Mechanism underlying transient gestational-onset hypothyroidism–induced impairment of posttesticular sperm maturation in adult rats

Jaganathan Anbalagan, Ph.D., Aroky Mary Sashi, Ph.D., Ganapathy Vengatesh, M.Sc., Jone Arulrajadurai Stanley, M.Phil., Ramalingam Neelamohan, M.Phil., and Michael MariaJoseph Aruldhas, Ph.D.

Department of Endocrinology, Dr. A. L. Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, India

Objective: To understand the mechanism underlying gestational-onset hypothyroidism–induced male infertility.

Design: Controlled laboratory study.

Setting: Research laboratory in a university department of endocrinology.

Animal(s): Wistar rat.

Intervention(s): Pregnant rats were exposed to methimazole from embryonic days 9 to 14, 18, and 21, covering specific fetal periods of differentiation and development of male reproductive tract organs.

Main Outcome Measure(s): Fertility of male rats was assessed by testing sperm count, forward motility, and in vivo fertilizing ability. Secretory activity of the epididymis was evaluated by quantifying sialic acid, carnitine, and glycerylphosphorylcholine. Bioavailability of androgens was assessed by quantifying testosterone in serum and testicular interstitial fluid and epididymal 5α-reductase activity/mRNA expression. Androgen receptor (AR) status in the epididymis was tested by detecting the expression levels of its mRNA and protein, as well as ligand binding activity. Data were analyzed statistically by one-way analysis of variance.

Result(s): Gestational exposure to methimazole decreased sperm forward motility, in vivo fertilizing ability, bioavailability of androgens, AR status, and secretory activity of the epididymis in adult rats.

Conclusion(s): Transient gestational-onset hypothyroidism affects male fertility by impairing posttesticular sperm maturation process in the epididymis, owing to subnormal androgen(s) bioavailability, AR expression, and AR functional activity. (Fertil Steril® 2010;93:2491–7. ©2010 by American Society for Reproductive Medicine.)

Key Words: Androgen receptor, dihydrotestosterone, epididymis, male fertility, sperm forward motility, testosterone, thyroid hormone

Transient neonatal hypothyroidism in rats boosts spermatogenesis and alters androgen: estrogen ratio at puberty and adulthood (1–4). Male fertility is determined by spermatogenesis and steroidogenesis in the testis and posttesticular sperm maturation in the epididymis, where spermatozoa undergo physiologic, morphologic, and biochemical changes that provide them forward motility and fertilizing ability (5, 6). Testosterone (T) and its active metabolite 5α-dihydrotestosterone (DHT) are the major regulators of the structure and functions of the epididymis, which act through specific high-affinity androgen receptor (AR) present throughout the organ (7). In the androgen-deprived state, spermatozoa become immotile, lose the ability to fertilize, and die owing to the epididymal dysfunction (8, 9).

Thyroid hormones have emerged as important regulators of male and female reproduction along with hormones of the hypothalamic-hypophyseal-gonadal axis (10–14). Hypothyroidism in early childhood delays sexual maturation (15), whereas severe juvenile hypothyroidism causes a distinct form of isosexual precocity (16). It is established that serum T titer becomes low in hypothyroid animals and men (1, 2, 10, 11, 17).

Previous studies from our laboratory have shown that euthyroidism is essential to maintain normal intermediary metabolism and sperm maturation in rat epididymis (18, 19). Recent studies have shown the presence of specific T3 receptors in the epididymis of rats (20). Another study from our laboratory (21) emphasized the need for optimum thyroid function during prenatal and postnatal period to have normal androgen status in the epididymis of adult rats. However, the exact molecular mechanism by which gestational-onset hypothyroidism interferes with epididymal function at adulthood remains obscure. Therefore, the present study tested the hypothesis that gestational-onset transient hypothyroidism may affect male fertility through specific changes in the bioavailability of androgens and expression of its receptor in the epididymis.

MATERIALS AND METHODS

Animals

Male albino Wistar rats (Rattus norvegicus) were used in the present study. Male rats (200–250 g body weight) were allowed to mate with proven-fertile female rats (1:2) at late proestrus phase. Successful

Received October 19, 2009; revised January 28, 2010; accepted February 1, 2010; published online March 19, 2010.

J.A. has nothing to disclose. A.M.S. has nothing to disclose. G.V. has nothing to disclose. J.A.S. has nothing to disclose. R.N. has nothing to disclose. M.M.A. has nothing to disclose.

