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Urocortinergic system in the epididymis of the normal and cryptorchid dogs

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Abstract

Cryptorchidism is associated with changes in the gonads and the spermatic duct system, which may cause infertility problems. Urocortin (UCN) is a corticotrophin-releasing hormone (CRH)-related peptide, which affects several functions of male genital organs. The aim of the present study was to investigate the expression of UCN and its receptors CRHR1 and CRHR2 using immunohistochemistry, western blotting and real-time reverse transcription polymerase chain reaction in tissues collected from the epididymis of normal and cryptorchid dogs. The lumen of the cryptic epididymal duct was found to be relatively smaller than that of the normal one, and interstitial tissue was abundant in the cryptic epididymis. In addition, only a few spermatids were observed in the lumen of the epididymal duct. Results showed that UCN, CRHR2 and CRHR1 were expressed in tissues collected from normal and cryptic epididymal ducts. Urocortin- and CRHR2-immunoreactivities (IRs) were detected in the principal cells of the caput, corpus and cauda of the normal and cryptic epididymides. CRHR1-IR was detected in vascular smooth muscles and fibromuscular cells surrounding epididymal tubules of the normal and cryptorchid dogs. Expression levels of UCN and CRHR2 mRNA were higher in cryptic epididymal ducts than that in normal epididymal ducts. These results suggest that UCN and its receptors might play a role in regulating the maturation and storage of spermatozoa. These findings indicated that the expression of these proteins could be modulated by the cryptorchidism condition.

KEYWORDS

cryptorchidism, epididymal duct, immunohistochemistry, urocortin

1 | INTRODUCTION

The present study investigated the presence of the urocortinergic system in the epididymis in order to extend our study to the entire male genital tract. Cryptorchidism is the failure of the scrotum of one or both testes to descend (Kawakami, Tsutsui, Yamada, & Yamauchi, 1984). The descent of testes is necessary to allow the normal process of spermatogenesis and, therefore, affects male fertility (Moon et al., 2014). Cryptorchidism is associated with changes in the gonads and spermatic duct system (D'Agostino, Campobasso, Spata, & Belloli, 1994), which might cause infertility problems (Nistal & Paniagua, 1996). Epididymal abnormalities observed in cryptorchidism include reduced diameter of the epididymal duct and decreased height of the epithelium within the duct, along with the absence of spermatozoa in the lumen of the duct (Garcia et al., 2011). Elevated

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temperatures in the cryptorchid state also affect the ionic and protein composition of the caudal fluid, which affects the caudal epithelium by abolishing the ability of the cauda to store and prolong the life of spermatozoa by promotion of rapid epididymal transport (Nieschalag, Behre, Mesched, & Kamischke, 2000). The epididymis is conventionally divided into three contiguous regions based on gross appearance: the caput, corpus and cauda epididymis. Differences between these segments affect the morphology and functions of the epithelial cells. In each of the epididymal segments, a specific set of proteins is expressed and secreted into the luminal fluid creating a dynamic luminal environment required for sperm maturation. Many genes have cell type and segment-specific expression patterns. Epididymal gene expression is regulated by endocrine, lumicrine and paracrine factors in a segment-specific manner providing the basis for epididymal sperm maturation (Sipilä & Björkgren, 2016).

Urocortin is a member of the corticotropin-releasing hormone (CRH)-related peptides that share a high degree of homology, and consists of 40 amino acid residues (Donaldson et al., 1995; Vaughan et al., 1995). The mammalian urocortin gene consists of one intron and two exons, with exon 2 encoding the relative peptide (Zhao, Donaldson, Smith, & Vale, 1998). The biological effects of CRH and UCNs are mediated by two distinct receptors, namely CRH receptor type 1 (CRHR1) and CRH receptor type 2 (CRHR2), respectively, which belong to the G-protein-coupled receptor superfamily of brain-gut neuropeptides (Vita et al., 1995). Urocortin and its receptors have been shown to be associated with a variety of physiological functions and detected in digestive, cardiovascular, immune, endocrine and genital tracts (De Luca et al., 2009; Lee, Braden, Kang, & Rivier, 2011; Oki & Sasano, 2004; Squillacioti et al., 2014, 2012; Squillacioti, Luca, Liguori, Paino, & Mirabella, 2011; Venkatasubramanian, Newby, & Lang, 2010; Yang et al., 2010). Specifically, UCN and CRHR expression has been observed in the testis of rat, mouse, human and dog (Lee et al., 2011; Squillacioti et al., 2016; Tao et al., 2007; Tezval et al., 2009), in the human prostate gland (Arcuri et al., 2002), and in the epididymis of the rat and alpaca (De Luca et al., 2014; Liguori et al., 2015). Urocortin and CRHRs have been considered to play a role in regulating spermatogenesis and steroidogenesis, sperm motility as well as prostatic and epididymal functions. In literature, no data are available regarding the presence and putative roles of UCN in the epididymis of normal and cryptorchid dogs. The present study was undertaken to investigate the presence of the urocortinergic system in the epididymis of dogs. In addition, the expression of the UCN and its receptors was observed in the cryptorchid condition to examine whether the urocortinergic system is involved in the maturation and storage of the spermatozoa.

