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The Effect of Tamoxifen on the Neonatal Development of Rat Penis

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Abstract: In rats penis development a seen two stages in prenatal life, the second stage is androgen hormone dependent chondrocyte and osteocyte differentiation period. To investigate Tamoxifane (Tx) administration on the developmental anomalies of penis. The newborn male rats were injected with 100 µg Tamoxifen subcutaneously from the day of birth to fifth day. This changes in Os penis and glans penis were investigated after the rats had been sacrificed and their penis were removed on the days 14.0, 21.0, 28.0, 35.0 and 60.0 Having been fixed in neutral formaline of 10%, the totally taken penis werembedded into paraffin blocks. The obtained paraffin sections were stained with Hematoxylene - Eosin, Verhoeff and Tripple. The structural changes in development of Os penis and glans penis were investigated.

In our study, while formation of hyaline cartilage, bone marrow and trabecula in proximal segment of Os penis was observed on the day 7.0 in the control group, a fibrocartilage tissue in distal segment of rats within control group was seen on days 28.0 - 60.0. In rats injected with Tx, it was observed that the hemapoetic tissue disappeared in the fourth week and the hylani ecartilage disappeared on the 60th day. It was also noticed that epidermal spindles in glans penis of rats injected with Tx as from the day 21.0 were gradually affected whereas epidermal spines and keratinization disappeared on the day 60.0.

As result, we identified the blockade of the development of fibrocartilagoneus tissue and hyaline cartilage in Os penis and also the blockade of the maturation of epidermal tips in rats.

Key Words: Tamoxifen, Os penis, Neonatal rat.

Introduction

There are several studies in the literature concerning the development of Os penis in rats and mice (1-5).

In adult rats, the penis is approximately 5 cm in length from symphysis pubis whereas Os penis is 1 cm in length. Two parts can be identified immature Os penis (Figure 1).

The tip of proximal (P- segment) is in membranous bone structure which is covered by hyaline cartilage. Distal segment (D - segment) is a fibrocartilage tissue. In the beginning of postnatal period, ossification could be observed in the proximal segment, whereas it could be observed later in the distal segment. Fibrocartilage structure gradually changes to bone structure in the distal segment (3, 6).

In rats, penis development is seen in two stages in prenatal life. In first stage, dense mesenchymal tissues occur irrespectively from androgen hormone in both sexes. However, chondrocyte differentiation is under the control of androgen hormone in the second stage (7). Procession of chondrogenesis and osteogenesis in Os penis and maturation of erectile tissue in corpus cavernosum basically depend on androgen hormones (1).

According to Murakami and Mizuno (1989), the rat penis is formed with cavernosum penis proximal segment and distal segment sequentially (Figure 1).

Corpus cavernosum penis is a trabecular and lacuner erectile tissue and is observed after the first week of postpartum. However, proximal segment is formed by fusion of hyaline cartilage and membranous bone (Enchondral ossification). Fibrocartilage distal segment is observed after the fourth week of postpartum and ossification starts in the tenth week (2).

In adult rats, formation and thicking of the epidermal tips which cover the glans penis start after the tenth day of postpartum. There are similarities between these epidermal tips shows dense keratinization (3). Tamoxifen

