ONCOLOGIA OGGI
Animali e Uomo Alleati Contro i Tumori

Venerdì 12 giugno 2009 ore 08.30 – 14.00

COMUNE DI ROMA
Palazzo Senatorio- Aula Giulio Cesare
P.zza del Campidoglio - Roma

I TUMORI UROTELIALI NEL BOVINO: UN MODELLO ANIMALE PER I TUMORI UMANI?

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I tumori della vescica sono piuttosto comuni, su scala mondiale infatti vengono segnalati oltre due milioni di casi occupando la settima posizione nell'incidenza epidemiologica delle neoplasie. L'Organizzazione Mondiale della Sanità stima in circa 260.000 nuovi casi per anno l'incidenza mondiale dei tumori uroteliali nel maschio e 76.000 nella donna. Nonostante i protocolli terapeutici siano negli ultimi anni notevolmente migliorati, tuttavia i tumori uroteliali manifestano una prognosi infausta in circa il 20% dei casi. Allo stato, esiste una percentuale di sopravvivenza a 5 anni intorno al 60-80%.

La carcinogenesi uroteliale della vescica è multifattoriale, ma i pathways molecolari responsabili dell''initiation', 'promotion' e 'progression' non sono ancora perfettamente conosciuti. Pertanto, una migliore conoscenza di questi meccanismi favorirebbe la messa a punto di nuovi protocolli terapeutici non invasivi, farmacologici. Per tale motivo sono stati proposti, di volta in volta, modelli animali per lo studio della cancerogenesi spontanea vescicale.

Recentemente, abbiamo proposto il bovino come modello animale per lo studio dei tumori uroteliale dell'uomo.

I bovini che vivono allo stato brado hanno un'alta incidenza di tumori della vescica nel cui determinismo un ruolo centrale viene svolto dall'azione sinergica tra ptaquiloside, un sesquiterpenoide contenuto nella felce (Pteridium aquilinum) e papillomavirus tipo 2, un virus a DNA particolarmente diffuso nell'apparato urinario del bovino. Ptaquiloside è ritenuto potenzialmente oncogeno anche per l'uomo, essendo coinvolto in alcuni istotipi di tumori dell'esofago e dello stomaco.

La morfologia dei tumori uroteliali del bovino, da noi osservata, è perfettamente sovrapponibile a quella riportata dall'Organizzazione Mondiale della Sanità per i tumori uroteliali nell'uomo. Inoltre, abbiamo dimostrato che alcuni pathways molecolari studiati *in vitro* e considerati importanti per la trasformazione neoplastica dell'urotelio nell'uomo, sono alla base della trasformazione neoplastica *in vivo* nel bovino.

I tumori uroteliali nel bovino: *'AN ANIMAL MODEL FOR HUMAN DISEASE'?*

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I tumori uroteliali nel bovino: *'AN ANIMAL MODEL FOR HUMAN DISEASE'*?

- Urinary bladder tumors have been reported very rarely in cattle in the absence of bracken fern accounting for only 0.01-0.1% of all bovine malignancies.

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Urinary bladder tumors are common in 4-12-year-old cattle grazing on bracken fern-infested land, with a prevalence as high as 90% in adult animals.

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The toxicity of bracken fern, *Pteridium* spp, one of the most common plants in the world, is very complicated as the plant elaborates several toxic substances that are implicated in different and severe pathological manifestations of animal livestock and represent an hazard for human health too. It is generally believed that its consumption is responsible for a number of well-recognized acute and chronic toxic syndromes in domestic animals (Smith, 1990) and increases the oncogenic risk in man as well (Fenwick, 1988; Potter and Baird, 2000).

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In cattle, the chronic poisoning of bracken fern causes neoplastic lesions of the alimentary canal as well as urothelial and mesenchymal tumours of the urinary bladder. An interaction of the bracken carcinogens with bovine papillomavirus type 4 (BPV-4) (Campo et al., 1980; Borzacchiello et al., 2003) and type 2 (BPV-2) (Campo et al., 1992; Borzacchiello et al., 2003, Roperto et al., 2008) is known to occur in alimentary and bladder tumours, respectively.

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The most important clinical manifestation of the latter ones is the presence of blood in the urine thus characterizing a syndrome known as bovine enzootic hematuria (BEH).



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Despite the fact that the toxicity and carcinogenicity of bracken fern was clearly documented by Evans and Mason as from the middle sixties, the carcinogenic fraction of bracken was uncertain until a norsesquiterpenoid glycoside, named ptaquiloside (PT) (Aquilide A), responsible for mutagenic, clastogenic and carcinogenic activity, was simultaneously isolated in Japan and the Netherlands (Niwa et al., 1983; Van der Hoeven et al., 1983).

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The carcinogenicity of PT was experimentally proven by Hirono et al. (1984) in rats suffering from mammary and ileum cancers as a consequence of intragastric PT administration.

Although, more recently, Castillo et al. (1998) were able to isolate a new toxic sesquiterpene glucoside, ptaquiloside Z, from *P. aquilinum* var. *caudatum,* the major carcinogen of bracken fern is known to be PT, concentrations of which are variable in the genotype of *Pteridium* and can be as high as 1.3% (12,945 µg/g) of the dry weight (Smith et al., 1994).

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It has been suggested that the different distribution and concentrations of PT in the genotype of bracken may be the reason for the geographically localized occurrence of BEH (Smith et al., 1988).

PT is believed to be carcinogenic via DNA alkylation only at specific organs and in a sequence selective fashion when it is activated to its unstable reactive dienone form – activated PT (APT) – under alkaline conditions (Prakash et al., 1996; Alonso-Amelot and Avendaño, 2002).

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Ileum and urinary bladder of cattle are known to be alkaline and provide ideal conditions for the formation of APT. It has been shown that in vivo in bracken-fed cattle APT alkylates DNA forming a number of DNA adducts in the ileum and urinary bladder which result in H-ras 1 mutation via initial adenine alkylation in codon 61 leading adenine to pyrimidine (T and C) transversion. The alkylating group present in APT compound is a cyclopropyl ring (Prakash et al., 1996).

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More recently, immunohistochemical expression of H-ras protein was detected in some pathological urinary bladders in cattle naturally exposed to bracken fern (Sardon et al., 2005).

In addition, mutations in codon 59 resulting in G to A transition or G to T transversion and consequent change from Ala to Thr or Ala to Ser are known to occur.

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They were shown in preneoplastic tissues of mammary glands of rats after dosing ATP by intravenous (iv) route suggesting that H-ras activation is an early event in the PT carcinogenesis (Shahin et al., 1998). The ras product p21 has GTPase activity and plays a role in the control of cell cycle.

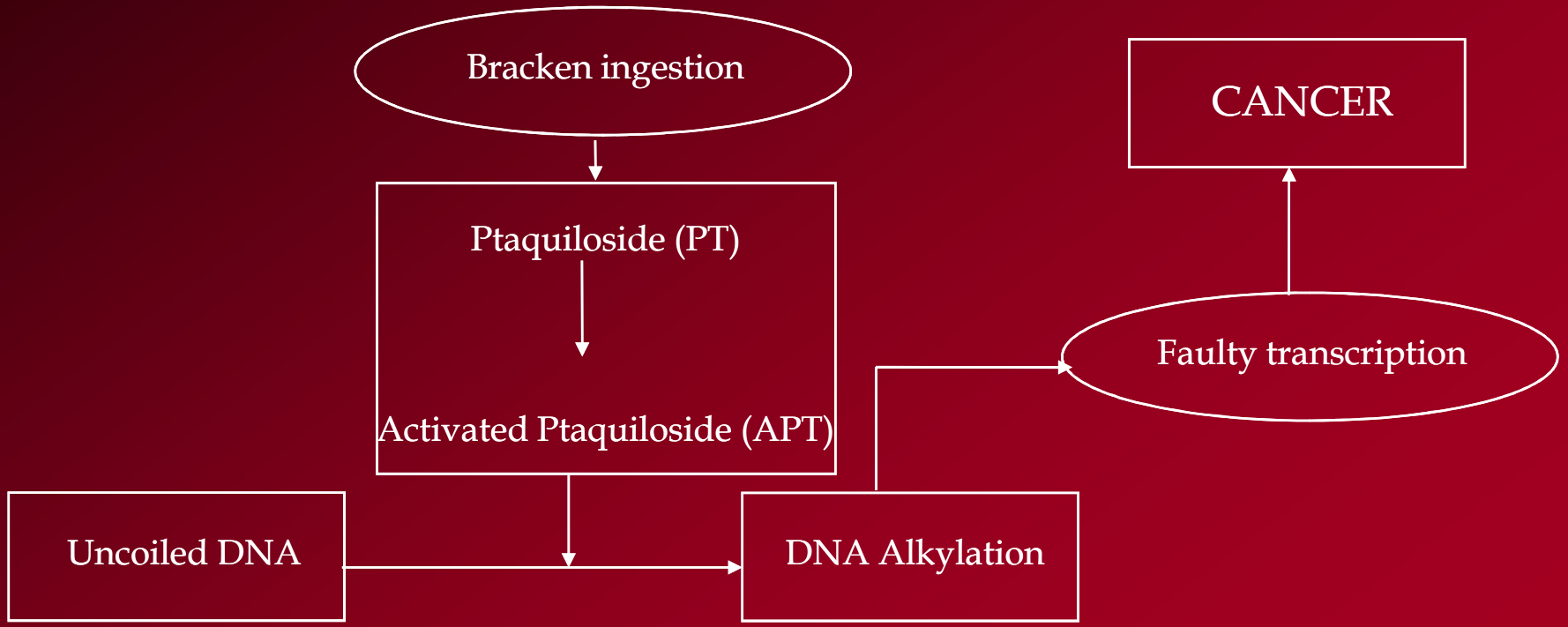
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In vitro mutagenesis studies have shown that H-ras mutations occurring in codons 12-13 and codons 59-63 are responsible for the alteration of the protein conformation thus reducing its activity (Benjamin and Vogt, 1991).

Interestingly, several DNA adducts were described in tissues of mice treated with *Pteridium aquilinum* subsp. *aquilinum*.

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Schematically, the following mechanism can elucidate the oncogenic potential of bracken.



Sixth IARC Monographs Advisory Group on priorities for future evaluations - Priority List of agents and exposures to consider in future IARC Monographs

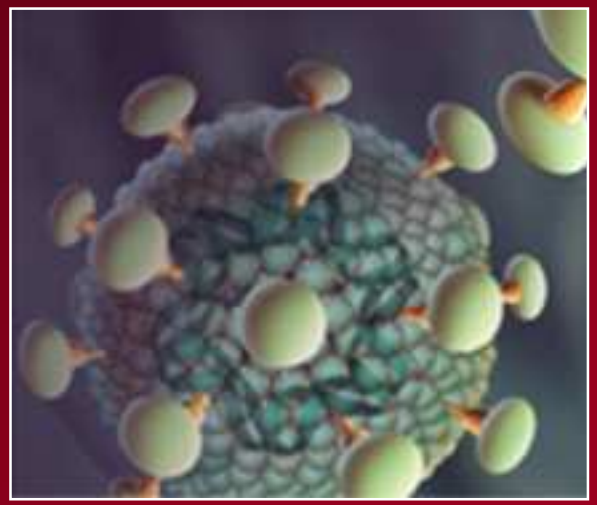
Food additives, contaminants and components		
Urethane (2B)	high	- new mechanistic data - NTP report will soon be available Within the framework of a re-evaluation of 'alcoholic beverages'
Naturally occurring substances		
Growth hormones (GH)	low	- children with growth deficiencies are given GH - possible human exposure because GH is given to cattle - animal data show increase in mammographic densities and prostatic hyperplasia in macaques
Methyl eugenol	low	- widespread but low human exposure - NTP animal carcinogenicity study
Nitrate, nitrite and endogenous nitrosation	high	- epidemiological studies on fertilizer workers and on nitrate in drinking water An *ad hoc* planning meeting is needed to define the scope of this Monograph
Ptaquiloside and bracken fern	high	- new animal carcinogenicity data and some epidemiology in relation to bracken fern
Insulin-like growth factors (IGF)	low	- increase in endogenous IGF concentration in serum would lead to increase in breast, prostate and colon cancer. This is supported by on-going *in vitro* studies - IGF-1 present in cows' milk, possibility of human exposure - data on transgenic animals are available

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Bracken fern

+



Bovine Papillomavirus type 2

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It is commonly believed that a synergism among toxic principles of bracken fern and some papillomaviruses, such as bovine papillomavirus type-1 (BPV-1) and, particularly bovine papillomavirus type-2 (BPV-2) is responsible for cell transformation.

Bovine papillomavirus type 2

In the new classification of Papillomaviruses (PVs) by de Villiers et al. (2004), BVP-2 is grouped in:

Delta-papillomavirus genus, species 4

Bovine papillomavirus type 2

At this genus, composed of four species, also belong:

- European elk papillomavirus (EEPV) and Reindeer papillomavirus (RPV), species 1;
- Deer papillomavirus (DPV), species 2;
- Ovine papillomavirus type 1 and type 2 (OvPV-1 and OvPV-2), species 3;
- Bovine papillomavirus type 1 (BPV-1), species 4.

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Shortly, three oncogenes of PVs E5, E6, and E7 modulate cell transformation, E1 and E2, that are genes coding regulatory proteins, modulate transcription and replication, and two genes, L1 and L2 code some structural proteins responsible for the formation of viral capsid.

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The E5 protein is localized in the Golgi Apparatus (GA), endoplasmic reticulum and occasionally the plasma membrane of the host cells (Ashrafi et al., 2005).

BPV E5 expression is responsible for the retention of major histocompatibility complexes class I in the Golgi and the inhibition of their transport to cell surface (Ashrafi et al., 2002; Marchetti et al., 2002).

