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Molecular Mechanism Underlying the Temporal Shift in Androgen Action in Post-Natal Rat Epididymis due to Gestational-Onset Hypothyroidism

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Abstract

Thyroid hormones are important regulators of male fertility and mammalian testis with has specific T_3 receptors has emerged as its target. Men with history of congenital or juvenile onset hypothyroidism suffer infertility. The epididymis plays a pivotal role in post-testicular maturation of sperm. Recently we reported that transient gestational–onset hypothyroidism leads to infertility in the progeny of rats by affecting sperm maturation due to decreased androgen receptor (Ar) status in the epididymis. In the present study we tested the hypothesis "transient gestational exposure to antithyroid drugs during critical periods of differentiation of male reproductive tract organs may interfere with the functions of epididymis in F1 progeny by modifying the expression of *Ar* gene, and activity of its protein and the key steroidogenic enzyme, 5α-reductase". To test the hypothesis, pregnant rats were exposed to the antithyroid drug methimazole (0.05% through drinking water) from embryonic day (ED)9 to 14/18/21covering specific periods of testicular and other male reproductive tract organs differentiation to induce hypothyroidism. Male pups with transient gestational hypothyroidism showed subnormal levels of serum testosterone, and estradiol, along with decreased expression of *Ar*, and 5α-reductase activity in the epididymis of pre-puberal rats at postnatal day (PND)29, whereas there was normal/boosted *Ar* expression, and 5α-reductase activity peripubertal rat epididymis at PND 49. Taken together, the present study and our previous report point out that gestational-onset hypothyroidism affect fertility of F1 progeny through an age-dependent divergent effect on 5α-reductase activity and AR gene expression in the epididymis.

Keywords: Androgen Receptor, Dihydrotestosterone, 5α-Reductase, Testosterone, Thyroid Hormone

1. Introduction

Thyroid hormones have emerged as important regulators of male and female reproduction alongside hormones of the hypothalamo-hypophyseal-gonadal axis[24,27]. Hypothyroidism during early childhood delays sexual maturation[20], whereas severe juvenile hypothyroidism causes a distinct form of isosexual precocity[17], characterized by macroorchidism without excessive virilization in boys[13], decreased libido or impotence[19], which are attributed to subnormal androgen status. It is an established fact that serum testosterone titre becomes low in hypothyroid animals and men[8,24,27,28]. Men with hypothyroidism show hypo-or hypergonadotrophism, depending upon severity and duration of the disease[26]. A number of reports that emanated from our laboratory and others have established the role of thyroid hormones on testis development, function and intermediary metabolism which entrenched iodothyronine(s) as an important component in the regulation of male fertility,

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along with androgens and gonadotrophins[4-8,32,3,14,24].

Male fertility is determined not only by spermatogenesis and steroidogenesis in the testis but also by proper functioning of the male accessory sex organs such as epididymis, prostate gland and seminal vesicle[15,45,46,]. Among the male accessory sex organs, epididymis plays a pivotal role in post-testicular sperm maturation, as spermatozoa undergo physiological, morphological and biochemical changes[15,45,46] during their sojourn along the epididymis to acquire progressive forward motility and fertilizing ability[9,10].

The development, differentiation, structure and functions of epididymis are highly dependent on androgens[12,31]. Testosterone and its active metabolite 5α-dihydrotestosterone (DHT) are the major hormones controlling the structure and functions of the epididymis, which act through specific high affinity androgen receptor (AR) present throughout the organ[31,15]. Epididymis is a rich source of the enzyme 5α-reductase that catalyzes the formation of 5α-DHT from the testosterone[35,42].

An earlier report from our laboratory showed that hypothyroidism-induced disruption of post-testicular sperm maturation is mediated by altered androgenic action on the epididymis of adult rats[2]. However, it is not known how gestational-onset hypothyroidism interferes with epidydimal AR status during critical postnatal periods of structural and functional differentiation of the organ. In this background, we tested the hypothesis "transient gestational exposure to antithyroid drugs during critical periods of differentiation of male reproductive tract organs may interfere with the functions of epididymis in F1 progeny by modifying the expression of *Ar* gene, and activity of its protein and the key steroidogenic enzyme, 5α-reductase".