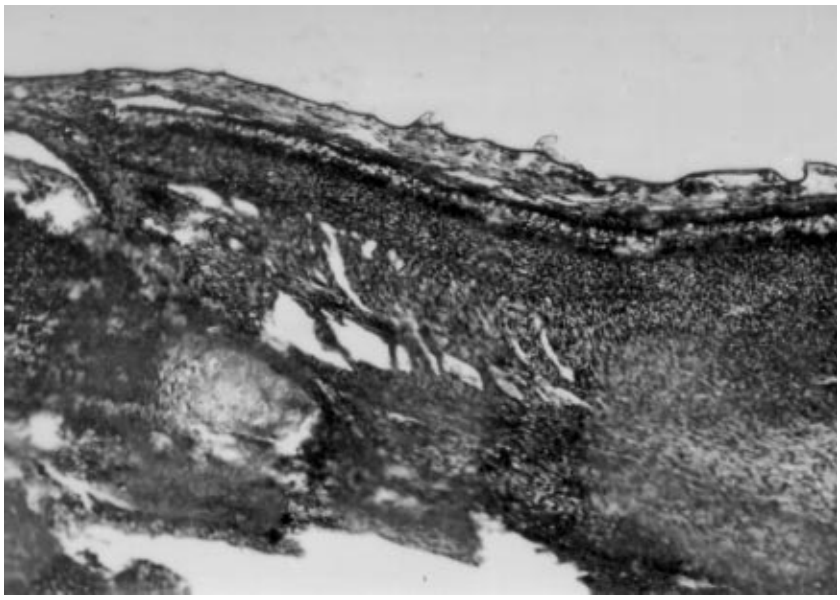

Figure 1. Increased heamopoietic tissue with enlargement of lacuna in the proximal segment (light arrow) and development of epidermal dips (dark at arrow) in the First control group (longitudinal section, Masson triple, original magnification X 16).

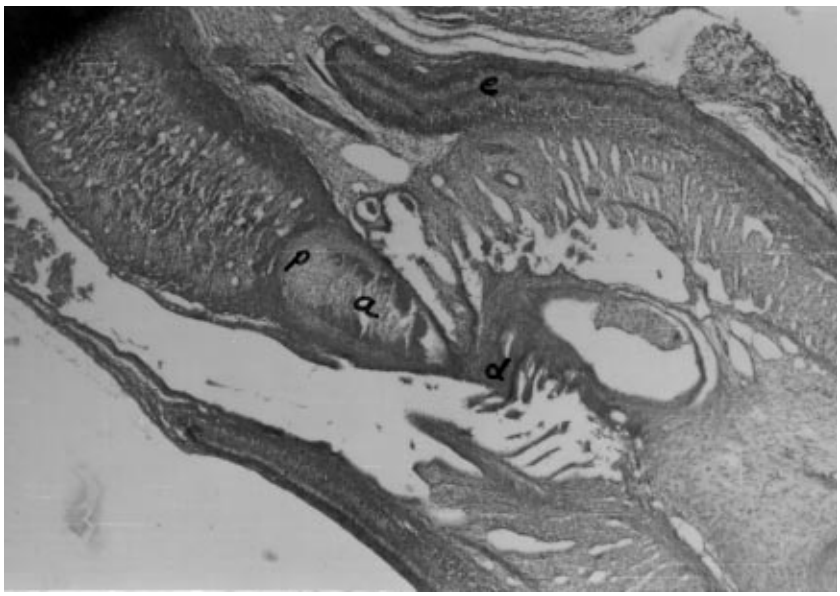

Figure 2. Rudimental (or rudimentary) proximal (p) and distal (d) segment and decreased of the length of epidermal tips (arrow) in the first experimental group (Verhoeff, original magnification X 16).

(Tx) is a dertivative of triphenylethlen group non - steroidal anti -estrogen drugs.

In experimental studes, some anomalies were seen in reproductive systems with Tx administration after birth. Delayed spermatogenesis and atrophy in testis and accessory glands were observed in male rats (9, 10). It has been postulated that Tx administration express the ossification of Os ischium and Os pubis in pelvis (9, 11). The aim present study was to investigate the developmental anomalies in Os penis and epidermal tips in glans penis with Tx, which is, anti-estrogen agent after birth. The clinical importance of this study on Tx is that Tx has a negative effect on the development of penis corpus cavernosum and glans penis.

Materials and Methods

Forty newborn male rats were gathered in this experiment. The male litters were obtained immediately after birth from the Department of Medical Science Application and Research Center of Dicle University. The male litters and their mothers were fed with milk and pellet food, respectively during the experiment.