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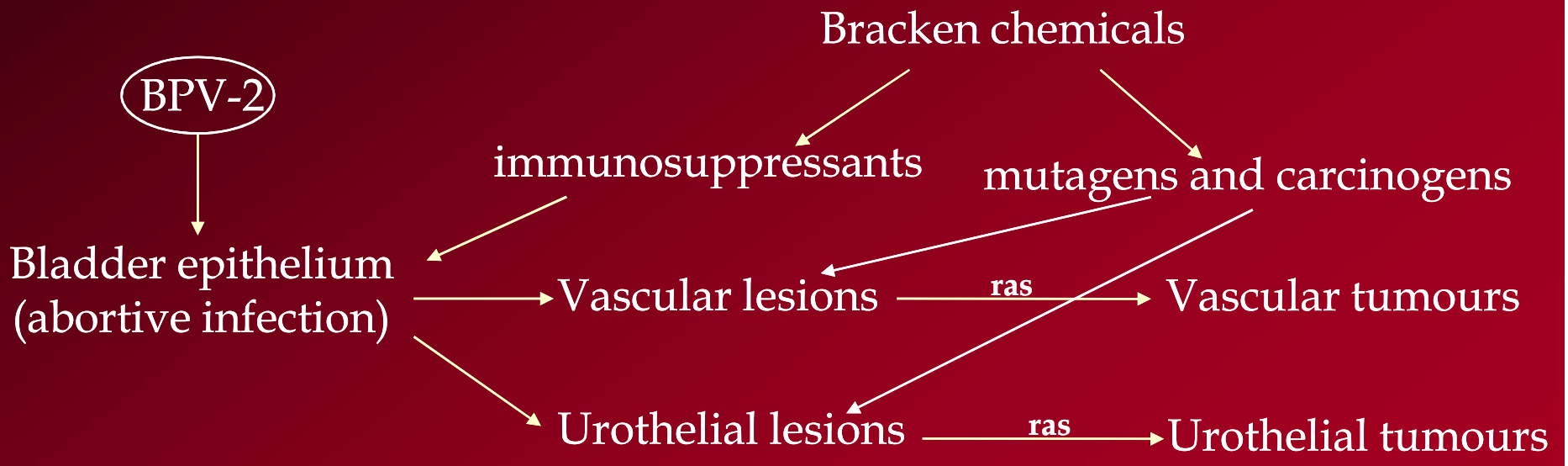
The main biological properties of BPV-2 are characterized by inducing cutaneous fibropapillomas; and, unique among PVs, BPV-2 can be responsible for a trans-species transmission causing equine sarcoids.

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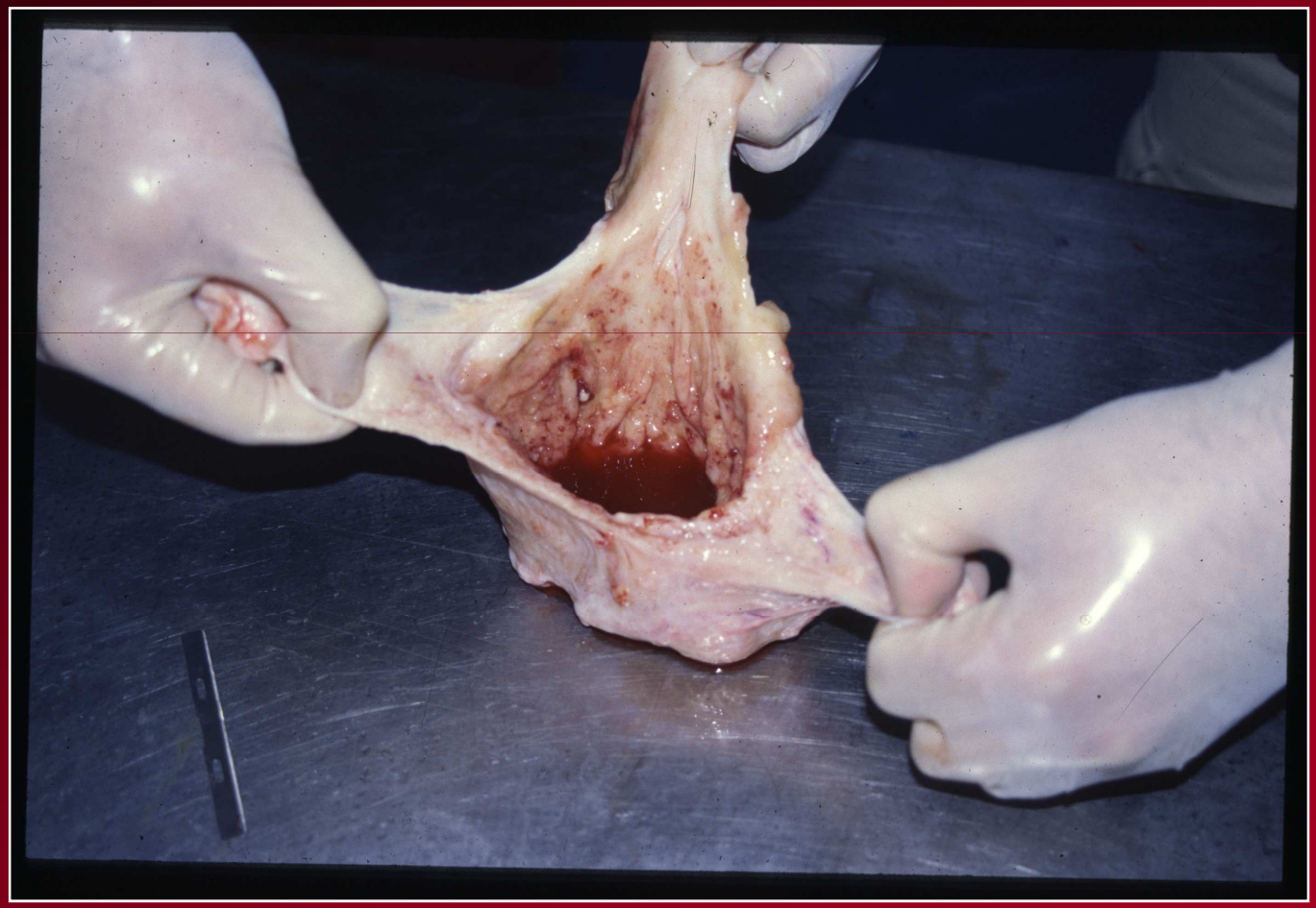
In spite of many molecular studies are under way to better understand pathogenetic mechanism(s) of disease of PVs, it is not well-known, step by step, how the BPV-2 works in inducing urinary bladder tumours.

Bovine papillomavirus type 2

It has been suggested the following scheme to justify the involvement of BPV-2 in urinary bladder carcinogenesis.



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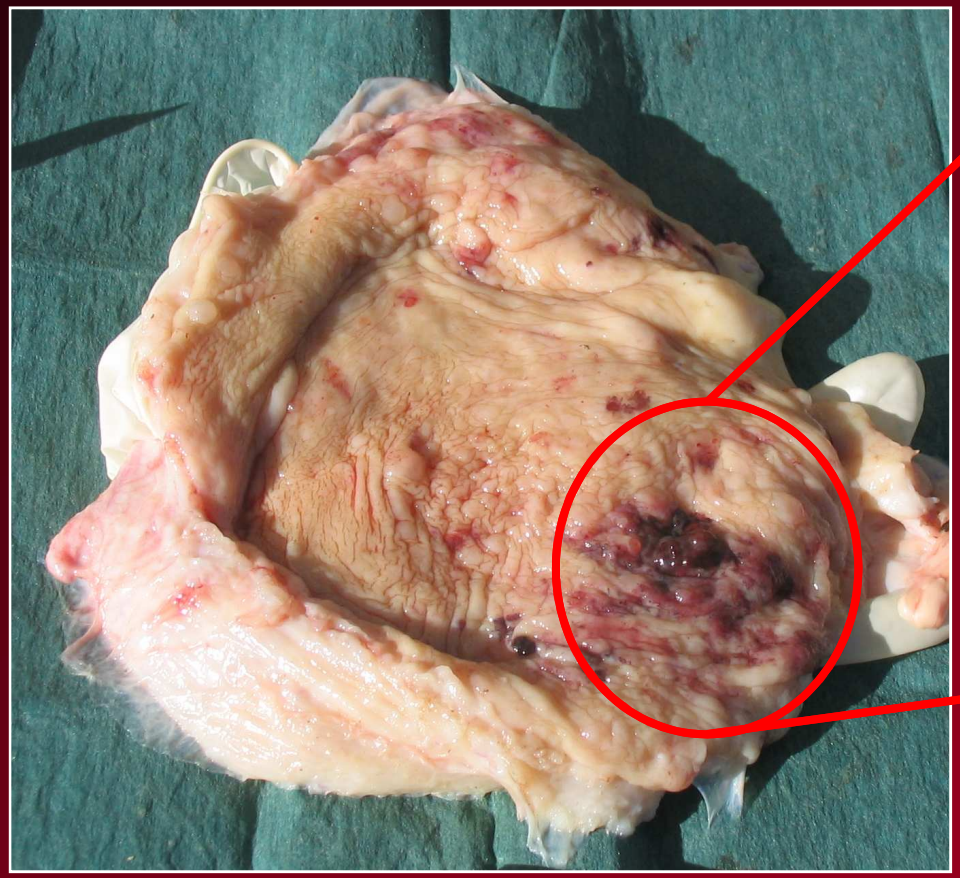
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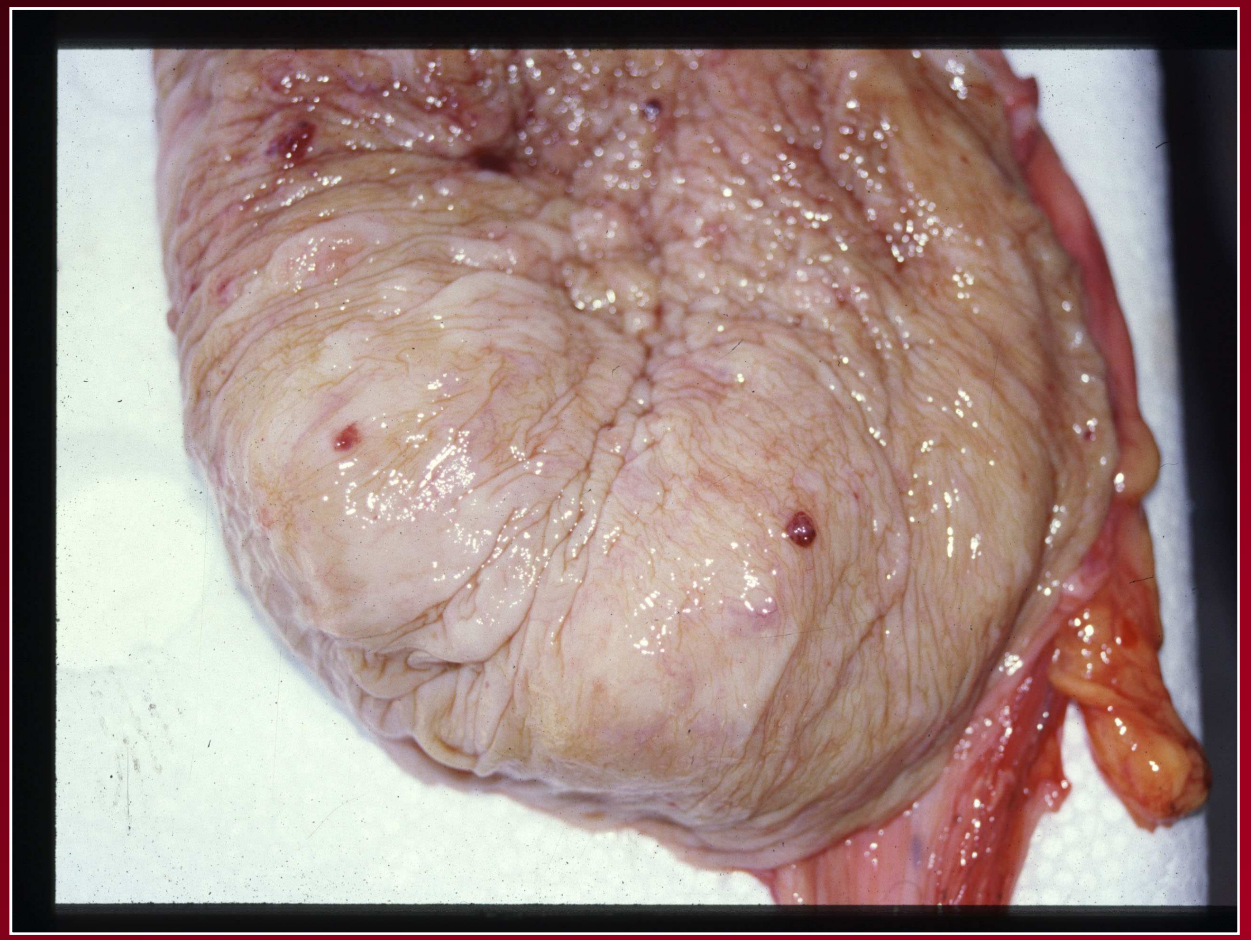


