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Relationship between Testis and Thymus during Postnatal Development in Swiss Mice

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Abstract:

In the present investigation we would like to find out the histological relationship between testis and thymus during postnatal development in Swiss mice, which also, depends on hormonal physiology. To find out the relationship between testis and thymus we use pregnant mice after confirming virginal plug. After parturition the testis and thymus of their pups are removed on 1st, 21st, 35th and 49th day, fixed, embed and sections are prepared for the histological study. Thymus and testis develops parallel until puberty (35th day) means cellular density of both organs is same. After puberty apparent cellular loss in the thymus of male mice is observed. From the present investigation we conclude that testis and thymus develops gradually up to puberty after that there is a retrogressive change in cellular numeral. This type of histological and cellular interrelationship occurs due to the physiological activities of gonadal hormone.

Keywords: Hormonal physiology, postnatal development, puberty, testis, thymus, Swiss mice

1.0 Introduction:

The development of an organism is an important process. The ontogeny of animal is a very complex process due to interrelationship between various organs such as parathyroid-thymus, kidney-adrenal gland and thymus and gonads. Pituitary-hypothalamus coordination regulates the development of all organs. The growth of organs in the albino rat is affected by gonadectomy (Hatai, 1915). The development of gonads also depends upon the follicle stimulating hormone and luteinising hormones which are secreted from anterior part of pituitary. The development of thymus is itself a mysterious process because of participation of gonads and pituitary gland. The thymus develops along with the thyroid and parathyroid, sharing the same pharyngeal pouch origin and migrates caudally. On 15th day thymus migrates into thorax and seperates from thyroid and parathyroid (Suster and Rosai, 1992). The rodent thymus develops from the endoderm of the third pharyngeal pouch and from the mesenchyme of the neural crest which is derived from the third and fourth pharyngeal pouches (Manley, 2000). Postnatally, the thymus increases in size and reaching a maximum size at around 10th week. Following sexual maturation, the thymus begins to shrink, and its weight and volume gradually decrease thereafter (Awaya and Oda, 1965; Bellamy *et al.,* 1976). Thymus is a main lymphoid organ where T-lymphocytes got there identification for proper functioning. The earliest known reference to the thymus is attributed to Rufus of Ephesus circa 100 AD. Thymus is one of the most mysterious organs in a mammalian animal body. The word thymus originates from the Latin derivation of the Greek word thymos, due to its resemblance with the flowers of the thyme plant (*thymus serpyllum*) (Jacobs *et al.,* 1999). The term thymus was introduced in human anatomy by Claudius Galenos of Pergamon in the second century AD (Kachlik *et al.,* 2008). Australian physician Jacques Miller recognized the function of thymus as a designer of immune system (Miller, 1961; Miller, 2002; Miller, 2004).

The reproductive organs are developed from the intermediate mesoderm. The permanent organs of the adult are preceded by a set of structures which are purely embryonic because almost the ducts disappear entirely before the end of fetal life, but there may be few exceptions. These embryonic structures are the Wolffian and Müllerian ducts, also known as mesonephric and paramesonephric ducts, respectively. The Wolffian duct remains as the duct in males, and the Müllerian as that of the female. The periphery of the testes is converted into the tunica albuginea. Cords of the central mass run together and form a network which

becomes the rete testis and another network develops in the seminiferous tubules. Via the rete testis, the seminiferous tubules connected with outgrowths from the mesonephros, which form the efferent ducts of the testis. Post-natal development of the testis depends on the action of the gonadotrophins (FSH and LH) which is secreted by the pituitary gland. FSH acts directly on the Sertoli cells while LH acts on the Leydig cells which secrete the androgen hormone. This androgen then acts on all cells expressing the androgen receptor (AR) in the testis (Willems *et al.,* 2010; De Gendt *et al.,* 2004; O'Shaughnessy *et al.,* 2010; O'Shaughnessy *et al.,* 2012). Due to this developmental complexity the present research paper is focused to evaluate the histological relationship between developing testis and thymus of Swiss mice.

2.0 Materials and Methods:

The proposed experiments were conducted in the Environmental and Developmental Toxicology Research Laboratory, Department of Zoology, University College of Science, Mohanlal Sukhadia University, Udaipur, Rajasthan, India to observe the histological relationship between developing testis and thymus of Swiss mice.

