SAR Journal of Anatomy and Physiology

Abbreviated Key Title: *SAR J Anat Physiol*
Home page: https://sarpublication.com/journal/sarjap/home
DOI: https://doi.org/10.36346/sarjap.2024.v05i03.002

OPEN ACCESS

ISSN 2707-7713 (P)
ISSN 2709-6874 (O)

Original Research Article

Histological Study of Development Structure of Testis and Epididymis in Gazelle *Gazelle Subgutturosa*

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Article History: | Received: 18.08.2024 | Accepted: 24.09.2024 | Published: 26.09.2024 |

Abstract: ***Objective***: The aim of this study for focused the light on the development structure of the testis of gazelle. ***Materials and Method***: 10 healthy male gazelle were used to study the histology structure of testis (5 pre- Puberty and 5 post- Puberty). ***Results:*** The testis is surrounded by a thick, irregular connective tissue capsule. Each lobule of the testis is composed of seminiferous tubules and interstitial tissue, and trabecular and interlobular septa divide the lobules apart. Neighboring the seminiferous tubules are Sertoli cells and spermatogenic cells. From spermatogenic cells, spermatozoa are produced. The first spermatogonia are small, spherical cells with black, spherical nuclei that reside on the basement membrane. Spermatogonia were earliest stages of the spermatogenic cells. Larger cells with frequently distinct chromatin are called primary spermatocytes. Secondary spermatocytes are rare because of their quick second meiotic division and the haploid spermatids that follow. Spermatids are round, pale-nucleated cells that are grouped close to the lumen of the seminiferous tubule. Prostatogenic cells outnumber Sertoli cells in the body. Oval or triangular in shape, the nucleus has a light coloration. In the connective tissue separating adjacent tubules, Leydig cells are present. Lines the epididymis are pseudostratified epithelium. Extensions of the cytoplasm into the lumen are called stereocilia. In different proportions, there is circular smooth muscle, and a connective tissue lamina propria supports the epithelium. The rete testis is connected to the ductus epididymidis by the efferent ductules, which are convolutions of tubules. Numerous valved veins were visible in the tunica albuginea, which covered the initial ascending portion of the epididymal head. Cuboidal epithelium was lining extratesticular rete testis. Three different cell types; migrating, basal, and columnar had epithelial membranes lining the efferent ductules. Many columnar, non-ciliated cells with many cytoplasmic vacuoles and tiny granules lined the epithelium of the first segment of the efferent ductules. ***Conclusion:*** In post-puberty age, the structure of the gazelle's testis exhibited a pattern resembling that of other ruminants; the seminiferous tubules contain adult sperm and a broad lumen.

Keywords: Developmental, Seminiferous Tubule's, Leydig Cells, Sertoli Cells, Epididymidis.

INTRODUCTION

The onset of ovulation and/or oestrous behavior in female and the first ejaculation with sperm release in males are considered the markers of puberty. Numerous environmental variables have an impact on puberty as nutrition and diseases [1-3]. The testicles, prepuce, genital duct, accessory gland, and epididymis make up the male reproductive system of ruminants. The paired, oval-to-rounded testes are situated outside abdominal cavity in the scrotum, a skin fold, and are in charge of secreting male sex hormones, particularly testosterone, and producing sperm [4, 5]. Because testicular function requires a temperature beneath the abdominal temperature, this testicular position is significant. The pigmentation progresses from white through yellow and is encased in a tight connective tissue capsule known as the tunica albuginea [6, 7]. The thick tunica albuginea is made up of collagen fibers that are arranged in a dense connective tissue architecture that is parallel to one another, providing the tissue minimal flexibility and greater strength. This is thickened on dorsal side, forming the mediastinum testis and splitting the organ into sections known as testicular lobes through the entry of fibrous septa [8]. Beyond that, this capsule is penetrated by testicular arteries and veins, indicating a highly vascularized structure, visceral layer of tunica vaginalis, which comprises of connective tissue containing fibroblasts and blood arteries, covers the tunica albuginea. The most prevalent part of testicular parenchyma, the seminiferous tubules, are made up of Sertoli and spermatogenic cells. They are encircled by fibroblast-formed connective tissue and a basement membrane [9]. myoid cell and immature germ cell known as spermatogonia are seen next to the basement membrane. These cells were found in the basal region of the seminiferous epithelium and were spherical cells with oval nuclei and one or more visible nucleoli [10-12]. These cells give rise to primary spermatocytes, which are big, spherical cells that are near the basal membrane and have a nucleus and obvious nucleolus, early spermatids, round containing sparsely pigmented nuclei, are derived from the secondary spermatocytes, which arise in succession and have smaller size than primary spermatocytes. Following these cell stages, the remaining spermatids have long tails that extend into lumen of seminiferous tubule and sperm, giving them a darker, smaller, oval, and/or elongated appearance [13-16]. There hasn't been much information released recently about the testis traits in gazelles. The purpose of this study was to shed light on the gazelle's testicles because this information is crucial for supplying data for other research projects.

