

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Role of Adhesion Molecules and Proliferation Hyperplastic, Pre Neoplastic and Neoplastic Lesions in Canine Prostate

¹M.M.P. Rodrigues, ²A. Rema, ^{2,3}M.F. Gartner and ¹R. Laufer-Amorim

¹São Paulo State University, Botucatu, Brazil

²Institute of Biomedical Sciences of Abel Salazar, University of Porto, Portugal

³Institute of Pathology and Molecular Immunology, University of Porto, Portugal

Abstract: E-cadherin and beta-catenin are component of adherens junctions in epithelial cells. Loss of these proteins have been associated with progression of prostatic diseases. We performed immunohistochemistry for E-cadherin, beta-catenin and Ki-67 on canine prostatic lesions. We analyzed the expression of these antibodies in benign prostatic hyperplasia (BPH, n = 22), in pre neoplastic lesions Prostatic Intra-epithelial Neoplasia (PIN), n = 3 and Prostatic Inflammatory Atrophy (PIA), n = 7 and prostate carcinoma (PC, n = 10). In this study, a membranous expression of E-cadherin and beta-catenin and nuclear expression of Ki-67 antigen were demonstrated. The proliferative index was statistically different between carcinomas and BPH and carcinomas and pre-neoplastic lesions. Like in men, the reduction of E-cadherin and increase of Ki-67 expression in neoplastic lesions in dog prostate may be related to the carcinogenic process in this gland.

Key words: BPH, β -catenin, canine, E-cadherin, prostate lesions

INTRODUCTION

The canine prostate gland shares many morphological and functional characteristics with the human prostate; dogs also are the only other large mammal that develops spontaneous prostate cancer (Bostwick, 1997). The canine prostate has a uniform morphology along the longitudinal axis and lacks zone demarcation based on glandular differentiation and epithelial cell morphology, as the human organ does (Foster and Ladds, 2007). Closely packed acini containing secretory epithelium comprise the major part of the canine prostate, from the periphery to the periurethral area (Lai *et al.*, 2008). The epithelial cells in the prostate gland are bound to one another, as well as to the Extracellular Matrix (ECM) (Mol *et al.*, 2007), through Cell Adhesion Molecules (CAMs) that are responsible for the maintenance of a normal tissue phenotype (Pontes-Junior *et al.*, 2009).

E-cadherin, a member of the cadherin family of CAMs, mediates lateral cell-cell adhesion in secretory tissues, including the prostate and mammary glands (Jaggi *et al.*, 2005). E-cadherin, a type-1 Ca^{2+} -dependent CAM, is a major component of adherens junctions in epithelial cells. E-cadherin is located at the cell membrane and binds cytoskeletal proteins through catenins (Jaggi *et al.*, 2005;

Matos *et al.*, 2007; Saha *et al.*, 2008). The expression of E-cadherin may play a role in the transition from noninvasive to invasive phenotype in prostate cancer disease (Verras and Sun, 2006). E-cadherin expression is strongly down-regulated in human prostate carcinoma, compared to benign prostate tissue in the same section (Jaggi *et al.*, 2005).

Beta-catenin, part of the cell membrane-bound adherens complex, binds to the cytoplasmic domain of cadherin and anchors the complex to the cytoskeleton. Beta-catenin is not only implicated structurally in the adherens junction complex but also acts as a key signaling molecule (Mol *et al.*, 2007). The loss of membranous beta-catenin expression has been associated with progression from benign to malignant prostate pathology in men (Jaggi *et al.*, 2005). More recently, nuclear beta-catenin signaling has been implicated in prostate carcinogenesis (Horvarth *et al.*, 2005). Multiple *in vivo* studies implicate beta-catenin in prostate cell growth and prostate carcinogenesis (Jaggi *et al.*, 2005; Verras and Sun, 2006; Matos *et al.*, 2006).

Loss of interaction between E-cadherin and beta-catenin not only disrupts intercellular adhesion in invasive cancer cells but also alters cell growth and differentiation signaling (Horvarth *et al.*, 2005; Gounari *et al.*, 2002; Whitaker *et al.*, 2008). E-cadherin, in

association with beta-catenin, controls cell-cell adhesion and influences cell migration (Horvarth *et al.*, 2005), cell growth and transformation and any disturbances in the system may promote neoplastic growth (Nowak *et al.*, 2007). The purpose of this study was to evaluate and compare the protein expression of both E-cadherin and beta-catenin and correlate with proliferation using Ki-67 antibody, in Benign Prostatic Hyperplasia (BPH), pre-neoplastic lesions (PIN and PIA) and Prostate Carcinoma (PC).

