The role of thymus graft on the onset of puberty in juvenile male rats

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SUMMARY

There exists a bidirectional relation between the thymus gland and the hypothalamo- hypophysial-gonadal axis. The thymus secretes various hormones, some of which can regulate the reproductive axis. So, it is worth to explore the possible role of an additional thymus on the onset of puberty in male albino rats.

Two groups of male rats aged 5 and 10 days were used for the study. Each subgroup consisted of 4 pups each in control and experimental group. Thymus glands for graft were obtained from newborn male rat pups. Depending on the age group, 5 day or 10-day-old male rat pup were anaesthetized, 2-3 mm incision was made, and a thymus gland was grafted to the axilla. Sham operations were performed to the control pups. Without handling, animals were observed daily for the onset of puberty. On the day of descent of testes, body weight of the animal was noted, blood was collected and used for radio immunoassay. All morphometric measurements were done using an occular micrometer. Volume fraction of seminiferous tubules, intertubular connective tissue of testes, cortex and medulla of the thymus were estimated by point count method.

In both the age groups thymus graft advanced the age of descent of testes, and increased body weight and organ weight. It also increased the serum hormone levels.

In conclusion, this study indicates that an additional thymus gland has a positive effect on the male reproductive system towards pro-gonadal action, and this effect is more pronounced in older-age groups of rats.

Key words: Descent of testes – Hormones – Onset of puberty – Thymus graft

INTRODUCTION

The relation between the thymus gland and the hypothalamo-hypophysial-gonadal axis is bidirectional. Several soluble factors produced by the thymus gland and the cells it regulates can modulate the immune system through reproductive neuroendocrine circuits indirectly.

Many studies have been conducted to evaluate the effects of partially purified thymic extracts in treating various reproductive disorders, as well as changes in gonadal tissue weights. Also, studies have focused on the chemical nature of the factors responsible for regulating reproductive function. The thymus secretes various hormones, some of which can regulate the reproductive axis. The hypothalamic-pituitary function is affected by the

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Submitted: February 19, 2023. **Accepted:** April 23, 2023

https://doi.org/10.52083/SURU3839

peptides derived from the thymus. In programming the hypothalamic-pituitary axis in the prepubertal period in rodents, the thymic hormones may play a vital role (Strich et al., 1985).

Thymosin beta 4, one of the thymosin hormones secreted by the tymus is a potent inducer of Luteinizing hormone-releasing hormone (LHRH) release, which in turn stimulates pituitary Luteinizing hormone (LH) release. Other factors like interleukin-1 (IL-1) have been found to inhibit the release of luteinizing hormone-releasing hormone and luteinizing hormone (LH). Further, it has been also found that IL-1 alters the expression of rat granulosa cells LH receptors. Also, the estrogen and progesterone release are suppressed by certain interferons (Hall et al., 1992). Despite of the many studies on the various aspects of interrelationship between the thymus gland and the reproductive axis, there is a lacuna on the role of an additional thymus gland in the reproductive system.

With the abovementioned facts in mind, the present study is designed to evaluate the role of allogeneic thymus gland graft on the onset of puberty in male juvenile rats.

MATERIALS AND METHODS

Inbred strains of Albino rats bred under controlled lighting using 12 hours of light and 12 hours of dark (12L: 12D) cycle were used as per ethical guidelines.

Animal groups

Two groups of male rats aged 5 and 10 days were used for the study. Each subgroup consisted of 4 pups in the control group and 4 corresponding pups in the experimental group. Newborn male pups were used as donors for allogeneic thymus graft.

Allogeneic thymus graft

Thymus glands for graft were obtained from new-born male rat pups. These pups were anaesthetized by ether, thoracic cavity was opened from sternum, intact thymus gland was removed carefully by dissecting, and then transferred immediately into Tyrode's solution. The entire procedure was carried out under sterile conditions.

Depending on the age group, 5-day-old or 10-day-old male rat pups were anaesthetized: 2-3 mm incision was made in the region of the axilla after sterilizing the region with absolute alcohol. The thymus gland from the Tyrode's solution was blotted on filter paper and grafted to the axilla of the recipient rat pup. The incision was closed by approximating and smearing with solution containing persplex dissolved in chloroform. Animals were monitored carefully and post-operative care was taken during their recovery period from anesthesia. They were placed in separate cages for 1-2 hours to avoid cannibalism, and finally transferred back to the cage with their mother.

Sham operations were performed to the control pups, except for the placement of the thymus gland: the entire procedure was carried out in the same way as with the experimental pups.