2.1 Animals

Healthy adult female Swiss mice 8-10 weeks old and 30gm average body weight were used for this study. Animals were obtained from the animal house of our department. Male and female mice in the 1:4 ratios were kept in the cages for mating. Female mice were examined every day in the morning and female showing vaginal plug were isolated and their gestation period were recorded. Presence of spermatozoa in the vagina the following morning was considered day one of gestation. Confirmed pregnant females were housed in polyvinyl chloride cages (270×220×140mm) wrapped with rice husk bedding, and maintained under standard laboratory conditions. The laboratory animals were kept in well ventilated animal room with relative humidity of 70-80%. The room lighting consisted of alternate 12 hours light and dark periods. The animals had free access to food (Amrut R & M Pallet purchased from Pranav Agro Industries Ltd. Plot No. 19, 20, Virat Estate, Near Samrat Petrol Pump, National Highway No. 8, Waghodia Chokadi, Vadodara, Gujarat, India) and water *ad labitum*. The maintenance and handling of the animals were done as per the guidelines of Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University (No. CS/Res/07/759).

2.2 Experimental Protocol

Females showing veginal plug were separated and their gestation period were recorded. After parturition the thymus and testis of their pups were removed on 1^{st}, 21^{st}, 35^{th} and 49^{th} day and these were subsequently fixed in Bouin's solution for 24 hours. Tissue transferred to 70% alcohol for prolonged washing to remove excess of picric acid. Tissues were dehydrated by treating with a series of different grades of alcohol, cleared in xylene and embedded in paraffin wax following routine procedure of block preparation according to Carleton *et al.,* (1967) method. After wax impregnation, the solid blocks of paraffin wax containing the tissues were prepared using Leuckhart's L pieces, placed on a metal plate serving as the base of the mould. The paraffin blocks were trimmed and mounted on the block holder. Routine 6 µ thick sections were cut with a rotator microtome and fixed on clear and albumenized slides. These slides containing sections were stained with haematoxylin and eosin. Appropriate sections were observed under the microscope. Photomicrographs of the desired section were obtained using digital research photographic microscope.

3.0 Result and Discussion:

3.1 Thymus and testis on 1^{st} day

On 1^{st} day well-developed cortical region covered with well-formed capsule is apparent. Shape, size and distribution of cells are appropriate. There are some differences in the distribution of epithelial cell in the regions of thymus. Some regions have denser cells in comparisons to others. The outer and denser region is cortex and inner and less dense region is known as medulla. There are no major differences found in the both regions on day 1^{st} (Fig. 1a and 1b). According to the microscopic and gross histological findings of Plećas-Solarović *et al.,* (2006) age dependent thymic changes in male rats of Wistar strain are similar with human thymus during aging. While in case of testis well-developed seminiferous tubules and regular distribution of cell types are apparent. Intertubular space present between the seminiferous tubules. Different developing stages of gonocytes are observed in the seminiferous tubules at the time of birth. Several gonocytes are present in irregular manner in centre of the tubule.

Sertoli cell precursors are found at the periphery of the tubules. Sertoli cells and spermatogonial cells are located near the basement membrane of the seminiferous tubules. Spermatogonial cells are arranged in layers which are further differentiating into another type of spermatogonia. At the periphery the cells present in larger number but smaller in size. While in the centre the number of cells is less and larger in size, it may be the precursor of sertoli cells (Fig. 1A).

In the mouse, spermatogenesis begins immediately after birth. Prospermatogonia is the only germ cell in the mouse testis which is located in the center of the seminiferous tubule (Nagano and Brinster, 1998; Shinohara *et al.,* 2001). These cells migrate to the basement membrane on 6th day of postpartum and produce undifferentiated type A spermatogonia, which begin to differentiate in a stepwise manner (McCarrey, 1993). Spermatozoa are not found in the seminiferous tubules until 35th days postpartum. During this period significant change occurs in the testicular microenvironment. Essential support for successful spermatogenesis is provided by the sertoli cells. These cells are the major somatic cells in the seminiferous tubules. Soon after birth, immature Sertoli cells begin to proliferate and continue to divide until 10th–12th days Postpartum (De Kretser and Kerr, 1994). The two gonadotropins, LH and FSH, have a key role in the differentiation and maturation of mammalian sexual organs and functions (Zhang *et al.,* 2001). Vergouwen *et al.* (1993) suggested that the numbers of spermatogonia A and sertoli cells mostly increase between 3rd to 25th day and the numbers of leydig cells increases from 11th day to 31st day in the testis.