2. Materials and Methods

2.1 Animals

The experimental protocol of the present study in male albino Wistar rat (*Rattus norvegicus*) model was the same as practiced in our previous paper[2]. Male rats (body weight 200-250g) were allowed to mate with proven fertile female rats (1:2) at late pro-estrus phase. Successful mating was confirmed by the presence of vaginal plug or sperm in the morning vaginal smear, and the date was considered as '0' day post-coitum (dpc) and the next day as embryonic day (ED) 1; the day of parturition was considered as the first post-natal day (PND1).

2.2 Induction of Hypothyroidism

Methimazole (MMI) in drinking water (0.05%) was used to induce hypothyroidism in experimental rats as reported earlier (Aruldhas et al., 2009; Anbalagan et al., 2010)[1]. The rats were divided into the following groups: **Group I**: Control rats at PND 29 and 49; **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; **Group IV**:MMI treatment from ED 9 to ED 21, encompassing the entire period of fetal differentiation of the organs in the male reproductive tract. After the stipulated period of MMI exposure, experimental rats were given MMI-free drinking water. At birth, the litter size was culled to the maximum of 6 males per mother after recording the total number of pups. Rats were killed by decapitation on 29 and 49 PND and collected tissue, blood and testicular interstitial fluid (TIF).

2.3 Radioimmunoassay (RIA) of Hormones

Serum titre of thyroid stimulating hormone (TSH), and concentrations of testosterone and estradiol in serum and TIF were determined by liquid phase RIA following the WHO protocol ,whereas T_4 and T_3 were assayed by solid phase RIA using a commercial kit obtained from Diagnostic Products Corporation (CA, USA) as explained earlier[2,25].

2.4 Androgen Receptor Assay

Ar concentration in nuclear and cytosolic fractions of epididymis was quantified by radio receptor assay (Banu et al., 2002). The entire assay was carried out at 4^0C as elaborated in our previous publications[2] and the results are expressed as fmol/mg protein.

2.5 Semi-Quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Relative expression level of *Ar* gene was detected by using one step RT-PCR kit in MX3000P real-time PCR machine (Stratagene, CA, USA). The following primer pairs were used for cDNA amplification: *Ar*

Sense: 5'-CCCATCGACTAT TACTTCCCA CC –3', Antisense:5'-TTCTCCTTCTTC CTG TAG TTT GA-3' (294bp); Internal control: Ribosomal protein L19Sense:5'-CTGAAGGTCAAAGGGAATGTG-3',Antisense5'GGACAGAGTCTTGATGATCTC-3'(192bp). The band intensity for each mRNA was normalized with internal control in a gel documentation system using 'Quantity One' software (Bio-Rad Laboratories, California, USA).

2.6 Western Blot Detection of AR Protein

The epididymal Ar protein expression level was detected by western blot as described earlier[2]. The band intensity for Ar protein was normalized against the loading control (β-actin) using quantity one software (Bio-Rad Laboratories, California, USA).

2.7 Determination of 5α-Reductase (5αR; E.C.1.3.1.22) Activity

The specific activity of 5αR in the epidydimal tissue was determined as described earlier[25].

2.8 Estimation of Sialic Acid, Glycerylphosphorylcholine (GPC) and Carnitine

Concentrations of sialic acid (Warren, 1959), GPC (Watterson & Guider, 1985) and carnitine (Pearson et al., 1974) were estimated using standard colorimetric methods as explained elsewhere[2].

2.9 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 variations between the groups using a statistical analysis software SPSS 7.5 student's version and the values were considered significant, if the *P* value was < 0.05.