MATERIALS AND METHODS

Thirty-seven prostates from dogs aged 1 to 15 years old (pure or mixed breed) were evaluated. The samples were selected from the archives of the Veterinary Pathology Service, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil. Tissues were fixed in 10% formalin and paraffin-embedded. Sections (2 µm) were cut and stained with hematoxylin and eosin (HE) for histological examination. From each tissue block at least three consecutive sections of 3 µm were cut for immunohistochemical studies. Histopathological analysis was conducted according to a previously published method (Foster and Ladds, 2007) for BPH (non-neoplastic lesion) and carcinoma (neoplastic lesion). For Prostatic Intraepithelial Neoplasia (PIN), the analysis was performed as described by Bostwick, 1997 and, for Prostatic Inflammatory Atrophy (PIA) as described by De Marzo *et al.* (1999) and Toledo *et al.* (2010). PIN and PIA were included in the pre-neoplastic group.

For immunohistochemistry exam tissue sections were deparaffinized and rehydrated and then incubated in citrate buffer (pH 6.0) for 3 min in a pressure cooker for antigen retrieval for the beta-catenin antibody and Ki-67 antibody and in Extran 0.05% (Merck) for 10 min in a 750 W microwave oven for the E-cadherin antibody. After cooling for 20 min at room temperature, the sections were immersed in 3% hydrogen peroxide (H₂O₂) for 10 min to block endogenous peroxidase activity. The sections were washed with Tris-buffered Saline (TBS) and incubation with the primary antibodies [E-cadherin, clone 4A2C7, 1:50 (Zymed); beta-catenin, 1:150, clone CAT5H10 (Zymed); Ki-67 clone MIB-1 (Dako) 1:50] for 90 min in a humid chamber at room temperature. The antibodies were detected using the Novolink Max Polymer (Novocastra). The slides were washed with TBS between each step. The color was developed for 3 min at room temperature with a freshly prepared solution of 3, 3' diaminobenzidine (DAB) (Dako), the sections were lightly counterstained with hematoxylin and then dehydrated and mounted. Adjacent normal prostate tissue was used as internal positive

control. Negative controls were carried out by replacing the primary antibody with mouse IgG₁ (Dako).

The immunostained tissue sections were reviewed independently by three observers (MMPR, FG and RLA), who were blind to any other feature of the tissue specimens. For E-cadherin and beta-catenin, positive staining was indicated by distinct brown membranous staining. The number of immunoreactive cells was assessed semi quantitatively as the percentage of positive cells (> 75%; 50-75%; 25-50%; < 25%), adapted from Matos *et al.* (2007), throughout the entire slide at 40x magnification. For the proliferative index (Ki-67), at least 1000 cells of each lesion were counted as positive or negative, at 40 x magnification, or in the case of PIN, the whole pre-neoplastic area was considered.

The results were subjected to statistical analysis using Graph-Pad InStat 3.0. Qui-square test (degree of freedom 1, p<0.01) was used to compare the number of cases that was positive for each antibody (E-cadherin and beta-catenin) and diagnosis (BPH, pre-neoplastic lesions and carcinomas). Tukey's multiple comparison test was used to verify the difference between the proliferative index and the diagnosis groups (p<0.05).

All procedures were performed under the guidelines and with the approval of the Ethics Committee for Animal Experimentation (CEEA/FMVZ/UNESP).

RESULTS

Twenty two (59.5%) prostates had BPH, 10 (27%) had pre-neoplastic lesions (three PIN and seven PIA) and five (13.5%) had neoplastic lesions. In some cases, different lesions were observed in the same gland but only a single lesion was evaluated. The normal prostate tissue showed membranous staining in epithelial cells, for E-cadherin and beta-catenin. All BPH scored >75% for E-cadherin (Fig. 1) and the beta-catenin (Fig. 2) score was higher than 50% of positive cells for this protein (one BPH scored 3 and the rest scored 4). In pre-neoplastic lesions E-cadherin (Fig. 3) score was: One case (PIA) showed less than 25%, two cases (PIN) between 25 and 50% and seven cases more than 75% of E-cadherin expression but for beta-catenin (Fig. 4) nine cases scored more than 75% of positivity and one case (PIN) between 50 and 75%. In neoplastic lesions of dog prostate (carcinomas) E-cadherin expression was: one case scored less than 25% (Fig. 5), other with 50-75% and three > 75%. Beta-catenin expression in carcinomas showed two samples with score 50-75% (Fig. 6) and three tumors with more than 75% of positive cells. None of the lesions scored less than 50% for beta-catenin (Table 1). We did not find cytoplasmic or nuclear stain for beta-catenin. Significant difference was