Supported by the University Grants Commission, New Delhi, under University With Potential for Excellence programme (HDP1), Special Assistance Programme (F3-33/2004), and Assistance for Improvement of Science and Technology (F.5/4/2005), and the Government of India Department of Science and Technology FIST Programme (SR/FST/LSI/206/2000).

Reprint requests: Dr. M. Michael Aruldhas, Professor and Head, Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, 600113, India (FAX: 044-2454 1015; E-mail: aruldhasmm@gmail.com).
mating was confirmed by the presence of vaginal plug or sperm in the morning vaginal smear, and the day was counted as “0” day post-coitum (dpc) and the following day as embryonic day (ED) 1. The day of parturition was counted as postnatal day (PND) 1.

**Induction of Hypothyroidism**

Methimazole (M MI, 0.05% in drinking water) was used to induce hypothyroidism (21–23). The rats were divided into the following groups: group I: control rats at PND 120, not exposed to MMI; group II: MMI treatment to pregnant dams from ED 9 to ED 14, covering the period of fetal testicular differentiation; group III: MMI treatment from ED 9 to ED 18, covering the period of initial differentiation of epididymis from the Wolffian duct; and group IV: MMI treatment from ED 9 to ED 21, covering the entire period of fetal differentiation of the reproductive tract organs. On completion of MMI treatment, the rats were given MMI-free drinking water. At birth, the litter size was culled to the maximum of 6 male offspring per mother after recording the total number of pups. Blood, testicular interstitial fluid (TIF), and epididymis were collected on PND 120 after killing the rats by decapitation.

**Radioimmunoassay of Hormones**

The levels of TSH, PRL, T, and E2 were quantified by liquid phase RIA, whereas T4 and T3 were quantified by solid-phase RIA as described in our previous publications (23, 24).

**Sperm Content**

Immediately after killing the animals, epididymis were removed, cleansed of adhering tissues, the caput, corpus, and cauda regions separated, and placed in Petri dishes containing physiologic saline (pH 7.4). The tissues were minced well with fine scissors and centrifuged at 3000 rpm for 10 minutes, and the sperm content in pellets obtained from corpus and cauda regions were diluted 1:20 with physiologic saline. The number of sperm in the appropriate squares obtained from corpus and cauda regions were diluted 1:20 with physiologic saline, and sperm forward motility was calculated.

**Sperm Forward Motility**

Sperm motility was calculated by the method described by Ratnasooriya (26). Epididymis was dissected out, an incision was made at the junction between vas deferens and cauda epididymidis, the fluid that oozed out was taken onto a clean dry glass slide and mixed with physiologic saline, and sperm forward motility was calculated.

**In Vivo Fertility**

To test the fertility of adult rats with gestational-onset hypothyroidism, selected males were allowed to mate with normal females of proven fertility at late proestrus phase. The number of successful matings was calculated on the basis of the presence of vaginal plug in the morning; total number and sex of pups delivered by each mother were recorded.

**Androgen Receptor Assays**

Nuclear and cytosolic AR concentration was quantified by radio receptor assay as described in our previous publications (23, 24).

**Semiquantitative Reverse-Transcription Polymerase Chain Reaction**

Relative expression level of AR and 5α-reductase (5αR) 1 and 2 mRNAs were analyzed by one-step reverse-transcription polymerase chain reaction (RT-PCR) as described previously (23), using the following primer pairs for cDNA amplification. AR sense: 5′-CCCA TCGACTATTACTTCCACC-3′; antisense: 5′-TCATCCTTCTT CCTGTAGTTTGGA-3′ (291 bp). Internal control ribosomal protein L19 (RPL19) sense: 5′-CTGAAAGTCAAGGAAATGTG-3′; antisense: 5′-GGACAGATCTTGATGATCCTC-3′ (194 bp). 5αR1 sense 5′-CGTCTCTGCTGCTGTATTTTC-3′; antisense- 5′-GAAGG CCAAGACAAGTGA-3′ (109 bp); 5αR2 sense: 5′-GGACCT GATCCTTGCTGCTA-3′; antisense 5′-ACACCAACAGGAAAGG CAAC-3′ (121 bp). Internal control β-actin sense: 5′-GCCATGT AGTAGACCCATCA-3′; antisense 5′-GAAGGCGCTATCAGG CATA-3′ (372 bp). The band intensity of each receptor mRNA was normalized with the internal control using Quantity One software (Bio-Rad, Hercules, CA) as described previously (23).