2 | METHODS

2.1 | Animals and tissue collection

This study was performed using five adult normal male dogs and five male dogs affected by unilateral cryptorchidism (two dogs with a gonad retained in the intra-abdominal region and three dogs with a gonad retained in the intra inguinal canal; in both the cases, the gonads were reduced in size if compared to the normal ones. All retained testes had the same histological characteristics) coming from the surgery unit of the Department of Veterinary Medicine and Animal Productions of the University of Naples "Federico II." All dogs were medium sized and aged between 2 and 8 years. Epididymides were collected immediately after bilateral orchiectomy using surgical techniques. All procedures for the collection of epididymides performed at the surgical unit were monitored by competent veterinary authorities and were approved by the Ethical Animal Care and Use Committee of University of Naples Federico II, Department of Veterinary Medicine and Animal Production, Naples, Italy (no. 0050377). The owner of the animals gave verbal consent to perform surgical procedures and for collection of samples; animals were not involved in any clinical trials or treatments. Tissue samples were divided into three groups: normal epididymis (epididymis from normal dogs), contralateral epididymis (scrotal epididymis from dogs affected by unilateral cryptorchidism) and cryptic epididymis (retained epididymis from the dogs affected by unilateral cryptorchidism). In addition, each epididymis was divided into three segments: caput, corpus and cauda. For western blot and real-time reverse transcription polymerase chain reaction (RT-PCR) analyses, fresh segments of epididymis were immediately frozen on dry ice and stored at −80°C. For immunohistochemical studies, fresh segments of epididymis were immediately fixed by immersion in Bouin's liquid (12–24 hr).

2.2 | Immunoprecipitation and western blotting

Protein extracts, immunoprecipitation and western blotting analysis were performed as described elsewhere (Pelagalli et al., 2016). The following primary antisera were used: polyclonal rabbit anti-UCN (U4757, diluted 1:1,000, Sigma); anti-CRHR1 (SAB4500465, diluted 1:1,000; Sigma); and anti-CRHR2 (SAB4500466, diluted 1:1,000; Sigma). The secondary antibody was anti-rabbit IgG conjugated to peroxidase (Vector Laboratories, diluted 1:2000). Proteins were visualized by an enhanced chemiluminescence kit (Amersham, Buckinghamshire, UK), and the images were acquired with the C-Digit Blot Scanner (Li-Cor). Marker proteins (coloured protein molecular weight markers; Prosieve, Lonza) were used to estimate the molecular weight of each band.

2.3 | Immunohistochemistry

Bouin-fixed tissue samples were processed for paraffin embedding in vacuum and sectioned at a thickness of 6–7 μm. Immunohistochemical staining was performed using EnVision system-horseradish antiperoxidase (HRP) (cod. K4002, Dako) (Squillacioti et al., 2016). Primary antisera were the same as described in the previous section, 1:1,000 diluted. The specificity of the primary immunoreactions was tested by replacing each antibody with a buffer or preabsorbing the antibody with an excess (100 μg antigen/ml antiserum as the final dilution) of the relative antigen

(Liguori et al., 2014, 2017). No immunoreaction was detected in control tests. The samples were observed using a Leica DMRA2 microscope (Leica Microsystems).

2.4 | RNA extraction and real-time RT-qPCR

RNA extraction, cDNA synthesis and real-time RT-qPCR were performed as reported in Squillacioti et al. (2016). The relative quantification method $2^{-\Delta\Delta Ct}$ (2–ddCt) was used for the normalization of gene expression, as described by Squillacioti et al. (2011). For statistical analyses, the data were expressed as mean ± *SD*. Significant differences in the UCN, CRHR1 and CRHR2 mRNA levels between the calibrator sample (normal epididymis) versus contralateral and cryptic epididymides were determined by one-way ANOVA followed by Tukey's HDS test for independent samples. The results were considered statistically significant for $p < 0.05$.

3 | RESULTS

3.1 | Western blot analysis

The results of the western blotting are shown in Figure 1. Tissue extracts of the normal, contralateral and cryptic epididymis of the dogs were reacted with the anti-UCN, anti-CRHR1 and anti-CRHR2 antibodies. Protein bands corresponding to UCN (16 kDa), CRHR1 and CRHR2 (both 55 kDa) were detected in caput, corpus and cauda of epididymis of the normal and cryptorchid dog.

3.2 | Immunohistochemistry

UCN-IR was detected in the epithelium of all the segments of epididymis of the normal and cryptorchid dogs (Figure 2). In the normal dogs, cross-sections of the epididymal duct were clearly observed and spermatids were located in the luminal portion. In the epididymis of the cryptorchid dogs, interstitial tissue was abundant and the lumen was smaller than that of the normal dogs. In addition, only few spermatids were observed in the lumen of the epididymal duct of the cryptorchid dogs. In the normal dogs, UCN-IR was found particularly in the apical portion of the principal cells and, in some samples, immunoreactive granules filled the entire profile of few cells of the caput (Figure 2a,b), corpus (Figure 2c) and cauda (Figure 2d). Immunopositive narrow cells were also detected in the caput of the epididymis of the normal and cryptorchid dogs (Figure 2a,e).

CRHR2-IR was observed in the epithelial principal cells of all the segments of epididymis of the normal and cryptorchid dogs (Figure 3). The immunostaining was distributed particularly in the basal portion of the principal cells of all the segments in both normal and cryptorchid dogs (Figure 3a–h). In addition, CRHR2-IR was also found in some apical cells of the caput of the epididymis of the normal and cryptorchid dogs (Figures 3a and 2e).

CRHR1-IR was found in many fibromuscular cells surrounding the epididymal duct throughout the organ and in the smooth musculature of the blood vessels of the normal and cryptorchid dogs (Figure 4a–h).

3.3 | Real-time RT-PCR

Results of real-time RT-PCR revealed that UCN, CRHR1 and CRHR2 were expressed in all segments of the epididymis of the normal and cryptorchid dogs (Figure 5). In the normal epididymis, the levels of UCN, CRHR1 and CRHR2 expression were higher in the cauda than in the caput and corpus (Figure 5a). The expression levels of UCN, CRHR1 and CRHR2 were higher in the cryptorchid epididymis than in the normal epididymis (Figure 5b–d).