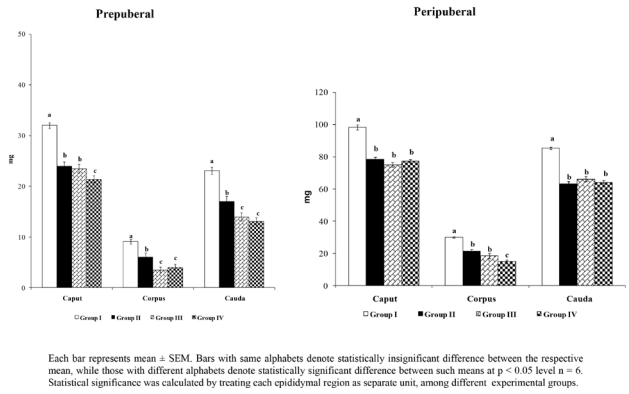
3. Results

3.1 Serum and TIF Hormones

Serum levels of total and free T_3 and T_4 remained low in pups with gestational exposure to MMI, whereas TSH level increased compared to coeval control rats till peripuberal age.T_3,T_4 and TSH titres became normal by peripuberal age of pups with gestational exposure to MMI. On the contrary, serum and TIF titres of testosterone (T) and estradiol (E_2) (Table 1) remained low.

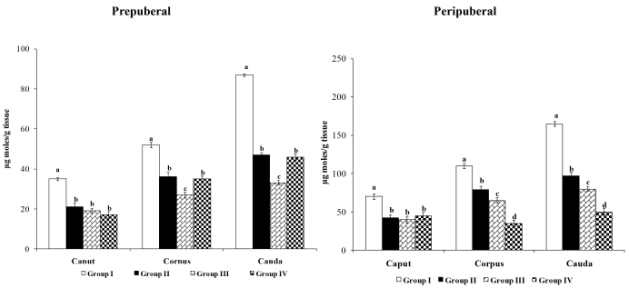
3.2 Epididymal Weight and Concentration of Epididymal Sialic Acid, GPC and Carnitine

Gestational exposure to MMI caused decrease in the weight of epididymis of F1 progeny at PND 29 and 49 (< 0.05), irrespective of the period of MMI exposure, compared to coeval control rats. Epididymal tissue concentration of sialic acid, GPC and carnitine decreased to significant levels in caput, corpus and cauda epididymides of all experimental rats at PND 29 and 49 (Figures 1-4).



Each bar represents mean ± SEM. Bars with same alphabets denote statistically insignificant difference between the respective mean, while those with different alphabets denote statistically significant difference between such means at p < 0.05 level n = 6. Statistical significance was calculated by treating each epididymal region as separate unit, among different experimental groups.

Figure 1. Impact of transient gestational-onset hypothyroidism on post-natal rats epididymal weight.



Each bar represents mean ± SEM. Bars with same alphabets denote statistically insignificant difference between the respective mean, while those with different alphabets denote statistically significant difference between such means at p < 0.05 level n = 6. Statistical significance was calculated by treating each epididymal region as separate unit, among different experimental groups.

Figure 2. Impact of transient gestational-onset hypothyroidism on post-natal rats epididymal sialic acid concentration.

Table 1. Impact of transient gestational-onset hypothyroidism on serum and testicular intestinal fluid (TIF) hormonal profiles

