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Presence of CRABP-1 and CA-IX in Human Testis Diagnosed with Klinefelter's and Sertoli Cell-Only Syndrome (SCOS): Effect on Spermatogenesis

Presencia de CRABP-1 y CA-IX en Testículo Humano Diagnosticado con Klinefelter y Síndrome de Sertoli Solo (SSS): Efecto en la Espermatogénesis

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RODRÍGUEZ, H.; ARAYA, J.C.; RODRÍGUEZ, N.; ARRIAZA, C. & ESPINOZA-NAVARRO, O. Presence of CRABP-1 and CA-IX In human testis diagnosed with Klinefelter's and Sertoli cell-only syndrome (SCOS): Effect on spermatogenesis. *Int. J. Morphol., 43(1)*:5-9, 2025.

SUMMARY: Complete spermatogenesis is clearly described in seminiferous tubules of normal adult human testis. In histological studies of testicular biopsies with a Klinefelter diagnosis, seminiferous tubules are shown with a total or partial absence ofspermatogenesis in the sustentacular cells, an anomaly known as Sertoli cell-only syndrome (SCOS). Testicular metabolism is sensitive to the regulation of retinoic acid and the acid-base balance. Retinoic acid (CRABP) and its metabolites appear as regulators of cell proliferation and differentiation. Carbonic anhydrase (CA or RCC) regulates a variety of cellular functions, including several processes in reproductive systems. The objective of this study was to demonstrate the interaction and presence of CRABP-1 and CA-IX in human testicular biopsies diagnosed with Secondary Klinefelter and SCOS and its possible effect on altered spermatogenesis. Testicular biopsies were processed with normal routine histology technique (PAS/hematoxylin). Mouse polyclonal antibody was used for the immunohistochemical (IHC) study of CA-IX and anti-CRABP-1 antibody was used for CRABP-1. Positive cells are characterized by having a red nucleus. The results show great positivity for CRABP-1 in Sertoli cells (sustentacular cells) while for CA-IX, positive cells are expressed in the interstitial compartment. The bar graphs (Mann Whitney test, $p \leq 0.05$) show significant statistical differences between the different germinal compartments under study. It is concluded that the metabolism of an acidophilic condition, due to the action of CRABP-1 and CA-IX in testes with a diagnosis of Klinefelter and SCOS, could be the causes of damage to spermatogenesis in men.

KEY WORDS: CRABP-1; Carbonic Anhydrase; IHC; Spermatogenesis; Testicle.

INTRODUCTION

Spermatogenesis is a continuous process that begins from the embryo-fetal stage, marking interrelationships between interstitial cells (Leydig) with peritubular cells (lamina propria) and sustentacular cells (Sertoli cells) (Rojas *et al*., 2017). Complete spermatogenesis sequence is precisely described in seminiferous tubules of normal adult human testis. However, in biopsies diagnosed with Klinefelter's disease, many areas of the seminiferous tubules show a complete or partial absence of spermatogenesis. Klinefelter syndrome, chromosome condition XXY, affects 1 in 600 men and is the most common cause of hypogonadotropic hypogonadism with testicles that are smaller than normal and therefore produce less testosterone (Høst *et al*., 2014). In cases of Sertoli cell-only syndrome (SCOS), the seminiferous tubules present a total absence of spermatogenesis, being responsible for almost 50% of cases of male infertility. This syndrome is also known as germ cell aplasia, characterized by azoospermia in which the seminiferous tubules of the testicular biopsy are lined only by sustentacular cells (Sharpe *et al*., 2003). Although SCOS can be a result of Klinefelter syndrome, most men with SCOS have a normal karyotype (Ghanami Gashti *et al*., 2021).

The metabolism of the cells of the different testicular compartments in both cases expresses changes in the regulation of retinoic acid uptake and the acidity balance (acid-base). Mezquita *et al*. (1999), show that bicarbonate

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production in the testis's intracellular and extracellular sources is essential for sperm motility. In skin, Glass & Guengerich (2021), describe cellular retinoic acid binding protein-1 (CRABP-1) and its metabolites as cell proliferation and differentiation regulators. Carbonic anhydrase (CA or RCC) catalyzes the reversible hydration of dissolved CO_2 to form bicarbonate ions and protons; active CA isoenzymes regulate a variety of cellular functions, including several processes in reproductive systems (Hynninen *et al*., 2004). CA belongs to a superfamily of metalloenzymes (iron) that maintains the balance of reactions between CO_2, bicarbonate, and H+ (Li *et al*., 2019). Carbonic anhydrase IX (CA-IX) regulates cell proliferation in response to hypoxic conditions and could be involved in oncogenesis and tumor progression. Previous studies have shown that it is absent in most normal tissues except gastric mucosal epithelial cells (Lam *et al*., 2005). The alteration of these proteins in the testis could become a significant factor in male infertility. Bernardino *et al*. (2019), suggest that CA is essential for mitochondrial biogenesis, glucose, and lipid metabolism in human Sertoli cells.