Observation on Descent of Testes and Blood collection

Without handling, animals were observed daily for the onset of puberty in male rats which is judged by descent of testes and is a conventionally accepted sign for the event (Relkin, 1971; Satya Prasad, 2019). On the day of the descent of testes, the body weight of the animal was noted andanaesthetized with ether; then blood was collected, and serum was separated and used for radio immunoassay.

Evaluation of graft acceptance

The acceptance of the graft was confirmed by the presence of neovascular network in and around the area of the grafted site in the experimental animals.

Collection of tissues

After collecting the blood, animals were sacrificed as per the international ethical guidelines (for animals); the organs, testes, epididymides and thymus were dissected and weighed. The testes and thymus were fixed in Bouin's fluid for histological studies. Tissues were processed and paraffin blocks were prepared. Sections of 5mm thickness were cut as described by Drury et al. (1967). For every 20 serial sections, only 5 were

Table 1. Effect of thymus graft on descent of testes and body, testes, epididymis, thymus weight and tail length in 5-day-old and 10-day-old rats. Cont, Control; Expt, Experimental; *P<0.05, **P<0.01

	5-day-old		10-day-old	
	Cont	**Expt**	**Cont**	**Expt**
Age on Descent of testes (Day)	30.75±0.48	26.25*±0.48	29.25±0.85	26.50*±0.87
Body weight (g)	43.75±1.03	44.75±1.84	40.00±0.83	44.00*±1.01
Testes weight (mg)	117.00±3.88	136.50*±8.19	129.10±0.77	146.90*±5.57
Epidydimides weight (mg)	19.38±0.38	20.13±0.43	15.75±0.78	19.50*±1.06
Thymus gland weight (mg)	139.00±4.73	146.75±4.73	123.75±5.21	142.75*±3.31
Tail length (cm)	7.88±0.09	8.45**±0.13	7.60±0.07	8.30**±0.12

selected and the remaining was discarded. The sections were later stained with Ehrlich's Haematoxylin and Eosin (Drury et al., 1967).

Histological parameters

For morphometric analysis, the cross section of the tubules that showed clear and well demarcated boundaries was selected. All morphometric measurements were done using an ocular micrometer. The volume fraction of seminiferous tubules, the intertubular connective tissue of testes, the cortex and medulla of the thymus were estimated by point count method (Karapetrovic 1995) using the eyepiece graticule. Further, cortico-medullary volume fraction ratio was calculated.

Radioimmune assay

Radioimmune assay for Luteinizing Hormone (LH) and testosterone was done using the kits procured from the Diagnostic Systems Laboratories, Inc., Texas, USA, while growth hormone (GH) was estimated by using the kit from ICN pharmaceuticals, inc., Costamesa CA 92626.

Statistical analysis

All data were entered, and Student's unpaired t-test was applied to assess the significant differences between the mean of the experimental and of the control group animals for each characteristic under study.

RESULTS

Gross parameters

Thymus graft (TG) advanced the age of descent of testes, which was statistically significant. Body weight in 10-day-old rats showed significant increase in TG compared to control group. TG increased the testes' weight in 5-day-old and 10-day-old rats, and it was statistically significant in both age groups. TG produced an increase in epididymides' weight, which was statistically significant in 10-day-old rats. TG increased the weight of in-situ thymus in both 5 and 10-day-old rats, and it was statistically significant in 10-day-old rats. TG significantly increased the tail length in both age rats (Table 1).

Histological parameters

Testis

TG increased the tubule diameter in both age groups, which was statistically significant. TG resulted in significant increase in tubule volume and significant decrease in intertubular connective tissue volume in both age groups (Table 2) (Fig. 1).

Thymus Gland

The in-situ thymus gland exhibited well-developed cortex and medulla. The in-situ thymus gland's cortical volume in the experimental groups was significantly higher when compared to the control group of rats. Similarly, TG resulted in significant reduction in medulla volume in all the experimental groups. Cortico-medullary volume ratio was significantly higher in all the experimental groups of rats (Table 3) (Fig. 2).