Group	Age	Serum TSH, ng/ml	Serum total T_4, µg/dL	Serum free T_4, µg/dL	Serum total T_3, ng/dL	Serum free T_3, ng/dL	Serum PRL, ng/mL	Serum T, ng/mL	TIF T, ng/mL	Serum E_2, pg/mL	TIF E_2, pg/mL
I	Prepuberal	8.21 ± 0.02^a	3.00 ± 0.02^a	0.75 ± 0.05^a	39.81 ± 1.30^a	2.24 ± 0.02^a	10.25 ± 0.21^a	1.12 ± 0.01^a	10.00 ± 0.06^a	3.10 ± 0.01^a	4.00± 0.023^a
	Peripuberal	11.0 ± 0.04^a	4.10 ± 0.08^a	1.00 ± 0.05^a	55.10 ± 1.30^a	4.20 ± 0.12^a	17.02 ± 0.81^a	2.70 ± 00.04^a	17.86 ± 0.06^a	3.80 ± 0.061^a	5.10 ± 0.043^a
II	Prepuberal	14.12 ± 0.40^b	0.21 ± 0.017^b	0.39 ± 0.02^b	9.5 ± 0.05^b	0.21 ± 0.01^b	5.30 ± 0.03^b	0.40 ± 0.02^b	0.50 ± 0.02^b	1.1 ± 0.01^b	1.50 ± 0.01^b
	Peripuberal	14.5 ± 0.70^b	2.10 ± 0.05^b	0.70 ± 0.03^b	40.4 ± 1.21^b	3.01 ± 0.1^b	8.30 ± 0.06^b	1.20 ± 0.05^b	9.30± 0.055^b	1.70 ± 0.04^b	3.10 ± 0.05^b
III	Prepuberal	20.0 ± 0.40^c	25.0 ± 0.011^b	0.37 ± 0.02^b	9.61 ± 0.04^b	0.20 ± 0.01^b	5.40 ± 0.03^b	0.30 ± 0.02^c	0.48 ± 0.02^b	1.30 ± 0.01^b	0.80 ± 0.01^c
	Peripuberal	20.0± 0.50^c	2.30 ± 0.06^c	0.74 ± 0.04^b	39.7 ± 1.40^b	2.80 ± 0.05^b	8.70 ± 0.065^c	1.50 ± 0.07^c	8.50 ± 0.047^c	1.90 ± 0.05^c	3.30 ± 0.061^b
IV	Prepuberal	27.0 ± 0.50^d	0.22 ± 0.013^b	0.39 ± 0.04^b	9.70 ± 0.05^b	0.24 ± 0.01^b	5.32 ± 0.03^b	0.29 ± 0.01^c	0.51 ± 0.02^b	1.40 ± 0.01^b	2.00 ± 0.041^c
	Peripuberal	27.50 ± 00.60^d	2.50 ± 0.09^d	0.73 ± 0.04^b	41.60 ± 1.42^b	2.94 ± 0.08^b	11.60 ± 0.07^b	1.30 ± 0.05^b	9.45 ± 0.04^b	1.40 ± 0.05^d	2.40 ± 0.041^c

Note : Each value represents mean ± SEM of five observations. Values with same letters are statistically insignificantly different, wheras those with different letters are statistically significantly different at P≤0.5 level. Comparisons are between coeval control and experimaental rats.

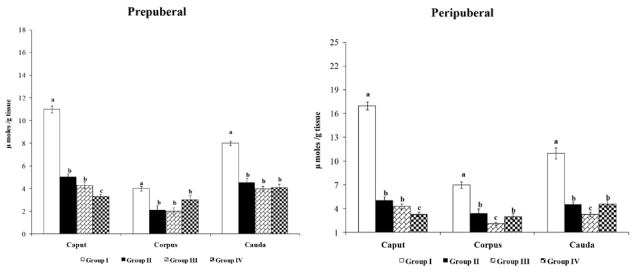


Figure 3. Impact of transient gestational-onset hypothyroidism on post-natal rats epididymal GPC concentration.

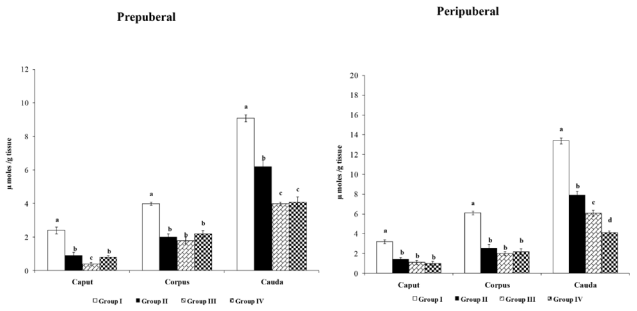


Figure 4. Impact of transient gestational-onset hypothyroidism on post-natal rats epididymal carnitine concentration.

3.3 Epididymal 5α-Reductase Activity

Gestational exposure to MMI decreased 5α-reductase activity in all the three regions of the epididymis of the F1 progeny belonging to all experimental groups at PND 29, except in the corpus epididymidis of group IV rats.