According to Zhang *et al*. (2022a), retinoic acid (RA or CRABP), one of the vitamin A derivatives, regulates spermatogonia proliferation and differentiation, meiosis, spermiogenesis, and spermiation. Additionally, they determine that there are several factors and possible transcriptional regulators for each step of spermatogenesis. It is also described that the development of normal spermatogenesis is associated with a balanced diet, where the metabolism of retinoids and carbonic anhydrase-type enzymes show a close relationship between the gut-testis axis and causes of impaired spermatogenesis (Zhang *et al*., 2022b). This study aimed to demonstrate the interaction and presence of CRABP-1 and CA-IX in biopsies diagnosed with Klinefelter's Syndrome and SCOS and the possible cause of impaired spermatogenesis.

MATERIAL AND METHOD

Tissue biopsies diagnosed as Klinefelter with SCOS fixed with alcoholic Bouin and embedded in paraffin (melting point 56-58 °C) were obtained from the Testicular Biopsy Bank of the Morphology Laboratory, Teaching Unit of Reproductive Biology, Faculty of Medicine, University of Chile. The photomicrograph of a normal testicle was obtained from the Histology Laboratory of the Faculty of Medicine of the University of Chile (PAS-Hematoxylin-Alcian Blue stain). Biopsies (n=12) were processed according to bioethical protocols for working with stored human tissues (safeguarding the policies of preservation of the identity of the patients and ensuring that the tissues will be used only for non-genetic scientific purposes). Sections of 5 μm thickness were obtained from the tissues and placed on glass slides in Silane® solution. The induced epitope was recovered with 10 mM Tris-HCl solution, pH 6.0. The samples were processed employing the standard routine histology technique of PAS/hematoxylin for nuclear staining. A standard avidin-biotin-peroxidase complex (ABC, hydrophilic developer protocol) technique was used for immunohistochemistry. For CA-IX, sections were treated with specific mouse polyclonal antibody (polyclonal IgG anti-carbonic anhydrase IX antibody, ab15086 at 1/500, Cambridge, MA 02139-1517 USA) and for CRABP-1, anti-CRABP-1 antibody [C-1], ab2816 at 1/1000, Cambridge, MA 02139- 1517 USA). Both antibodies were incubated for 1 hour. The reaction-positive cells, using both primary antibodies and contrasted with hematoxylin (nuclei staining), are characterized by intense red in the nuclear area.

The results were assessed by quantifying the number of positive cells (red nucleus) per seminiferous tubule compartment, peritubular compartment, and testicular interstitial section area (0.17 mm^2). The representation of bar graphs was developed with averages and standard deviations. The statistical differences in the number of cells between compartments were performed through the Mann-Whitney test ($p \leq 0.05$). Digitized microphotographs were obtained with a resolution of a minimum of 300 dpi.

RESULTS

Routine histology. Figure 1 shows a normal histology of the testicle. The three compartments under study are observed with a seminiferous tubule with various stages of spermatogenesis. The peritubular compartment shows normal histology. Similarly, the interstitial compartment is observed, with interstitial cells. PAS/H-Alcián blue stain.

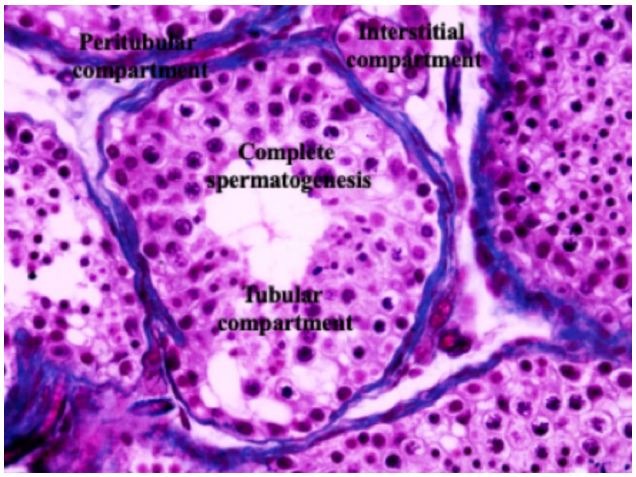

Fig. 1. Normal histology photomicrograph of normal human testis. PAS/H/ Alcián blue stain. 40x.