MATERIALS AND METHODS

Study Animals

The study was performed on 10 healthy male gazelle *Gazelle subgutturos* (5 pre- Puberty in age 4-8 month and 5 pos- Puberty in age 9-12 month), After giving the animals an overdose of ketamine and xylazine to induce unconsciousness, the animals were slaughtered, and the testicles were taken for histological examination [10].

Histological Technique

Xylene to cleaning, paraffin for penetration and embedding, ethyl alcohol for dehydration, and a microtome for sectioning, then Hematoxylin and Eosin (H @ E) [17].

Statistical Analysis

The research was analyzed at the 5% significant level using the one-way analysis of variance (ANOVA) test. Utilizing statistical tools for social science, data were managed and analyzed [18].

RESULTS AND DISCUSION

The testis is encased in a thick, irregular connective tissue capsule. Trabecular and interlobular septa divide the testis' lobules, which are composed of the seminiferous tubules and the an interstitial tissue. The seminiferous tubules are surrounded by Sertoli cells and spermatogenic cells. Spermatogenic cells give rise to spermatozoa, earliest stages of the spermatogenic cells are called spermatogonia, and they are small, spherical cells that reside on the basement membrane. They have black, spherical nuclei. In the spermotogonia, primary spermatocytes; larger cells with frequently different chromatin; are produced during mitosis. Secondary spermatocytes are rarely observed because to their quick second meiotic division and consequent haploid spermatid production. Spermatids were spherical cell having pale nuclei that are grouped close to the lumen of the seminiferous tubule. Spermatogenic cells outnumber Sertoli cells, also known as sustentacular cells, The nucleus has a big nucleolus and is pale in color, oval in shape. Between adjacent tubules in connective tissue are Leydig cells. In pre-puberty ages the spermatic cords small in diameter, then in post-puberty the has wider diameter (Fig. 1-5) (Table 1).

Through the process of spermatogenesis, which takes place in the complex system ducts from seminiferous tubules to vas deferens, the testes generate sperm cells. An intricate component of the testis supports the development of sperm cells. The seminiferous tubules' lumen will be approached by sperm cells that have developed as a result of spermatogenesis. After passing through rete testis tissues and the ductuli efferentes, the sperm cells will reach the epididymis where they will begin the process of developing [6-19].

The earliest cells in spermatogenic lineage were called spermotogonia. In the spermotogonia, primary spermatocytes; larger cells with frequently different chromatin; are produced during mitosis. The bigger primary spermatocytes undergo the first meiotic division, which yields secondary spermatocytes. Secondary spermatocytes are rarely observed due to their quick second meiotic and subsequent haploid spermatid formation. Spermatid was spherical cell with pale nucleus that are grouped close to the lumen of the seminiferous tubule (Fig.6) [20], also outlined the anatomical foundation of goat spermatogenesis. The testicle is composed of a capsule known as the tunica albuginea, which contains fibroblasts and collagen fibers. It also has tubules seminíferos, which have well-developed stratified epithelium with light and dark areas, as well as visible support cells or sertoli that have an irregular shape, developed interstitial or tubular tissue, and vascular compartments that contain Leydig cells (Fig. 4-6).