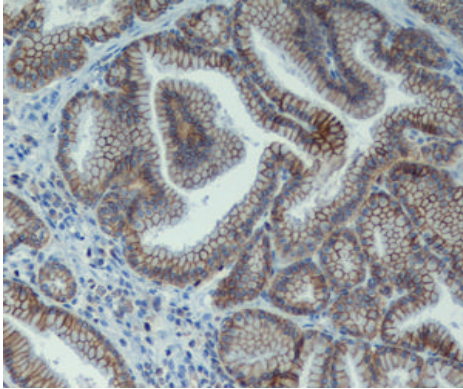


Fig. 1: BPH of canine prostate, Membranous E-cadherin expression, Immunohistochemistry, Novolink MaxPolymer, E-cadherin, DAB, hematoxylin counterstain 20 x

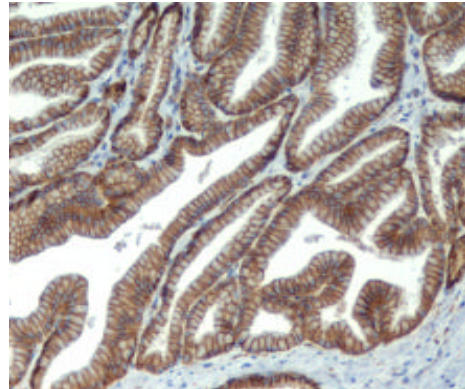


Fig. 2: BPH of canine prostate, Membranous beta-catenin expression, Immunohistochemistry, Novolink MaxPolymer, beta-catenin, DAB, hematoxylin counterstain 20 x

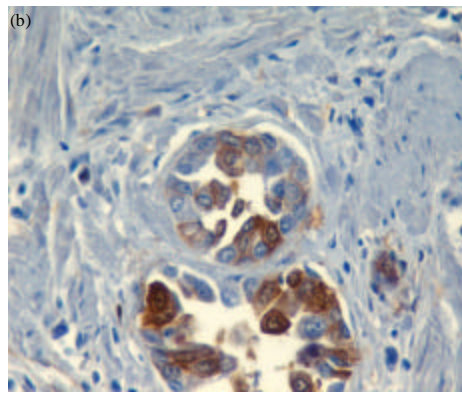
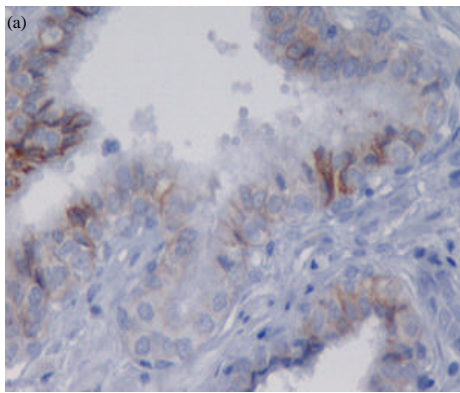


Fig. 3(a-b): Pre-neoplastic lesions in the canine prostate, Membranous E-cadherin expression in 25-50% of epithelial dysplastic cells, Immunohistochemistry, Novolink MaxPolymer, E-cadherin, DAB, hematoxylin counterstain (a) 20 x and (b) 40 x

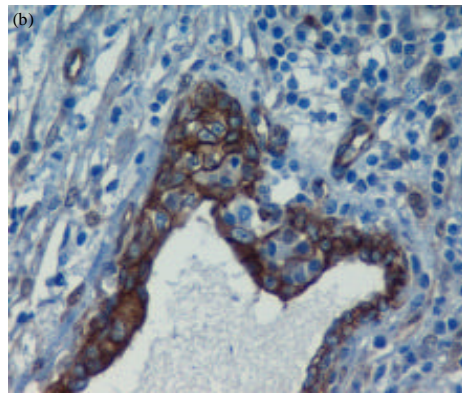
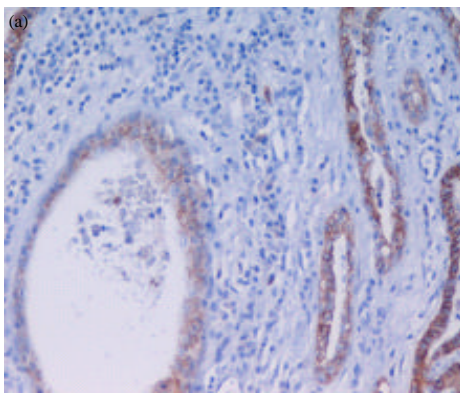