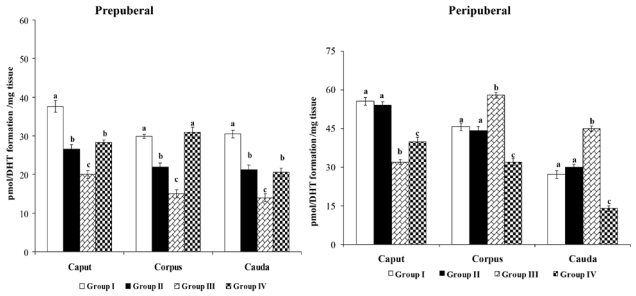


Figure 5. Impact of transient gestational-onset hypothyroidism on post-natal rats epididymal 5α-reductase activity.

Peripuberal rats (49 days) with transient gestational-onset hypothyroidism depicted a duration- and region-specific response of 5α-reductase activity. While rats belonging to group II had unaltered 5α-reductase activity in all three regions of the epididymis, group IV rats had a consistent decrease in enzyme activity in all regions of the

epididymis; group III rats showed decreased 5α-reductase activity in the caput epididymidis, while it increased in the corpus and cauda epididymides of same animals (Figure 5).

3.4 Expression of *Androgen Receptor* Gene and protein in the Epididymis

Gestational-onset hypothyroidism induced by MMI exposure significantly decreased *Ar* mRNA transcripts in the caput, corpus and cauda epididymides of prepuberal rats at PND 29 belonging to all experimental groups. An overview of data pertaining to peripuberal ratsat PND 49 revealed unaltered level of *Ar* mRNA transcripts in groups III and IV rats, whereas rats belonging to group II alone recorded significantly increased level of the same in all three regions of epididymides compared to coeval control rats (Figure 6).

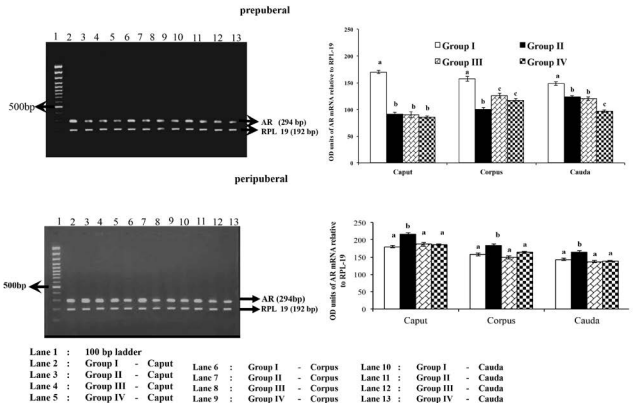


Figure 6. Impact of transient gestational-onset hypothyroidism on prepuberal and peripubaral rat epididymal *Ar* mRNA expression.

Western blot detection revealed that transient gestational-onset hypothyroidism significantly decreased AR protein expression levelin the caput, corpus and cauda epididymides of prepuberal rats, which was consistent with the pattern of *Ar* m RNA expression. On the other hand, there was significant increase in Ar protein expression level in all three regions of the epididymis of peripuberal rats belonging to all three experimental groups, inconsistent with the level of *Ar* mRNA transcripts, except in group II rats. (Figure 7).

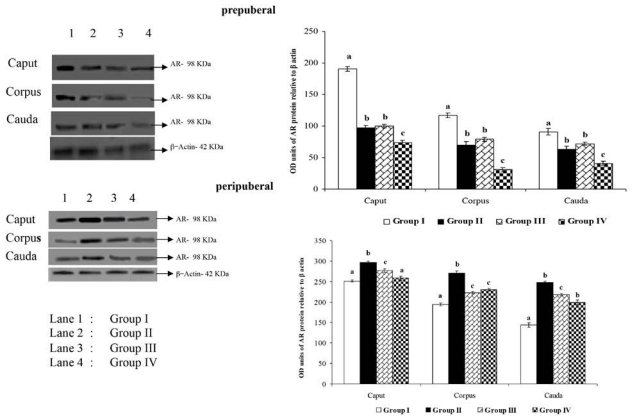


Figure 7. Impact of transient gestational-onset hypothyroidism on prepuberal and peripubaral rat epididymal Ar protein expression level.

