STUDIES ON PROLIFERATION AND AgNOR INDICES IN CUTANEOUS AND SUBCUTANEOUS NEOPLASMS OF DOGS.

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STUDIES ON PROLIFERATION AND AgNOR INDICES IN CUTANEOUS AND SUBCUTANEOUS NEOPLASMS OF DOGS.

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Affectionately dedicated to

my Almighty, Appa, Amma, Roopa, Suguna Madam,

Gurus, Mentors and Well wishers.

DEPARTMENT OF VETERINARY PATHOLOGY
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CERTIFICATE

This is to certify that the thesis entitled "STUDIES ON PROLIFERATION AND AgNOR INDICES IN CUTANEOUS AND SUBCUTANEOUS NEOPLASMS OF DOGS" *submitted by* Mr. DAYANANDA, T.S., *for the award of degree of* MASTER OF VETERINARY SCIENCE *in* VETERINARY PATHOLOGY *to the Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, is a record of bona-fide research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associate ship, fellowship or other similar titles.*

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LIST OF ABBREVATION

AgNOR	Argyrophilic Nucleolar Organizer Region
ANOVA	Analysis of Variance
APES	Aminopropyl triethoxy silane
BrdU	Bromodeoxyuridine
CCH	Canine cutaneous histiocytoma
DAB	Diamine benzidine tetrahydrochloride
HIER	Heat induced epitope retrieval
HRPO	Horse Radish peroxidase
IHC	Immunohistochemistry
N: C	Nuclear to cytoplasmic ratio
NORs	Nucleolar Organizer Regions
PBS	Phosphate buffer saline
PCNA	Proliferating Cell Nuclear Antigen
PTEN	Phosphatase and tensin homologue
rRNA	Ribosomal RNA
TVT	Transmissible venereal tumour

Introduction

1. INTRODUCTION

Cancer, a fatal malignant disturbance of growth, is one of the major causes of mortality in canines. Neoplasms in dogs are twice more frequent in comparison with man, which progress more rapidly and bear similar anatomical and physiological properties proving them as an excellent animal model for understanding human cancers.

The skin is a complex organ consisting of many tissues accounting for more than 25 morphologically distinct primary neoplasms of which, some are common and has frequent occurrences.

Tumours of the skin and subcutaneous tissue are the most common tumours affecting the dog, accounting for approximately one third of all tumours encountered in this species, followed by that of mammary gland.

Tumour growth depends on cellular proliferation and cell death. Cell proliferation is regarded as one of the most important biological mechanisms in oncogenesis and is considered as a principal determinant of tumour progression and prognosis. In recent years, measurements of tumour proliferation have shown a good correlation with biological behaviour in certain tumours in man providing valuable additional prognostic information.

Determination of cell proliferation in tumours recently has been focused on demonstration of proliferation associated antigens and one such antigen is Ki 67. Ki 67 antigen is a nuclear protein expressed during all active stages of cell cycle (G_1, S, G_2, and M phases) but absent in resting, noncycling cells (G_0 phase) and is an excellent marker for determining growth fraction or proliferation index of a given cell population. Recently, a mouse monoclonal antibody Ki 67(clone MIB-1) against Ki 67 antigen has been applied

as a useful proliferative cell marker in many human neoplasms. Evaluation of growth fraction by the Ki 67 proliferative index is highly predictive of biological behaviour of various canine neoplasms.

One more factor that could be adapted to measure proliferation rate is AgNOR. Nucleolar organizer regions (NORs) are loops of ribosomal DNA (rDNA) occurring in all nucleoli of cells. Silver stained NORs are referred to as AgNORs, which appear as black dots in the nucleus. Their quantity increases progressively when a resting cell enters mitotic cycle from G_1 to S phase. The AgNOR quantity is strictly related to the rapidity of cell proliferation and higher the AgNOR quantity, the faster will be the cell proliferation and neoplastic mass expansion. Quantitative analysis of interphase NORs has proved to be valuable in tumour pathology for distinguishing malignant from benign lesions of the same origin.

In India, although there is a high incidence of canine cutaneous and subcutaneous tissue tumours accounting for a substantial death rate, a study on various aspects of tumours including prognostic factors such as proliferation antigen and AgNOR has not been carried out in a comprehensive way. Hence the present work was taken up with the following objectives.