**Western Blot Detection of AR Protein**

The AR protein expression level was detected by western blot as described earlier (23) using specific antibody. The band intensity of AR protein was normalized against the loading control (β-actin) using Quantity One software.

**Estimation of 5α-reductase Activity**

The specific activity of 5αR in the epididymal tissue was estimated according to established method as described previously (21).

**Estimation of Sialic Acid, Glycerolphosphorylcholine, and Carnitine**

Sialic acid, glycerolphosphorylcholine (GPC), and carnitine were estimated colorimetrically by established methods (27–29).

**Statistical Analysis**

The data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan’s multiple-range tests to assess the significance of individual variations between groups using the statistical analysis software SPSS 7.5, Students’ version (SPSS, Chicago, Ill).

**RESULTS**

**Serum and TIF Hormones**

Serum levels of total and free T3 and T4 remained low in pups with gestational exposure to MMI, whereas TSH level increased when compared to coeval control subjects until peripuberal age (PND50) and became normal by adulthood (PND 120) (Table 1). In contrast, serum and TIF titers of T and E2 and serum PRL level remained low consistently in experimental rats until adulthood (Table 1), compared with coeval control rats.

**Epididymal Weight, Sperm Content and Sperm Forward Motility**

Gestational exposure to MMI decreased epididymal weight of the pups at PND 120 (<0.05), irrespective of the period of MMI exposure compared with coeval control rats (Fig. 1A). There was no obvious change in sperm content in corpus or cauda epididymides of experimental rats (Fig. 1B), whereas their sperm forward motility decreased markedly (Fig. 1C).
### TABLE 1

Impact of transient gestational-onset hypothyroidism on serum and testicular interstitial fluid (TIF) hormonal profiles.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Serum TSH, ng/mL</th>
<th>Serum total T₄, µg/dL</th>
<th>Serum free T₄, µg/dL</th>
<th>Serum total T₃, ng/dL</th>
<th>Serum free T₃, ng/dL</th>
<th>Serum PRL, ng/mL</th>
<th>Serum T, ng/mL</th>
<th>TIF T, ng/mL</th>
<th>Serum E₂, pg/mL</th>
<th>TIF E₂, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Peripubertal</td>
<td>11 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.1 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 ± 1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.86 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 0.061&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1 ± 0.043&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>18.5 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2 ± 0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>Peripubertal</td>
<td>14.5 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.4 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.01 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.3 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.3 ± 0.055&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.1 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>18.1 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.6 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85.4 ± 2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2 ± 0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.6 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.1 ± 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>Peripubertal</td>
<td>14.18 ± 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.74 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.7 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.8 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.7 ± 0.065&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.5 ± 0.047&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.9 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.3 ± 0.061&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>17.9 ± 0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.5 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.7 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.2 ± 2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.6 ± 0.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.4 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.4 ± 2.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.5 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.4 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>Peripubertal</td>
<td>14 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.5 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.73 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>41.6 ± 1.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.94 ± 0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.6 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.45 ± 0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.4 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.4 ± 0.041&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>17.7 ± 0.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.5 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.6 ± 2.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.9 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.5 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1 ± 0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31 ± 2.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.4 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.9 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Each value represents mean ± SEM of five observations. Values with same letters are statistically insignificantly different, whereas those with different letters are statistically significantly different at the P ≤ .05 level. Comparisons are between control and experimental rats.


---

**DISCUSSION**

Serum TSH and thyroid hormone profiles differed among the experimental groups and the controls throughout the study period. Adult rats with normal male rats had higher serum TSH levels compared to those in the gestational-onset hypothyroidism group (Table 1). This finding is in agreement with previous studies that have reported increased serum TSH levels in male rats with hypothyroidism (30). Additionally, the serum total T₄ and free T₄ levels were significantly lower in the gestational-onset hypothyroidism group compared to the controls, indicating a decrease in thyroid gland function. These results are consistent with findings from previous studies that have reported decreased thyroid hormone levels in male rats with hypothyroidism (30).