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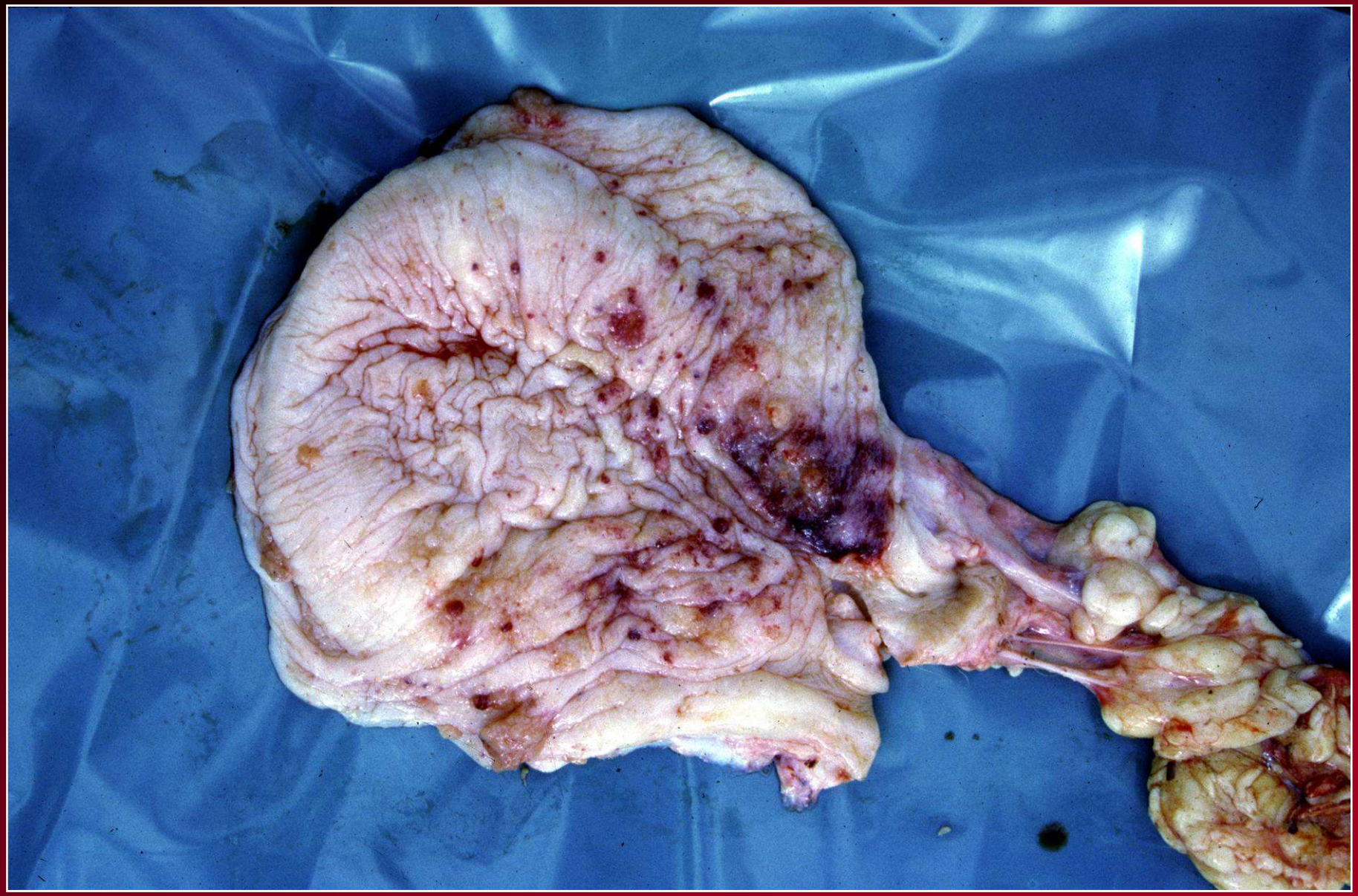


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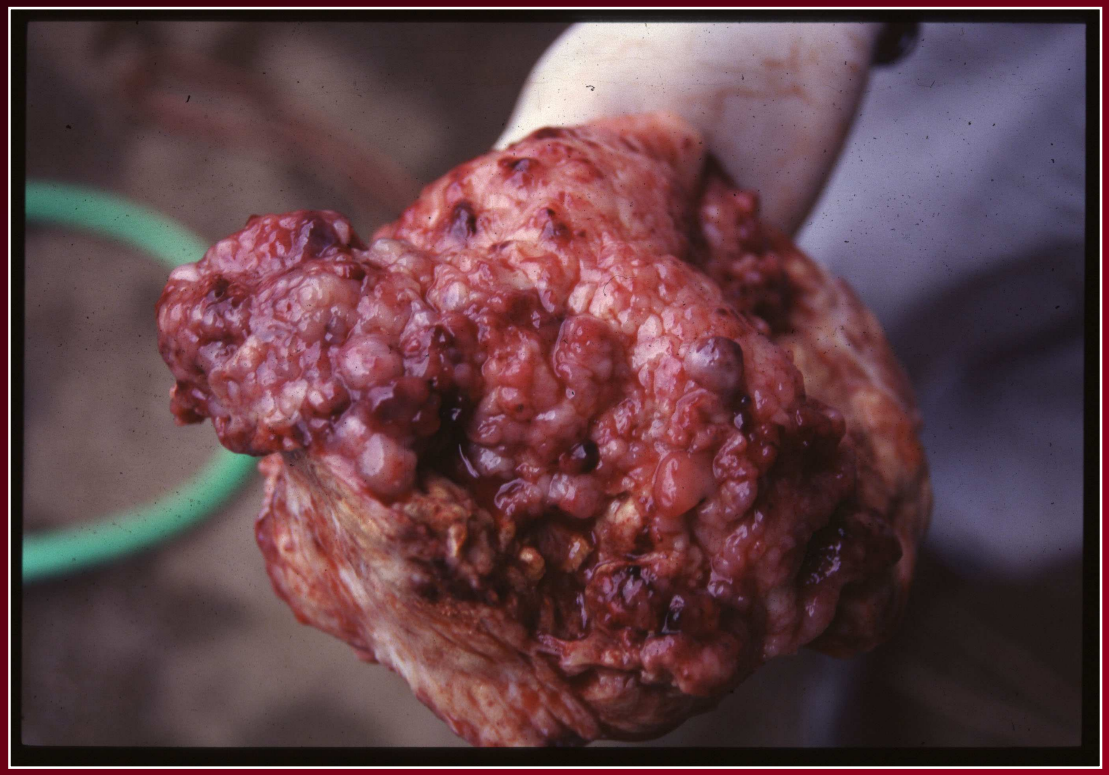


Roperto – Roma, 12 Giugno 2009



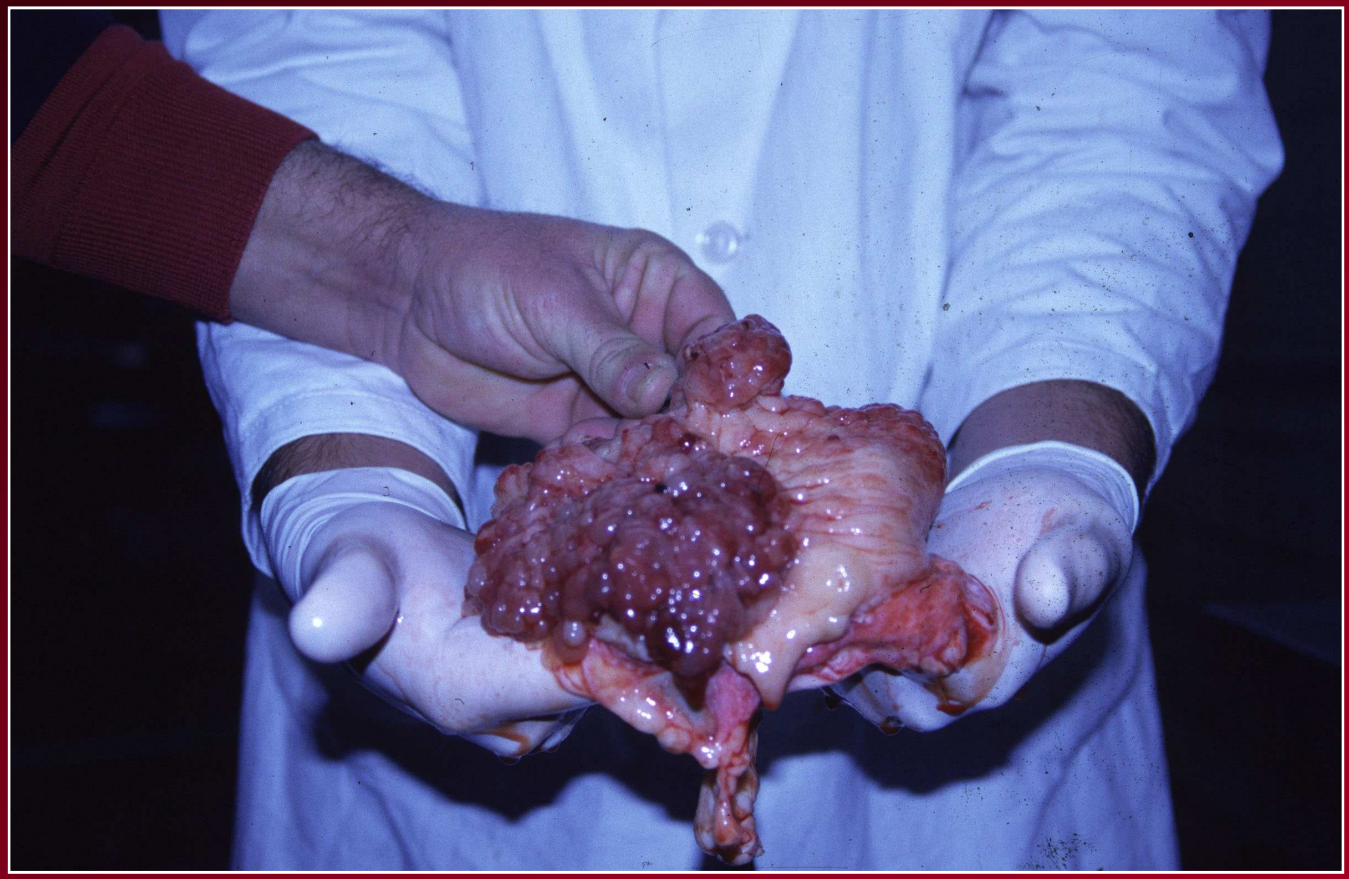
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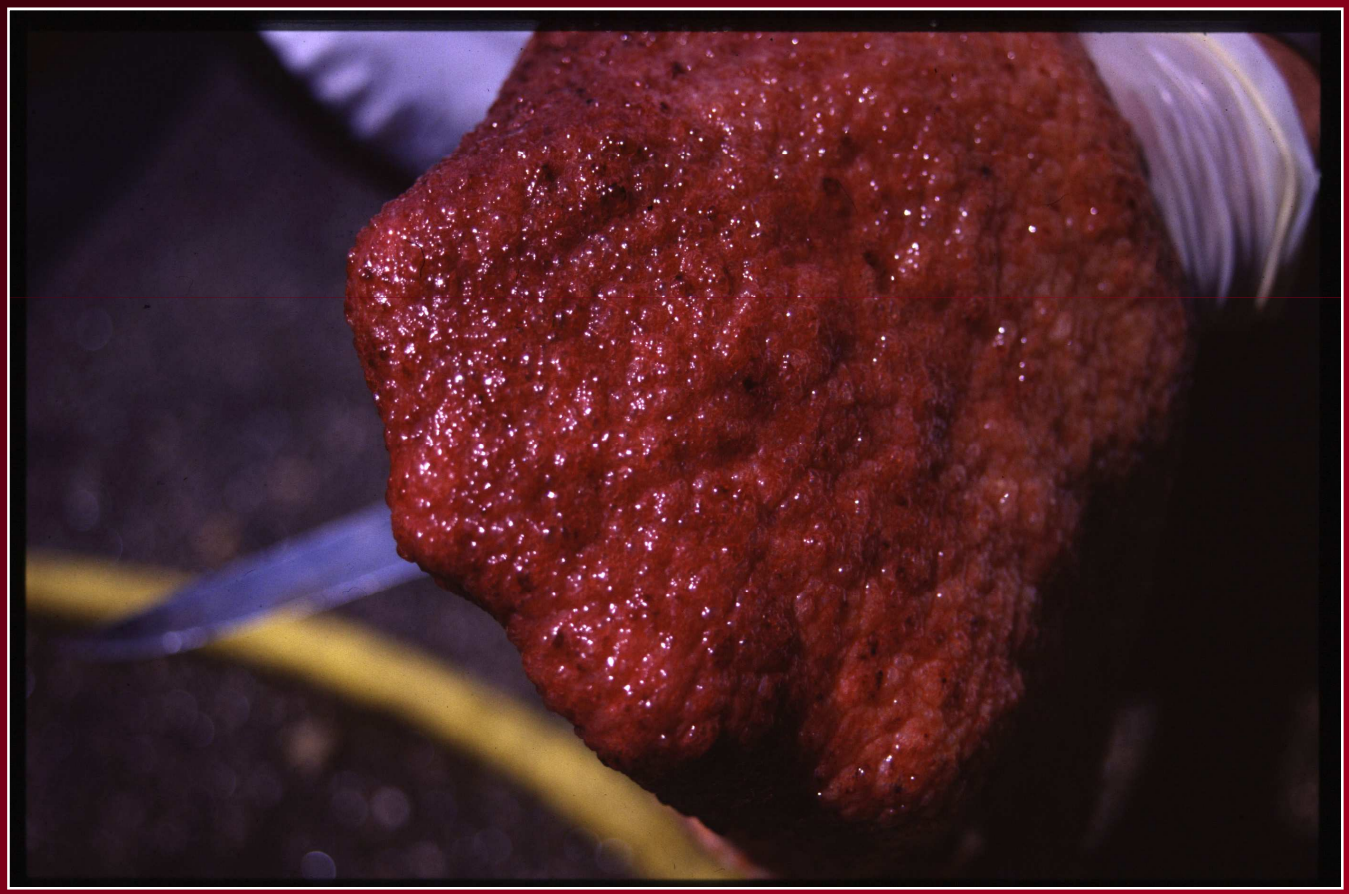


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J. Comp. Path. 2006, Vol. 135, 190–199

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Classification of Canine Urinary Bladder Urothelial Tumours Based on the World Health Organization/ International Society of Urological Pathology Consensus Classification

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[*]MPI Research, Mattawan, Michigan 49071, [†]Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, Michigan 48910, and [‡]Armed Forces Institute of Pathology, Department of Genitourinary Pathology, Washington, DC, 20306, USA

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Histological patterns of bovine urothelial tumours are superimposible to human counterpart ones; unlike urothelial carcinomas in man, bovine urothelial cancers metastatize more rarely (~ 10%). We found that GM3 ganglioside content is increased in bovine urothelial tumours (S. Roperto et al., 2007) whereas is decreased in human counterpart.