All litters were separated into ten groups, five control

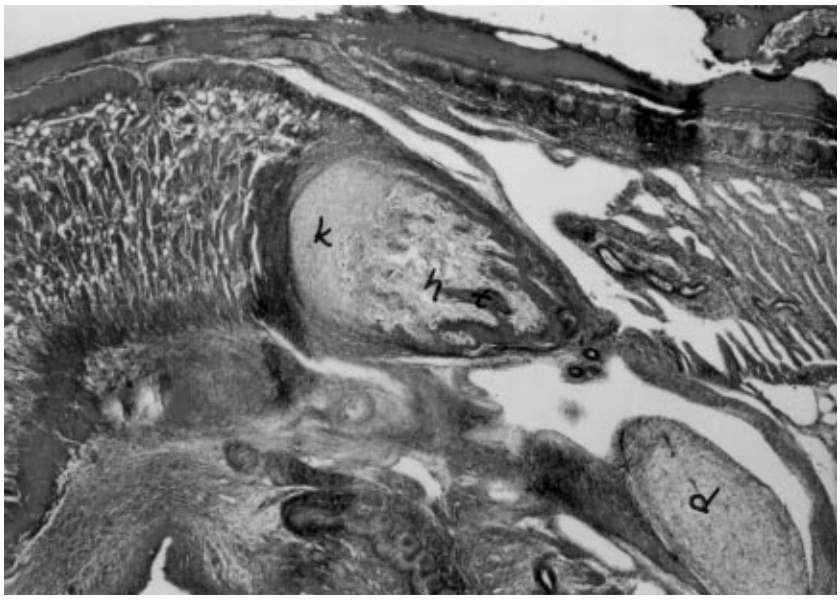

Figure 3. The trabecullae, thicking of proximal segment (light arrow) and heamopoietic tissue increasing (dark arrow). Development of fibrocartilagine tissue in the distal segment and hyaline cartilage (arrowheads). Mature appearances of epidermal tips and keratinization (arrows) in glans penis and prepuce epidermis of the second control group (Masson triple, original magnification X 16).

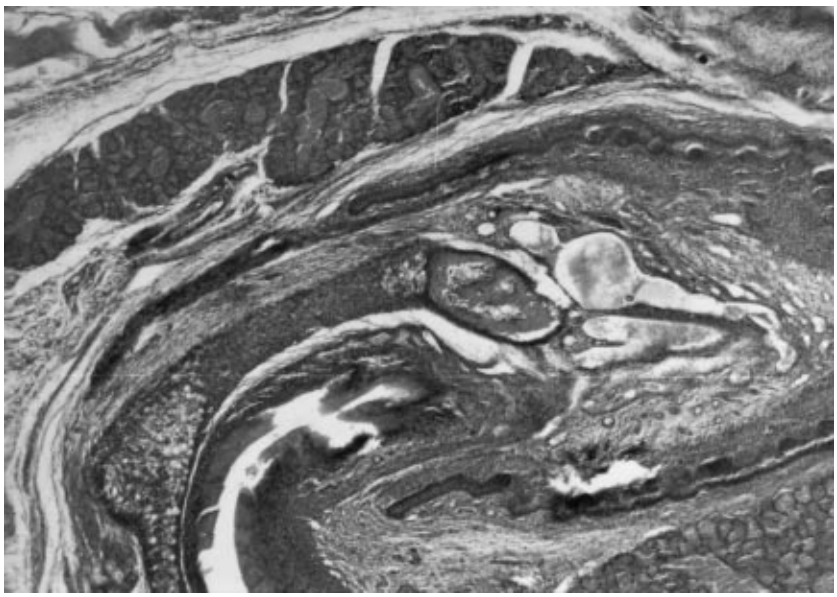

Figure 4. Rudimentary Os penis (light arrow) and affected lacunas of the erectile tissue (dark arrow) in the second experimental group (H & E, original magnification X 16).

and five experimental groups. The control group litters were given only saline whereas experimental litters were treated daily, from the birth to the fifth day with 100 µ g (Tx) Tamoxifen citrate in 0,02 ml saline subcutaneously. Four litters in each group were anesthezied and dissected for their penises totally on the sacrificing days (Table 1).