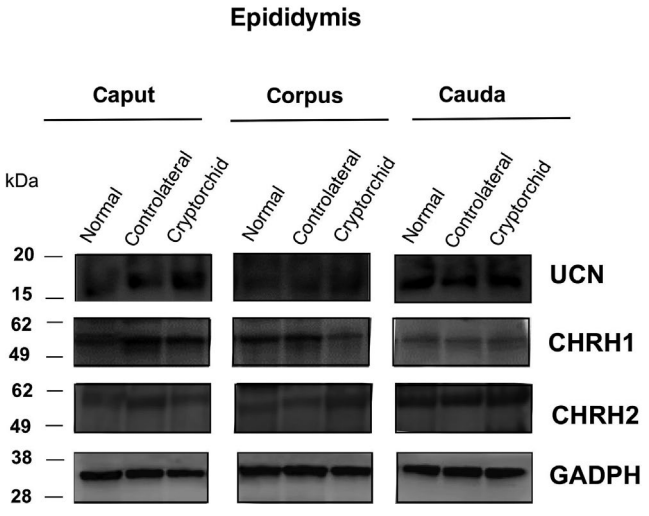

FIGURE 1 Detection of UCN, CRHR1 and CRHR2 in the normal, contralateral and cryptic epididymis of dogs by western blotting. UCN (16 kDa), CRHR1 (55 KDa) and CRHR2 (55 KDa) were detected in the normal, contralateral and cryptic epididymis. GAPDH (36 KDa) was used as a loading control

4 | DISCUSSION

The results of the present study show that UCN, CRHR1 and CRHR2 are expressed in the epididymis of the normal and cryptorchid dogs, which is in agreement with previous findings. Western blotting analysis detected a protein band of approximately 16 kDa consistent with the mammalian UCN precursor, which is a 122-aa protein (Donaldson et al., 1995; Vaughan et al., 1995). In addition, 55 kDa protein bands, consistent with mammalian CRH receptors, were also detected (Perrin, Grace, Riek, & Vale, 2006).

Urocortin- and CRHR2-IRs were found in the epithelial cells of all the segments of the epididymis of the normal and cryptorchid dogs. UCN-IR and CRHR2 were distributed in the principal cells of all the segments of the normal and cryptic epididymis. In addition, some narrow cells were immunopositive for UCN and some apical cells were immunopositive for CRHR2 of the caput epididymis of the normal and cryptorchid dogs. The presence of UCN and CRHR2 was previously described in the rodent (De Luca et al., 2014) and alpaca epididymis (Liguori et al., 2015). In these

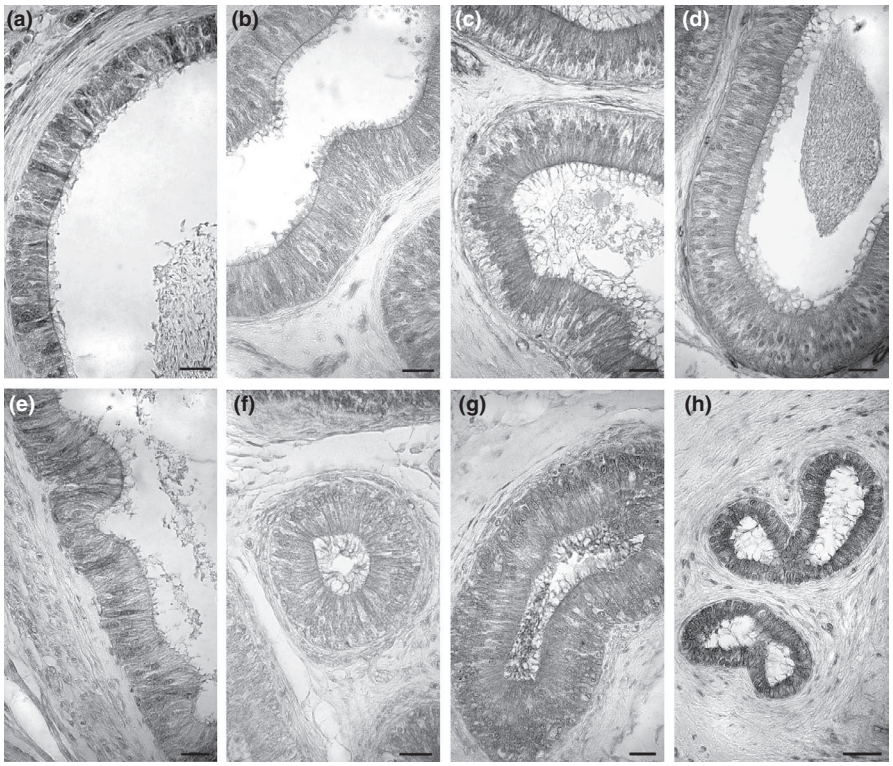

FIGURE 2 Distribution of UCN-IR in the epididymis of the normal (a–d) and cryptorchid dog (e–h). UCN-IR was found in the principal cells of the caput (a, b and e, f), corpus (c, g) and cauda (d, h). Immunopositive granules were observed in the apical portion of the caput (a, b), and in some samples, these granules were present throughout few cells (a), corpus (c) and cauda (d) of the normal epididymis. In the epididymis of the cryptorchid dogs, the immunopositive signal was detected in the principal cells of caput (e, f), corpus (g) and cauda (h). Few positive narrow cells were described in the caput epididymis of both the normal and cryptorchid subjects (a, e). Bars: 25 μm