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It has been suggested that the amount of GM3 expressed may serve as an indicator of the invasion potential of bladder cancer.
There is an inverse correlation of the amount of GM3 expressed with invasive potential in spontaneous human primary bladder tumour and that exogenously given GM3 suppresses invasion potential in some human bladder tumour cell lines.

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In addition GM3 overexpression induced apoptosis and reduced malignant potential in murine bladder cancer. It is well known that GM3 inhibits cell motility/invasiveness when complexed with CD9 or CD82.

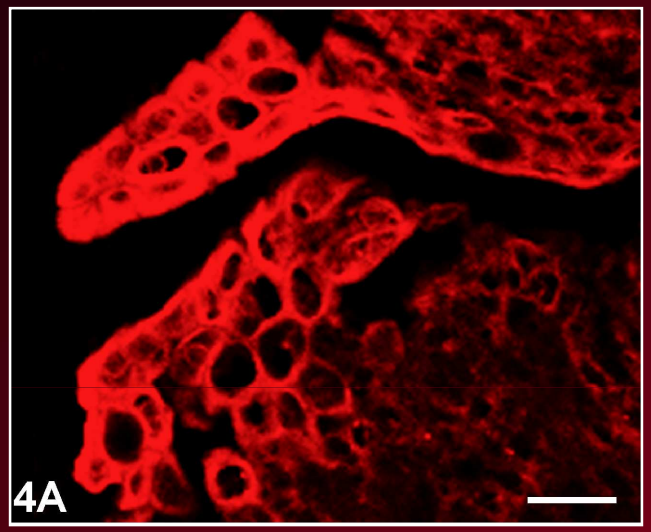
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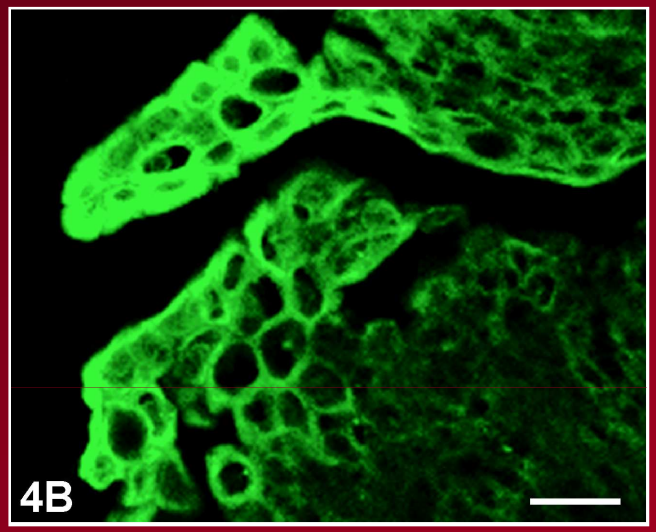
It is reasonable to believe that GM3 ganglioside may be one of factors responsible for different biological behaviour (S.Roperto et al., 2007).

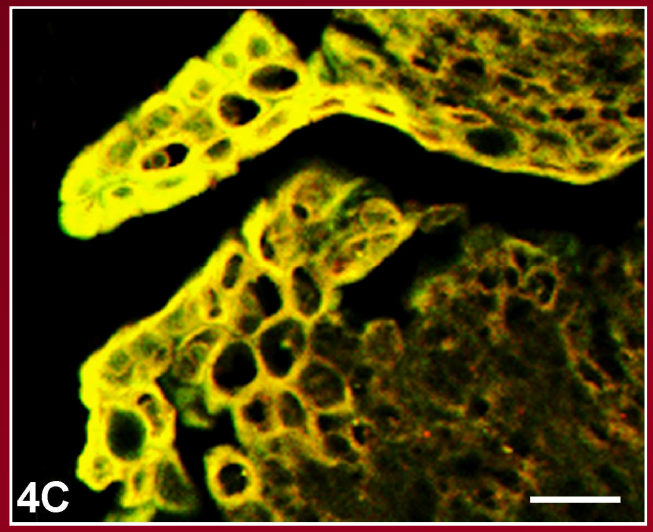
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Furthermore, we demonstrated that the expression and colocalization of cyclossigenase I and II in bovine urothelial cancers is superimposible to Cox expression and co-localization known in man (Borzacchiello et al., Vet Pathol, 2003).
Finally, uroplakin expression overlaps in bovine and human urothelial cancers (Ambrosio et al., Vet Pathol, 2001; S. Roperto et al., Vet Pathol, 2005).

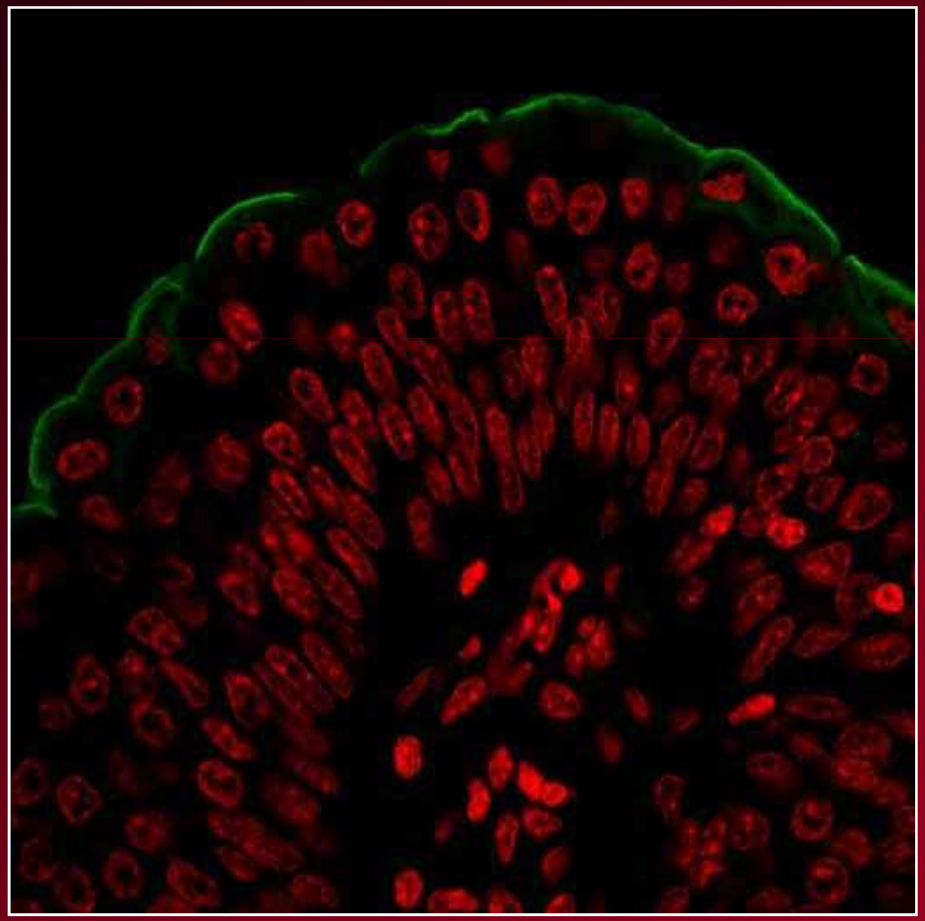
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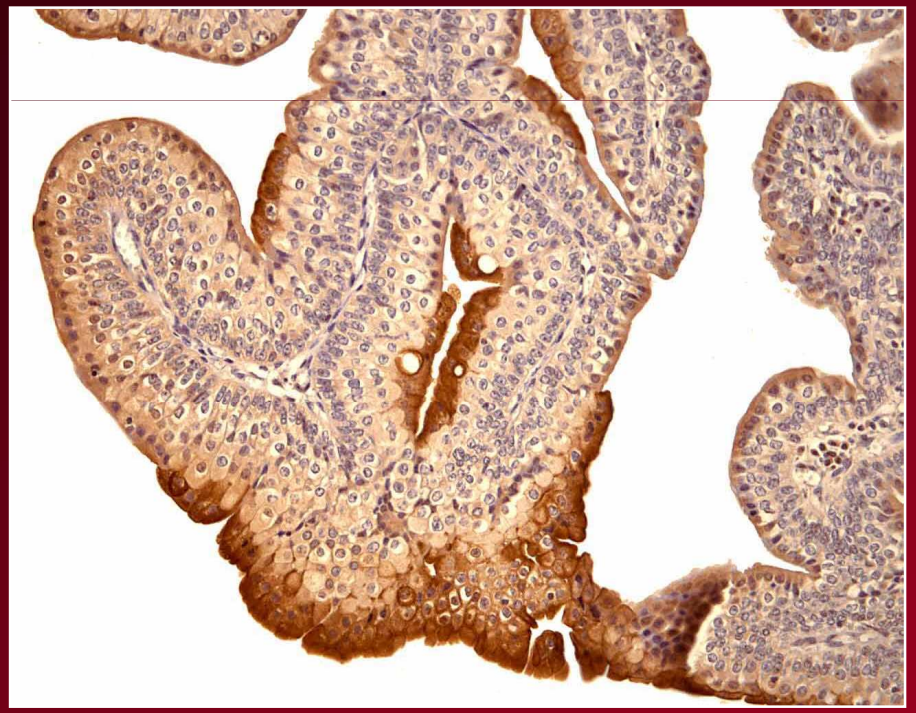


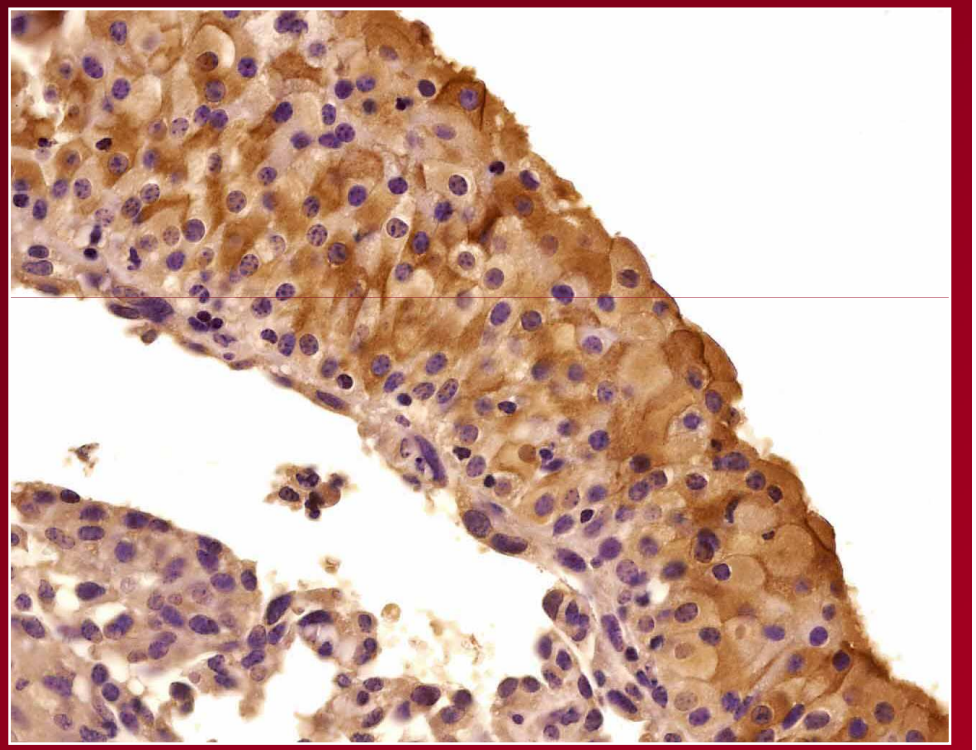
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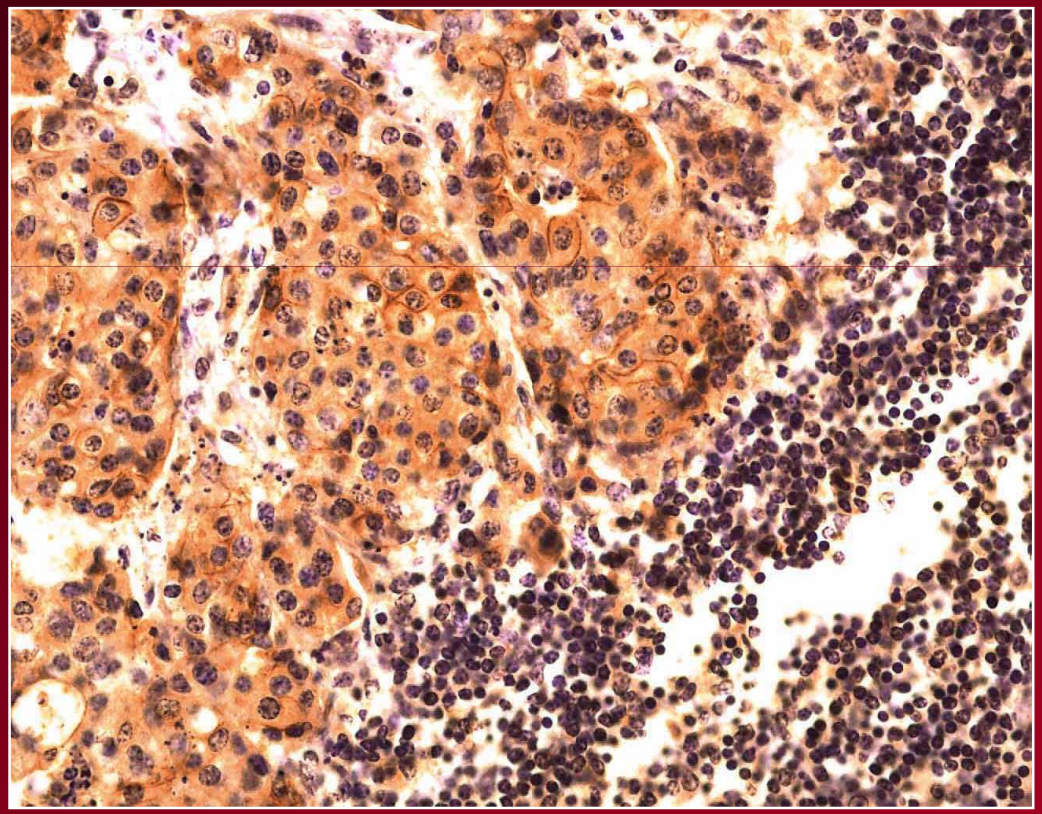