- To study and classify cutaneous and subcutaneous neoplasms cytologically and histologically.
- To determine AgNOR index.
- To determine proliferation index immunohistochemically.
- To correlate histological type, proliferation index and AgNOR index in canine cutaneous and subcutaneous neoplasms.

Review of Literature

2. REVIEW OF LITERATURE

Perusal of literature has shown several reports of work being carried out on cutaneous and subcutaneous tissue tumours in dogs. The available literature on gross pathology, cytology, histopathology, AgNOR index, Ki 67 proliferation index of cutaneous and subcutaneous tissue tumours of dogs has been reviewed as follows.

The cutaneous and subcutaneous tissue neoplasms have been reported to be the most common tumours accounting for approximately one third of all the tumours encountered in dogs. (Dorn *et al.,* 1968, Bostock 1986, Chu *et al.,* 1992, Rostami *et al.,* 1994 and Bolos and Baba 2005).

Er and Sutton (1989), Chu *et al.,* (1992), Chiti and Amber (1992), and Bolos and Baba (2005) in their study on canine cutaneous neoplasms observed an increased prevalence in the occurrence of skin tumours in aged dogs and also indicated that the incidence increased with age with most occurring in dogs aged above six years.

Glavits (1977) observed among skin tumours, occurrence of malignant tumours at a higher frequency with a ratio of 1:3 of benign to malignant tumours in dogs. Similar observations have also been made by other workers (Gomes 1987, Swak *et al.,* 1993 and Solcan *et al.,* 1998). However, many workers have also reported the occurrence of more number of benign than malignant skin tumours in canines (Frese *et al.,* 1982, Rostami *et al.,* 1994, Kolodzieyski *et al.,* 1998, Sevcik *et al.,* 2000 and Bolos and Baba 2005).

In an analysis of skin neoplasms in dogs carried out by Marchevsky *et al.,* (1980) revealed higher prevalence of epithelial tumours over other types. Similar observations were also made by Frese

et al., (1982), Giesel (1987), Mukhopadhayay and Som (1992), Kolodzieyski *et al.,* (1998) and Mukaratirwa *et al.,* (2005).

2.1 Gross, cytology and histopathology of cutaneous and subcutaneous tissue tumours.

2.1.1 Round cell tumours

2.1.1.1 Mast cell tumours

Grossly, the appearance of mast cell tumour in dogs was described as encapsulated or nonencapsulated, soft to firm raised nodular mass with white creamy, light yellow, brown coloured cut surface depending up on degree of degranulation (Bostock, 1986; Dean, 1988; Simoes *et al.,* 1994, Yager and Wilcock 1994, Goldschmidt and Hendrick 2002 and Mathur 2004).

A retrospective study of cytology of canine cutaneous round cell tumours carried out by Duncan and Prasse (1979) on 64 cases revealed 25 cases of mast cell tumours. They noticed cytologically round to slightly oval shaped nuclei with aggregated chromatin, indistinct nucleoli, cytoplasmic metachromatic granules and occasional cytoplasmic vacuoles which they considered as diagnostic of mast cell tumours. Similar cytological descriptions of mast cell tumours have also been reported by Pelt *et al.,* (1986), Dean (1988), Tvedten (1994), Dunn and Villers (1998), Alleman and Bain (2000), Raskin (2001) and Nesbit *et al.,* (2002). However, Dorn *et al.,* (1968), Pelt *et al.,* (1986), Dean (1988) and Tvedten (1994)) observed presence of eosinophils in the cytological preparations and indicated that the eosinophils in cytological preparations might be a clue for diagnosis of mast cell tumours. Govier (2003) reported that mast cell tumours exfoliate well and show distinct cytological features.

Histopathologically, Herman *et al.,* (1967), Lund and park (1978), Patnaik *et al.,* (1982), Gross *et al.,* (1992), Simoes *et al.,* (1994), Kessler *et al.,* (1997), Chenier and Dore (1998) and Kaldrymidou *et al.,* (2002), ozaki *et al.,* (2002) and Mathur (2004) observed round to oval shaped pleomorphic cells, scanty to abundant granular cytoplasm, vesicular round to oval shaped nuclei with large prominent nucleoli. In addition Pelt *et al.,* (1986), Gross *et al.,* (1992), Lund and Park (1978), Chenier and Dore (1998) also noticed a few to numerous metachromatically stained cytoplasmic granules with abundant eosinophils among the neoplastic cells and around small vessels within the mass in toluidine blue stained sections.

