in failure of conception. However, females inseminated with semen of male goats of control group showed 100 % conception. These observations indicate that, thyroid hormones play a key role in maintaining the normal reproductive procecess of male goats.

(Key Words: Hypothyroidism, Testosterone, Fertility, Male Goats)

INTRODUCTION

Information on the role of thyroid dysfunction in reproductive physiology of either sex among farm animals, in general, and goats in particular is meagre. Hypothyroidism has been reported to be associated with some cases of subfertility (Reddi, 1982). A subclinical hypothyroid state can cause subtle degenerative changes in the reproductive organs in livestock (Reddi and Rajan, 1986), leading to subfertility and even infertility causing serious losses to both production and reproduction. It has been established in laboratory rodents (Aruldhas et al., 1982; Pereira et al., 1983; Valle et al., 1985, Jannini et al., 1994), quails (Peczely et al., 1979), men (Buitrago and Diez, 1987) and rams (Chandrasekhar et al., 1985a, b; 1986) that hypothyroidism leads to decreased androgenesis and testosterone levels. Goats are more susceptible to hypothyroidism and there has not been any in depth studies reported describing about the changes in the characteristics of semen in hypothyroidism in goats. Since the thyroid appears in most species to have the function of modulating gonadal performance and also it's relationship with fertility, it was considered important to study the effect of thiourea - induced hypothroidism (Gupta et al., 1991) on the reproductive performance in male goats.

MATERIALS AND METHODS

Ten adult healthy male Black Bengal goats proven to fertile were maintained under normal husbandry conditions. Animals were housed in a well ventilated shed and were fed on a standard ration as recommended by Ranjhan (1980). The animals were divided equally into treated and control groups.

Induction of hypothyroid state

Thiourea (BDH/Glaxo, Bombay) was used as the antithyroid compound. A stock solution of thiourea was prepared by dissolving 1,000 mg/11 ml of sterile distilled water, and was stored at 4℃. The daily dose was 100 mg/kg BW for the initial 15 days and 66.7 mg/kg for the subsequent 15 days. The dose was administered by subcutaneous injection of three equal sub-doses per day (Gupta et al., 1991) for 30 consecutive days. Controls were injected with sterile distilled water.

Collection of blood samples and hormone analysis

Blood samples were collected by jugular vein

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puncture once daily, on three cosecutive days, prior to the start of thiourea treatment, every 4 days from day 4 to day 16, and then every 2 days until the last day (day 30) of treatment.

Plasma was separated and stored at −20℃(± 5℃) until the hormone analysis. Plasma testosterone was analysed by radio-immunoassay (Hall and Sufi. 1981). Antisera to testosterone was supplied by Dr. G. D. Niswender, U. S. A. The lebelled testosterone tracer was purchased from Amersham, International PLC, Amersham, U. K. Cross - reactivity of testosterone antiserum was 100% for testosterone, 50% for dihydrotestosterone and 1% or less for other related steroids. The recovery of testosterone at two different concentrations i.e. at 50 pg and at 125 pg in 0.5 ml of steroid free plasma, was 96 and 98% respectively. Intra and inter assay coefficients of variations were 9.4% and 15.3% respectively. The minimum detectable level was 5 pg.

Tri iodo-thyronine (T3) and thyroxine (T4) were estimated with specific kits purchased from Bhabha Atomic Research Centre, Bombay, India. All samples were assayed in a single assay to avoid inter - assay variation. The intra - assay variation. The intra - assay variation was 10%. Cross-reactivity of anti -T3 with T4 10 was 0.01%, and that of anti -T4 with T3 was 5%. The minimum detectable levels of T3 and T4 were 10 pg and 50 pg, respectively.