The columnar pseudostratified on a basement membrane filled lined the epididymal ducts. This epithelium is composed of various cells, with cylindrical cell design stereocilia toward the epididymal duct lumen, whose interior duct light found occasionally some, and spermatogenic cells in large numbers relative to Sertoli cells (sustentacular cells). Their nuclei have irregular cleft-like infoldings, a big nucleolus, and an oval or triangular shape. They are pale. Although they are lengthy cells that extend from the basement membrane of the tubule to its lumen, it is difficult to distinguish their borders in typical histologic preparations (Fig.7). The results corroborated those of [21], who discovered that Sertoli cells could be distinguished by their unique fewer tall columnar cells and irregular nuclei and smaller size, testicles were tubular structures with cytogenic and endocrine properties (Fig.4,5,8). The production of spermatozoa and testosterone depends on these processes. The majority of animals, such as rams, bulls, vicuna, deer, have complex tubular structures called tunica albuginea, which is surrounded by a thick capsule of dense, irregular connective tissue. An abundance of fibroblasts, white, reticular, and elastic fibers, as well as a small number of blood vessels, are present in this capsule. The testis is the site of production of male germ cells, while the epididymis acts as its chief duct system in addition to providing the nutrients and environment needed for their growth and maturation [22, 23].

The testis plays combined endocrine and exocrine functions since it is the main organ that produces spermatozoa and testosterone. Seminiferous tubules, which generate spermatozoa for the continuation of spermatogenesis, Leydig cells, which release testosterone for male sexuality, make up the parenchyma of the testicle [24, 25], mention because the animals have a unique and limited breeding season that fluctuates depending on geographic and genetic makeup, these seasonal shifts are further influenced by ecological site, management practices, and meteorological conditions. The breeding season is when spermatogenesis is observed to be at its peak. Researchers concur that the activity of Leydig cells and the morphology of the testicular tissue are both clearly influenced by the season.

Vaginal and albuginea tunicae, which is composed of a lot of collagen fibers and strong connective tissue, coated the testes. Convoluted seminiferous tubules within the organ were encircled by a basement membrane that contained myoid and Sertoli cells as well as germinative epithelium made up of the interstitial tissue lies between the seminiferous tubules (Fig.2,4), consisting of Leydig cells arranged in a polyhedral form, connective tissue, blood, and lymph vessels. Stereocilia were positioned on a basement membrane that was packed with myoid cells in the pseudostratified columnar epithelium of the epididymal ducts (Fig.5,6). Our observations of the structures closely resemble those reported for other mammals [26-31], visceral layer of vaginalis encased a thick layer of tunica albuginea, encircling the epididymis. One could distinguish between an inner loose fibrous layer displaying numerous blood vessels and an exterior thick fibrous layer in the ascending area of the epididymal head's tunica albuginea. Furthermore, smooth muscle fibers were only visible in the blood vessel wall. Numerous valved veins could be seen in the albuginea covering the ascending first area of the head. Additionally, intralobular fibrous connective tissue septa, which divide the epididymal duct and efferent ductule into lobules of varying sizes, extended from the deeper part of the albuginea (Fig.5,6).

Loose connective tissue made up the intralobular stroma, which contains the loops of the epididymal duct and the efferent ductules. It revealed blood capillaries in a profusion of amorphous ground substance, as well as some connective tissue cells and small collagenous fibers. The intralobular cells were macrophages, fibroblasts, and lymphocytes. The extratesticular portion of the rete testis and the loops of efferent ductules were encompassed by the ascending initial area of the epididymal head. Convolutions of the epididymal duct's tubules and efferent ductules were visible in the second section. The testis's excurrent duct system was thought to include the efferent ductules. The rete testis and the ductus epididymidis are connected by these tubule convolutions (Fig.5,6). This similar to [8].

Numerous valved veins were visible in the tunica albuginea, which covered the first ascending region of epididymal head. The extratesticular portion of the rete testis and efferent ductule convolutions were found in the first region, whereas the tubules of the first segments of epididymal duct and efferent ductules were found in the second region, rete testis was bordered by cuboidal epithelium. There was dramatic alteration in epithelium lining the efferent ductules. Three different cell types; migrating, basal, and columnar; had epithelial membranes lining the efferent ductules. Many columnar, non-ciliated cells with many cytoplasmic vacuoles and tiny granules lined the epithelium of the first segment of the efferent ductules (Fig.5), this agree with [4-6].