3.2 Thymus and testis on 21st day

On day 21st cortical and medullary regions are clearly seen. The cortex contains cortical thymic epithelial cells and precursor of lymphocyte. The medulla has abundant medullary thymic epithelial cells, thymocytes and lymphocytes of medium and large size (Fig. 1c and 1d). On the same day in testis well-developed seminiferous tubules are visible. Basement membrane of seminiferous tubules is in proper manner. Different developing stages of germ cells are observed like spermatogonia, intermediate spermatogonia, primary spermatocytes and secondary spermatocytes. Sertoli cells are also visible near the basement membrane of seminiferous tubule. Lumen of the tubule is not clearly visible on 21st day of postnatal development (Fig. 1B). Vergouwen *et al.,* (1993) demonstrated that the proliferarive activites of sertoli cells was high at birth and decrease up to 17th day. Leydig cells show low proliferation activities during 21 days after birth. Hardy *et al.* (1989) evaluated the different type of cells in testis during postnatal development. The number of Leydig cells per testis was found to be low during the first three weeks after birth and then increase rapidly. The results of vergouwen *et al.* (1993) indicate that the spermatogonia A and sertoli cells reach their adult population size within the first three weeks after birth.

3.3 Thymus and testis on 35th day

At the time of puberty (35th day) the sections of thymus shows infra structurally well-developed compartment of thymus. At this time cortical and medullary epithelial cells got their maximum number, largest size and appropriate shape. There is slight difference in the number of cortical and medullary epithelial cells (cortical region have more density of cells). Different stages of developing T-lymphocytes in cortical and medullary region are also found (Fig. 1e and 1f). In case of testis basement membrane of seminiferous tubule is clearly visible on 35th day of postnatal development. Interstitial cells (leydig cells) are visible just outside the basement membrane. Spermatogonia, primary spematocyte, secondary spermatocytes and spermatids are clearly visible in well developed manner. Primary spermatocytes are characterized by a large, spheroidal or elliptical nucleus with fine moderately condensed chromatin but secondary spermatocytes have a well stained nucleus. Less number of sertoli cells is present in comparison to other cells. Lumen is clearly seen in the centre of tubule (Fig. 1C). Study of Khattab (2007) showed the normal structure of control testis which is completely covered by a thick capsule and tunica albuginea. The structural components of the testis are the seminiferous tubules and interstitial tissues. The seminiferous tubules have two types of cells, the Sertoli cells which are found near the basement membrane and the spermatogenic cells. Spermatogonic cells are the spermatogonia, primary and secondary spermatocytes; spermatoids and finally mature spermatozoa. Same author also reported that the 5 week old testis shows A and B types spermatogonia, sertoli cell and primary spermatocytes.

Vergouwen *et al.* (1993) reported that spermatogenesis had proceeded up to the pachytene spermatocyte on 18th day and some round spermatids could be seen in the testis at day 21st. At days 28th and 31st, elongated spermatids were seen.

Spermatogenesis was completed on 35th day. Thereafter, the gain in testis weight decreased slowly as only the developing interstitial cell population remained to increase testis weight. Erdost *et al.,* (2009) demonstrated that the wall of seminiferous tubules in 35th day old testis contains the spermatogenic cell lines. First cell line has the spermatogonia and sertoli cells. The second cell line has the spermatocytes and round spermatids. In the 35 day-old, the wall of the seminiferous tubules contains spermatogenic cell lines. Spermatozoa are not found in the seminiferous tubules until 35th day's postpartum (Shinohara *et al.,* 2001).