Collection and evaluation of semen samples

Semen samples were collected by using artificial vagina for sheep and goat. Semen characteristics were recorded 3 days before the treatment and on days 8, 16, 20, 26 and 30 during the treatment, and then on days 2, 4 and 6 after the treatment. Ejaculate volume was noted directly from the graduated collection vial. Motility of sperms was assessedy examining a small drop of semen diluted with Na citrate dehydrate immediately after collection under a microscope at 37℃. Sperm concentration was measured using heamocytometer. Perecentage of live sperms was estimated by differential staining method using Nigrosin-Eosin stain as described by Bloom (1950). All the animals were observed daily for clinical symptoms and for libido at the time of semen collection. Hypothyroidism was confirmed in the treated group by estimating the plasma T3 and T4 levels.

Fertility of individual goats of both control and treated males was evaluated by inseminatiog 10 untreated normal cycling female goats of proven fertility. The females were inseminated at 16 h after the onset of estrus and twice more 10 h apart. Pregnancy in inseminated females was confirmed by the absence of estrus for three consecutive cycles and also by plasma progesterone profile follwing insemination. Semen used for insemination was evaluated before inseminating the females.

Management of the females was same as the for males. Throught the experiment, males were separated from females.

Statistical Analysis

Paired 't' test was used for comparisions between the days within the same group. Analysis of Varience (ANOVA) was used for comparisions of more than two treatments.

RESULTS

Induction of hypothyroidism in thiourea treated goats was confirmed by monitoring plasma T3 and T4 levels. During the entire experimental period, (from 4 th to 30 th day) profile of both T3 and T4 (table 1) in treated animals was lower ($p < 0.01$) than pretreatment value and corresponding levels control group. The peripheral plasma testosterone levels in thiourea-treated animals lower ($p < 0.01$) than corresponding levels in controls. Plasma testosterone levels during the first 22 days of the treatment fluctuated between 0.28 and 0.93 ng/ml (i.e. 13.45% of the pre treatment level). During the last 6 days of treatment, testosterone fluctuated at lower levels (0.10-0.20 ng/ml, less than 9% of the pretreatment values). Further, withdrawal of thiourea treatment was followed by a rise in the T3, T4 and testosterone levels within 2-6 days.

Sperm concentration was 2,819 and 2,736 (millions/ml) on 20 th and 26 th day of the treatment respectively, as compared to 3,695 and 3,648 (millions/ml) in the controls showing a significant ($p < 0.01$) reduction. Percentage of motile sperm in thiourea-treated animals declined ($p < 0.01$) continuously (table 2) during treatment from pretreatment values (90.80).

Percentage live spermatozoa (75%) was less ($p < 0.01$) during the treatment than before. In the control group viability was 90.80 and did not differ significantly between collections. Insemination using semen from control males (five male goats) resulted in a 100% conception. Whereas, none of the does inseminated with semen from (five) thiourea-treated males conceived.

DISCUSSION

Table 1. Plasma triiodothyronine (T3), Thyroxine (T4) and testosterone levels in control and thiourea - treated male goats