They displayed apocrine secretion symptoms, columnar ciliated cell grew toward the epididymal duct along the efferent ductules. A small number of tall, slender, dark cells emerged in the epithelium close to the epididymal duct junction, smooth muscle layer encircled the camel's efferent ductules, and these layers became more frequent as one approached the epididymal duct. Reactivity of the alkaline phosphatase enzyme was seen in the blood vessels and subepithelial connective tissue. The acid phosphatase enzyme showed strong granular activity over the whole epithelium (Fig.6). Mammals' epididymis is regarded as a crucial component of the extra-testicular sperm pathway. It is a highly coiled tube that is closely inserted along lateral surface of testicle to form a compact mass. It is the main site of sperm maturation and storage, as well as the site where sperm acquire motility. The epididymis is separated into the head, body, and tail. These observation in mammals are consistent with this description [2]. The epididymis is lined with pseudostratified epithelium. Long structures that reach into the lumen are called stereocilia. The amount of circular smooth muscle varies, and a connective tissue supports the epithelium (Fig.7), this agree with [13].

Table 1: Measurements of left and right testis of gazelle, µm ($\bar{X}$ ± S.E)

Measure Age	Capsule		Diameter of Seminfeorus		Diameter epididymis ducts
	Left	Right	Left	Right	
4-6 month	67.2±0.7A	60.3±0.8A	123.4±0.1A	112.1±0.3A	88.7±0.2A
7-8 month	81.8±1.6A	75.2±1.7A	157.2±0.5A	145.6±0.2A	117.5±0.5A
9-11 month	90.9±0.5A	83.7±0.3A	184.6±0.2A	172.3±0.7A	134.6±0.4A
12-16 month	102.3±0.4A	96.4±0.2A	229.6±0.5A	218.5±0.4A	189.2±0.6A

The values columns with capital letters in same column denote for significant difference (P>0.05)

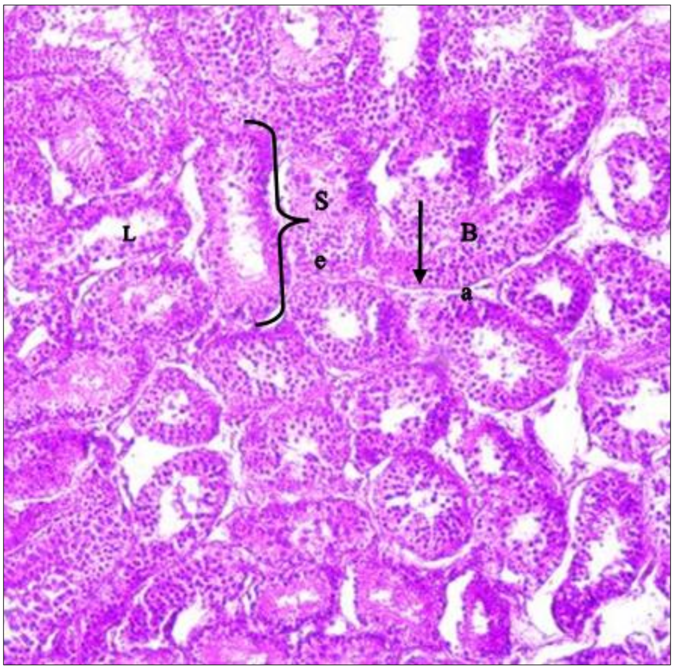

Fig. 1: photomicrograph of pre-puberty testis (4 months age); Spermatic cord (S e), basement membrane (B a), lumen of Spermatic cord very small (L), H@E,400X.