Figure 2 shows a testicular biopsy with SCOS. Sustentacular cells are observed as a cylindrical lining epithelium with irregular luminal margins and absence of germ cells were observed in the apical two-thirds, and they show a prominent pyramidal-shaped nucleus with the apex directed towards the lumen of the tubule and a central red nucleolus (PAS/H).

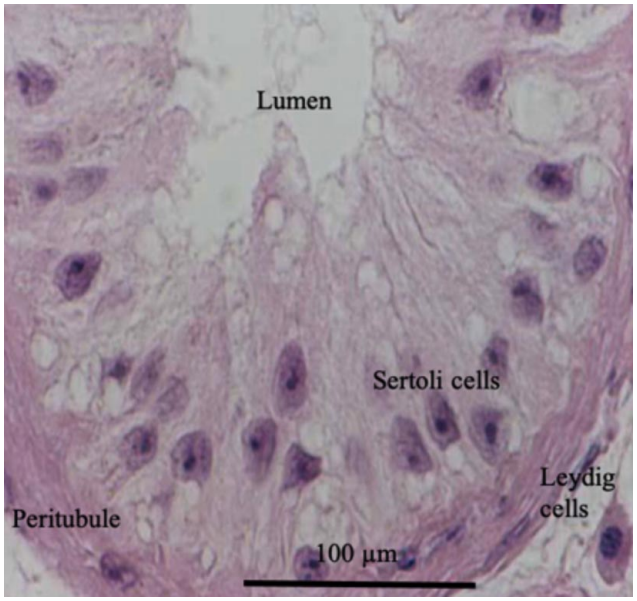

Fig. 2. Human testicular biopsy photomicrograph with Klinefelter diagnosis. PAS/hematoxylin stain. 100x.

Immunohistochemistry

Cellular Retinoic Acid Binding Protein - 1 (CRABP-1). Figure 3, Diagnosis of Klinefelter's biopsy, shows the immunohistochemistry of the three testicular compartments, with cells positive for CRABP-1 (red-brown nuclei). The tubular compartment shows the nuclei of sustentacular cells. However, in the peritubular compartment, only some cells react positively to the staining technique used. The same is observed in the interstitial compartment with interstitial cells.

Figure 4 shows the results of quantifying CRABP-1 positive cells (biopsy with Klinefelter's diagnosis). The number of positive sustentacular cells is significantly higher than positive cells from peritubular and interstitial compartments ($p \leq 0.05$).

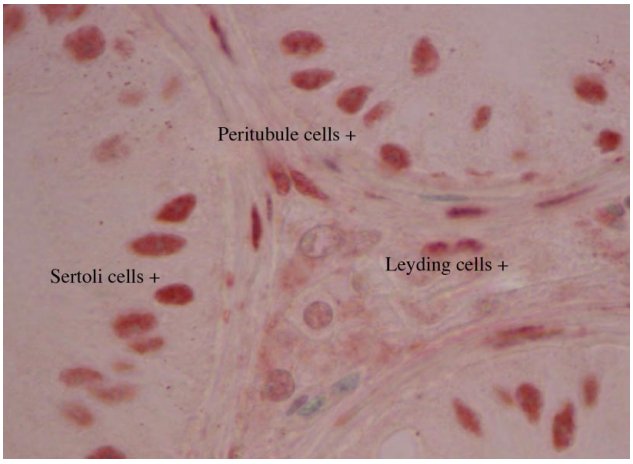

Fig. 3. Human testicular biopsy photomicrograph with a diagnosis of Klinefelter's syndrome. Positive reaction to CRABP-1 (red nucleus) in sustentacular, peritubular, and interstitial cells.

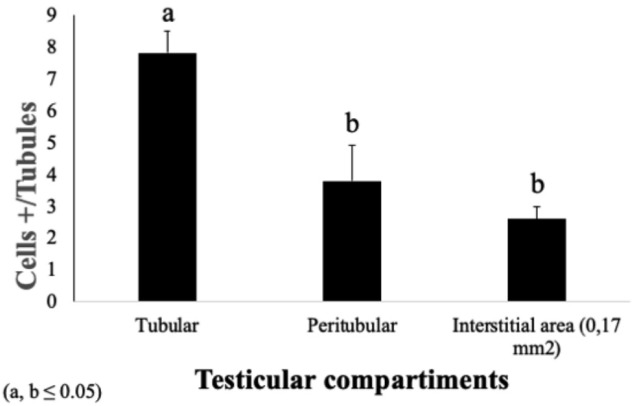

Fig. 4. Bar graph: Quantification of CRABP-1 positive cells by IHC/AEC in the three testicular compartments with Klinefelter's diagnosis. Field area, 0.17 mm^2 ($p \leq 0.05$).