Plate 1

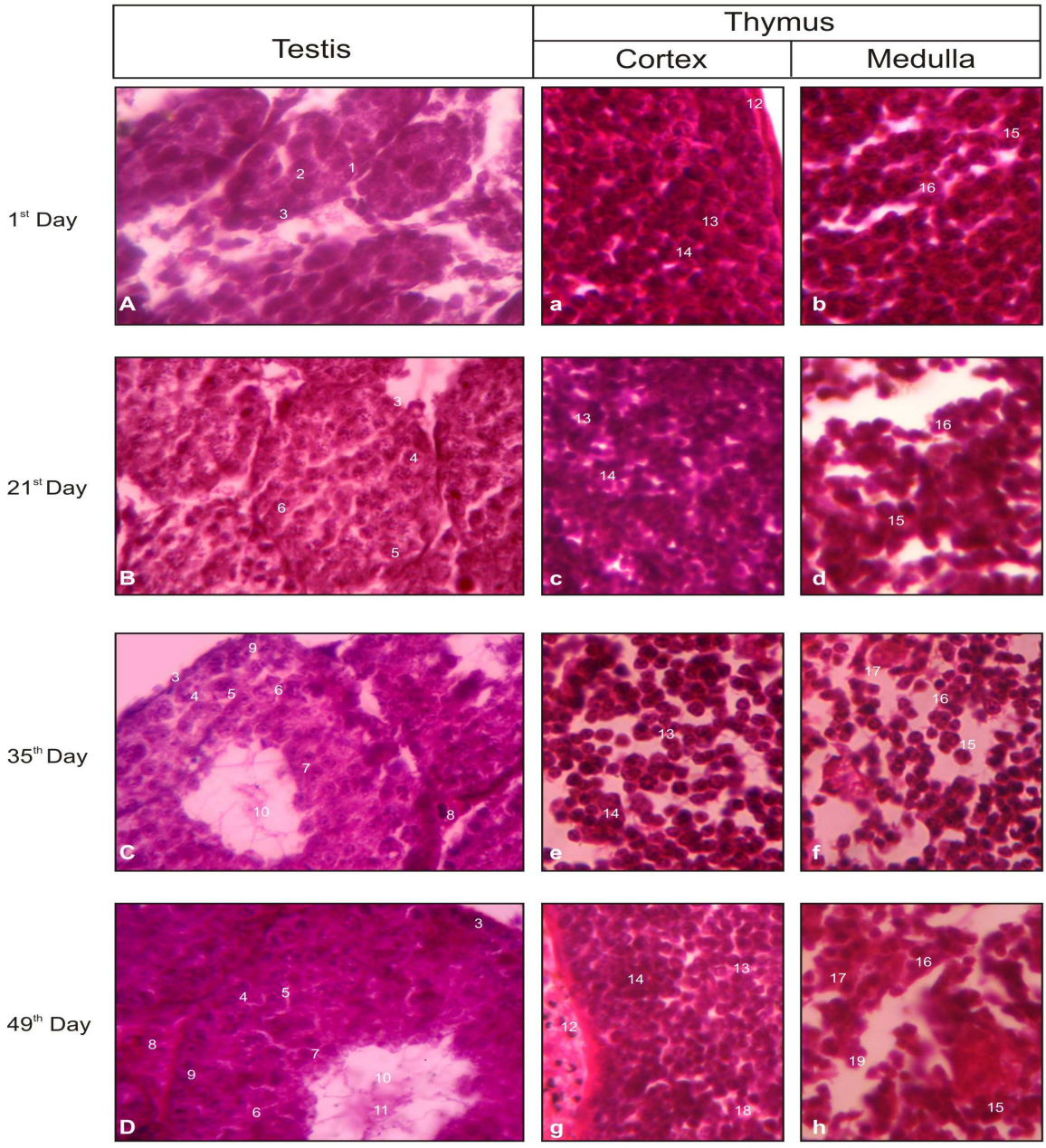

Fig. 1: Show the histology of testis and thymus on different developing days. Fig. A, B, C and D shows the histology of testis on 1st, 21st, 35th and 49th day. Fig. a, b, c, d, e, f, g, h, shows the histology of thymus on respective days. In this plate 1- Precursor of gonocyte, 2- Precursor of sertoli cell, 3- Basement membrane, 4- Primary spermatocyte, 5- Secondary spermatocyte, 6- Sertoli cell, 7- Spermatids, 8- leydig cell, 9- spermatogonia, 10- Luman, 11- Sperm, 12- Capsule, 13- Cortical thymocyte, 14- Cortical epithelial cell, 15- Medullary thymocyte, 16- Medullary epithelial cell, 17- Hassall's corpuscles, 18- Modified cortical epithelial cell, 19- Modified medullary epithelial cell.