Based on morphological features such as invasiveness, cellularity, cellular morphology, mitotic index and stromal reaction, Bostock (1973), Patnaik *et al.,* (1984), Simoes *et al.,* (1994), Jaffe *et al.,* (2000), Strefezzi *et al.,* (2003) graded mast cell tumours in to grade I (well differentiated), grade II (intermediate differentiation) and grade III (poorly differentiated) and correlated them with the survival time.

Chenier and Dore (1998) correlated the expression of P-selection an adhesion molecule in blood vessels of mast cell tumour to the infiltration of eosinophils in to the tumour mass.

2.1.1.2 Histiocytoma

Goldschmidt and Bevier (1981), Raskin (2001), and Goldschmidt and Hendrick (2002) described histiocytoma as solitary or multiple, well circumscribed, raised, smooth, pinkish to red coloured masses with alopecic skin covering, occurring most commonly in head region, especially ear pinnae.

Histiocytomas were reported to be characterized cytologically by round to oval shaped cells with minimal anisocytosis and anisokaryosis,

abundant clear to lightly basophilic cytoplasm, round, oval or indented nuclei with fine chromatin and indistinct nucleoli by Duncan and Prasse (1979) and Raskin (2001).

Histopathologically, dermal infiltrate of densely packed, mildly pleomorphic, round shaped cells consisting of moderately eosinophilic cytoplasm, hyperchromatic nuclei, arranged in cords or sheets with little or no stroma and frequent mitosis indicative of histiocytoma was described by Kelly (1970), Weiss (1974), Glick *et al.,* (1976), Bostock (1986), Raskin (2001) and Goldschmidt and Hendrick (2002).

2.1.1.3 Malignant melanoma

Bostock (1986), Bolon *et al.,* (1990), Goldschmidt (1994), Monteros *et al.,* (2000), Roels *et al.,* (2001), Goldschmidt and Hendrick (2002), Smith *et al.,* (2002), Mathur (2004) and Sapierzynski (2005), reported that macroscopically melanomas occurred as dark brown, blue or black coloured, soft or moderately hard to firm, round to ovoid, dome shaped raised masses of varying sizes from inconspicuous black macule to large growing mass.

Typical cytological features of malignant melanoma such as highly pleomorphic spindle shaped, epitheloid or admixture of both the cells with abundant fine, brownish black to green melanin pigment, have been described by Coles (1986), Alleman and Bain (2000), Raskin (2001), Smith *et al.,* (2002) and Mathur (2004).

Histopathologically, malignant melanomas were reported to be characterized by epitheloid, spindle shaped or admixture of both cell, dendritic and whorled types, with varying degree of melanin pigment formation by Conroy (1967), Weiss and Frese (1974), Turk and Leathers (1981), Wilcock and Peiffer (1986), Smith *et al.,* (2002) and Mathur

(2004). In addition they also indicated that silver stain was essential to differentiate melanotic and amelanotic type of tumours.

2.1.2 Epithelial tumours

2.1.2.1 Squamous cell carcinoma

Macroscopic appearance of squamous cell carcinoma was described by Nielsen and Cole (1960), Barrie *et al.,* (1982), Bostock (1986), Yager and Wilcock (1994), Mathur (2004), Girish (2004) as solitary or multiple, proliferative, pink to light brown coloured, raised, firm, round, ovoid to cauliflower like irregular masses with ulceration, necrosis and secondary bacterial infection.

Cytologic features of squamous cell carcinoma such as large number of pleomorphic round, caudate, tad pole shaped malignant squamous epithelial cells displaying wide range of maturation, anisocytosis, anisokaryosis, binucleation, multinucleation, deeply basophilic cytoplasm of nonkeratinized and bluish green cytoplasm of keratinized cells after staining with giemsa, numerous polymorphonuclear cells, extracellular pinkish coloured keratinized material were reported by Griffiths *et al.,* (1984), Garma-Avina (1994), Felizzola *et al.,* (1999), Andersen *et al.,* (2001), Burkhard *et al.,* (2001), Raskin (2001), Mathur (2004) and Girish (2004). Further Mathur (2004) observed spindle shaped cells with variable nuclear to cytoplasmic ratio in spindle cell type of squamous cell carcinoma.