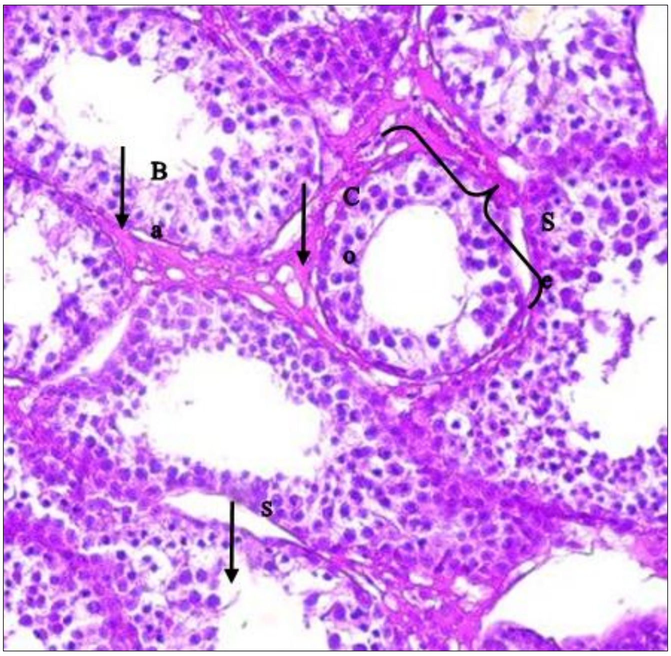

Fig. 2: photomicrograph of post-puberty testis (7 months age); seminiferous tubules (S e), basement membrane (B a), connective tissue (Ca), adult Sperm (S), H@E,400X.

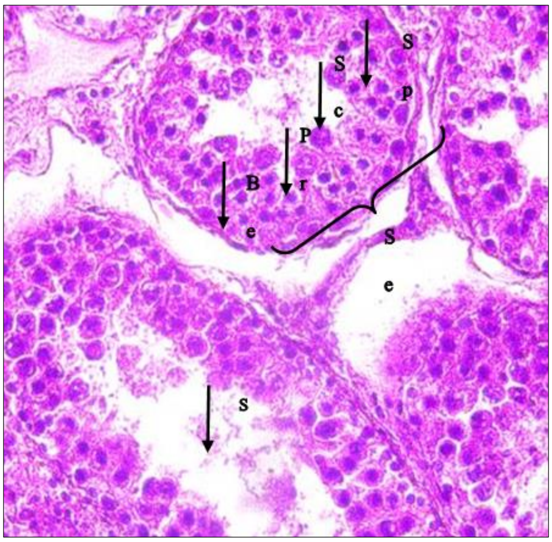

Fig. 3: photomicrograph of post-puberty testis (8 months age); seminiferous tubules (S e), basement membrane (B e), primary spermatocyte (P r), secondary spermatocyte (S c), spermatogonia (S p), sperm (S), H@E,400X.

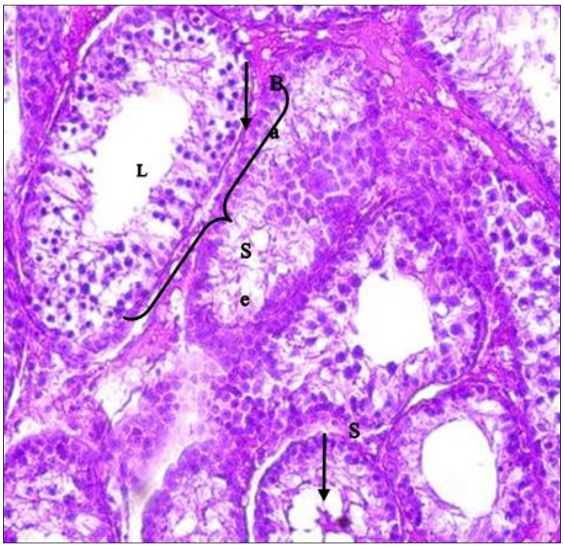

Fig. 4: photomicrograph of post-puberty testis (9 months age); seminiferous tubules (S e), basement membrane (B a), Lumen (L), adult Sperm (S), H@E,400X.

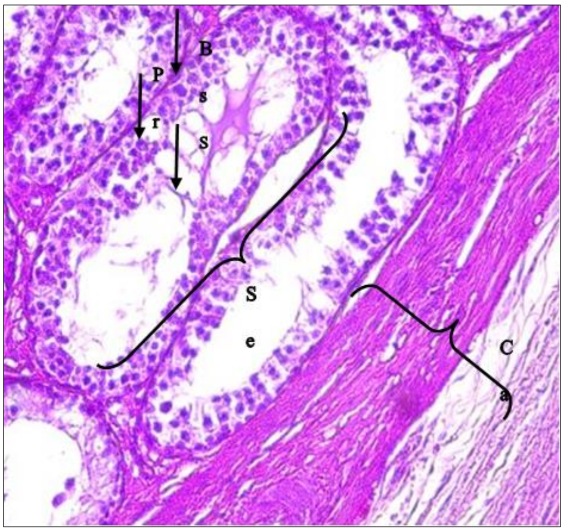

Fig. 5: photomicrograph of post-puberty testis (10 months age); seminiferous tubules (S e), basement membrane (B s), capsule (Ca), primary spermatocyte (P r), adult Sperm (S), H@E,400X.