3.4 Thymus and testis on 49th day

At the termination of experiment (on 49th day) demarcation of cortex and medulla starts disappearing, which had seen on 35th day. Now medullary region have more cells in comparison to 35th day medulla and cortex have less number of cells in comparison to 35th day cortex. Enlarged cells are found in both regions with lesser numbers as compared with 35th day thymus. Different developing T- lymphocytes are present but immature and apoptotic cells are also found (Fig 1g and 1h). On the other hand in testis well-developed seminiferous tubules are visible which covered by basement membrane. Leydig cells are present in intertubuler space (space between seminiferous tubules). Spermatogonia primary spermatocyte, secondary spermatocyte and spermatids are clearly visible in the tubule. Sperms are present in lumen. Sertoli cells are visible in between the different developing stages of spermatogenesis (Fig. 1D). Study of Shioya *et al.,* (2000) demonstrated that castration induced hypertrophic changes in the thymus which was observed only in rats over 31st day of age. Because the immature testis unable to secrete a sufficient amount of androgen to inhibit the growth of thymus. Weight gain of thymus continues under inhibitory regulation by the testis after 36th day.

Studies of Patil and patil (2011) show that cross section of testis of control mice on 49th day showing spermatogonia, spermatocytes, spermatozoa and healthy Leydig cells. Studies of Erdost *et al.* (2009) also demonstrated that seminiferous tubules on 50th day has a cells stage composed of spematogonia, spermatocytes and two types of spermatids (elongated and rounded). Spermatozoa are also seen in the lumen of seminiferous tubules. Development of testis is also studied by Garu *et al.* (2011), Sharma *et al.* (2012) and Sharma and Garu (2013) in Swiss mice, there they also observed the same pattern of postnatal development observed previously by other authors According to Pejcić-Karapetrović *et al.* (2001) the gonadectomy of adult rats indicates sex and age dependent changes in the modulatory role of gonadal hormones in regulation of T-cell maturation, and possibly immune response. They also show that gonadectomy, in both sexes' rats, influences, but not abolish the sexual dimorphism in thymus size and cellularity, as well as that in thymocyte subsets composition.

A direct or indirect relationship between the pituitary gland and the thymus has been also supported by the observation that the thymus atrophies following injection of anti-pituitary serum into young mice (Pierpaoli and Sorkin, 1967a). Pierpaoli and Sorkin (1967b) postulated that hypophysis-thymus axis regulates the sexual maturation in the development of the peripheral lymphatic tissue, possible body growth and differentiation.

The thymus gland of male mice is enlarged under conditions of androgen deficiency or in mice with altered androgen action because the function and anatomy of thymus are very sensitive to androgens (Olsen *et al.,* 1991a; Olsen *et al.,* 1991b). The thymus develops gradually parallel to other organs till puberty but after puberty it goes to dramatic changes known as thymic involution. In histological view involution is reduction in the size, decrease cortical lymphocyte, irregular cortex, increase in tangile body macrophages and demarcation of cortico medullary zone. The thymus body ratio is highest during perinatal period but the organ continues to increase in absolute size until about puberty, after which it tends to gradually decline. Olsen *et al.* (200) also reported that androgen receptor in the thyme epithelium modulate the thymus size and thymocyte development. According to Kuper *et al.,* (2002) when there is a decrease in thymic size and cellularity one should use the term "reduced number of cortical lymphocyte and increased number of macrophages". These changes may be identified as "atrophy or involution". After puberty dramatic retrogressive changes occurs in size and cellularity of the thymus known as thymic involution or age associated thymic atrophy (Sharma and kantwa, 2011).

4.0 Conclusion:

During postnatal development there is a direct relationship between the testis and thymus in Swiss mice which depends on hormonal status of the organism. Postnatal development of testis depends on gonadotrophins, and the concentration of these gonadotrophins fluctuates during various developmental stages. Gonadotrophin regulates the concentration of androgens secreted by Leydigs cells. The cellular components of thymus are very sensitive to the concentration of these androgens. In the present investigation we also observed the parallel development of testis and thymus up to 35th day of postnatal development. A gradual regression in the thymus after 35th day is noted when testis approaching maturity. We concluded that at the time of birth when the levels of gonadotropins are present in low concentration the thymus is in

normal condition but when the levels of gonadotropins are high at 35th day the thymus starts regressing. The present findings are in accordance with the above hypothesis approved for the integrated development of thymus and testis.

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