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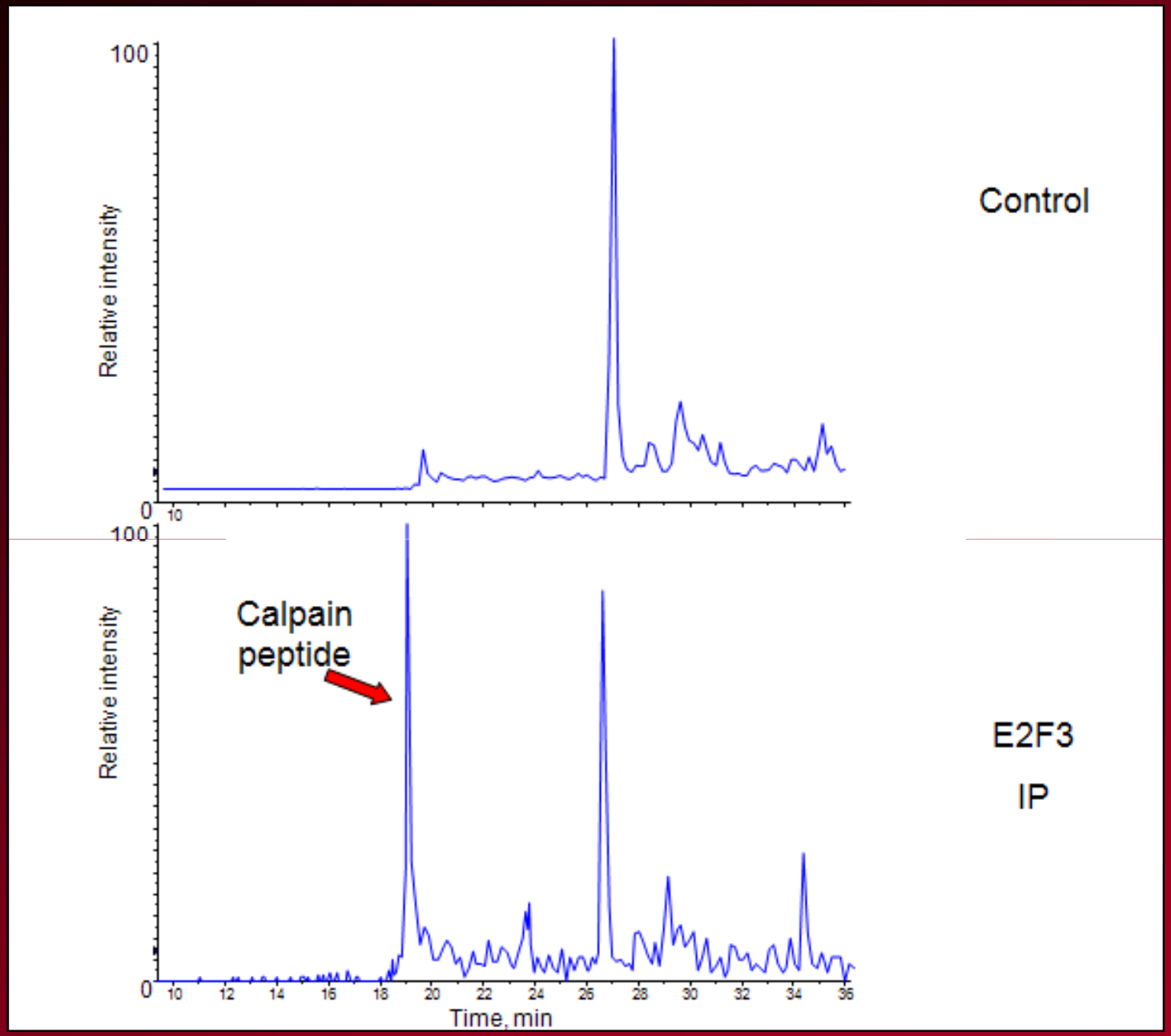




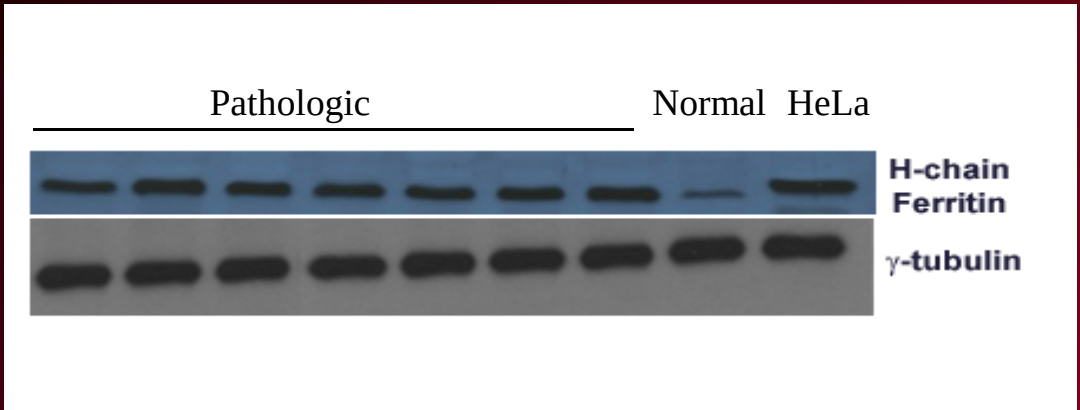
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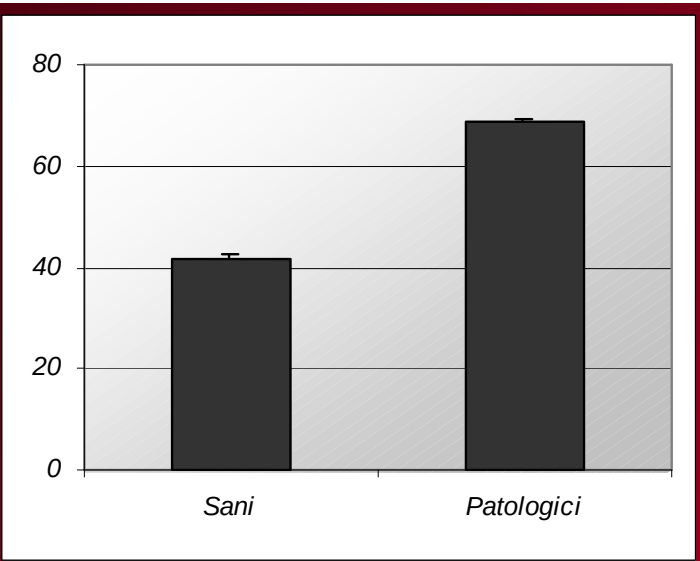


I


100
Relative intensity
0
10
Control
100
Relative intensity
Calpain
peptide
0
10
12
14
16
18
20
22
24
26
28
30
32
34
36
Time, min
E2F3
IP

Total Ferritin Heavy Chain





P<0,002

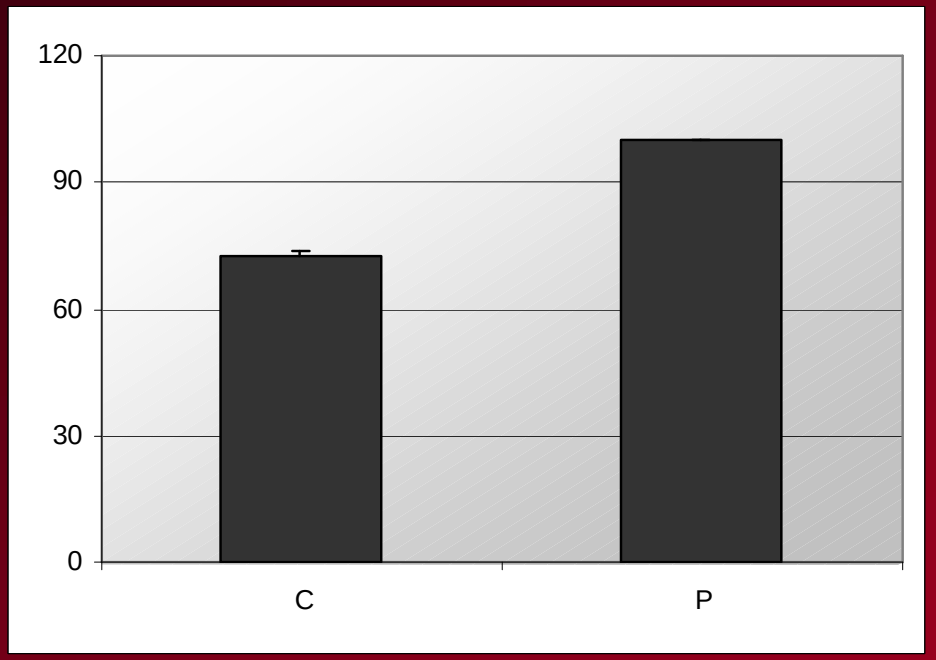
FIG.2

Immunoblot showing the expression pattern of ferritin Heavy Chain in bladder carcinoma's affected cattle. As positive control was loaded 30 µg of HeLa's totale extract, as negative control The same quantity of normal tissues.

Histograms were extracted by misuration with Scion Image Program and analyzed by T Student.

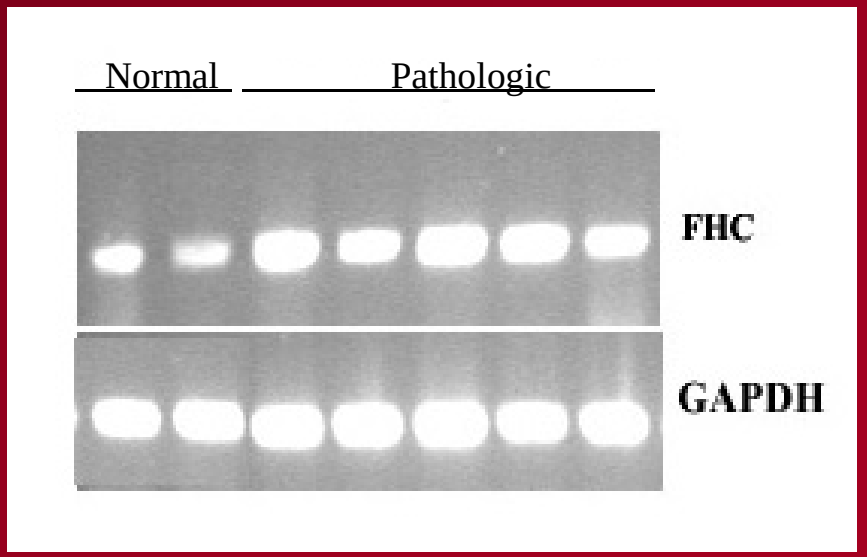
RT-PCR

FIG.1

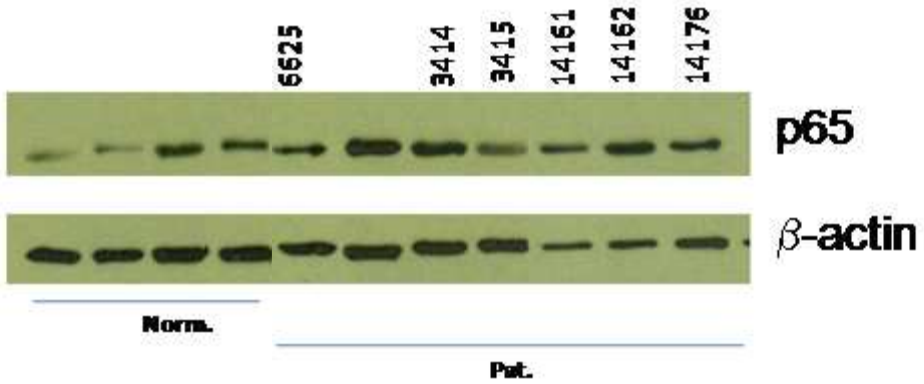


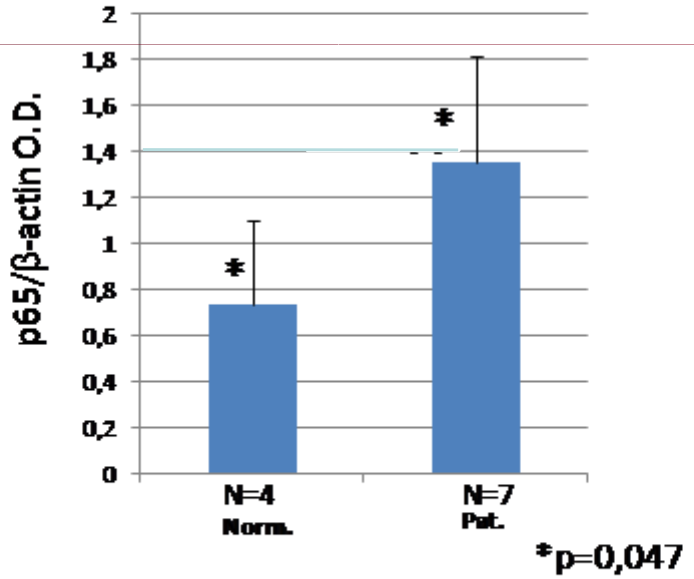
P<0,001

RT-PCR analysis for FHC and GAPDH in bovine normal and pathologic bladder tissues. The sizes were 429 bp for GAPDH and 382bp for FHC. The sequence of the PCR product completely coincided with those reported in databanks, as shown in fig.