The tissues were put into 10% formaldehyde fixative solution after dehydration and embedded into paraffine blocks then, they were cut into 5 µm thickness with microtome. After sectioning, they were stained with Hematoxylene - Eosin (H - E), Masson triple and Verhoeff stains (12, 13). Histopathological were carried out under a light microscope.

Results

The development of Os genis and prepuce were compared in control and experimental rats.

In the first control group (sacrificed at 14 day after birth).

Hemopoetic tissues increased with enlargement of the lacunes in the proximal segments. Fibrous tissues were observed in distal segments. Development of epidermal tips were noticed in the first control group (Figure 1).

In the first experimental group (sacrificed at 14

Tablo 1.

Control Groups (Days of sacrificed)	Saline-Administration (Subcutaneous)	Indices
14.0	0.02 ml	4
21.0	0.02 ml	4
28.0	0.02 ml	4
35.0	0.02 ml	4
60.0	0.02 ml	4
Experiment Group (Days of sacrificed)	**Tamoxifen (Tx)-Administration (Subcutaneous)**	**Indices**
14.0	100 µg	4
21.0	100 µg	4
28.0	100 µg	4
35.0	100 µg	4
60.0	100 µg	4

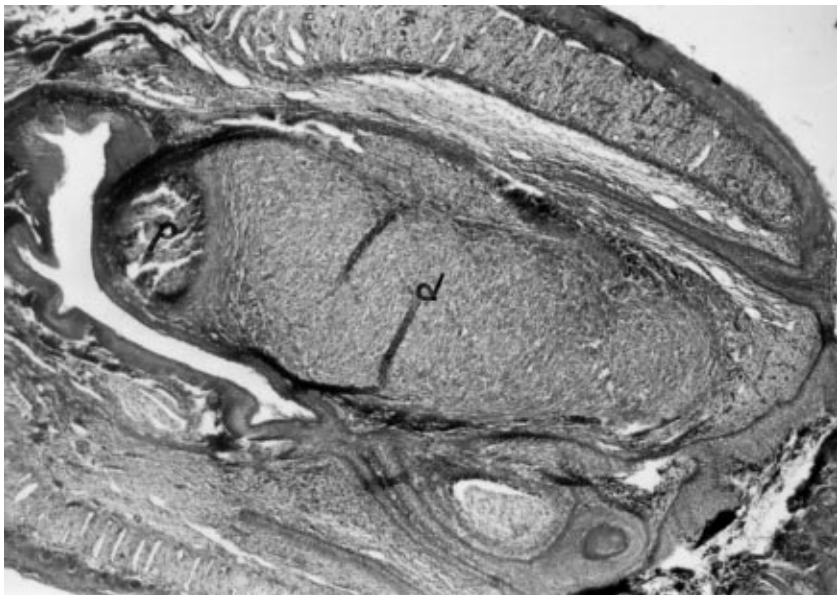

Figure 5. Progressive endochondral ossification of the proximal segment (p) and development of fibrocartilage tissue in the distal segment (d) the third control group (Masson triple, original magnification X 16).

day after birth).

The half below of proximal segment and the distal segment were observed to be rudimentary. A decrease were observed in the length and density of epidermal tips (Figure 2).

In the second control group (sacrificed at 21 day after birth).

Hemopoetic tissues and trabecullar thicking of lamellae bone of Os penis were observed clearly in the proximal segment. However, hyaline cartilages covered some parts of the proximal segment which was adjacent to the cavernous tissues (Figure 3).

In the second experimental group (sacrificed at 21 day after birth).

Rudimentary Os penis and affected lacunas in the erectile tissue were noticed (Figure 4).

In the third control group (sacrificed at 28 day after birth).

Progressive endochondral ossification was seen in the proximal segment. Fibrous materdial changed to fibrocartilage tissue in the distal segment (Figure 5).