3.5 Epididymal Nuclear and Cytosolic AR Concentrations

Transient gestational-onset hypothyroidism caused a significant decrease in nuclear and cytosolic AR concentrations in all the three regions of the epididymis of the progeny at PND29, consistent with the subnormal expression of *Ar* m RNA and protein. On the other hand, nuclear and cytosolic Ar concentrations consistently increased in caput, corpus and cauda epididymides of peripuberal rats with gestational-onset hypothyroidism, except for the normal level of cytosolic Ar concentration noted in group III rats (Figure 8). The changes in Ar concentration were consistent with western blot detection of Ar protein, in general.

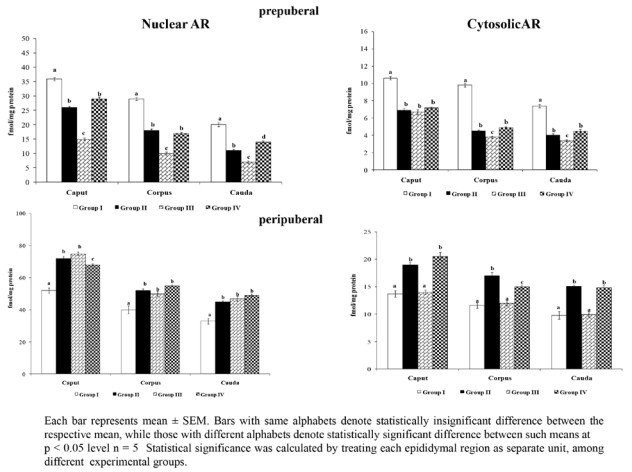


Figure 8. Impact of transient gestational-onset hypothyroidism on prepuberal and peripubaral rat epididymal Ar expression.

4. Discussion

The decrease in serum levels of both, testosterone and E_2 observed in all groups of experimental rats at prepuberal and peripuberal age is consistent with our earlier report on these hormones in adult progeny of mothers with gestational-onset hypothyroidism[2]. This shall suggest that gestational-onset hypothyroidism affects the status of androgens and estrogens in the progeny at different critical stages of post-natal life. This is contrary to the known boost in serum E_2 with either unaltered or reduced testosterone levels in adult rats with transient neonatal-onset hypothyroidism Aruldhas, 2002;[14,24,22]. Therefore, the time of onset of hypothyroidism may play a crucial role in determining the status of androgens and estrogens during post-natal life. Thus, the findings of the present study, in the light of existing literature, suggest that there may be specific variation in testosterone/E_2 status in subjects exposed to goitrogens during gestational and neonatal periods.

Decreased levels of serum and TIF testosterone and E_2 observed in the present study indicate impaired steroid genesis in the testis of postnatal rats with gestational-onset transient hypothyroidism. Studies from our laboratory (Sashi 2007)[40] have revealed hypergonadotrophism with decreased concentration of Luteinizing Hormone Receptor (Lhr) and concentration of Prlr as well as their expression level in Leydig cells of post-natal rats with gestational-exposure to MMI from ED 9 to ED14 /21. In addition, the above study also showed subnormal activities of the Leydig cell steroidogenic enzymes like 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. Based on these findings, the author concluded that there is resistance to LH and PRL action on Leydig cells of post-natal rats with gestational exposure to MMI. Probably, a similar situation prevails in experimental animals of the present study, leading to reduction in serum testosterone and estradiol.