Fig. 4(a-b): Pre-neoplastic lesions in the canine prostate. Membranous beta-catenin expression in 25-50% of epithelial dysplastic cells. Immunohistochemistry, Novolink MaxPolymer, beta-catenin, DAB, hematoxylin counterstain (a) 20 x and (b) 40 x

Table 1: E-cadherin and beta-catenin expression in canine prostatic lesions (BPH, pre-neoplastic lesions and carcinoma)

	BPH		Pre neoplastic		Carcinoma	
	E-cadherin	beta-catenin	E-cadherin	beta-catenin	E-cadherin	beta-catenin
<25%	0	0	1 (10%)	0	1 (20%)	0
25-50%	0	0	2 (20%)	0	0	0
50-75%	0	22 (100%)	0	1 (10%)	1 (20%)	2 (40%)
>75%	22 (100%)	0	7 (70%)	9 (90%)	3 (60%)	3 (60%)

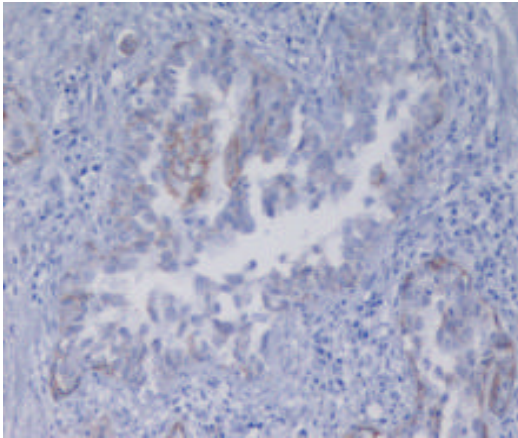


Fig. 5: Neoplastic lesions in the canine prostate, Membranous E-cadherin expression is observed in fewer than 25% of epithelial tumor cells, Immunohistochemistry, Novolink MaxPolymer, E-cadherin, DAB, hematoxylin counterstain 20 x

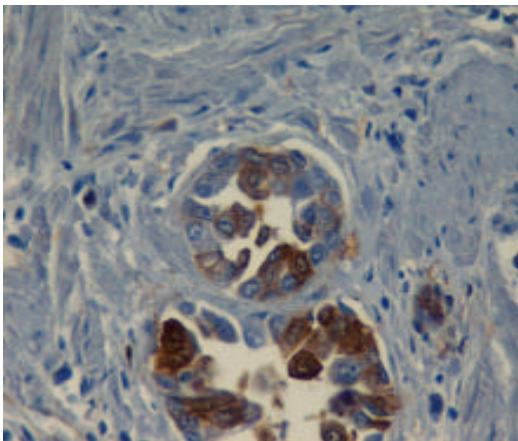


Fig. 6: Neoplastic lesions in the canine prostate, Membranous beta-catenin expression is observed in fewer than 25% of epithelial tumor cells. Immunohistochemistry, Novolink MaxPolymer, beta-catenin, DAB, hematoxylin counterstain 40 x

observed in BPH number of positive cases (>75%) for E-cadherin when compared to carcinomas (p = 0.01). For

beta-catenin, carcinomas and pre-neoplastic lesions have higher number of cases with >75% of positive cells than BPH (p<0.001).

The mean proliferative index for BPH was 5.3±4.5; for pre-neoplastic lesions 5.9±5.2 and for carcinomas 46.1±35.6. There was statistical difference in the proliferative index between carcinomas and BPH (p<0.001) and carcinomas and pre-neoplastic lesions (p<0.001). The samples that did not have a positive internal control (at least one positive cell for Ki-67 in any part of the tissue) weren't considered in the statistics, so two samples of carcinoma and five of HPB were taken out of the analysis.