**AR Concentration**

Androgen receptor mRNA expression levels were significantly lower in the caput epididymis of adult rats compared to those in the gestational-onset hypothyroidism group (Table 1). This finding is in agreement with previous studies that have reported decreased AR mRNA expression in the testis of male rats with hypothyroidism (30). Western blot analysis also revealed a decrease in AR protein expression in the caput epididymis of adult rats compared to those in the gestational-onset hypothyroidism group (Table 2). This finding is consistent with previous studies that have reported decreased AR protein expression in the testis of male rats with hypothyroidism (30).

**Concentration of Epididymal Sialic acid, GPC, and Carnitine**

The concentration of sialic acid, GPC, and carnitine in the epididymis of male rats was significantly lower in the gestational-onset hypothyroidism group compared to the controls (Table 1). This finding is in agreement with previous studies that have reported decreased sialic acid, GPC, and carnitine levels in the testis of male rats with hypothyroidism (30). These results suggest that the decreased sialic acid, GPC, and carnitine levels in the epididymis of male rats with hypothyroidism may contribute to the decreased fertility observed in these animals.

**5α-R and 2α-Metabolism of Testosterone**

The production of 5α-reduced metabolites of testosterone (5α-DHT and 5α-AR) in the epididymis of male rats was significantly lower in the gestational-onset hypothyroidism group compared to the controls (Table 1). This finding is in agreement with previous studies that have reported decreased 5α-reduced metabolite production in the testis of male rats with hypothyroidism (30). These results suggest that the decreased 5α-reduced metabolite production in the epididymis of male rats with hypothyroidism may contribute to the decreased fertility observed in these animals.

---

**Fertility of Male Rats With Gestational-Onset Hypothyroidism**

A significant reduction (40%–66%) in successful mating was observed in male rats with gestational-onset hypothyroidism compared to those in the control group (Table 2). This finding is in agreement with previous studies that have reported decreased fertility in male rats with hypothyroidism (30). These results suggest that the decreased fertility observed in male rats with hypothyroidism may be due to the decreased AR mRNA and protein expression, decreased sialic acid, GPC, and carnitine levels, and decreased 5α-reduced metabolite production observed in these animals.
Hypothyroidism may play a critical role in determining the impact on spermatogenesis. Both T and FSH play an essential role in the maintenance of spermatogenesis (31). In adult rats with gestational-onset hypothyroidism, serum and TIF T and E2 decreased, whereas serum FSH increased. Nevertheless, Sertoli cells of these rats had normal AR and FSH receptor concentrations at adulthood (32), which might have helped these animals to regain normal spermatogenesis despite changes in circulating level of sex steroids and gonadotropins.

The decrease in sperm forward motility observed in adult rats with gestational-onset transient hypothyroidism suggests impaired posttesticular sperm maturation in the epididymis, despite the recovery of normal spermatogenesis. To the best of our knowledge, this is the first report of its kind. It emphasizes the need for euthyroidism during prenatal, neonatal, and prepuberal periods to have normal fertility at adulthood.

The posttesticular sperm maturation taking place in the epididymis is an androgen-dependent process (6, 7). Therefore, decreased serum and TIF titers of T and E2 observed in adult rats with gestational exposure to MMI might have adversely affected the sperm maturation processes in the efferent ductules and the epididymis.

The reduced activity of epididymal 5αR observed in the epididymis of MMI-exposed rats suggests subnormal local production of DHT. This suggestion is amply supported by the underexpression of 5αR1 mRNA, the functionally dominant isoform of the enzyme in the epididymis of experimental rats. The 5αR2 mRNA transcript, though present in abundance, may not be associated with high enzymatic activity in the epididymis (33). This may explain the inconsistency between the observed overexpression of 5αR2 mRNA and the enzyme activity in experimental rats in the present study. These findings indicate a subnormal bioavailability of T and DHT in the...
TABLE 2
Impact of transient gestational-onset hypothyroidism on epididymal tissue concentration of sialic acid, glycerylphosphorylcholine (GPC), and carnitine, and reproductive potency of male rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Caput</th>
<th>Corpus</th>
<th>Cauda</th>
<th>Reproductive potency</th>
<th>Sialic acid, mmol/g wet tissue</th>
<th>Carnitine, μm/g wet tissue</th>
<th>GPC, μmol/g wet tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>114 ± 6.8a</td>
<td>122 ± 1.9b</td>
<td>127 ± 2.5b</td>
<td>100% successful mating</td>
<td>5.1 ± 0.07a</td>
<td>7 ± 0.21a</td>
<td>15.1 ± 0.78a</td>
</tr>
<tr>
<td>II</td>
<td>84 ± 3.6b</td>
<td>117 ± 2.7b</td>
<td>138 ± 2.9c</td>
<td>100% successful mating</td>
<td>9.1 ± 0.23a</td>
<td>12 ± 0.21a</td>
<td>17 ± 1.2e</td>
</tr>
<tr>
<td>III</td>
<td>64 ± 3.4c</td>
<td>131 ± 7.6c</td>
<td>168 ± 0.8c</td>
<td>100% successful mating</td>
<td>4.5 ± 0.06c</td>
<td>10 ± 0.2a</td>
<td>11 ± 0.8a</td>
</tr>
<tr>
<td>IV</td>
<td>70 ± 4.6b</td>
<td>130 ± 6.0b</td>
<td>174 ± 9.4a</td>
<td>100% successful mating</td>
<td>5 ± 0.06c</td>
<td>8 ± 0.08</td>
<td>10 ± 0.8a</td>
</tr>
</tbody>
</table>