Days	T3 (ng/ml)		T4 (ng/ml)		Testosterone (ng/ml)	
	Control	treated	Control	treated	Control	treated
Pretreatment period						
−3	1.45 ± 0.2[a]	1.22 ± 0.1[a]	24.29 ± 1.6	26.07 ± 1.3	1.53 ± 0.2	2.05 ± 0.04
−2	1.34 ± 0.1[a]	1.22 ± 0.1[a]	25.31 ± 1.0	22.85 ± 0.6	1.75 ± 0.2	1.82 ± 0.14
−1	1.23 ± 0.2[a]	1.10 ± 0.0[a]	24.80 ± 1.8	25.72 ± 2.0	1.49 ± 0.1	1.94 ± 0.05
During the treatment						
4	1.19 ± 0.3[a]	0.67[a]**	24.93 ± 1.5[a]	20.40 ± 2.0[b]**	1.55 ± 0.1	0.93 ± 0.02**
8	1.43 ± 0.3[a]	0.04[b]**	26.01 ± 1.8[a]	4.88 ± 0.5[b]**	1.67 ± 0.9	0.85 ± 0.04**
12	1.32 ± 0.0[a]	0.00[b]**	25.54 ± 2.5[a]	1.94 ± 0.5[b]**	1.63 ± 0.2	0.76 ± 0.02**
16	1.36 ± 0.1[a]	0.00[b]**	23.71 ± 2.3[a]	1.09 ± 0.2[b]**	1.48 ± 0.1	0.74 ± 0.02**
18	1.44 ± 0.1[a]	0.00[b]**	26.15 ± 1.6[a]	1.99 ± 0.2[b]**	1.26 ± 0.1	0.60 ± 0.02**
20	1.40 ± 0.2[a]	0.01[b]**	26.11 ± 2.3[a]	1.24 ± 0.2[b]**	1.39 ± 0.1	0.36 ± 0.03**
22	1.34 ± 0.1[a]	0.00[b]**	27.31 ± 3.2[a]*	0.47 ± 0.1[b]**	1.43 ± 0.2	0.28 ± 0.03**
24	0.93 ± 0.0[a]	0.00[b]**	24.52 ± 0.7[a]	0.50 ± 0.2[b]**	1.11 ± 0.0	0.20 ± 0.00**
26	1.08 ± 0.0[a]	0.04[b]**	26.98 ± 1.9[a]*	1.75 ± 0.5[b]**	1.41 ± 0.2	0.11 ± 0.01**
28	1.22 ± 0.0[a]	0.04[b]**	25.47 ± 2.1[a]	1.80 ± 0.3[b]**	1.61 ± 0.0	0.11 ± 0.01**
30	1.32 ± 0.1[a]	0.04[b]**	27.14 ± 0.0[a]	2.07 ± 0.3[b]**	1.17 ± 0.1	0.10 ± 0.02**
Post treatment						
2	1.19 ± 0.1[a]	0.91 ± 0.0[a]**	24.69 ± 0.8[a]	16.58 ± 2.9[a]	1.31 ± 0.0[a]	0.32 ± 0.01[b]**
4	1.74 ± 0.1[a]	1.18 ± 0.2[b]	26.09 ± 2.0[a]	18.32 ± 2.0[b]**	1.37 ± 0.0[a]	0.33 ± 0.02**
6	1.05 ± 0.1[a]	1.02 ± 0.1[a]	26.45 ± 2.4[a]	17.23 ± 0.6[b]	1.27 ± 0.1[a]	0.40 ± 0.02[b]**

Analysis of varience of data for between the treatments. Mean with different superscripts (a & b) in a column differ significantly from controls at ($p < 0.05$). Mean in a row with asterik differ significantly from their corresponding pretreatment levels at * ($p < 0.05$) ** ($p < 0.01$).

Table 2. Seminal characteristics of control and thiourea - treated male goats

Days	Sperm Concentration (millions/ml)		Motility (%)		Viability (%)	
	Control	Treated	Control	Treated	Control	Created
−3	3,516.00 ± 42.32[a]	3,742.20 ± 51.90[a]	90.80 ± 1.84[a]	90.40 ± 1.82[a]	90.80 ± 1.84	91.00 ± 1.44[a]
8	3,518.80 ± 112.44[a]	3,151.20 ± 48.15[b]*	90.80 ± 1.80[a]	86.00 ± 1.65[b]*	89.60 ± 1.79[a]	86.60 ± 0.92[b]*
16	3,673.00 ± 59.75[a]	3,038.60 ± 13.92[b]*	89.60 ± 1.79[a]	77.00 ± 1.04[b]*	90.40 ± 1.82[a]	77.00 ± 1.04[b]*
20	3,695.60 ± 79.06[a]	2,819.00 ± 29.25[b]*	90.40 ± 1.83[a]	72.80 ± 0.86[b]**	91.00 ± 1.85[a]	75.40 ± 1.07[b]**
26	3,648.60 ± 53.39[a]	2,736.40 ± 10.83[b]**	90.60 ± 1.79[a]	69.40 ± 1.20[b]**	90.60 ± 1.83[a]	73.00 ± 1.70[b]**
30	3,672.00 ± 58.96[a]	2,692.40 ± 32.77[b]**	91.00 ± 1.78[a]	67.00 ± 1.04[b]**	91.60 ± 1.87[a]	72.80 ± 1.39[b]**

Mean in a column with different superscripts (a & b) differ significantly from their controls ($p < 0.05$).
Mean in a row with asterik differ significantly from their pretreatment levels. * ($p < 0.05$) ** ($p < 0.01$).