DISCUSSION

Extensive role of adhesion molecules in neoplastic progression in humans was proved in studies with breast cancer that analyzed E-cadherin expression (Asgeirsson *et al.*, 2000). In this study, E-cadherin expression was down-regulated in canine prostatic carcinomas (two out of five carcinoma scored less than 75% of positivity) compared to prostate non-neoplastic lesions (BPH), as described by Jaggi *et al.* (2005) in human. E-cadherin and beta-catenin expression affecting intercellular adhesion function has been documented in normal prostate tissue in humans (Saha *et al.*, 2008) but not in dogs and as in humans, canine BPH had positive immunostaining in more than 75% of the neoplastic cells. Pre-neoplastic lesions also had 30% (3/10) of the samples with less than 50% of E-cadherin expression, showing similar behavior as carcinomas. These data indicate that the loss of adherens junctions occurs early in tumor development and in pre-neoplastic lesions (Jaggi *et al.*, 2005). Loss of E-cadherin expression is associated with several factors that correlate with poor prognosis, both in human (Pontes-Junior *et al.*, 2009; Jaggi *et al.*, 2005; Saha *et al.*, 2008) and in canine mammary tumors (Matos *et al.*, 2007) but there are no reports in canine prostatic lesions. In addition, alterations in CAMs have been found in primary prostate tumors in men (Pontes-Junior *et al.*, 2009; Jaggi *et al.*, 2005; Saha *et al.*, 2008).

When evaluating E-cadherin and beta-catenin expression in the human prostate (Saha *et al.*, 2008)

observed that HPB cells exhibited homogenous membranous expression for E-cadherin, accompanied by similar beta-catenin expression. Whitaker *et al.* (2008) verified nuclear beta-catenin expression in HPB, with a gradual loss of nuclear distribution in human prostate cancer. In contrast, we observed marked membranous expression in E-cadherin and beta-catenin with no nuclear distribution and there wasn't a reduction of membranous beta-catenin in pre-neoplastic lesions and carcinomas.

Beta-catenin is regulated by APC and the loss of APC/beta catenin interaction or WNT signaling leads to accumulation of nuclear beta-catenin, that loses its tumor suppressor activity and promote cell proliferation (Huang *et al.*, 2010). In humans prostate carcinomas, this complex (APC/beta-catenin) continue to act but an increase level of beta-catenin is frequently observed (Huang *et al.*, 2010). To our knowledge this is the first time that beta-catenin expression is studied in canine prostatic lesions and it seems that the protein pathway involving beta-catenin is not involved in cellular proliferation, either in benign proliferative lesions or in cancer, since we did not find nuclear immunolabeling in any tissue and pre-neoplastic lesions and carcinomas had more samples with more than 75% of the cells positive than BPH.

There was no correlation between E-cadherin and beta-catenin with proliferative index (Ki-67) expression in canine prostate lesions, in accordance with previous results that demonstrated no correlation between these proteins in bitch mammary tumors (Nowak *et al.*, 2007). An interesting finding in canine prostatic carcinomas was less E-cadherin expression than HPB and higher proliferative index, what demonstrate the interaction between epithelial cell adhesion by E-cadherin and cellular proliferation.

CONCLUSION

E-cadherin expression is lower in carcinomas than in BPH, which is consistent with loss of cell adhesion and proliferation, since carcinomas had the highest proliferative index of all lesions. The role of beta-catenin pathways should be more investigate in canine prostatic lesions, correlating them to cell proliferation, since we found more beta-catenin in pre-neoplastic and neoplastic lesions of canine prostate, than in BPH.

ACKNOWLEDGMENTS

We thank Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP) (n°06/61523-1 and n°06/61814-1) and Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Portugal.