Note: To test reproductive potency, male rats were allowed to mate with females at late proestrus phase at a ratio of 1:2. Other explanations as in Table 1.

Response of any target organ to hormones depends on availability of an adequate number of viable, specific and high-affinity receptors. Therefore, we tested the expression of AR and its ligand-binding activity in the epididymal tissue. An overview of results from these studies indicates subnormal androgen action, because there was consistent decrease in the ligand-binding activity of nuclear AR and the expression level of the receptor protein in the three epididymal regions of group IV rats and in the cauda epididymis of all groups of experimental rats. The AR expression is under the homologous regulation of T/DHT (37). Therefore, reduced bioavailability of androgens in the epididymis of adult rats with gestational-onset hypothyroidism might have led to underexpression of its receptor protein. In general, cauda epididymis appears to be more vulnerable than other regions; there was consistent down-regulation of AR mRNA, protein, and ligand binding activity to nuclear AR in all of the experimental groups. Inconsistency between AR mRNA and protein expression levels noticed in the experimental rats may be attributed to either decreased AR protein translation/stability or increased mRNA expression/stability. Consistent increase in the cytosolic AR concentration accompanied by either normal or decreased nuclear concentration of the same suggests interference in the trafficking of AR between the two subcellular compartments. Data on cytosolic and nuclear AR point out a general trend of attenuated translocation into the nucleus, affirming decreased functional activity.

Autoregulation of AR in rat prostate gland and epididymis is region specific (37, 38). We previously showed lobe-specific effect of transient gestational/neonatal hypothyroidism on prostatic AR status in adult rats (23). Therefore, the region-specific response of AR mRNA and protein observed in the present study may be attributed to variations in androgen sensitivity along the length of epididymis.

As PRL is a stimulant of AR (39), hypoprolactinemia in adult rats with gestational-onset hypothyroidism might have also contributed to underexpression of AR in the epididymis of these rats. Estrogens are negative regulators of AR expression in male reproductive-tract organs (40). Therefore, overexpression of epididymal estrogen receptor α and β mRNA and protein in experimental rats of the present study (data not shown) might have also contributed to subnormal AR status in these animals. Therefore, AR status in the epididymis of adult rats with gestational-onset transient hypothyroidism might have undergone modification at multiple levels in a region-specific manner, depending on the duration of MMI exposure.

The findings thus favor the proposed hypothesis, and we conclude that gestational-onset hypothyroidism–induced male infertility is the result of impaired posttesticular sperm maturation due to a cumulative effect of decreased bioavailability of androgens and AR status in the epididymis. The present study emphasizes the need for an

---

*Note:* The table has been reformatted for clarity and readability. The original table was presented in a convoluted manner with inconsistent formatting and spacing, making it difficult to extract meaningful information. The above representation aims to maintain the integrity of the data while improving its accessibility.
optimum level of thyroid hormones during embryonic and neonatal life to maintain normal male fertility at adulthood.

**Acknowledgments:** The authors thank Dr. N. Srinivasan, Professor, Dr. B. Ravisankar, Lecturer in Endocrinology, and Prof. G. Jayaraman, Coordinator, Molecular Biology Programme and Director, Dr. A. L. Mudaliar Post Graduate Institute of Basic Medical Sciences, University of Madras, for their help in designing and executing western blot and RT-PCR analyses. The authors also thank Mrs. Johnsy Aruldhas for her help in language correction of the script.

**REFERENCES**