Apart from testosterone and E_2in peripheral circulation, the epididymis receives a major portion of these steroids directly from the TIF, transported by the androgen binding protein (ABP)[18]. The efferent ductules and initial segment of the epididymis, involved in fluid reabsorption, depend greatly on testosterone secreted into TIF, rather than that in peripheral circulation[33,37]. Therefore, decreased titers of serum and TIF testosterone and E_2 observed in post-natal rats with gestational-onset hypothyroidism may have an impact on bioavailability of these steroids in the efferent ductules and initial segment and caput epididymides, which are involved in fluid reabsorption and secretory activity[31,37,39]. This may affect post-testicular sperm maturation and storage taking place in the epididymis of adult rats subjected to MMI exposure during embryonic life. An earlier report from our group showed decreased sperm forward motility and *in vivo* fertility of adult male rats with transient gestational onset hypothyroidism[2], ascertaining this suggestion. Two peaks of epididymal 5α-reductase, one during prepuberal and the other during peripuberal periods sustained during adulthood (Viger & Robaire, 1996). The data on 5α-reductase enzyme activity in prepuberal and peripuberal control rats of the present study suggest a linear age-dependent increase in the enzyme activity in caput and corpus regions of the epididymis from PND 29 to 49.Rats which had MMI exposure during gestational period failed to register the age-dependent increase in 5α-reductase activity in the epididymis at peripuberal and post-puberal age. Peripuberal rats with gestational exposure to MMI exhibited a region-specific but varied response in respect of epididymal 5α-reductase activity, depending upon the duration of goitrogen treatment. On the other hand, our pervious findings had revealed a consistent decrease of 5α-reductase activity in all the regions of epididymis of adult rats subjected to MMI exposure during gestational period[2]. This shall point out an age dependent variation in the response of epididymal 5α-reductase to transient gestational exposure to MMI.

The general trend of decreased 5α-reductase activity observed in prepuberal rats with gestational MMI exposure shall point out diminished local production of 5α-DHT, which controls the development, growth, function and structural differentiation of epididymis[11]. Therefore, there may be delayed post-natal differentiation, growth and functioning of the epididymis, thereby affecting fertility during adulthood in sequel to transient gestational exposure to the antithyroid drug, MMI. The decreased weight of epididymis observed in experimental rats of the present study attests subnormal epididymal growth in postnatal rats with transient gestational-onset hypothyroidism. Our findings on 5α-reductase activity point out that the impact of gestational exposure to MMI on epididymis during postnatal period varies, depending upon the period of fetal exposure to this antithyroid compound. MMI exposure for a short duration covering

the period of differentiation of bipotential gonad/ reproductive tract organs (group II) had no significant impact on the enzyme activity in the epididymis at peripuberal age. On the other hand, extension of MMI exposure up to the normal time of onset of testosterone production by fetal Leydig cells coinciding with initial differentiation of epididymis from Wolffian duct (group III), recorded a specific augmentation in 5α-reductase activity in the corpus and cauda epididymides at peripuberal age. Contrary to these findings in peripuberal and prepuberalrats, adult rats with transient gestational exposure to MMI[2] revealed subnormal epididymal structural development and function. This differential response of 5α-reductase activity in response to gestational-onset hypothyroidism suggests a temporal and age-dependent shift in the response of epididymal 5α-reductaseactivity to gestational-onset hypothyroidism.

The parallel decrease in epididymal 5α-reductase activity and the bioavailability of serum and TIF testosterone observed in experimental rats of the present study during prepuberal and peripuberal age suggests decreased transport of testosterone to the epididymis as the possible reason for subnormal activity of 5α-reductase in the progeny of mothers with gestational-on set hypothyroidism. Earlier studies from our laboratory have shown that hypothyroidism during early pre-natal or neonatal periods results in decreased ABP concentration in serum, TIF and epididymal tissues[25,29,47] suggesting that transport of testosterone from testis to epididymis suffers due to hypothyroidism. The experimental rats of the present study remained in hypothyroid state till peripuberal age, which might have affected the production of ABP, leading to diminished transport of testosterone from testis to epididymis and, therefore, there could have decreased 5α-reductase activity. LH is the known positive regulator of 5α-reductase activity in Leydig cells (Murona & Washburn, 1990). LHR and FSHR are present in epididymis[16]. FSH is a positive regulator of 5α-reductase activity in Sertoli cells since it stimulates both isoforms of 5α-reductase[34]. We found increased titers of LH as well as FSH in all experimental rats of the present study[1] suggesting hypergonadotrophismin the progeny of mothers with gestational-onset hypothyroidism. Transient neonatal hypothyroidism decreased of Lhr concentration in Leydig cells of puberal[29,30] and adult[47] rats. As discussed vide supra gestational exposure to MMI lead to decreased expression of *Lhr* in Leydig cells of postnatal rats[40]. Therefore, modified LH signaling in the epididymis may also be a reason for the changes in 5α-reductase activity, apart from reduced bioavailability of testosterone, the substrate for the enzyme.