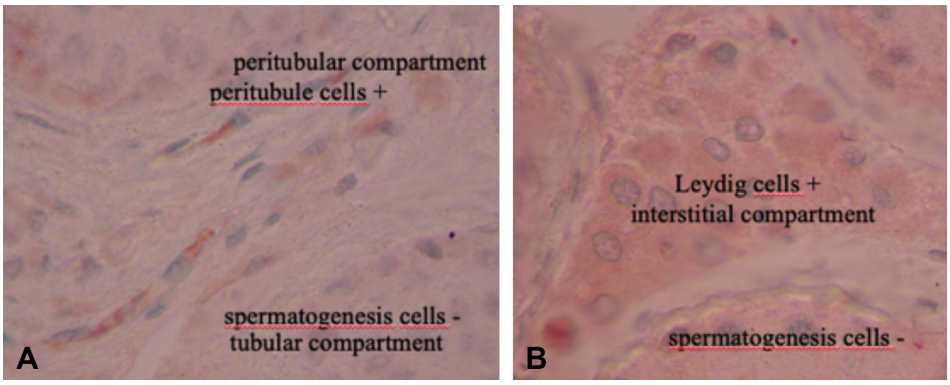

Fig. 5. Testicular biopsy photomicrograph. IHC- AEC staining (red nucleus). Positive reaction to CRABP-IX in interstitial and peritubular cells.

Carbonic anhydrase (CA-IX). Figure 5 (A and B) shows a diagnosis of Klinefelter's disease. Cells of the interstitial and peritubular compartments are observed. interstitial and peritubular cells show positive reactions in the presence of CA-IX. No positive reaction is observed in the cells of the tubular compartment.

The bar graph in Figure 6 shows the results of quantifying AC-IX-positive cells, highlighting an increased number of positive interstitial cells ($p \leq 0.05$).

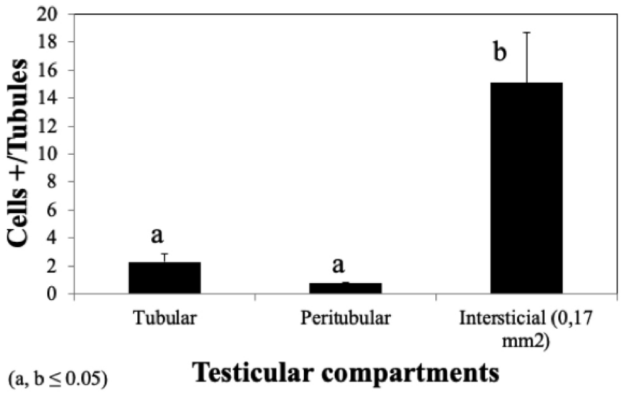

Fig. 6. Bar graph: Quantification of AC-IX positive cells by IHC/AEC in the three testicular compartments (with Klinefelter's). Area of 0.17 mm^2. ($p \leq 0.05$).

DISCUSSION

Harry Klinefelter described in 1942, nine cases of the syndrome that bears his name, initially composed of gynecomastia, tall stature, sparse facial and body hair, reduced sperm count, and small testicular size. The current definition of Klinefelter syndrome, or XXY aneuploidy, consists of achromosomal alteration due to the presence of an extra X chromosome in the male. Histological analyses in these males show many areas of the seminiferous tubules with total or partial absence of spermatogenesis with SCOS (Del Castillo *et al*., 1947). According to Livera *et al*. (2002), the metabolism of the different testicular compartments' express changes in the regulation of retinoic acid uptake, and the acid-base balance, altering the signaling pathways of numerous factors secreted in the sustentacular cells. Low interstitial vascularization and peritubular sclerosis may account for hypoxic determinants of spermatogenesis alterations. Although acidosis is mainly attributed to excessive lactic acid production, it also involves CA-IX mediated conversion of CO_2 into an extracellular proton and a bicarbonate ion transported to the cytoplasm (Bernardino *et al*., 2019). CA-IX is predominantly expressed in tumors with poor prognosis, and its transcription and activity are induced by hypoxia (Ihnatko *et al*., 2006). Aspatwar *et al*. (2023), highlight that these enzymes play an essential role in human sustentacular cells, contributing to spermatogenesis.