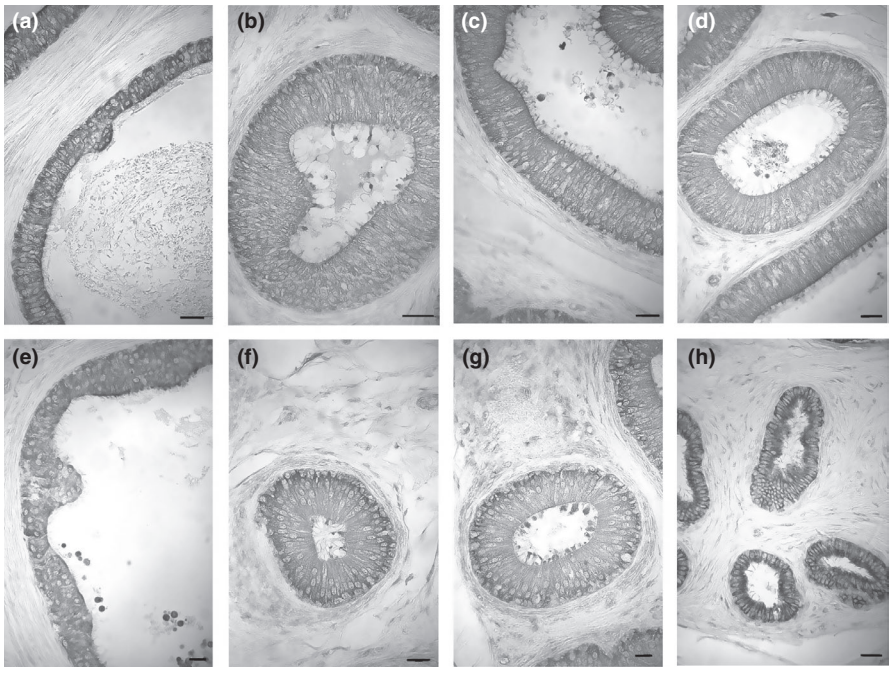

FIGURE 3 Distribution of the CRHR2-IR in the epididymis of the normal (a–d) and cryptorchid dogs (e–h). CRHR2-IR was observed in the principal cells of the caput (a, b and e, f), corpus (c, g) and cauda (d, h). The basal portion of the principal cells was positive for CRHR2 (a–h). Only few reactive apical cells were found (a, e). Bars: 25 μm

reports, it was hypothesized that the role for UCN is modulation of growth and hormonal secretion of the epididymal epithelial cells via CRHR2 by an autocrine mechanism. Based on the results of the present study, it could be argued that this mechanism may exist in the dog epididymis. Further, it could be hypothesized that UCN plays a role in the cell–cell interactions, particularly between principal, apical and narrow cells in the epididymal duct of both normal and cryptorchid dogs. These different cytotypes have several functions. Principal cells are responsible for the bulk of the proteins that are secreted into the lumen and are directly involved in the control of the luminal protein concentrations (Robaire, Hinton, & Orgebin-Crist, 2006). Apical cells are related to sperm quiescence and regulation of the pH in the lumen through the production of enzymes of the carbonic anhydrase family (Hermo et al., 2005). Narrow and apical cells are involved in the intracellular transport between the lumen and epithelial

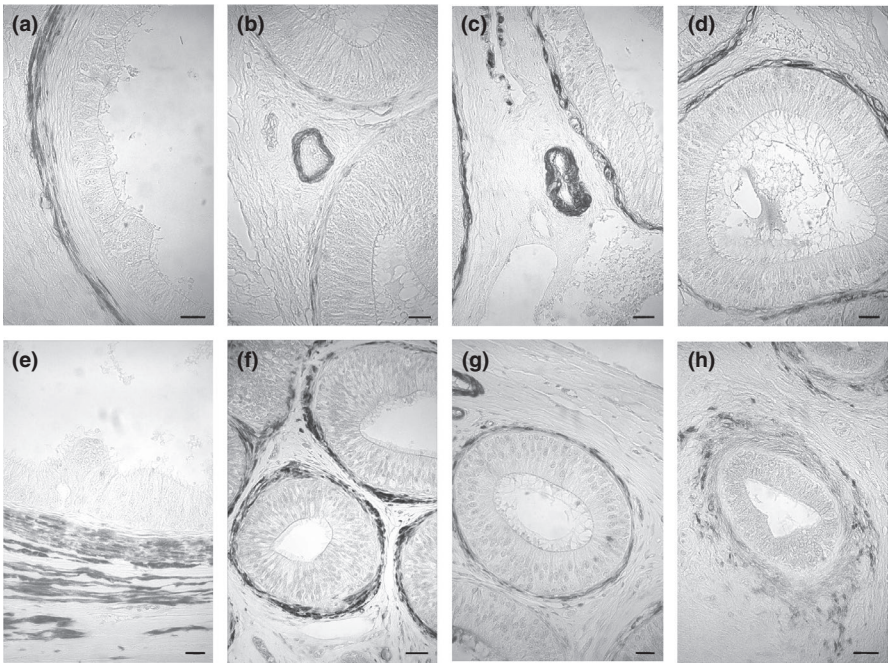

FIGURE 4 Distribution of CRHR1-IR in the epididymis of the normal (a–d) and cryptorchid dog (e–h). CRHR1-IR was found in the fibromuscular cells surrounding epididymal tubules and in the musculature of the blood vessels of the caput (a, b and e, f), corpus (c, g) and cauda (d, h). Bars: 25 µm

