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Relationship between Developing Ovary and Thymus in Swiss Mice

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Abstract:

In the present investigation the relationship between developing ovary and thymus in Swiss mice was studied. To find out the relationship between ovary and thymus pregnant mice after confirming virginal plug were used. After parturition the ovary and thymus of their pups were removed on 1st, 21st, 35th and 49th day, fixed, embed and sections were prepared for the histological study. Thymus and ovary develops parallel until puberty (30th day) means cellular density of both organs is same. After puberty apparent cellular loss in the thymus of female mice is observed. While in case of males the inception of puberty takes place after 35th day of postnatal life. From the present investigation we conclude that ovary and thymus develops gradually up to puberty after that there is a retrogressive change in cellular numeral. The levels of gonadotropins are responsible for these changes; therefor onset of puberty in females occurs earlier in comparison to males. In the same species as the sexual maturity occurs on different postnatal days so the process of thymic involution is also takes place accordingly.

Keywords: Ovary, Thymus, Puberty, Female Mice, Pups

1.0 Introduction:

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 development of gonads also depends upon the follicle stimulating hormone and luteinising hormones which are secreted from anterior part of pituitary. In mice both gonads develops from paired genital ridges which are arises at the ventro-medial surface of the mesonephroi nearly 10th day of gestation. Shortly after the expression of male determining gene (sex determining region of chromosome Y) is initiated in the XY gonad, genital ridges differentiate into tests (Koopman *et al.,* 1991; Sekido *et al.,* 2004). If *Sry* (sex determining region of chromosome Y) is absent or fails to act in time, the indifferent genital ridges differentiates into an ovary, which is driven by a different gene expression program (Chen *et al.,* 2012).

Hypothalamus–hypophyseal system, GnRH and GnRHR mRNA is also extensively expressed in the thymus, the spleen and in peripheral blood lymphocytes, which are identical to that of the hypothalamus (Azad *et al.,* 1993; Chen *et al.,* 2002; Tanriverdi *et al.,* 2004; Izvol'skaia *et al.,* 2010; Marchetti *et al.,* 1989; Standaert *et al.,* 1992). Therefore, GnRH appears to not only have a central effect in the process of reproduction, but is also involved in the regulation of the immune response. The presence of GnRH and its receptors in the thymus suggests a physiological role for intrathymically produced GnRH in the regulation of immune function in an autocrine or paracrine manner (Min *et al.,* 2009; Tanriverdi *et al.,* 2005). The suppressive influence of sex steroids on the thymus is a result of their direct binding to receptors in thymoctyes (Hince *et al.,* 2008; Li and McMurray, 2006; Olsen *et al.,* 2001). However, many studies show that GnRH can regulate involution of the thymus through its mediation of the immune effect of sex steroids (Ataya *et al.,* 1989; Jacobson and Ansari, 2004). Thus, there may be two ways by which GnRH regulates involution of the thymus: via GnRH and GnRHR, as well as via GnRH-LH/FSH-sex steroid–sex steroid receptor signaling pathways (Calder *et al.,* 2011), thereby altering hormone and receptor signals pathways to affect reproductive hormone immune function (Su *et al.,* 2013).

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). 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). There for in the present study we want to find out the relationship between developing ovary and thymus on the 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. The present study was focused to evaluate the histological relationship between developing ovary and thymus to compare it with development of male gonads in Swiss albino mice. The main target of the present study was to find out that levels of gonadotropins affect the development of thymus in specific sex or not?

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 ratio 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 ovary and thymus of their pups were removed on 1st, 21st, 35th and 49th 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 6µ 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 Results and Discussion:

3.1 Ovary and Thymus on 1st Day:

At the time of birth ovary is compact organ covered by surface epithelium. Ovary has only two types of cells, oocytes and stroma cells. Differentiation between cortex and medulla is not very apparent as both types of cells are distributed through the ovary (Fig.1A). On the other hand thymus is also compact with epithelial cells. Cortical and medullary regions are easily identified. Cortical region (Fig. 1a) is denser than medullary region (Fig. 1b).

Development of ovary from birth to puberty is a complex process. It develops from simple organ to highly differentiate multiform organ. At the time of birth only two types of cells are found in ovary, oocytes and stroma cells. After it stroma cells differentiate into granulosa cells, theca cells and lutein cells. In this time interval ovary does not grow only in size but also forms complicated bodies like follicles (Peters, 1969). At the time of birth the ovary is filled with germ cells and stroma which are covered by the surface epithelium.