Existing information clearly suggests that DHT favors post-testicular sperm maturation by modulating the synthesis and secretion of epididymis-specific proteins and glycoproteins and other bio-molecules including sialic acid, carnitine and GPC[37,39,38]. Therefore, decreased epididymal concentration of carnitine, sialic acid and GPC in post-natal rats with transient gestational-onset hypothyroidism may be attributed to subnormal local production of DHT.

Data on epididymal *Ar* mRNA expression revealed an age- and region-dependent specific temporal differential response to gestational-onset hypothyroidism. *Ar* mRNA expression pattern indicates an age-dependent shift from the subnormal level in prepuberal age to normal/near normal level by peripuberal age in rats with transient gestational-onset hypothyroidism. In our previous report, we showed a general trend of decreased expression of *AR* mRNA in the cauda epididymidis of adult progeny rats with transient gestational-onset hypothyroidism at adult age[2]. Thus, the peripuberal period appears to be a transition point from where the shift in response of *Ar* expression occurs in rats subjected to gestational-onset hypothyroidism. As discussed earlier, the experimental rats remained in severe hypothyroid state during prepuberalage, and marginal at peripuberal age, returning to euthyroid state in the adults. This transition state of thyroid hormone profiles is, probably, reflected in *Ar* gene transcription level in the epididymis as well, despite a consistent decrease in androgens. Thus, thyroid functional state appears to be a major regulator of *Ar* mRNA expression in postnatal rat epididymis, a process which is under the regulatory control of androgens.

Diminution of *Ar* mRNA expression seen throughout the epididymis of prepuberal rats with gestational-onset hypothyroidism is reflected in its protein level and activity too. This may have serious repercussion on the androgen-dependent post-natal development and differentiation of epididymis[44]. During prenatal and neonatal periods, Fetal type Leydig Cells (FLCs) secrete testosterone, which would be taken over by Adult type Leydig Cells (ALCs), subsequently. In rats, this shift in the site of testosterone production from FLCs to ALCs occurs by PND28[43] coinciding with the onset of histological differentiation

of epididymis (Sun & Flickinger, 1979). Testosterone secreted from FLC is essential for masculinization and differentiation of Wolffian duct into the epididymis, vasdeferens and seminal vesicles[44]. This is the reason for a very high level of testosterone seen from late gestational to neonatal periods in rats, which falls sharply thereafter as the FLCs undergo degeneration during the post-natal life[41]. Therefore, subnormal bioavailability of testosterone from birth onwards might be the reason for the consistent decreased expression of *Ar* and its activity in the epididymis of prepuberal experimental rats of the present study. Subnormal expression of *Ar* gene observed in the epididymis of prenatal rats with gestational exposure to MMI may be expected to have delayed/impaired developmental differentiation of epididymis. The decreased organ weight found in hypothyroid rats of the present study and reduced lumen diameter, cell number and size found throughout the epididymis of these rats[1] support this inference.

The happenings in the epididymis of peripuberal rats are not consistent with those of prepuberal rats with gestational-onset hypothyroidism as there was either normal or over-expression of *Ar* gene, as evinced by PCR and western blot data. Nuclear Ar concentration also depicted a temporal spurt in peripuberal rats from the low level in pre-puberal rats with gestational-onset hypothyroidism, On the other hand, its expression level and nuclear concentration decreased in similar rats at adulthood[2]. Thus, there appears to be an age-dependent shift in Ar status in the epididymis of post-natal rats with gestational-onset hypothyroidism, which may be attributed to specific changes in its expression and stability, depending on the thyroid hormone status. Thus, the present study points out that the impact of gestational-onset hypothyroidism on the epididymal Ar status is temporal and there may be a shift in the response of *Ar* gene expression and the ultimate concentration/activity of the receptor protein from the prepuberal to adult age. Thus, the findings favor the hypothesis proposed and we conclude that gestational-onset hypothyroidism exerts an age-dependent divergent effect on 5α-reductase activity and *Ar* gene expression in the epididymis, depending upon the period of gestational exposure to antithyroid drugs and thus, affecting post-testicular sperm maturation events in the epididymis of F1 progeny.

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