During the initiation of testicular meiosis, cellular retinoic acid allows interaction with cytochrome P450 synthesis enzymes, receptors, and degradation enzymes (Griswold *et al*., 2012). Zhang *et al*. (2022b), describe that the action of retinoic acid is associated with endocrine function and testicular cellularity related to FSH and testosterone regulating spermatogenesis, mitosis, and meiosis through several regulators of gene expression (Stra8, Kit, GDNF, BMP4). Gewiss *et al*. (2020), report inhibiting spermatogonial self-renewal through DMRT, GDNF, and cyclin, regulating seminiferous epithelial cycle initiation. According to Schleif *et al*. (2022), retinoic acid is a transcriptional regulator and regulator of cell differentiation in spermatogenesis, including maintenance of the blood-vesicle barrier. Steinhoff *et al*. (2022), report the importance of retinol homeostasis in health and disease. Also, Sá *et al*. (2023), confirm that, in the description of SCOS with Klinefelter, almost the totality of their seminiferous tubules presents a total absence of germline and a tubular compartment only with Sertoli cells and areas of vacuolization. The peritubular compartment is slightly thickened with variable sclerosis, while the intertubular interstitium is observed with abundant lax connective tissue with low cellular density and little vascularization, generally with azoospermia and only sometimes oligozoospermia (Fig. 2).

The different metabolic conditions due to the action of CRABP-1 and CA-IX, with the acidophilic condition, could be the causes of the damage of spermatogenesis in males with a confirmed diagnosis of Klinefelter's disease, added to the probable variable dysfunction of interstitial cells (Maia *et al*., 2002). A genetic condition of the XXY type with alterations of carbonic anhydrase metabolism could affect the retinoic acid binding protein, expressing the disappearance of spermatogonial cells in patients with this genetic diagnosis. This study provides evidence that CRABP-1 is present mainly in the sustentacular cells of the tubular compartment, with a minor distribution in the peritubular and interstitial compartments (Figs. 3 and 4).

CONCLUSION

It is concluded that the metabolism of an acidophilic condition, due to the action of CRABP-1 and CA-IX in testes with a diagnosis of Klinefelter and SCOS, could be the causes of damage to spermatogenesis in men. Sustentacular cells show a strong positive reaction to the detection of the CRABP-1 factor, while interstitial cells show a positive reaction to CA-IX.

RODRÍGUEZ, H.; ARAYA, J.C.: RODRÍGUEZ, N.; ARRIAZA, C. & ESPINOZA-NAVARRO, O. Presencia de CRABP-1 y CA-IX en testículo humano diagnosticado con Klinefelter y síndrome de Sertoli solo (SSS): Efecto en la espermatogénesis. *Int. J. Morphol., 43(1)*:5-9, 2025.

RESUMEN: La espermatogénesis completa se describe claramente en túbulos seminíferos de testículo humano adulto normal. En estudios histológicos de biopsias testiculares con diagnóstico de Klinefelter, se muestran túbulos seminíferos con ausencia total o parcial de espermatogénesis en las células sustentaculares, anomalía conocida como síndrome de Sertoli solo (SSS). El metabolismo testicular es sensible a la regulación del ácido retinoico y del equilibro acido-base. El ácido retinoico (CRABP) y sus metabolitos se presentan como reguladores de la proliferación y diferenciación celular. La anhidrasa carbónica (CA o RCC) regulan una variedad de funciones celulares, incluidos varios procesos en los sistemas reproductivos. El objetivo de este estudio fue demostrar la interacción y presencia de CRABP-1 y CA-IX, en biopsias de testículo humano con diagnóstico de Síndrome de Klinefelter y Sertoli Secundario (SSS) y su posible efecto en una espermatogénesis alterada. Biopsias testiculares fueron procesadas con técnica de histología de rutina normal (PAS/hematoxilina). Para el estudio inmunohistoquímico (IHC) de CA-IX se utilizó anticuerpo policlonal de ratón y para CRABP-1 anticuerpo anti-CRABP-1. Las células positivas se caracterizan por presentar núcleo rojo. Los resultados muestran una gran positividad para CRABP en células de sustentaculares mientras que para CA-IX, las células positivas se expresan en el compartimento interticial. Los gráficos de barra (Test de Mann Whitney, $p \leq 0,05$), muestran diferencias estadísticas significativas entre los diferentes compartimentos germinativos en estudio. Se concluye que el metabolismo de condición acidófila, debido a la acción de CRABP-1 y CA-IX en testículos con diagnóstico de Klinefelter y SSS, podrían ser las causas del daño a la espermatogénesis en los hombres.

PALABRAS CLAVE: CRABP-1; Anhidrasa Carbónica; IHC; Espermatogenesis; Testículo.

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