The significantly lower levels of T3 and T4 in thiourea-treated males, compared to controls, confirmed the successful induction of hypothyroidism in mals goats in confirmity with that reported by Gupta et al. (1991). Decreased peripheral testosterone levels in the present study during induced hypothyroidism is in agreement with previous reports obtained in rams (Chandrasekhar et al., 1985 a, b; 1986) and goats (Gupta et al., 1991), although

Kalland et al. (1978) found no effects of hypothyroidism on the plasma testos terone levels in the rats. Chandrasekhar et al. (1985b, 1986) suggested that decreased testosterone levels in hypothyroid rams were due to either depressed pituitary response to luteinizing hormone releasing hormone (production of LH) or decreased testicular response to gonadotropin.

Decreased testosterone levels in goats during hypothyroid period may be explained similarly. Another possible reason may be a decrease in the number of testicular receptors for gonadotropins, as reported in hypothyroid rats by Valle et al. (1985). This indicates thyroid hormones can act at both pituitary gland and testes to influence reproductive function in animals. Quick recovery of thyroid and testosterone hormones (Reddi and Rajan, 1986) after discontinuation of treatment may possibly be due to rapid metabolism and excretion of thiourea allowing thyroid function to return to normal thus releasing inhibitory effects on the pituiary and testes.

Sperm concentration, spermatozoal motility and viability in control animals were comparable to those from earlier reports on the same breed (Singh et al., 1985). Anotable deerease in the quality of semen in thiourea treated goats is evidence for effects of hypothyroidism on the reproductive performance and is modulated through decreased testosterone in goats. Furthermore, reduced testosterone secretion in the present study during hypothyroid period indicated that the normal function of Leydig cells depends on an adequate level of thyroid hormones as has been previously suggested in rams (Chandrasekhar et al., 1986). Decrease in circulatory testosterone could explain the significant decrease in the ejaculate volume through an effect of impaired secretory activity of the accessory sex glands as observed by Buitrago and Diez (1987) in human males and Reddi and Rajan (1986) in goats during hypothyroidism. During the treatment period sperm concentration, motility and viability were significantly lower than during pre treatment. similar findings were reported by Reddi and Rajan (1986) in goats, suggests a positive correlation between thyroid status and percentage of live spermatozoa. This may be due to the production of increased number of non-viable spermatozoa resulting from the absence of adequate testosterone action/production.

The difference in conception rate (100% for does inseminated by control vs 0% from treated males) might be related to decline in the properties of sperm detected. Fur thermore, it is possible that the sperm produced might have reduced fertilizing capacity too (Maqsood, 1951). The deleterous effects on seminal characteristics observed within one month of the transient hypothyroidism might be due to the decreased testosterone action in accessory sex glands which ultimately affected the fertility of the thiourea-treated male goats.

Although the exact mechanism by which thyroid hormones affects sex libido, seminal characteristics have not been lelucidated, decreased level of thyroxine is considred to produce a change in the sensitivity of the gonads to the gonadotrophic hormones in a number of species (Nalbandov 1976). It has also been suggested by Aruldhas et al. (1982) and Periera et al. (1983) that hypothyroidism alters key enzymes of testes and epididymis, which interfere with androgenesis, testicular sperm production and epididymal sperm maturation, the process that confers the forward progressive motility to spermatozoa.

CONCLUSION

In conclusion hypothyroidism resulted in lower level of testosterone and could be an important factor associated with poor quality of semen which ultimately resulted in infertility, thus establishing a key role of thyroid hormones in goat reproduction.

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