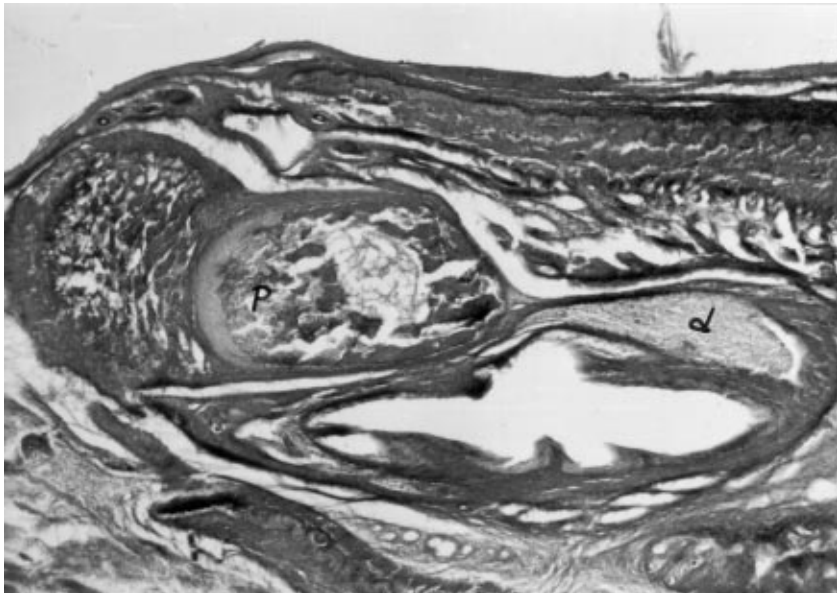

Figure 6. Disappeared heamopoietic tissue of the proximal segment (p) and distal segment atrophy (d) in the third experimental group (Verhoeff, original magnification X 16).

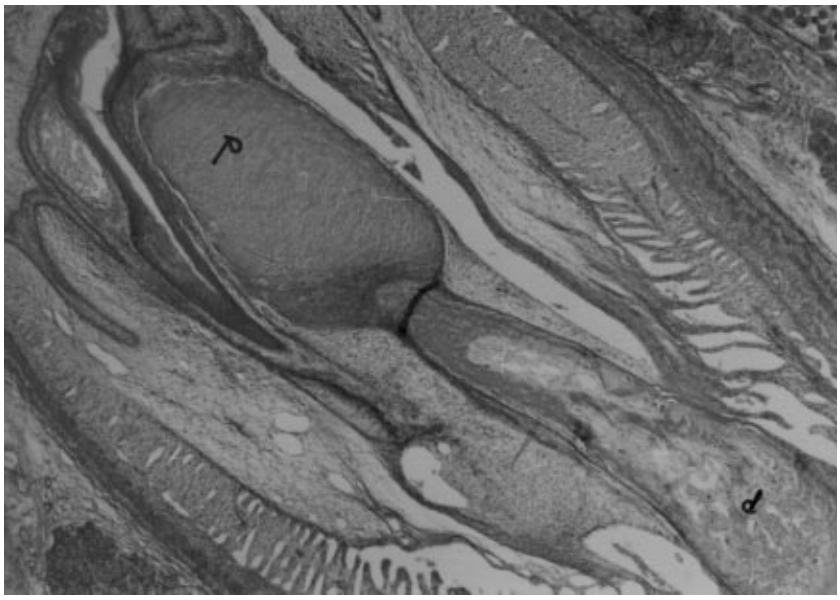

Figure 7. Proximal segmet ossification (p) and chondrocyte maturation of the distal segment (d) in the fourth group (Masson triple original magnification X 16).

In the third experimental group (sacrificed at 28 day after birth).

Bone marrow compactly disappeared in the proximal segment, and distal segment atrophy was seen (Figure 6).

In the fourth control group (sacrificed at 35 day after birth).

