

**The presence of immunoreactive cyclooxygenases in the ductuli efferentes and epididymis of prepubertal and adult alpaca (*Lama pacos*)**

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**ABSTRACT**

In this study, we report the cell specific expression of cyclooxygenase (COX) enzymes, COX1 and COX2, in the ductuli efferentes and epididymis of prepubertal and adult alpaca. COX1 weakly stained the cytoplasm of epithelial cells lining the ductuli efferentes and the epididymal corpus, whereas these cells were immunonegative in the epididymal caput and cauda. In adults, we observed an increase in the immunosignals for COX1 in the cytoplasm of epithelial cells lining the ductuli efferentes and all the epididymal regions. In prepubertal alpaca, immunoreactivity for COX2 was not revealed in the epithelial cells lining the ductuli efferentes and epididymal regions, whereas it was evidenced in adult animals. The apical rich mitochondria cells immunoreacted only with COX1 in the epididymis of prepubertal animals, whereas they expressed both COX1 and -2 in the adult alpaca. Our results suggest that COXs may play a role in the pubertal development of the excurrent duct system of the alpaca.

**Key words:** alpaca, adult, cyclooxygenase, genital tract, immunohistochemistry, prepubertal

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**Introduction**

It has been established that prostaglandins (PGs) are involved in male reproductive physiology, such as in sperm motility, growth of male accessory sexual glands, and contractility of the smooth muscle layers in the testis (KIRSCHENBAUM et al., 2000; STANFIELD and KHAN, 2003). PGs are produced from the catalytic conversion of arachidonic acid by cyclooxygenase (COX) enzymes, COX1 and COX2 (LAZARUS et al., 2002). COX1 is considered the constitutive isoform, and is expressed in many tissues

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and organs including the kidney, gastrointestinal tract and platelets, where it regulates the production of PGs necessary for homeostatic physiologic processes. Conversely, COX2 is an inducible enzyme that is regulated by a variety of cytokines and mitogens, generating PGs that mediate cell growth and inflammation (WATANABE et al., 2002). Several studies have been performed demonstrating the expression patterns and importance of the COXs in the male and female genital organs of mammalian species (KIRSCHENBAUM et al., 2000; NEERAJA et al., 2003; STANFIELD and KHAN, 2003; BALAJI et al., 2007; WACLAWIK et al., 2008).

The male alpaca (*Lama pacos*), a South American camelid species, has unique reproductive characteristics, which cause their poor breeding performance compared with other domestic species (TIBARY and VAUGHAN, 2006). Some basic descriptions exist of the histology, histochemistry and immunohistochemistry of the male genital tracts of alpacas (PARILLO et al., 2009a,b,c; 2012a, 2013a) and also some aspects of the male reproductive physiology have been examined (CATONE et al., 2010; PARILLO et al., 2011, 2013b; ZERANI et al., 2011).

In order to add new information about the male reproductive biology of this camelid, we report here the cell specific expression of COX1 and COX2 in the ductuli efferentes and epididymis of prepubertal and adult alpaca.

### Materials and methods

*Animals and tissue collection.* Six adult (4-8 years) and four prepubertal (8-12 months) male alpacas from the “Maridiana” farm (Umbertide, Umbria, Italy) were used. The castration technique was performed following the description provided by FOWLER (1998), to prevent aggressive behaviour or to allow housing with non-pregnant females. Immediately after castration at the Veterinary Teaching Hospital of the University Camerino, the testes and epididymides were promptly removed, trimmed of excess tissue, and weighed separately.

*Immunohistochemistry of COX1 and COX2.* Ductuli efferentes and epididymides (divided into caput, corpus, cauda) were processed for immunohistochemical investigation according to procedures previously described (PARILLO et al., 2012b, 2013c; ZERANI et al., 2012, 2013a,b). The slides were incubated with the following primary antibodies: rabbit polyclonal anti-COX1 (1:50, Abcam, Cambridge) and goat polyclonal anti-COX2 (1:50, Abcam, Cambridge). Then, the slides were incubated with biotinylated goat anti-rabbit or rabbit anti-goat secondary antibodies (Santa Cruz Biotechnology, CA, USA), exposed to avidin-biotin complex (ABC kit, Vector Laboratories), and the peroxidase activity sites were visualized using the DAB kit (Vector Laboratories) as chromogen (PARILLO et al., 2013d,e). The sections were counterstained with Mayer’s haematoxylin. Tissue sections, in which the primary antibody was omitted or substituted by rabbit or goat IgG, were used as negative controls of non-specific staining.