Surface epithelium does not contribute in the formation of germ cells (Franchi *et al.,* 1962).
Cui *et al.* (2004) reported that bipotential gonad arises in the mouse from the coelomic epithelium of the urogenital ridges, and differentiation of a functional ovary is a complex process (Tevosian *et al.,* 2002). Morale *et al.* (2013) confirm the relationship between hypothalamus-pituitary-gonadal axis and thymus development. They also demonstrated that LHRH involved in the maturation of thymus and development of cell mediated immune response. So it was confirm that LHRH plays a bidirectional role in the programming of neuroendocrine and immune functions. Grossman (1985) also reported that immune system was regulated by the gonadal steroids. Such interactions were mediated through hypothalamic-pituitary-gonadal-thymic axis.

3.2 Ovary and Thymus on 21st Day:

At the end of lactation (on 21st day) the ovary of mice shows the growing and large oocytes in developing follicles. Most of cortex is occupied by developing follicles so cortex and medulla of ovary can be easily identified. Primary follicles with granulosa cells, secondary and tertiary follicles with zona pellucida are visible. At this stage ovary is completely developed but ovulation does not occurs (Fig. 1B). On the other side thymus is also develops gradually with ovary. Cortical and medullary epithelial cells and developing T-lymphocytes are also visible in both regions of the thymus. Numbers of cells are higher in cortex than medulla (Fig. 1c and 1d). Most of the changes in the ovary take place during the 3rd and 4th weeks of life. Most characteristics morphological changes occur in the centre of the organ (Peters, 1969). Similar to these findings it is also observed in the present study that as ovary gradually develops after birth the developing follicles confined to the cortical region. Medullary region of ovary contain stromal cells in between plenty of blood vessels. Jones and Krohn, (1961a and b) and Peters and Levy (1964) reported about the 3rd and 4th week ovary. They observed that outer cortex contains the small oocytes in type 2 or 3a follicles. They also found the growing and large oocytes in the developing follicles which are occupy the greatest part of the ovarian cortex. Follicle development is continuous process so small, medium and large size follicles are seen in the cortex of ovary.

Morris and Sass (1966) describe about the central part of the juvenile ovary which contains the blood capillaries and vessels of irregular shapes and dilatations, which might belongs to lymphatic system of the ovary. Lindner *et al.* (1964) and Wenzel (1966) describe the role of lymphatic vessels in the transport of estrogens. Richards and Pangas (2010) describe the classical view of ovarian follicle development. They suggested that development of ovarian follicles is regulated by the hypothalamic-pituitary-ovarian axis. In this mechanism gonadotropin releasing hormone which is secreted from the hypothalamus, regulates the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH which are secretes from pituitary, regulates the formation and secretion of ovarian steroids. Concentration of these ovarian steroids further controls the development of thymus and maturation of T-lymphocytes.

3.3 Ovary and Thymus on 35th Day:

At time of puberty fully developed ovary is observed. Cortex of ovary is covered by germinal epithelium. Various follicles are observed which are in different developing stages. Medulla of ovary is also in normal state (Fig. 1C). At the same time in the thymus epithelial cells are visible in cortex (Fig.1e) and medulla (Fig. 1f) but some of them are with irregular cellular boundaries (arrow head). In our previous study on relationship between developing testis and thymus in Swiss mice (Sharma *et al.,* 2014) we observed that sexual maturity in male mice takes place after 35th day of postnatal development. While in the present study it is observed that sexual maturity in female mice takes place before 35th day. On the basis of these finding it can be accomplished that sexual maturity in male and female mice depends on the internal hormonal environment of the organism. Study of DeFalco and Capel (2009) on different vertebrate spices also showed that there is a significant amount of plasticity in the adult gonads that can be influenced by hormones. According to Alten and Groscurth (1975) more atretic follicles are found in the homozygous females than those of their heterozygous littermates. They also reported 5th-7th week's old female's ovaries contain few corpora lutea. These results indicate that ovulation occurs before 35th day of postnatal development. Thus in homozygous nude females delay in the onset of sexual maturity is observed due to the less number of corpora lutea therefor levels of ovarian gonadotropins are also reduced so the retrogressive changes in thymus are also delayed.