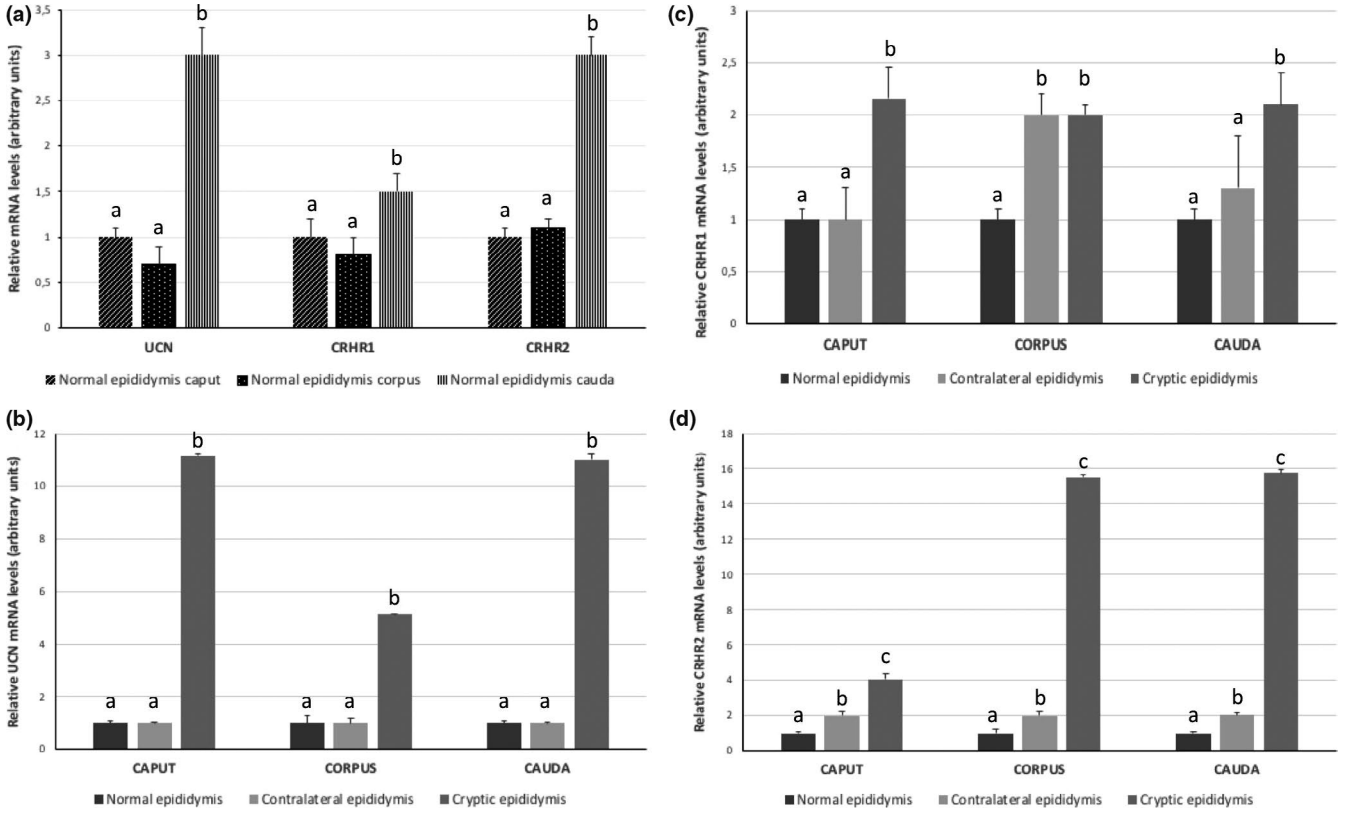

FIGURE 5 Expression of UCN, CRHR1 and CRHR2 in the epididymis of the normal and cryptorchid dogs detected by real-time RT-PCR. (a) The statistical differences in the expression levels of UCN, CRHR1 and CRHR2 between the calibrator sample (caput) and the corpus and cauda epididymis of the normal dogs were determined by one-way ANOVA followed by Tukey's HDS tests. Different letters depict significant differences between the examined groups ($p < 0.05$). (b–d) The statistical differences in the expression levels of UCN, CRHR1 and CRHR2 between the calibrator sample (normal epididymis) and the contralateral and cryptic epididymis were determined by one-way ANOVA followed by Tukey's HDS tests. Different letters depict significant differences between the examined groups ($p < 0.05$)

cells, degradation of specific proteins and carbohydrates within their lysosomes, and protecting spermatozoa from a changing environment of harmful electrophiles (Robaire et al., 2006). Each cytotype may express different proteins within the distinct epididymal regions. This epididymal regionalization, which is attributed to the diverse patterns of gene expression, is critical to

the formation and maintenance of the functions of the epididymal duct (Belleannè, Thimon, & Sullivan, 2012).

CRHR1-IR was found in many fibromuscular cells surrounding the epididymal duct throughout the organ and in the smooth muscles of the blood vessels of the normal and cryptorchid dogs. The localization of CRHR1 in the cryptic epididymal duct was similar to that in the normal. This suggests that cryptorchidism does not induce changes in the localization of CRHR1. The presence of CRHR1 in these cytotypes was described in the rat (De Luca et al., 2014) and alpaca epididymis (Liguori et al., 2015), suggesting a role for UCN in the regulation of the contractility of this duct by a paracrine mechanism via CRHR1. Based on the results of this study, this research proposes that this mechanism may also be present in dog epididymis. Among reproductive organs, CRH-related peptides have been demonstrated to modulate uterine contractility during pregnancy (Grammatopoulos, 2007; Linton et al., 2001). Moreover, evidence supports that, although the epididymis has a rich innervation, other local, non-neuronal factors participate in the nerve-independent epididymal contractility (Corona, Jannini, Vignozzi, Rastrelli, & Maggi, 2012). In particular, the cauda epididymis, in which the fibromuscular pattern is higher than in the caput and corpus, is the major site for storage of spermatozoa in the male reproductive tract. It might be hypothesized that CRH-related peptides facilitate the storage of spermatozoa in the cauda by inhibiting the contractility of epididymal fibromuscular stromal cells via CRHR1. The localization of CRHR1 in the smooth musculature of blood vessels suggests its role in modulating the local blood flow. This hypothesis is corroborated by the finding that UCN is a dilator of arteries (Lubomirov et al., 2006).