### Results and discussion

Our results, for the first time, displayed a different expression pattern of COXs in prepubertal and adult alpaca. Indeed, in prepubertal animals, COX1 weakly marked the cytoplasm of epithelial cells lining the ductuli efferentes (Fig. 1A) and the epididymal corpus (1C), whereas these cells were immunonegative in the epididymal caput and cauda (Fig. 1C, G). In adults, we observed an increase of the immunosignals for COX1 in the cytoplasm of epithelial cells lining the ductuli efferentes (1B) and all the epididymal regions (Fig. 1 D, F, H). In prepubertal alpaca, immunoreactivity for COX2 was not detected in the epithelial cells lining the ductuli efferentes and epididymal regions, but was largely evidenced in the intertubular connective tissue (Fig. 2A, C, E, G). Conversely, in the adult animals, COX2 immunosignals were moderately observed in epithelial cells of efferent ducts and epididymal regions (Fig. 2 B, D, F, H). The apical rich mitochondria cells, which we previously described in the alpaca epididymis (PARILLO et al., 2009a), were detected in all epididymal tracts of the prepubertal animals, whereas they were evidenced in the caput and corpus, but not in the cauda, of the adult epididymis. These cells immunoreacted only with COX1 in the epididymis of prepubertal animals (Fig. 1C, E, G), whereas they expressed both COX1 and -2 in the adult alpaca (data not shown). Taken together, all these results suggest that the expression of COXs is age-related, as reported previously in the rat (STANFIELD and KHAN, 2003). These authors hypothesized that COX2 expression in the epididymis may be positively regulated by androgens. Additionally, although COX2 is considered an inducible isoenzyme, it has been suggested that it is constitutively expressed in immature and mature rat (NEERAJA et al., 2003) and alpaca (PARILLO et al., 2011) testis, in male mouse reproductive organs (LAZARUS et al., 2002), and in other non-reproductive tissues, such as rat and human neurons (BREDER et al., 1995) and rat kidneys (BEUCKMANN et al., 2000). Our immunohistochemical results agree with these studies, suggesting the presence of a constitutive COX2 isoform in the adult and prepubertal alpaca ductuli efferentes and also in the epididymis.

The peritubular myoid cells, present in the subepithelial layer of the ductuli efferentes and the epididymal duct, were immunostained with both COXs in prepubertal and adult alpaca. In this context, it is well known that PGs are modulators of smooth muscle function and growth in various organs (WATANABE et al., 2002), suggesting that COXs may play a functional role in favouring sperm progression along the alpaca genital tracts.

In conclusion, the increasing of COX1 expression in the epithelial lining cells during the development of the excurrent duct system, and the presence of COX2 in the same cells, but only in adult alpaca, led us to hypothesize that COXs and derived PGs may be implicated in the pubertal development of the ductuli efferentes and epididymis in this species.

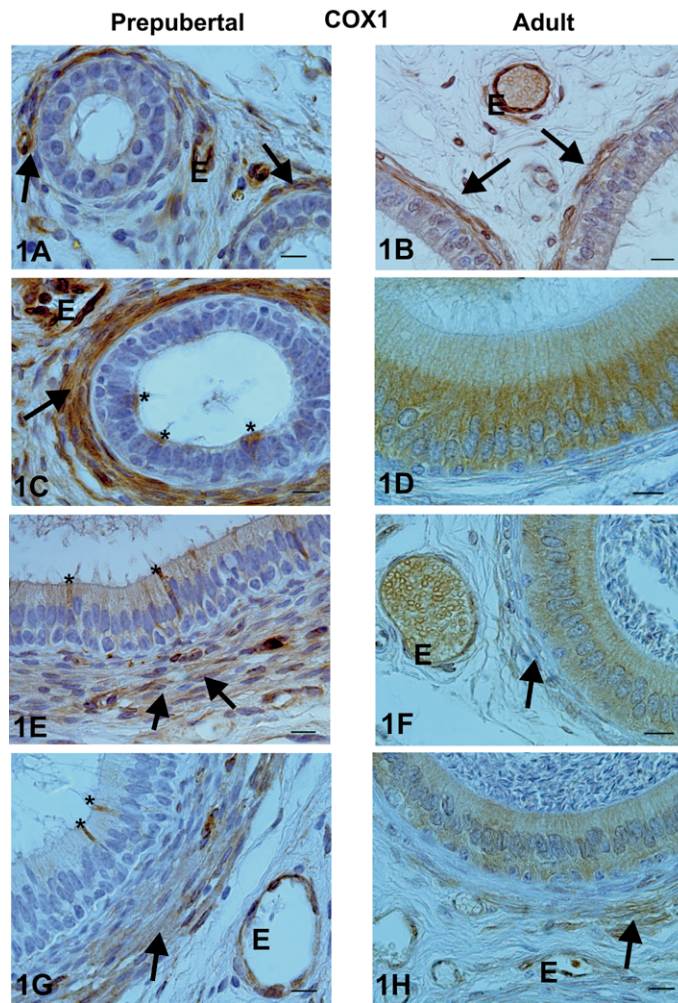


Fig. 1. COX1 - A-B Ductuli efferentes. A) prepubertal alpaca, the cytoplasm of epithelial lining cells weakly reacts; B) adult alpaca, epithelial cells are moderately stained. C-D caput epididymis. Epithelial lining cells are immunonegative in C) prepubertal alpaca and moderately stained in D) adults; E-F corpus epididymis. Immunosignals are weakly localized in epithelial lining cells in E) prepubertal and F) adult animals. G-H cauda epididymis. Epithelial lining cells are immunonegative in G) prepubertal alpaca and moderately stained in H) adults. Peritubular myoid cells (arrows), apical rich mitochondria cells (asterisks), endothelial cells (E). Scale bars = 10  $\mu$ m.