P65 protein levels in nuclear extracts





*p=0,047

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                ....|....| ....|....| ....|....| ....|....| ....|....|
                        210        220        230        240        250
835_FER_H_      ---------- ---------- ---------- -----GCGGT TTTTTCGGCC
mRNA Bos T      AGGACTCGGA GGCCGCCATC AACCGCCAGA TCAACCTGGA GCTCTACGCC
mRNA H FER      AGGACTCAGA GGCCGCCATC AACCGCCAGA TCAACCTGGA GCTCTACGCC


                ....|....| ....|....| ....|....| ....|....| ....|....|
                        260        270        280        290        300
835_FER_H_      TCCTATGTCC TACCTGTCCA TGTCGTACTA TTTTGACCGT GATGATGTGG
mRNA Bos T      TCCTATGTC- TACCTGTCCA TGTCGTACTA TTTTGACCGT GATGATGTGG
mRNA H FER      TCCTACGTT- TACCTGTCCA TGTCTTACTA CTTTGACCGC GATGATGTGG


                ....|....| ....|....| ....|....| ....|....| ....|....|
                        310        320        330        340        350
835_FER_H_      CTTTGAAGAA CTTTGCCAAA TACTTTCTTC ACCAATCTCA TGAGGAGAGG
mRNA Bos T      CTTTGAAGAA CTTTGCCAAA TACTTTCTTC ACCAATCTCA TGAGGAGAGG
mRNA H FER      CTTTGAAGAA CTTTGCCAAA TACTTTCTTC ACCAATCTCA TGAGGAGAGG


                ....|....| ....|....| ....|....| ....|....| ....|....|
                        360        370        380        390        400
835_FER_H_      GAACATGCTG AGAGACTGAT GAAGCTGCAG AACCAGCGAG GCGGCCGAAT
mRNA Bos T      GAACATGCTG AGAGACTGAT GAAGCTGCAG AACCAGCGAG GCGGCCGAAT
mRNA H FER      GAACATGCTG AGAAACTGAT GAAGCTGCAG AACCAACGAG GTGGCCGAAT
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                ....|....| ....|....| ....|....| ....|....| ....|....|
                       410        420        430        440        450
835_FER_H_      CTTCCTTCAG GATATCAAGA AACCAGACCG TGATGACTGG GAGAATGGGC
mRNA Bos T      CTTCCTTCAG GATATCAAGA AACCAGACCG TGATGACTGG GAGAATGGGC
mRNA H FER      CTTCCTTCAG GATATCAAGA AACCAGACTG TGATGACTGG GAGAGCGGGC

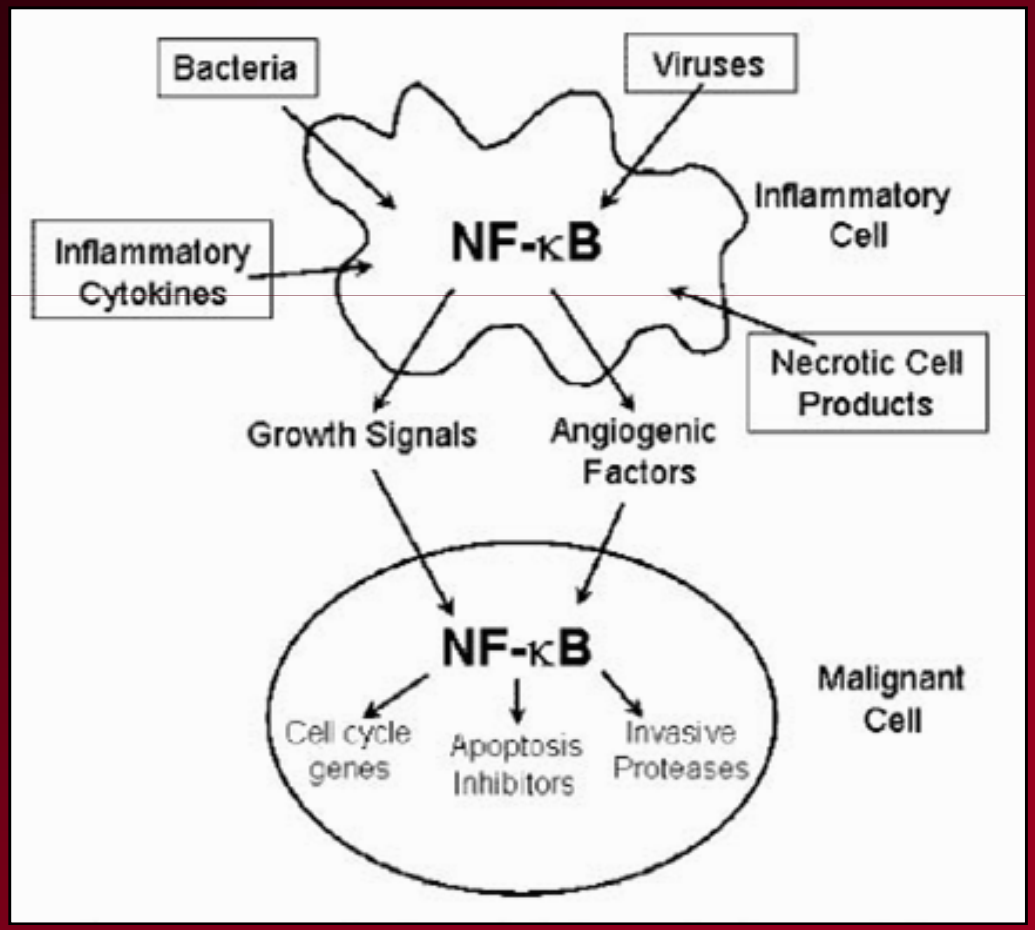
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                       460        470        480        490        500
835_FER_H_      TGACTGCAAT GGAATGTGCG CTGTGCTTGG AGAGAAGTGT GAATCAGTCA
mRNA Bos T      TGACTGCAAT GGAATGTGCG CTGTGCTTGG AGAGAAGTGT GAATCAGTCA
mRNA H FER      TGAATGCAAT GGAGTGTGCA TTACATTTGG AAAAAAATGT GAATCAGTCA

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       510        520        530        540        550
835_FER_H_      CTACTGGAAC TGCACAAACT GGCCACTGAA AAAAATGATC CCCATCTGTG
mRNA Bos T      CTACTGGAAC TGCACAAACT GGCCACTGAA AAAAATGATC CCCATCTGTG
mRNA H FER      CTACTGGAAC TGCACAAACT GGCCACTGAC AAAAATGACC CCCATTTGTG

                ....|....| ....|....| ....|....| ....|....| ....|....|
                       560        570        580        590        600
835_FER_H_      TGATTTC-TT GAGACCTCTT ACCTGAATGA GCAGGTG-AA GCCATTC---
mRNA Bos T      TGATTTCATT GAGACTCATT ACCTGAATGA GCAGGTGGAA GCCATCAAAG
mRNA H FER      TGACTTCATT GAGACACATT ACCTGAATGA GCAGGTGAAA GCCATCAAAG
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Allineamento effettuato mediante il programma BioEdit Sequence Alignment Editor versione 7.0

I tumori uroteliali nel bovino: *'AN ANIMAL MODEL FOR HUMAN DISEASE'*?



We examined 108 urine samples from cattle with bladder tumours. In all of them we were able to isolate an important mixture of numerous bacterial pathogens (Roperto et al., 2008).

I tumori uroteliali nel bovino: *'AN ANIMAL MODEL FOR HUMAN DISEASE'*?

I prefer the following histological classification:

Flat/Sessile Urothelial Lesions

- **Inverted papilloma**
- **Urothelial dysplasia (low grade intraurothelial neoplasia)**
- **Urothelial carcinoma in situ (CIS)**

I tumori uroteliali nel bovino: *'AN ANIMAL MODEL FOR HUMAN DISEASE'*?

Papillary Urothelial Neoplasias

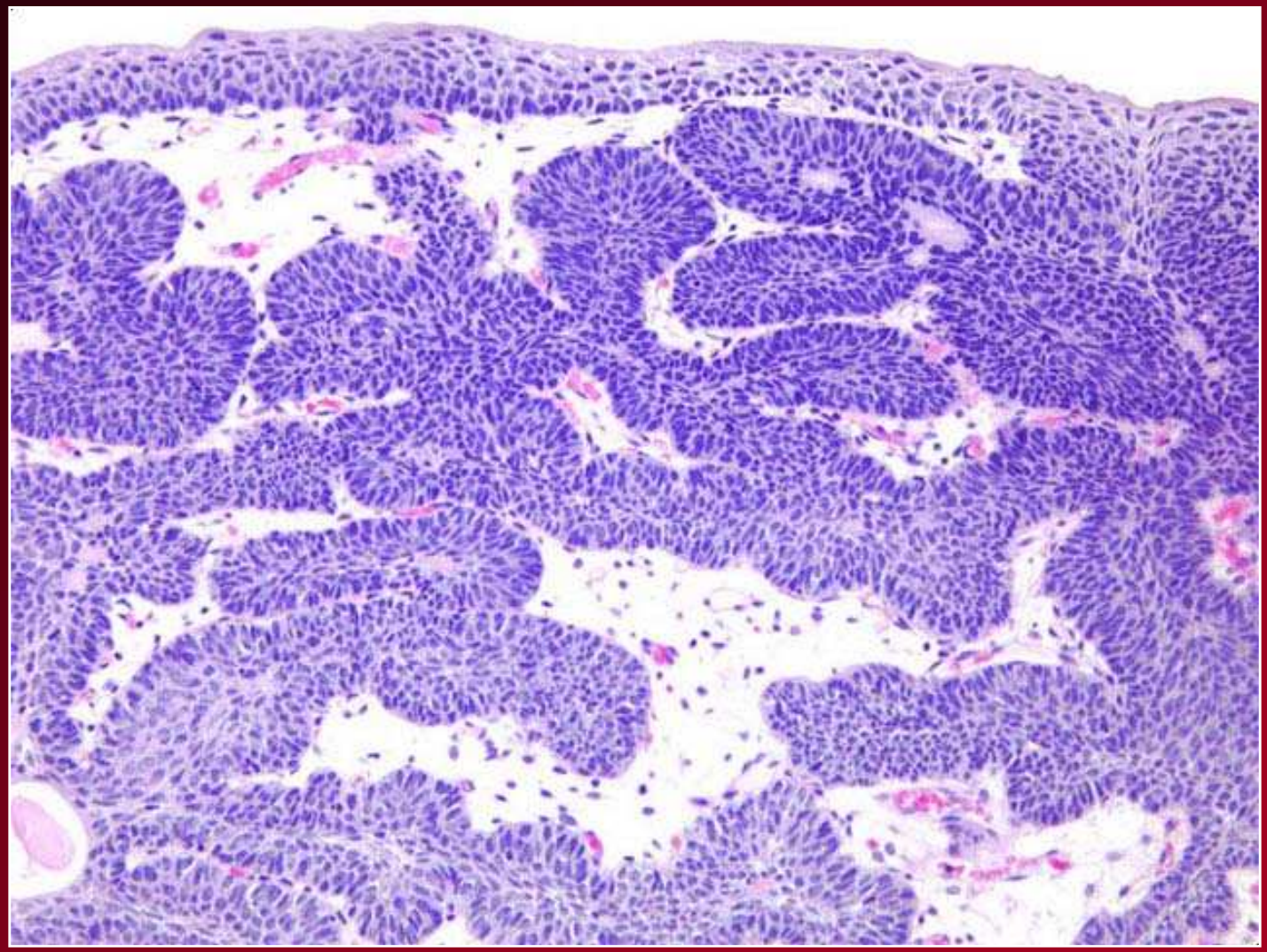
- Exophytic papilloma
- Papillary urothelial neoplasm of low malignant potential (PUNLMP)
- Low-grade papillary carcinoma
- High-grade papillary carcinoma

I tumori uroteliali nel bovino: *'AN ANIMAL MODEL FOR HUMAN DISEASE'*?