An increase maturation was observed in chondrocytes of the distal segment and progressive ossification was seen in the proximal segment in Os penis (Figure 7).

In the fourth experimental group (sacrificed at 35 day after birth).

Rudimental proximal and distal segments were noticed in Os penis (Figure 8).

In the fifth control group (sacrificed at 60 day after birth).

Membranous bone structure was prominent in the proximal segment (Figure 9).

In the fifth experimental group (sacrificed at 60 day after birth).

Conspicuous atrophy was seen in both segments and

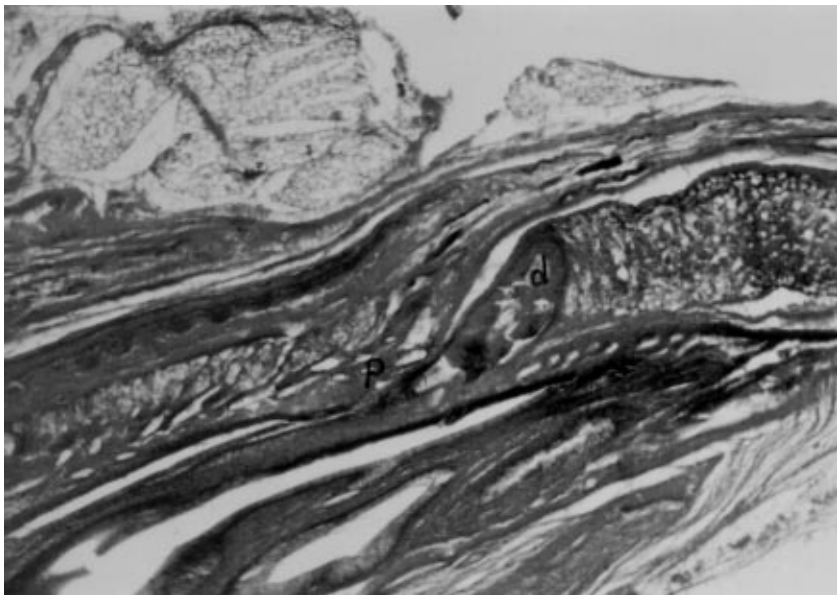

Figure 8. Rudimentary proximal (p) and distal (d) segment of the fourth experimental group (Verhoeff, original magnification X 16).

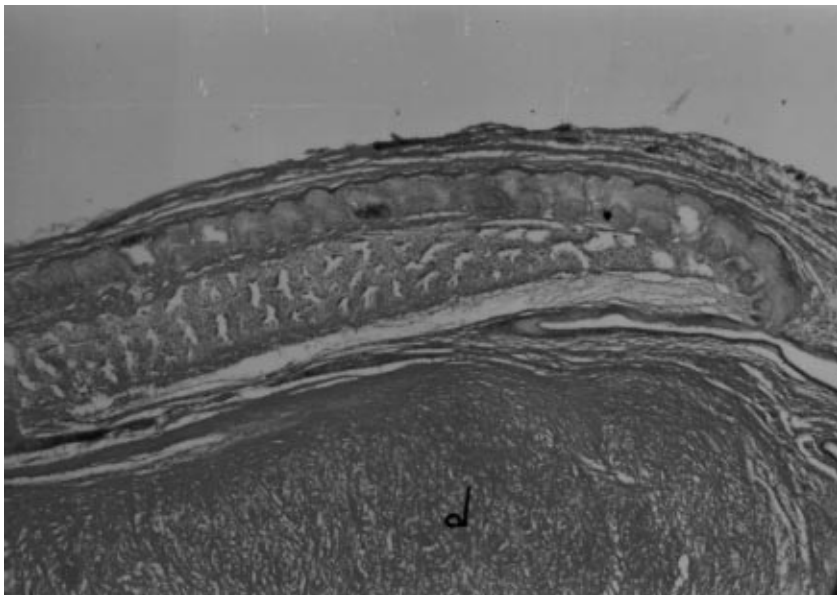

Figure 9. Development of fibrocartilage tissue in the distal segment (d) of the fifth control group (Verhoeff, original magnification X 16).

there was no fibrocartilage tissue in the distal segment (Figure 10).