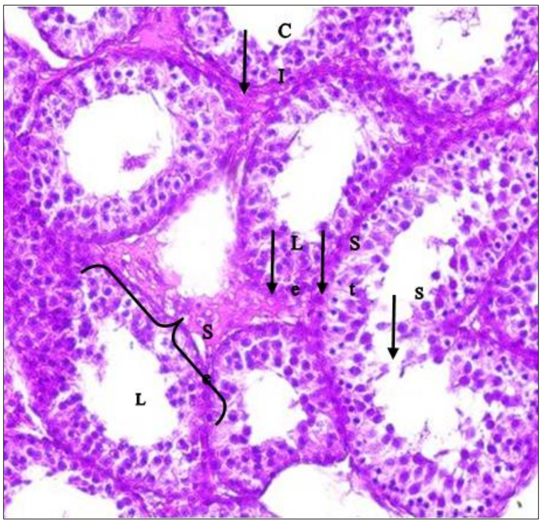

Fig. 6: photomicrograph of testis (12 months age); seminiferous tubules (S e), leydig cell (L e), Lumen (L), sertoli cell (S t), adult Sperm (S), H@E,400X.

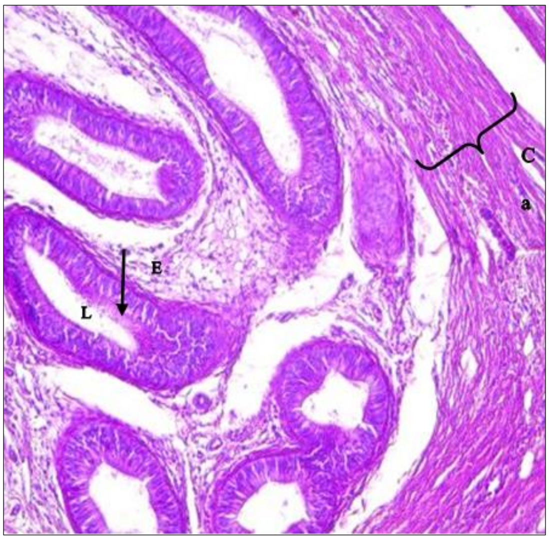

Fig. 7: photomicrograph of post-puberty epididymis (16 months age); capsule (C a), epithelium (E), duct of epididymis (Ca), H@E,400X.

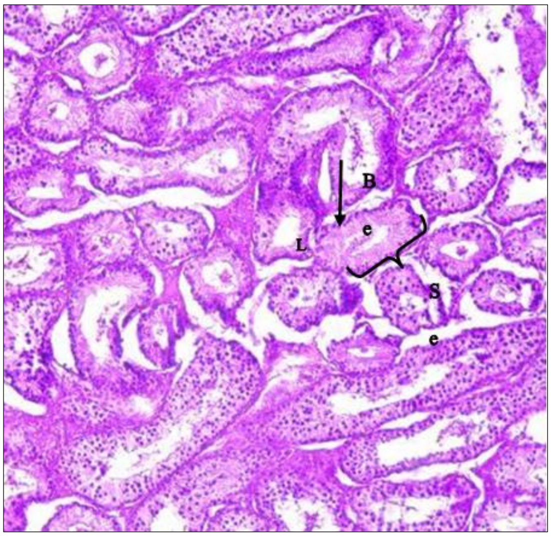

Fig. 8: photomicrograph of pre-puberty testis (16 months age); seminiferous tubules (S e), basement membrane (B a), lumen of tubules (L), H@E, 400X

CONCLUSION

The structure of the gazelle testis showed a similar pattern to that described for other ruminant.

Funding: Rather than obtain outside the funding, author finances this study on the independent basis

Availability of Data and Materials: Available data to the current study upon reasonable request.

Competing Interest: There are no conflict of the interest between authors.

Ethical Consideration: Ethical guidelines to study conduct had been adhered to within the AL-Qasim Green University, Iraq.

Author Contributions: All authors contribute equally.

ACKNOWLEDGEMENTS

Especially want to express our gratitude to Histology Department in Veterinary Medicine College, AL-Qasim Green University, in Iraq for their diligent efforts and commitment to our initiative.

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