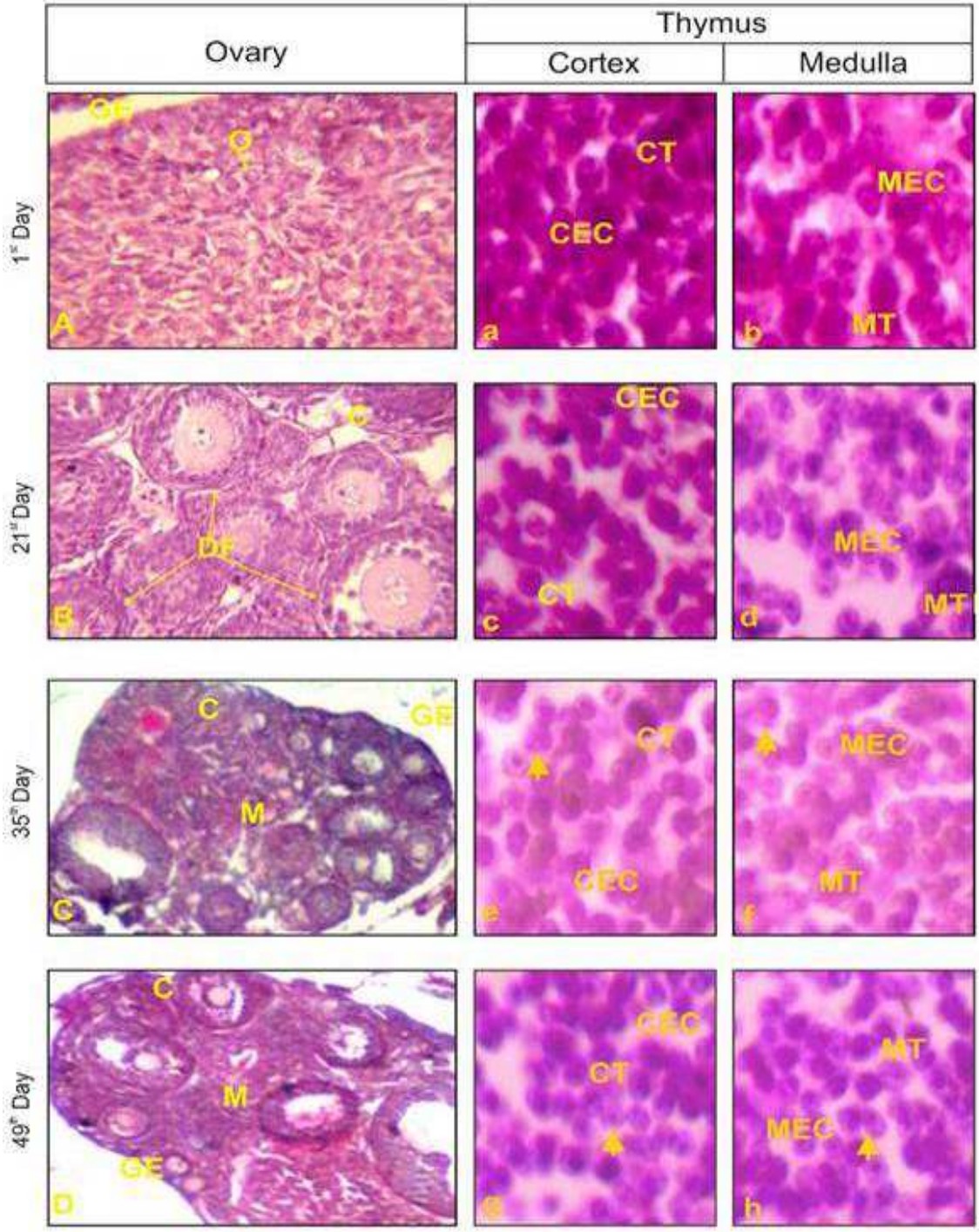

Fig. 1: GE- Germinal Epithelium, O- Oocyte, C- Cortex, M- Medulla, DF- Developing Follicles, CEC - Cortical epithelial Cells, CT - Cortical thymocyte, MEC - Medullary epithelial cells, MT - Medullary epithelial Cells, Arrow Head- Cells with irregular cellular boundaries.

3.4 Ovary and Thymus on 49th Day:

At the termination of experiment (on 49th day) well-developed ovary shows the normal structure and distribution pattern of various follicles. Germinal epithelium, cortex and medulla are observed along with corpus luteum (Fig. 1D). At the periphery of ovary primordial follicles are visible and primary, secondary and tertiary follicles are inserted in the inner side of the cortex. On the other hand in case of thymus degenerated epithelial cells (arrow head) are observed in cortex (Fig. 1g) and medulla (Fig. 1h) with normal cellular architecture. T-lymphocytes with disintegrated cellular boundaries are visible. Alten and groseurth

(1975) reported that 6^{th}-7^{th} week old ovaries of nude mice consist of primary, secondary and tertiary follicles. They also suggested that degenerating corpora lutea are found in the deeper layer of ovary. Levels of gonadotropins are high after sexual maturity so the degeneration of thymus occurs and the maturation of T-lymphocytes are also affected. Similar type of phenomenon is also reported by Sharma *et al.* (2014) in male mice but in case of females onset of puberty occurs earlier so this kind of degeneration occurs earlier in females.

4.0 Conclusions:

There is an inverse relationship between the cellularity of thymus and gonads in developing mice. As the ovary develops after birth there is progression in the development of various types of follicles and clear cut demarcation in cortical and medullary regions. With the advancement of maturity in ovary there is regression in the thymus which is known as the thymic involution. In the ovary and thymus histological and cellular interrelationship occurs due to the physiological activities of gonadal hormones. The concentration of gonadotropins play pivotal role in the histological modification of the thymus and ovary. Comparing the two gonads the onset of female maturity transpires earlier in females.

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