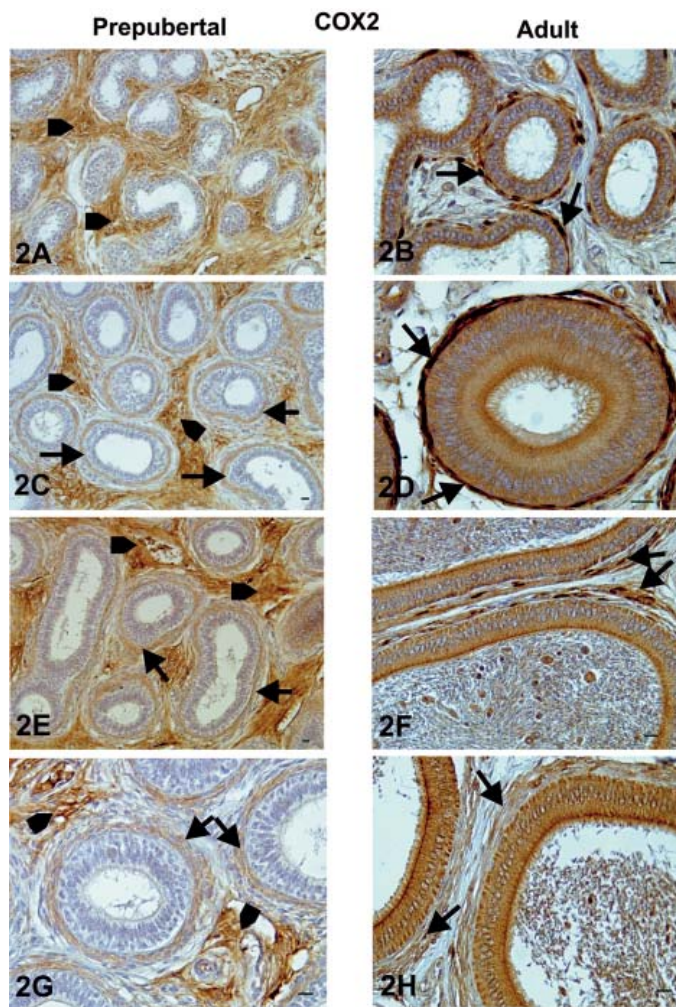


Fig. 2. COX2 - A-B Ductuli efferentes. A) prepubertal alpaca, epithelial lining cells are negative; B) adult alpaca, epithelial cells are moderately stained. C-D caput epididymis, E-F corpus epididymis, G-H cauda epididymis: epithelial lining cells are immunonegative in prepubertal alpaca (C, E, G) and moderately immunostained in adults (D, F, H). Peritubular myoid cells (arrows), intertubular connective tissue (arrowheads). Scale bar = 10  $\mu$ m.

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**PARILLO, F., G. CATONE, C. CAPEZZONE, M. ZERANI: Prisutnost imunoreaktivnih ciklooksigenaza u sjemenskim kanalčićima i nuzjajima u alpaka prije spolne zrelosti i odraslih alpaka (*Lama pacos*). Vet. arhiv 83, 677-684, 2013.**

**SAŽETAK**

Istražena je stanično specifična ekspresija enzima ciklooksigenaza COX1 i COX2 u sjemenim kanalčićima i nuzjaju u alpaka prije spolne zrelosti i odraslih alpaka. Slaba aktivnost COX1 dokazana je u citoplazmi epitelnih stanica sjemenih kanalčića i tijela nuzjaja dok su iste stanice bile imunološki negativne u glavi i repu nuzjaja. U odraslih je opaženo povećanje imunosnog signala za COX1 u citoplazmi bazalnih epitelnih stanica sjemenih kanalčića i cijelog područja nuzjaja. U alpaka prije spolne zrelosti, imunoreaktivnost za COX2 se nije vidjela u epitelnim stanicama sjemenih kanalčića i nuzjajima kao što je to bilo kod odraslih životinja. Apikalne stanice bogate mitohondrijima reagirale su samo na COX1 u nuzjajima životinja prije spolne zrelosti dok je ekspresija i COX1 i COX2 opažene u odraslih alpaka. Rezultati ukazuju na moguću ulogu ciklooksigenaza u razvoju razgranatog sustava kanalčića prije spolne zrelosti u alpaka.

**Ključne riječi:** alpaka, ciklooksigenaza, spolni sustav, imunohistokemija, prepubertet

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