Real-time RT-PCR analysis demonstrated that UCN, CRHR1 and CRHR2 are expressed in all segments of the epididymis of the normal and cryptorchid dogs. In the normal epididymis, expression levels of UCN, CRHR1 and CRHR2 were higher in the cauda than in the caput and corpus. Expression levels of UCN, CRHR1 and CRHR2 were higher in the cryptorchid than in the normal epididymis. The expression levels of these mRNAs in the normal dog epididymis were different from those in the rat (De Luca et al., 2014). In the normal rat epididymis, the expression levels of UCN and CRHR2 were higher in the caput and corpus than that in the cauda. In previous papers, it has been suggested that UCN and CRHR2 may play an important role in the epithelial cell-mediated maturation of spermatozoa that initially occurs in the caput and corpus (De Luca et al., 2014). Urocortin may influence the maturation of spermatozoa via different indirect mechanisms such as regulating the growth of epididymal epithelial cells and their hormonal secretion. In the normal dog epididymis, a higher expression level has been demonstrated in the cauda if compared to the other epididymal segments. The cauda of the epididymis is the major site of spermatozoa storage in the male reproductive tract. The mechanisms of sperm survival in the distal epididymis are poorly understood. The spermatozoa are at a risk during transit and during the period of storage within the epididymis because of their susceptibility to lipid peroxidation by reactive oxygen species (ROS). To avoid such damage as well as bacterial attacks, specific proteins released from epididymal epithelium appear to play a protective role (Gatti et al., 2004; Robaire et al., 2006; Robaire, Syntin, & Jervis, 2000). Urocortin may be involved in this mechanism. Urocortin has potent endothelial anti-oxidative properties and functions as a counter regulator of oxidative stress in inflammatory lesions (Honjo et al., 2006) and prevents mitochondrial permeability transition in response to reperfusion injury indirectly, by reducing oxidative stress (Townsend et al., 2007). The difference observed in the mRNA levels of UCN and its receptors in these two animal species (rat and dog) with different breeding behaviour probably reflect the sperm storage conditions in the epididymal cauda. The importance of the epididymal microenvironment has been well recognized and, in particular, the environment in the caudal portion should favour maximal survival time of the spermatozoa stored there for relatively long periods without losing their ability to fertilize and motility (Robaire et al., 2006; Rodriguez & Hinton, 2003).

In addition, the cryptorchid condition affects the expression level of UCN and its receptors in all segment of epididymal tract, inducing an increase in these levels. Epididymis is known to possess distinct gene expression profiles, ensuring different epididymal functions essential to the various steps of sperm maturation (Belleannè et al., 2012; Sipilä & Björkgren, 2016). Factors such as steroid hormones, lumicrine factors and temperature affect the gene expression pattern in the epididymis (Sipilä & Björkgren, 2016). The increase in the temperature in the cryptorchid condition may affect the gene expression and physiology of the epididymis. For instance, temperature regulates sperm storage and survival in the cauda epididymis and epididymal epithelial cell secretion (Esponda & Bedford, 1986; Regalado, Esponda, & NietoA, 1993).

Moreover, specific gene expression in the epididymis is controlled by temperature (Esponda & Bedford, 1986; Pera, Ivell, & Kirchoff, 1996; Regalado et al., 1993). In particular, bcl-2 and bax mRNAs have been shown to be overexpressed following cryptepididdidymis, suggesting a role in the temperature-dependent apoptosis observed in the cauda epididymis (Jara, Esponda, & Carballada, 2002). It has been hypothesized that UCN plays a role in the apoptosis in the cryptic epididymis. This hypothesis is corroborated by the finding that UCNs induce macrophage apoptosis via CRHR2. Following their activation, macrophages undergo apoptosis as a mechanism to contain inflammation. For instance, UCNs exert an anti-inflammatory effect via inducing macrophage apoptosis (Tsatsanis et al., 2005). In addition, we cannot exclude a possibility that the higher expression of UCN and its receptors in the cryptic epididymis than in normal epididymis could be a compensatory mechanism in order to replace the normal condition.

In conclusion, the present study demonstrates the difference in the expression of UCN and its receptor between epididymides of normal and cryptorchid dogs. These findings indicate that the expressions of these proteins were altered in the pathologic condition of cryptorchidism. Further investigations regarding the roles of UCN on cryptorchidism are necessary.

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CONFLICT OF INTEREST

We confirm that there are no known conflicts of interest to declare, and there has been no significant financial support for this research.

AUTHOR CONTRIBUTIONS

CS, AP and NM contributed to the experimental design, interpretation of results and critical review of the manuscript draft; AD, GL and SA collected samples, performed experiments, discussed results and contributed to write the manuscript draft. All the authors approved the final version of the manuscript.

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REFERENCES

Arcuri, F., Cintorino, M., Florio, P., Floccari, F., Pergola, L., & Romagnoli, R. (2002). Expression of urocortin mRNA and peptide in the human prostate and in prostatic adenocarcinoma. *Prostate*, *52*, 167–172. https://doi.org/10.1002/pros.10094

Belleannè, C., Thimon, V., & Sullivan, R. (2012). Region-specific gene expression in the epididymis. *Cell and Tissue Research*, *349*, 717–731. https://doi.org/10.1007/s00441-012-1381-0

Corona, G., Jannini, E. A., Vignozzi, L., Rastrelli, G., & Maggi, M. (2012). The hormonal control of ejaculation. *Nature Reviews. Urology*, *9*, 508–519. https://doi.org/10.1038/nrurol.2012.147c

D'Agostino, S., Campobasso, P., Spata, F., & Belloli, G. (1994). Cryptorchidism: Anomalies of excretory ducts and azoospermia. *Pediatria Medica E Chirurgica*, *16*, 509–512.

De Luca, A., Liguori, G., Squillacioti, C., Paino, S., Germano, G., Alì, S., & Mirabella, N. (2014). Expression of urocortin and its receptors in the rat epididymis. *Reproductive Biology*, *14*, 140–147. https://doi.org/10.1016/j.repbio.2014.01.007

De Luca, A., Squillacioti, C., Pero, M. E., Paino, S., Langella, E., & Mirabella, N. (2009). Urocortin-like immunoreactivity in the primary lymphoid organs of the duck (*Anas platyrhynchos*). *European Journal of Histochemistry*, *53*, 167–176. https://doi.org/10.4081/ejh.2009.167

Donaldson, C. J., Sutton, S. W., Perrin, M. H., Corrigan, A. Z., Lewis, K. A., & Rivier, J. (1995). Cloning and characterization of human urocortin. *Endocrinology*, *137*, 3896.