Radioimmune assay

TG had increased higher serum LH levels; it was statistically significant in both age groups (Fig. 3). TG resulted in significant increase in the levels

Table 2. Effect of thymus graft on histomorphometry of testes in 5-day-old and 10-day-old rats. Cont, Control; Expt, Experiment; *P<0.05, **P<0.01, ***P<0.001

	5-day-old		**10-day-old**	
	Cont	**Expt**	**Cont**	**Expt**
Seminiferous tubule diameter (µm)	130.60±4.33	151.90**±1.58	136.0±1.03	146.40**±1.72
Seminiferous tubule volume fraction (mm^3/ mm^3)	0.81±0.01	0.87***±0.01	0.80±0.01	0.86**±0.01
Intertubular connective tissue volume fraction (mm^3/ mm^3)	0.19±0.01	0.13***±0.01	0.20±0.01	0.14*±0.01

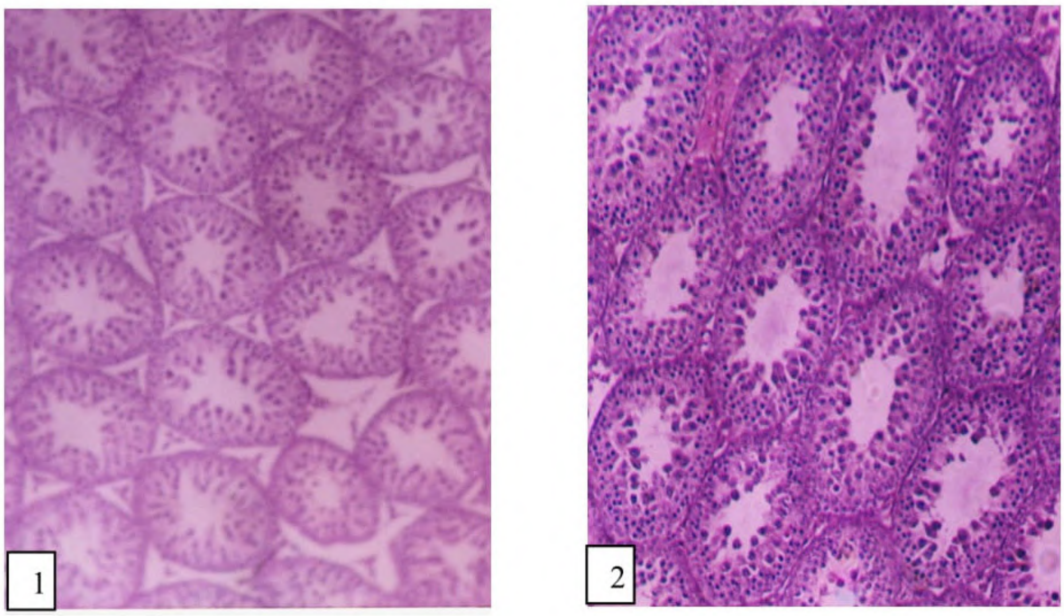

Fig. 1.- (1) Control rat testis showing normal tubule diameter, less compactness of tubules and normal interstitial connective tissue (H&E stain, X 100). **(2)** Experimental rat testis showing increased tubular diameter, compactness of tubules and reduced interstitial connective tissue (H&E stain, X 100).

Table 3. Effect of thymus graft on histomorphometry of in-situ thymus gland in 5-day-old and 10-day-old rats. Cont, Control; Expt, Experiment; *P<0.05

	5-day-old		**10-day-old**	
	Cont	**Expt**	**Cont**	**Expt**
Cortex volume fraction (mm^3/ mm^3)	0.77±0.01	0.85*±0.03	0.79±0.01	0.88*±0.03
Medulla volume fraction (mm^3/ mm^3)	0.23±0.01	0.15*±0.03	0.21±0.01	0.12*±0.02
Cortico-medullary volume fraction ratio (mm^3/ mm^3)	3.31±0.09	6.55*±0.53	3.68±0.20	8.98*±0.41

of testosterone in both age groups (Fig. 4). TG increased serum GH concentration that was found to be statistically significant in 10-day-old rats (Fig. 5).

DISCUSSION

Descent of testes normally occurs around 40 days of postnatal life in male rats (Bergh et al., 1978). The event is considered as the signal for the onset of puberty. The gain in tbody weight of the rat also directs for earlier sexual maturity. The result of the present study indicates that TG was able to advance the onset of puberty in experimental animals. The classical studies of Kennedy and Mitra established that the weight and composition of the body is well associated with the timing of sexual maturation (Kennedy et al., 1963).