REFERENCES

- Asgeirsson, K.S., J.G. Jonasson, L. Tryggvadottir, K. Olafsdottir, J.R. Sigurgeirsdottir, S. Ingvarsson and H.M Ogmundsdottir, 2000. Altered expression of E-cadherin in breast cancer: Patterns, mechanisms and clinical significance. *Eur. J. Cancer*, 36: 1098-1106.
- Bostwick, D.G., 1997. Neoplasms of the Prostate. In: *Urologic Surgical Pathology*, Bostwick, D.G. and J.N. Eble (Eds.). Mosby Inc., St. Louis, USA., pp: 343-421.
- De Marzo, A.M., V.L. Marchi, J.I. Epstein and W.G. Nelson, 1999. Proliferative inflammatory atrophy of the prostate: Implications for prostatic carcinogenesis. *Am. J. Pathol.*, 155: 1985-1892.
- Foster, R.A. and P.W. Ladds, 2007. Male Genital System. In: *Pathology of Domestic Animals*, Jubb, K.V.F., P.C. Kennedy and N. Palmer (Eds.). Elsevier Limited, St. Louis, USA., pp: 590.
- Gounari, F., S. Signoretti, R. Bronson, L. Klein and W.R. Sellers *et al.*, 2002. Stabilization of β -catenin induces lesions reminiscent of prostatic intraepithelial neoplasia but terminal squamous transdifferentiation of other secretory epithelia. *Oncogene*, 21: 4099-4107.
- Horvarth, L.G., S.M. Henshall, C.S. Lee, J.G. Kench and D. Golovsky *et al.*, 2005. Lower levels of nuclear β -catenin predict for a poorer prognosis in localized prostate cancer. *Int. J. Cancer*, 113: 415-422.
- Huang, S.P., W.C. Ting, L.M. Chen, L.C. Huang and C.C. Liu *et al.*, 2010. Association analysis of Wnt pathway genes on prostate-specific antigen recurrence after radical prostatectomy. *Ann. Surg. Oncol.*, 17: 312-322.
- Jaggi, M., S.L. Johansson, J.J. Baker, L.M. Smith, A. Galich and K.C. Balaji, 2005. Aberrant expression of E-cadherin and β -catenin in human prostate cancer. *Urologic Oncol.: Semin. Original Invest.*, 23: 402-406.
- Lai, C.L., R. Van den Ham, G. van Leenders, J. van der Lugt and E. Teske, 2008. Comparative characterization of the canine normal prostate in intact and castrated animals. *Prostate*, 68: 498-507.
- Matos, A.J.F., C. Lopes, J. Carvalheira, M. Santos, G.R. Rutteman and F. Gartner, 2006. E-cadherin expression in canine malignant mammary tumours: Relationship to other clinico-pathological variables. *J. Comp. Pathol.*, 134: 182-189.
- Matos, A.J.F., C.C.C. Lopes, A.M.R. Faustino, J.G.V. Carvalheira, G.R. Rutteman and M.D.F.R.M. Gartner, 2007. E-cadherin, β -catenin, invasion and lymph node metastases in canine malignant mammary tumours. *APMIS*, 115: 327-334.

- Mol, A.J.M., A.A. Geldof, G.A. Meijer, H.G. van der Poel and R.J.A. van Moorselaar, 2007. New experimental markers for early detection of high-risk prostate cancer: Role of cell-cell adhesion and cell migration. *J. Can. Res. Clin. Oncol.*, 133: 687-695.
- Nowak, M., J.A. Madejand and P. Dziegiel, 2007. Expression of E-cadherin, β -catenin and Ki-67 antigen and their reciprocal relationships in mammary adenocarcinomas in bitches. *Folia Histochem. Cytobiol.*, 45: 233-238.
- Pontes-Junior, J., S.T. Reis, M. Dall'Oglio, L.C.N. de Oliveira and J.Cury *et al.*, 2009. Evaluation of the expression of integrins and cell adhesion molecules through tissue microarray in lymph node metastases of prostate cancer. *J. Carcinogenesis*, Vol. 8.
- Saha, B., A. Arase, S.S. Imam, D. Tsao-Wei and W.Y. Naritoku *et al.*, 2008. Overexpression of E-cadherin and β -catenin proteins in metastatic prostate cancer cells in bone. *Prostate*, 68: 78-84.
- Toledo, D.C., M.B.R. Faleiro, M.M.P. Rodrigues, G.W. Di Santis, R. Laufer-Amorim and V.M.B.D. de Moura, 2010. Caracterizaco histomorfolgica da atrofia inflamatoria proliferativa na prostata canina [Histomorphological characterization of proliferative inflammatory atrophy in canine prostate]. *Ciencia Rural*, 40: 1372-1377.
- Verras, M. and Z. Sun, 2006. Roles and regulation of Wnt signaling and β -catenin in prostate cancer. *Cancer Lett.*, 237: 22-32.
- Whitaker, H.C., J. Girling, A.Y. Warren, H. Leung, I.G. Mills and D.E. Neal, 2008. Alterations in β -catenin expression and localization in prostate cancer. *Prostate*, 68: 1196-1205.