Esponda, P., & Bedford, J. M. (1986). The influence of body temperature and castration on the protein composition of fluid in the rat cauda epididymidis. *Journal of Reproduction and Fertility*, *78*, 505–514. https://doi.org/10.1530/jrf.0.0780505

Garcia, P. V., Arroteia, K. F., Joazeiro, P. P., Mesquita, S. F. P., Kempinas, W. G., & Pereira, L. A. V. (2011). Orchidopexy restores morphometric-stereologic changes in the caput epididymis and daily sperm production in cryptorchidic mice, although sperm transit time and fertility parameters remain impaired. *Fertility and Sterility*, *96*, 739–744. https://doi.org/10.1016/j.fertnstert.2011.06.036

Gatti, J. L., Castella, S., Dacheux, F., Ecroyd, H., Métayer, S., Thimon, V., & Dacheux, J. L. (2004). Post-testicular sperm environment and fertility. *Animal Reproduction Science*, *82–83*, 321–339.

Grammatopoulos, D. K. (2007). The role of CRH receptors and their agonists in myometrial contractility and quiescence during pregnancy and labour. *Frontiers in Bioscience*, *12*, 561–571. https://doi.org/10.2741/2082

Hermo, L., Chong, D. L., Moffatt, P., Sly, W. S., Waheed, A., & Smith, C. E. (2005). Region – and cell – specific differences in the distribution of carbonic anhydrases II, III, XII, and XIV in the adult rat epididymis. *The Journal of Histochemistry and Cytochemistry*, *53*, 699–713. https://doi.org/10.1369/jhc.4a6575.2005

Honjo, T., Inoue, N., Shiraki, R., Kobayashi, S., Otsui, K., & Takahashi, M. (2006). Endothelial urocortin has potent antioxidative properties and is upregulated by inflammatory cytokines and pitavastatin. *Journal of Vascular Research*, *43*, 131–138. https://doi.org/10.1159/000090132

Jara, M., Esponda, P., & Carballada, R. (2002). Abdominal temperature induces region-specific p53-independent apoptosis in the cauda epididymidis of the mouse. *Biology of Reproduction*, *67*, 1189–1196. https://doi.org/10.1095/biolreprod67.4.1189

Kawakami, E., Tsutsui, T., Yamada, Y., & Yamauchi, M. (1984). Cryptorchidism in the dog: Occurrence of cryptorchidism and semen quality in the cryptorchid dog. *Nihon Juigaku Zasshi*, *46*, 303–308. https://doi.org/10.1292/jvms1939.46.303

Lee, S., Braden, B., Kang, S. S., & Rivier, C. (2011). Urocortins are present in the rat testis. *Neuropeptides*, *45*, 131–137. https://doi.org/10.1016/j.npep.2010.12.006

Liguori, G., Paino, S., Mirabella, N., Squillacioti, C., De Luca, A., & Vittoria, A. (2014). Expression of orexin A and its receptor 1 in the epididymis of the South American camelid alpaca (*Vicugna pacos*). *Anatomia Histologia and Embryologia*, *43*, 42–47. https://doi.org/10.1111/ahe.12046

Liguori, G., Pavone, L. M., Assisi, L., Langella, E., Tafuri, S., Mirabella, N., … Vittoria, A. (2017). Expression of orexin B and its receptor 2 in rat testis. *General and Comparative Endocrinology*, *242*, 66–73. https://doi.org/10.1016/j.ygcen.2015.11.015

Liguori, G., Squillacioti, C., De Luca, A., Ciarcia, R., Vittoria, A., & Mirabella, N. (2015). Presence and distribution of urocortin and its receptors in the epididymis of alpaca (*Vicugna pacos*). *Anatomia Histologia Embryologia*, *44*, 66–71. https://doi.org/10.1111/ahe.12110

Linton, E. A., Woodman, J. R., Asboth, G., Glynn, B. P., Plested, C. P., & Bernal, A. L. (2001). Corticotrophin releasing hormone: Its potential for a role in human myometrium. *Experimental Physiology*, *86*, 273–281.

Lubomirov, L. T., Reimann, K., Metzler, D., Hasse, V., Stehle, R., Ito, M., … Schubert, R. (2006). Urocortin-induced decrease in Ca^{2+} sensitivity of contraction in mouse tail arteries is attributable to cAMP-dependent dephosphorylation of MYPT1 and activation of myosin light chain phosphatase. *Circulation Research*, *98*, 1159–1167.

Moon, J. H., Yoo, D. Y., Jo, Y. K., Kim, G. A., Jung, H. Y., Choi, J. H., … Jang, G. (2014). Unilateral cryptorchidism induces morphological changes of testes and hyperplasia of Sertoli cells in a dog. *Laboratory Animal Research*, *30*, 185–189. https://doi.org/10.5625/lar.2014.30.4.185

Nieschalag, E., Behre, H. M., Mesched, D., & Kamischke, A. (2000). Disorders at the testicular level. In E. Nieschalag, & H. M. Behre (Eds.), *Andrology: Male reproduction health and dysfunction* (pp. 143–176). Berlin, Germany: Springer. ISBN: 3-540-67224-9.

Nistal, M., & Paniagua, R. (1996). Non-Neoplastic diseases of the testis. In D. G. Bostwick, & J. N. Eble (Eds.), *Urologic SURGICAL PATHOLOGY* (pp. 457–565). St Louis, MO: Mosby-Year Book.

Oki, Y., & Sasano, H. (2004). Localization and physiological roles of urocortin. *Peptides*, *25*, 1745–1749. https://doi.org/10.1016/j.peptides.2004.06.023

Pelagalli, A., Squillacioti, C., De Luca, A., Pero, M. E., Vassalotti, G., Lombardi, P., … Mirabella, N. (2016). Expression and localization

of aquaporin 4 and aquaporin 5 along the large intestine of colostrum-suckling buffalo calves. *Journal of Veterinary Medicine Series C: Anatomia Histologia Embryologia*, *45*, 418–427. https://doi.org/10.1111/ahe.12210

Pera, I., Ivell, R., & Kirchoff, C. (1996). Body temperature (37 C) specifically down-regulates the messenger ribonucleic acid for the major sperm surface antigen CD52 in epididymal cell culture. *Endocrinology*, *137*, 4451–4459.