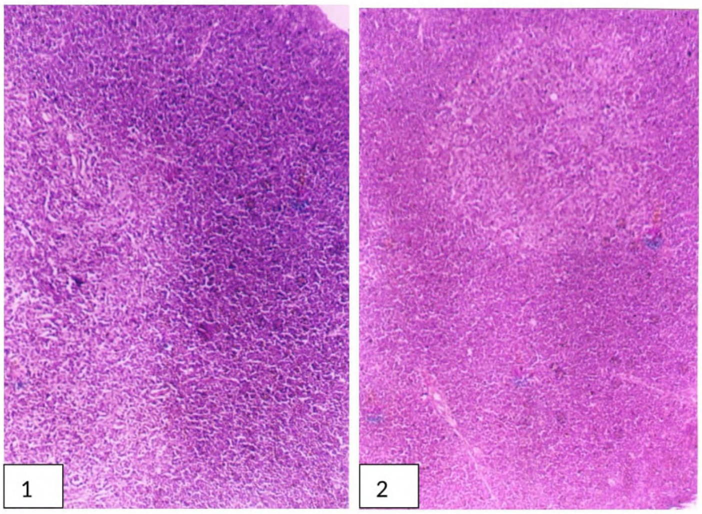

Fig. 2.- (1) In situ thymus gland of control rats (H&E stain, X 125) showing normal central medulla and peripheral cortex. **(2)** In situ thymus gland of experimental rats showing increased, dense, wider cortex and lesser medulla (H&E stain, X 125).

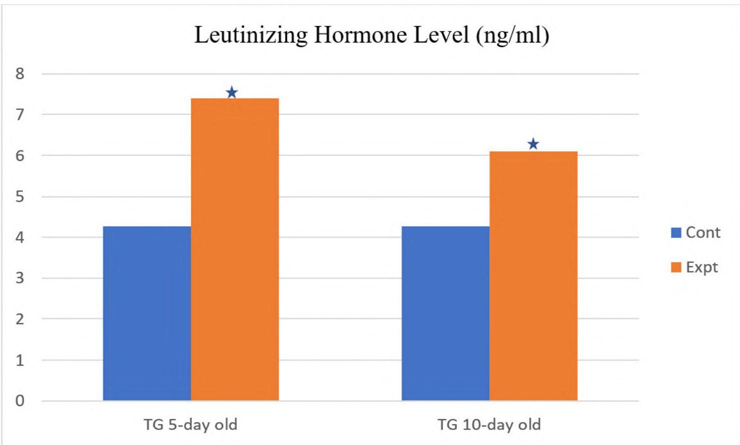

Fig. 3.- Effect of thymus graft on luteinizing hormone in 5-day-old rats and 10-day-old rats. P < 0.05 - *

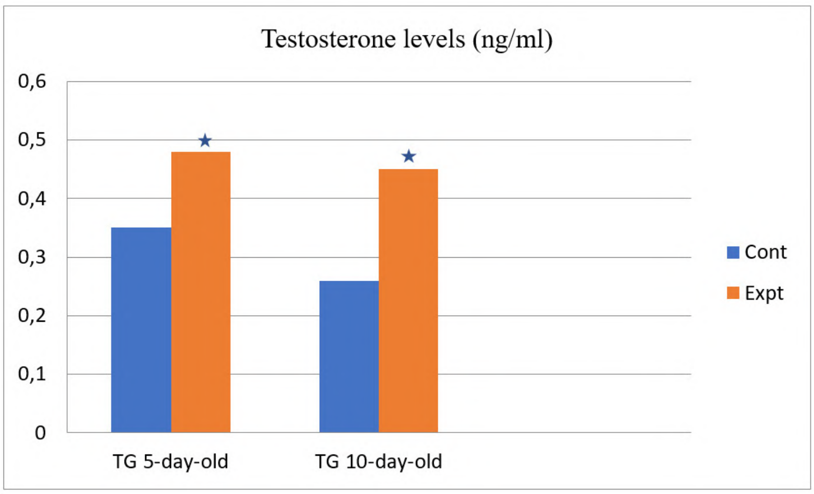

Fig. 4.- Effect of thymus graft on testosterone in 5-day-old rats and 10-day-old rats. P < 0.05 - *

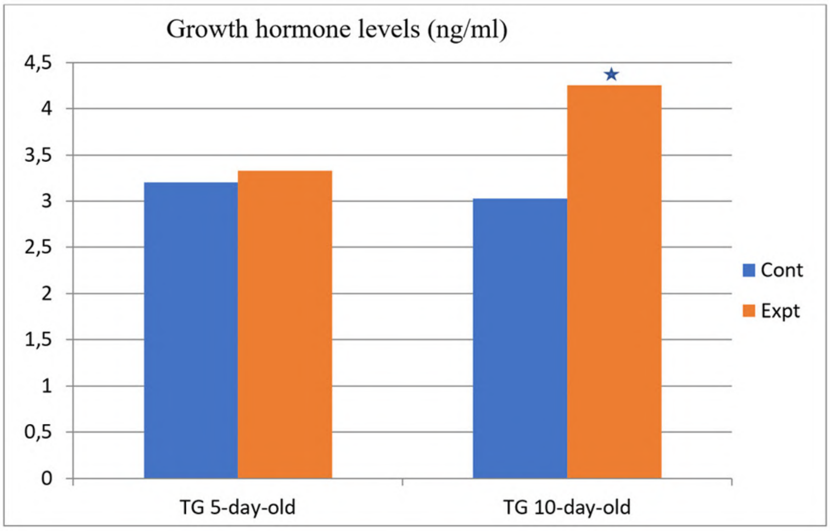

Fig. 5.- Effect of thymus graft on growth hormone in 5-day-old rats and 10-day-old rats. P < 0.05 - *