Discussion

A number of studies have been carried out on the normal development of rat penis (1, 2, 4). In our study, the proximal segment of Os penis differentiated to compact bone with heamopoetic tissue during the second and third week of postpartum. At the same period, a fibrous material was seen in the extracellular matrix of segment. In the fourth week distal segment differentiated to typical fibrocartilage tissue (Figure 5). Our histopathologic findings were similar to the previous studies (13, 1, 3, 6). In the experimental group, heamopoetic tissues disappeared in one month and hyaline cartilage tissues were effected in two months. In our study, the maximum area of proximal segment was observed at smallest in the fifth experimental group. The decrease in the fifth experimental group. The decrease in the volume of proximal segment was observed particularly in the fifth experimental group (60.0 day).

It has been suggested that the inhibitor effects of Tx on maturation of proximal segment are superior to other durgs (14). In an experimental study, Gluckman et al (13), immediately after the birth, anti - androgen

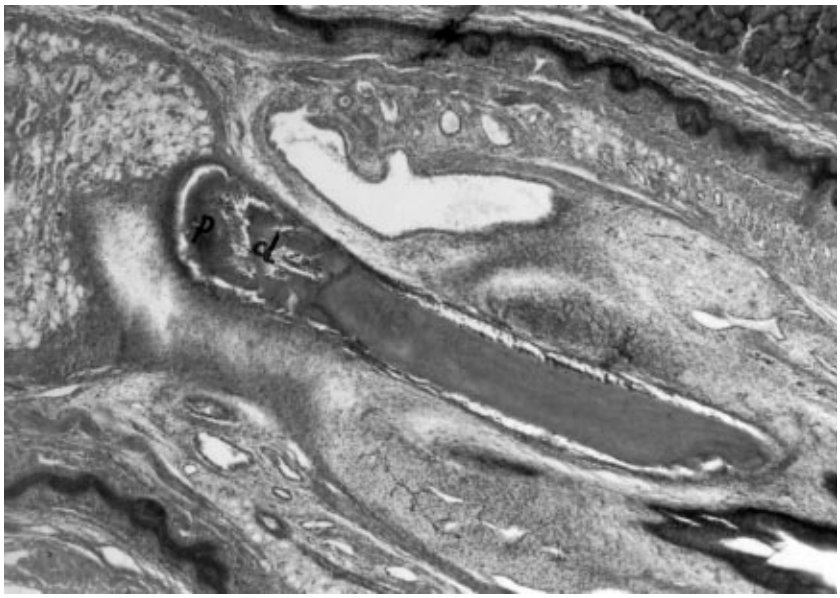

Figure 10. Proximal (p) and distal (d) segment atrophy of the fifth experimental group (H & E, original magnification X 16).

treatments were found to delay the maturation and ossification of distal segment. However, the maturation was totally blocked by anti - androgen treatments in the castration of animals. In our study, a fibrocartilage tissue was observed in the distal segment of the third, fourth and fifth control group. Furthermore, the volume of distal segment was less in the experimental group Iguchi et al (14). Also observed similar abnormalities in Os penis due to anti - estrogen effects of Tx.

These findings supported the concept that androgens enhance the development of fibrocartilage tissue: in our study, there was no difference in the epidermal tips between the first control and experimental group. The epidermal tips start to disappear gradually in the second experimental group, whereas gross atrophy and lack of keratinization in epidermis were observed in the fifth experimental group the previous studies (14, 8). We also observed the unseparated prepuce and epidermis of glands penis in our study. Immediately after birth, Tx treatment blocked the development of fibrocartilage tissue and hyaline cartilage in Os penis. It also blocked the maturation of epidermal tips in rats. Although Tx has an anti - oestrogen effect we suggest that it prevents the estrogen action on target organs by binding to estrogen receptors.

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