Perrin, M. H., Grace, C. R., Riek, R., & Vale, W. W. (2006). The three-dimensional structure of the N-terminal domain of corticotropin-releasing factor receptors: Sushi domains and the B1 family of G protein-coupled receptors. *Annals of the New York Academy of Sciences*, *1070*, 105–119. https://doi.org/10.1196/annals.1317.065

Regalado, F., Esponda, P., & NietoA,, (1993). Temperature and androgens regulate the biosynthesis of secretory proteins from rabbit cauda epididymidis. *Molecular Reproduction and Development*, *36*, 448–453. https://doi.org/10.1002/mrd.1080360407

Robaire, B., Hinton, B. T., & Orgebin-Crist, M. C. (2006). The epididymis. In Knobil & J. Neil (Ed.) *The physiology of reproduction* (pp. 1071–1148). New York, NY: Elsevier. EUA

Robaire, B., Syntin, P., & Jervis, K. (2000). The coming of age of the epididymis. In B. Jégou, C. Pineau, & J. Saez (Eds.), *Testis*, *epididymis and technologies in the year 2000* (pp. 229–262). Berlin, Germany: Springer.

Rodriguez, C. M., & Hinton, B. T. (2003). The testicular and epididymal luminal fluid microenvironment. In D. Tulsiani (Ed.), *Introduction to Mammalian Reproduction* (pp. 61–77). Norwell, MA: Kluwer Academic.

Sipilä, P., & Björkgren, I. (2016). Segment-specific regulation of epididymal gene expression. *Reproduction*, *152*, R91–R99. https://doi.org/10.1530/rep-15-0533

Squillacioti, C., De Luca, A., Alì, S., Ciarcia, R., Germano, G., Vittoria, A., & Mirabella, N. (2014). Presence and distribution of urocortin and corticotrophin-releasing hormone receptors in the bovine thyroid gland. *Anatomia Histologia and Embryologia*, *43*, 429–434. https://doi.org/10.1111/ahe.12094

Squillacioti, C., De Luca, A., Alì, S., Paino, S., Liguori, G., & Mirabella, N. (2012). Expression of urocortin and corticotropin-releasing hormone receptors in the horse thyroid gland. *Cell Tissue Research*, *350*, 45–53. https://doi.org/10.1007/s00441-012-1450-4

Squillacioti, C., De Luca, A., Liguori, G., Alì, S., Germano, G., Vassalotti, G., … Mirabella, N. (2016). Urocortinergic system in the testes of normal and cryptorchid dogs. *Annals of Anatomy – Anatomischer Anzeiger*, *207*, 91–96. https://doi.org/10.1016/j.aanat.2016.05.004

Squillacioti, C., De Luca, A., Liguori, G., Paino, S., & Mirabella, N. (2011). Expression of urocortin and corticotropin-releasing hormone receptors in the bovine adrenal gland. *General and Comparative Endocrinology*, *172*, 416–422. https://doi.org/10.1016/j.ygcen.2011.04.004

Tao, J., Lin, M., Sha, J., Tan, G., Wah Soong, T., & Li, S. (2007). Separate locations of urocortin and its receptors in mouse testis: Function in male reproduction and the relevant mechanisms. *Cell Physiol and Biochem*, *19*, 303–312.

Tezval, H., Merseburger, A. S., Serth, J., Herrmann, T. W., Becker, J. U., Jahn, O., & Kuczyk, M. A. (2009). Differential expression of urocortin in human testicular germ cells in course of spermatogenesis: Role of urocortin in male fertility? *Urology*, *73*, 901–905.

Townsend, P. A., Davidson, S. M., Samantha, J., Khaliulin, C. I., Carroll, C. J., Scarabelli, T. M., … Halestrap, A. P. (2007). Urocortin prevents mitochondrial permeability transition in response to reperfusion injury indirectly, by reducing oxidative stress. *American Journal of Physiology. Heart and Circulatory Physiology*, *293*, H928–H938.

Tsatsanis, C., Androulidaki, A., Dermitzaki, E., Charalampopoulos, I., Spiess, J., Gravanis, A., & Margioris, A. N. (2005). Urocortin 1 and Urocortin 2 induce macrophage apoptosis via CRFR2. *FEBS Letters*, *579*, 4259–4264.

Vaughan, J., Donaldson, C., Bittencourt, J., Perrin, M. H., Lewis, K., Sutton, S., … Vale, W. W. (1995). Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotrophin-releasing factor. *Nature*, *378*, 287–292. https://doi.org/10.1038/378287a0

Venkatasubramanian, S., Newby, D. E., & Lang, N. N. (2010). Urocortins in heart failure. *Biochemical Pharmacology*, *80*, 289–296.

Vita, N., Laurent, P., Lefort, S., Chalon, P., Lelias, J. M., Kaghad, M., … Ferrara, P. (1995). Primary structure and functional expression of mouse pituitary and human brain corticotrophin releasing factor receptors. *FEBS Letters*, *335*, 1–5.

Yang, L. Z., Tovote, P., Rayner, M., Kockskamper, J., Pieske, B., & Spiess, J. (2010). Corticotropin-releasing factor receptors and urocortins, links between the brain and the heart. *European Journal of Pharmacology*, *632*, 1–6. https://doi.org/10.1016/j.ejphar.2010.01.027

Zhao, L., Donaldson, C. J., Smith, G. W., & Vale, W. W. (1998). The structures of the mouse and human urocortin genes (Ucn and UCN). *Genomics*, *50*, 23–33. https://doi.org/10.1006/geno.1998.5292

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