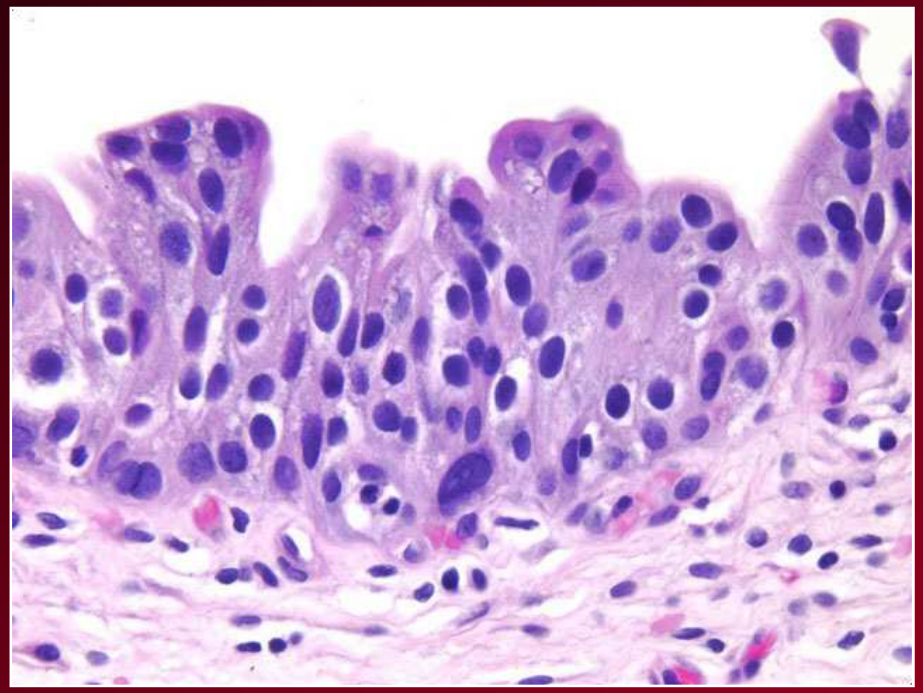
Invasive Urothelial Carcinoma

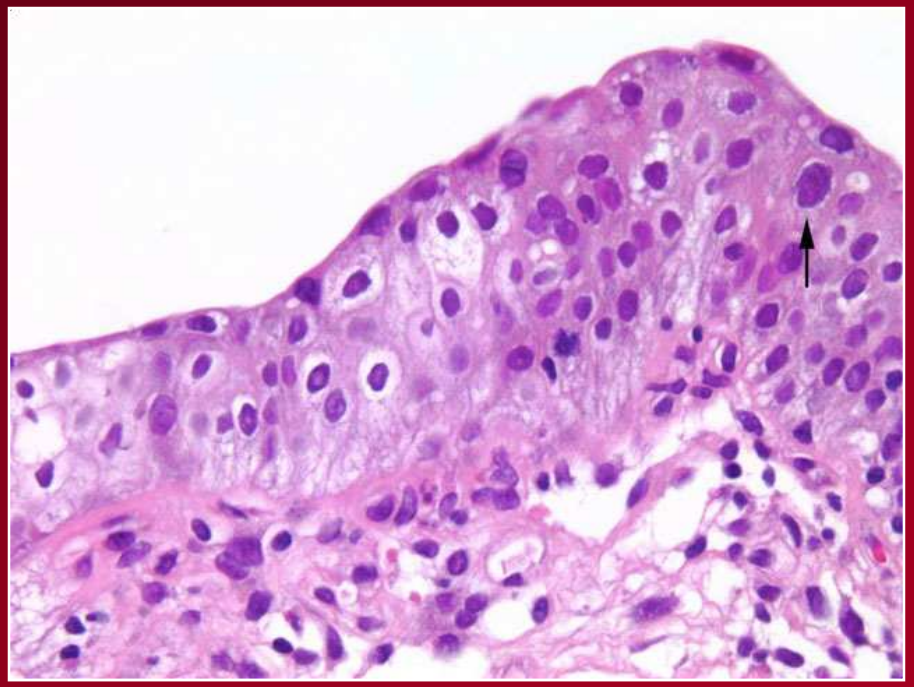
- Low-grade invasive carcinoma
- High-grade invasive carcinoma
- Histological Variants of Invasive Urothelial Carcinomas

Inverted Papilloma

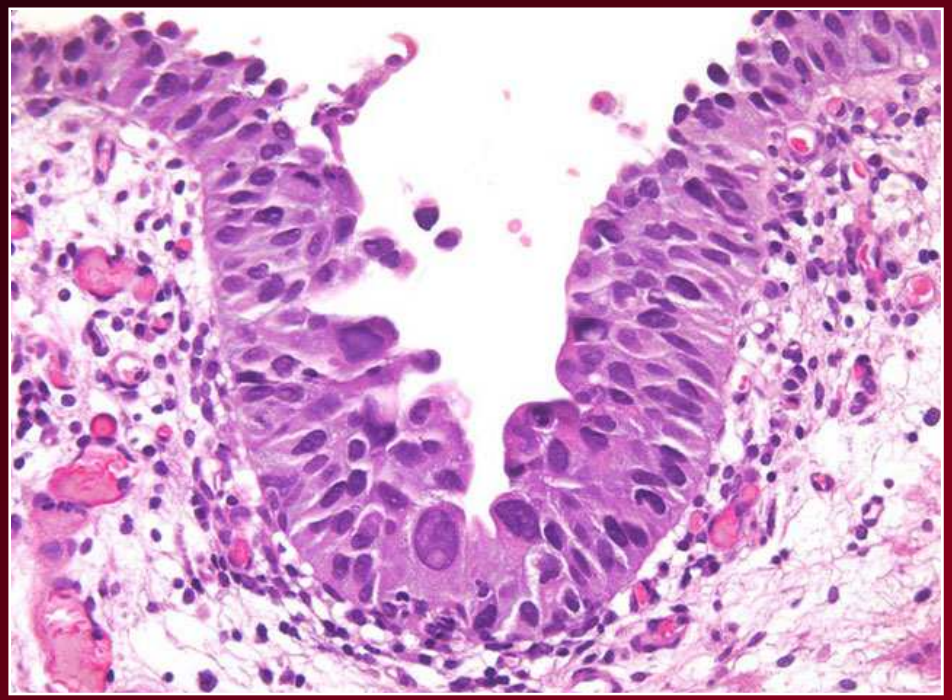


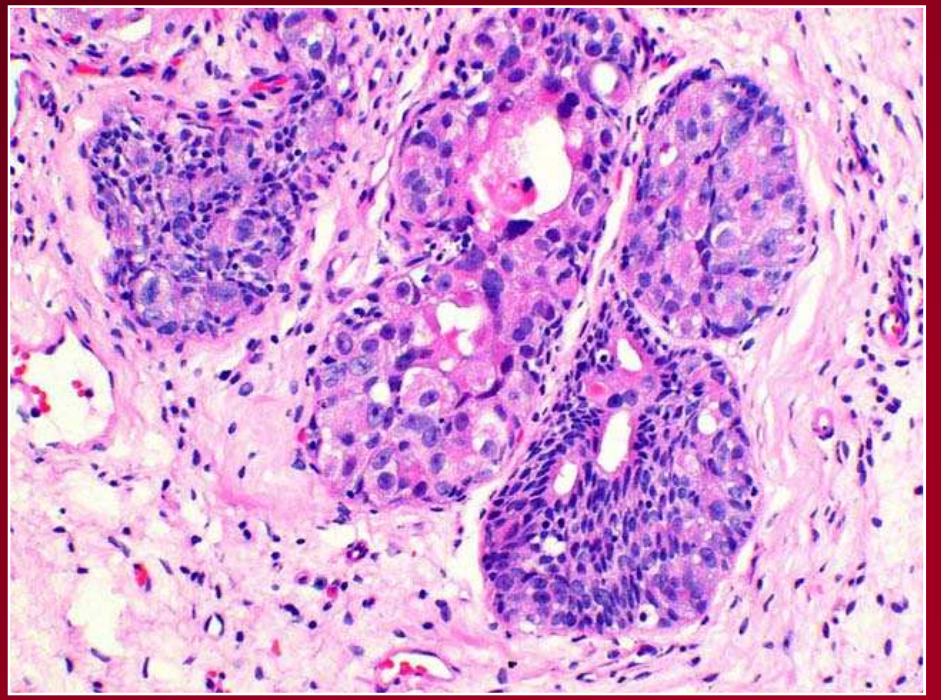
Urothelial Dysplasia



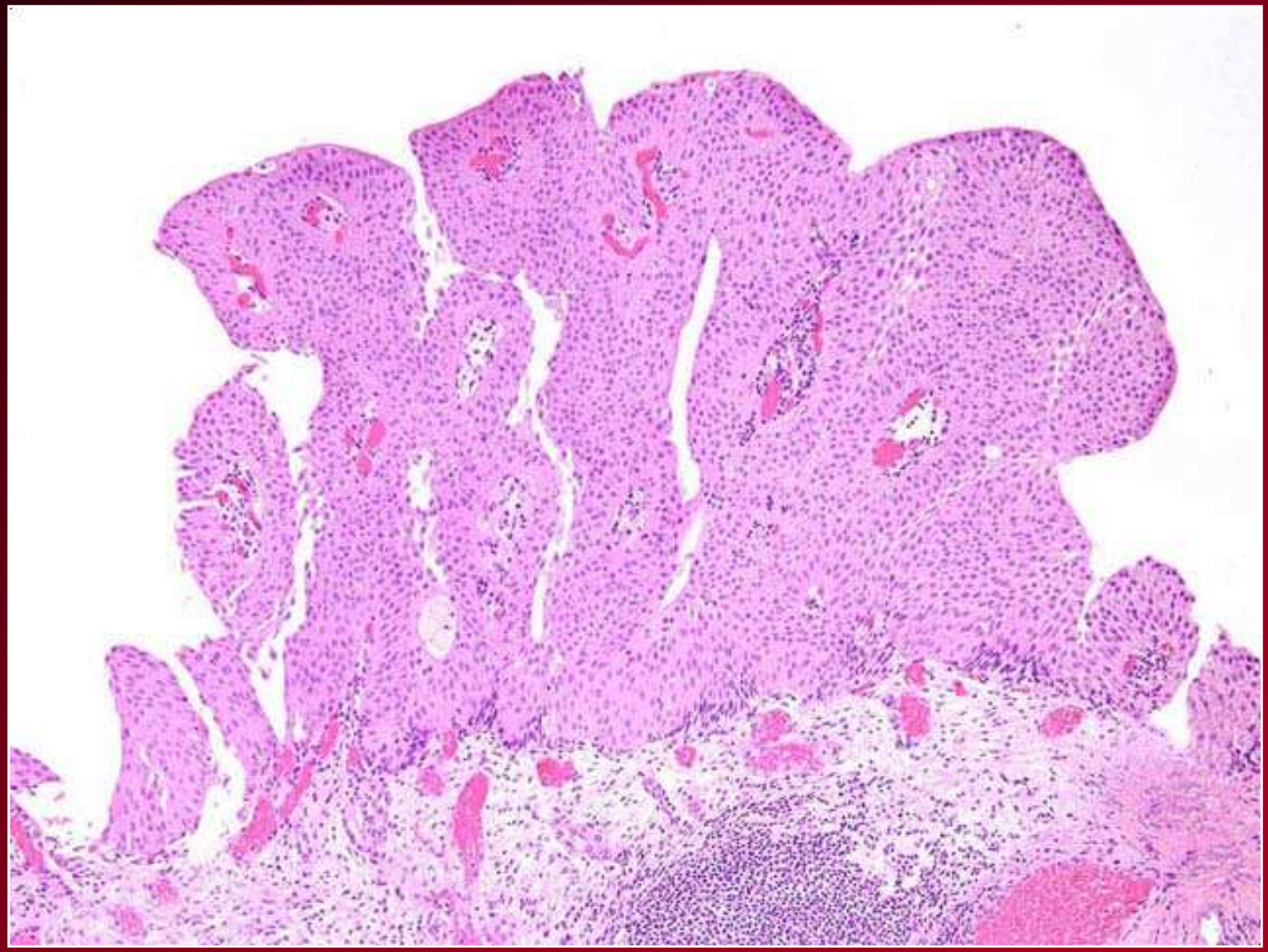


Carcinoma in Situ (CIS)