In the present study, TG increased body weight. Increase in body weight is more evident when the age of descent is taken into consideration, which is earlier compared to the control group. Also, it was found that TG increased tail length significantly, indicating the positive effect and supporting the studies of Kennedy and Mitra (1963). Interestingly, TG increased the testes' weight significantly, thus revealing that TG may be capable of exerting pro-gonadal effect, but the action may be local. TG also increased epidydimes' weight, which was statistically significant in 10-day-old rats, indicating the probable pro-gonadal effect at the individual organ level. TG increased in-situ thymus gland weight; the increased thymus may further exert a stimulatory effect on the hypothalamus and have results in the pro-gonadal effect. But, in the present study, the increase was significant in 10-day-old rats, substantiating the fact that TG was able to advance the onset of puberty by acting individually at the different organ levels, especially more effective in older-age groups.

Histological Parameters

TG resulted in significant increase in tubule diameter in both age groups, which corresponded with the weight of the testes. This may be due to the stimulatory action of TG. The increased tubule diameter of the testes in the experimental rats is indicative of the action of TG on higher centers, especially on the pituitary gland, which through its secretions brings about the changes in the histomorphometric aspects of the testes, which further accounts for the gross changes. The action at the organ level cannot be ruled out with the fact that testicular morphometry depends on the local secretions too. Increased seminiferous tubule volume and decreased intertubular connective tissue volume in corresponding groups supported the increased testes' weight in experimental groups.

The volume fraction of in-situ thymus gland cortex was significantly increased, and medullary volume fraction was decreased in experimental rats when compared to control rats. These changes possibly reflect the increased weight of in-situ thymus. The increased cortical volume fraction observed in the present study may account for the increased activity of the cortical cells, thereby increasing the secretions of the same, which may be responsible for bringing about the pro-gonadal action by acting at the higher centers as well as at the organ level. The results of cortico-medullary volume fraction ratio account for the increased thymus weight in the respective experimental groups. The thymus secretes various hormones, some of which can regulate the reproductive axis. The hypothalamic-pituitary function is affected by the peptides derived from the thymus. In programming the hypothalamic-pituitary axis in the prepubertal period in rodents, the thymic hormones may play a vital role (Strich et al., 1985). Also, there is an influence of age on the release of the active fraction of the thymus. During the neonatal period the inhibitory effect is high, and it is declined as the onset of puberty approaches (Reyes-Esparza et al., 1989). This is substantiated by the results of the present study, which indicate the higher pro-gonadal action in the older-age group of rats.

Radioimmuno Assay

Stimulatory action of TG on LH release goes well with the report of Hall *et al*., who stated that the thymus secretes thymosin beta-4, a potent inducer of LHRH release from the hypothalamus, thus stimulating the pituitary for LH release (Hall et al., 1992). TG had influenced LH levels significantly, which may be due to additional thymosin beta-4 secretion. Correspondingly TG was able to alter the testosterone concentration significantly, which resulted in the advancement of cytoarchitecture of testis, and was probably responsible for the pro-gonadal action when compared with control rats.

TG increased GH levels. The GH concentrations may probably result in the increased secretion of IGF-1. This is hypothesized with the background of the report stating that the deficiency of GH results in a low state of IGF-1, thereby affecting translational efficiency and secretory responses of both the hormones (Jevdjovic et al., 2005). The increase in GH levels seen in the present study may be connected to the increase in body weight and other histological parameters of reproductive organs concerned partially, which was stated to be associated with the sexual maturity as described by Kennedy and Mitra (1963), accounting for the pro-gonadal form of action. It can be concluded from the present study that an additional thymus gland has a positive effect on the male reproductive system towards the advancement of the onset of puberty, indicating probable pro-gonadal effect; and this was more pronounced in older groups of rats.

ACKNOWLEDGEMENTS

The author expresses gratitude to the JIPMER, Pondicherry for the facilities to carry out this work.

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