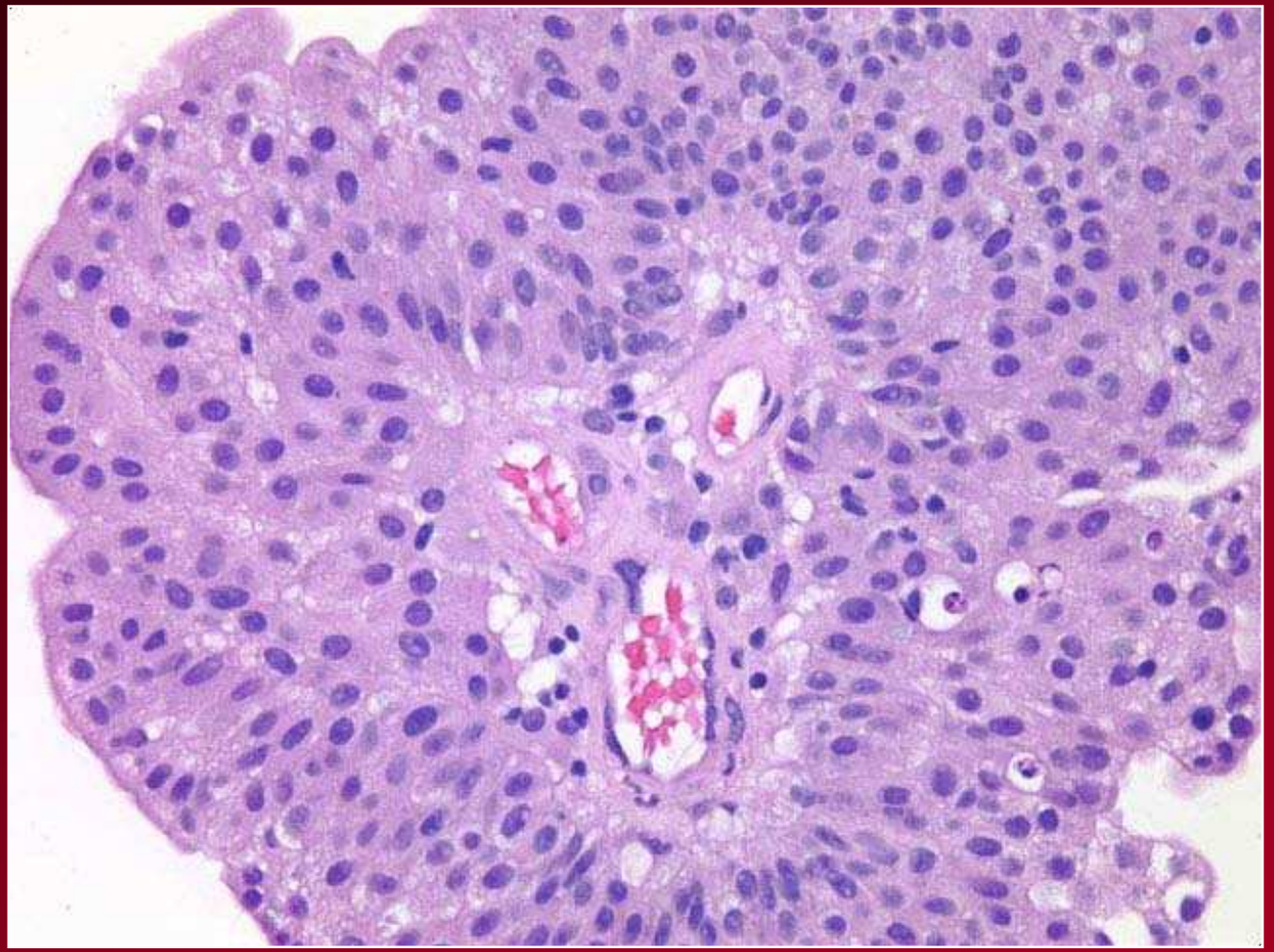




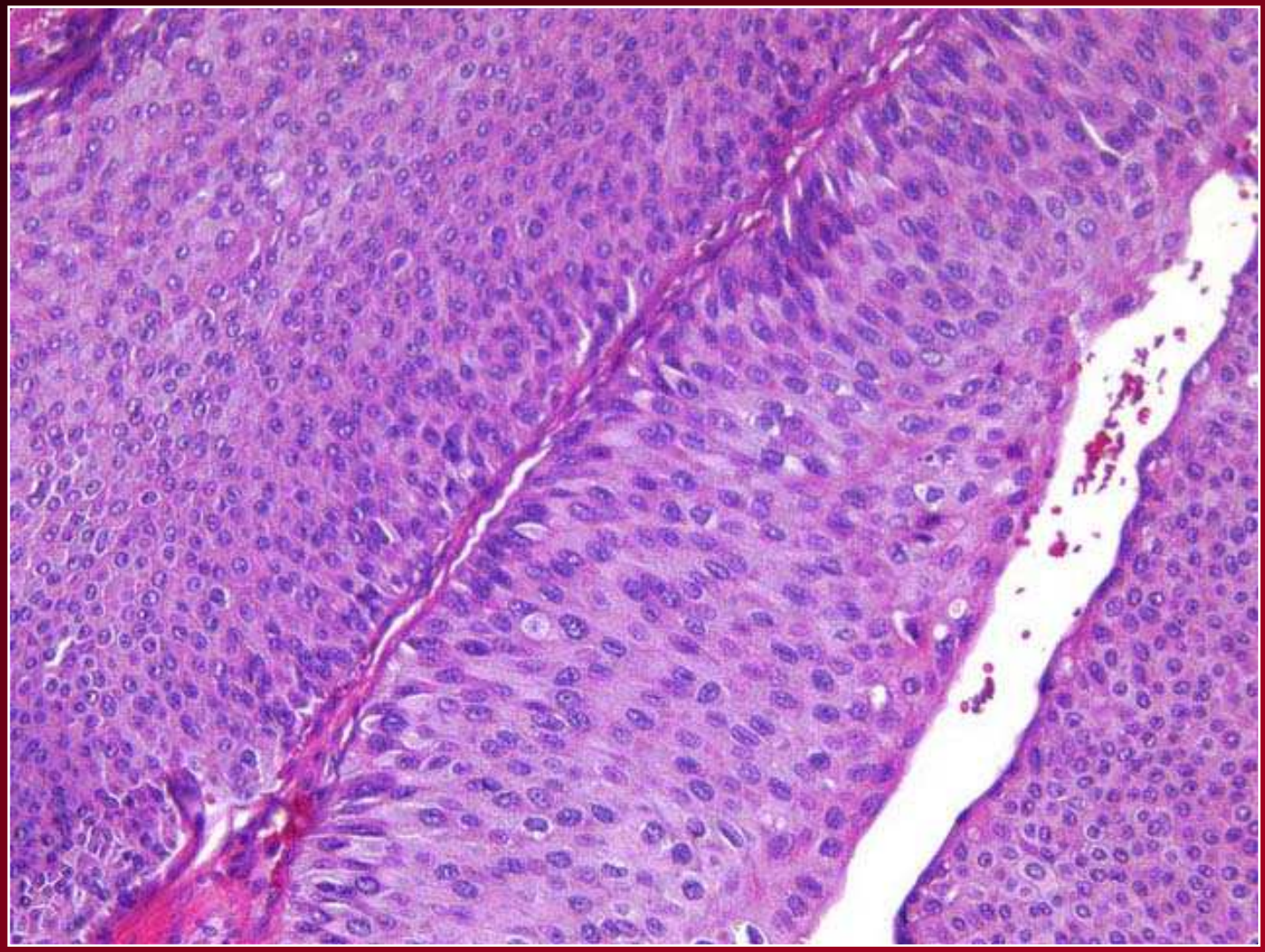
Exophytic Papilloma



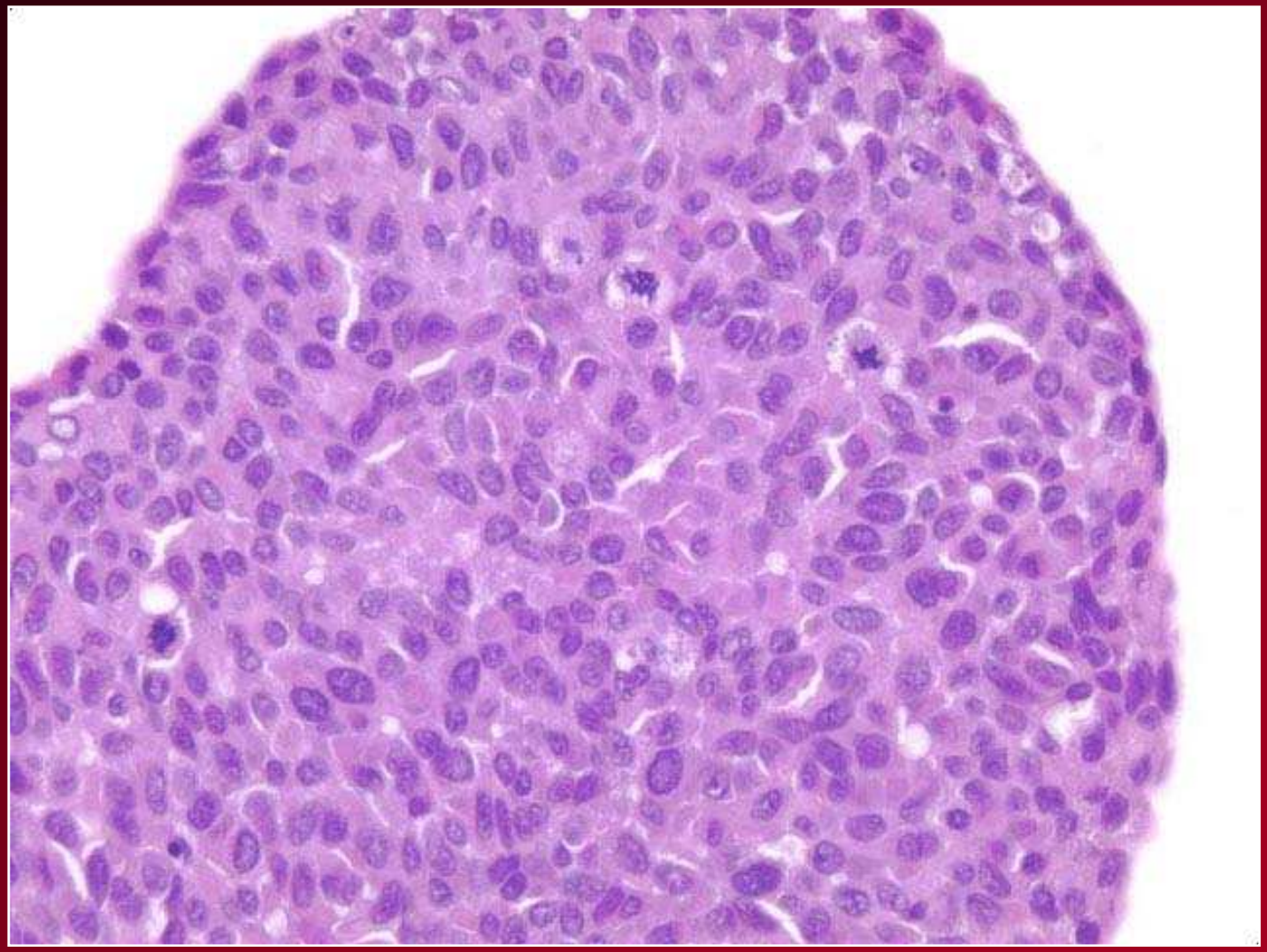
Papillary Urothelial Neoplasm of Low Malignant Potential



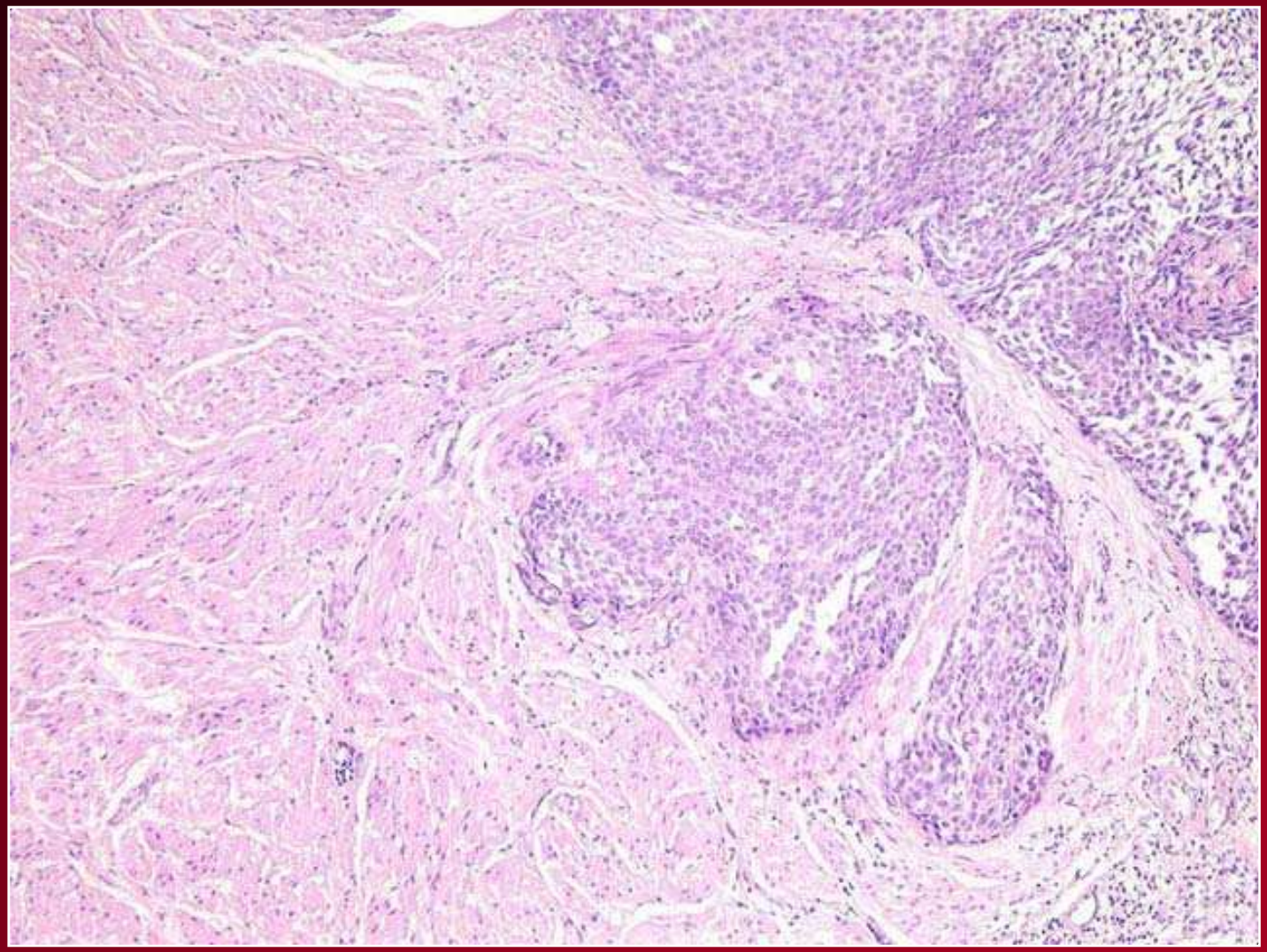
Low-grade Papillary Carcinoma



High-grade Papillary Carcinoma



Low-grade Invasive Carcinomas



High-grade Papillary Carcinoma