Histologically, squamous cell carcinoma was reported to be characterized by proliferating neoplastic squamous epithelial cells with formation of well lamellated keratin pearls in case of well differentiated type and minimal degree of keratinization with high cellular anaplasia and pleomorphism in case of moderate to poorly differentiated squamous cell carcinoma by Weiss and Frese (1974), Yager and Scott (1985),

Viswanath *et al.,* (1998) and Raskin (2001), Mathur (2004), and Girish (2004). Further Goldschmidt and Hendrick (2002) and Mathur (2004) reported that spindle type of squamous cell carcinoma was a uncommon variant of squamous cell carcinoma, characterized by spindle shaped cells with vesicular nuclei and numerous mitotic cells.

2.1.2.2 Basal cell carcinoma.

Macroscopically, basal cell carcinomas have been reported to occur as well demarcated, raised round intradermal masses, firmly attached to the skin with a smooth alopecic surface [(Nielson and Cole, 1960, Seiler, 1981, Raskin, 2001, Goldschmidt and Hendrick, 2002, Helan *et al.,* 2003)].

Cytologically, Raskin (2001) reported that basal cell tumours were characterized by small cells with high nuclear to cytoplasmic ratio, monomorphic nuclei and basophilic cytoplasm, arranged in clusters or in rows.

Histologically, basal cell carcinomas were reported to be characterized by hyperchromatic epithelial cells arranged in ribbon like, medusoid, solid, trabecular and rosette types with varying amount of connective tissue stroma by Nielson and Cole (1960), Weiss and Frese (1974), Diters and Walsh (1984), Bostock (1986), Enjung *et al.,* (1995) and Goldschmidt and Hendrick (2002). In addition Seiler (1981) and Goldsmidt and Hendrick (2002) described granular basal cell tumours comprising of neoplastic cells with granules in the cytoplasm.

2.1.2.3 Hepatoid gland adenocarcinoma

Yager and Scott (1985), Anilkumar *et al. (*1997), Withrow (2001), Goldschmidt and Hendrick (2002) and Mathur (2004) described adenocarcinoma of hepatoid gland morphologically as moderately firm,

roughly spherical masses with pink coloured cut surface occurring commonly in perianal, preputial and tail skin.

Tvedten (1994) cytologically in perianal gland tumours observed, cells resembling hepatocytes with square or polyhedral shape, abundant granular cytoplasm, round, single or multiple nuclei and one or more prominent nucleoli.

Tvedten (1994) and Alleman and Bain (2000) stated that a cluster of large hepatoid appearing cells with low nucleolar to cytoplasmic ratio surrounded by the smaller reserve cells with high N:C ratio was suggestive of perianal gland tumour.

Raskin (2001) indicated that hepatoid gland carcinoma were infrequently encountered and were characterized by marked nuclear pleomorphism.

Histologically Weiss and Frese (1974), Head (1976), Anilkumar *et al.* (1997), Villalobos (2002) and Mathur (2004) reported compactly arranged sheets of highly cellular mass containing polygonal to spindle shaped cells, interspersed with reserve cells along with malignant features as characteristic of hepatoid gland adenocarcinoma.

2.1.2.4 Sebaceous gland carcinoma

Raskin (2001) and Goldschmidt and Hendrick (2002) based on gross observation of sebaceous gland carcinoma noted that they occurred as rapidly growing, poorly circumscribed, solitary, elevated multinodular intradermal masses with pale yellow to white coloured cut surface.

Raskin (2001) described cytologic features of sebaceous gland adenocarcinoma and reported that it consisted of pleomorphic glandular epithelium displaying malignant nuclear features like

anisokaryosis, prominent nucleoli and frequent atypical mitotic figures with finely vacuolated cytoplasm suggesting sebaceous differentiation.

Histopathologically, sebaceous gland carcinoma was reported to be characterized by undifferentiated polymorphous cells predominantly contained in lobule of varying size with varying degree of lipidization. (Weiss and Frese, 1974 and Goldschmidt and Hendrick, 2002)

2.1.2.5 Sweat gland adenocarcinoma

Macroscopically, sweat gland carcinoma was reported to be characterized by solitary, raised, well circumscribed, solid nodular intradermal or subcutaneous masses with cut surface showing multiple lobulations and infrequent cyst formation by Raskin (2001) and Goldschmidt and Hendrick (2002).

Cytologically, Raskin (2001) reported ductular epithelium occurring in clusters, displaying malignant characteristics along with fibroblasts in sweat gland adenocarcinoma of dogs.

Nielsen and Cole (1960), Goldschmidt and Hendrick (2002) indicated that sweat gland adenocarcinoma were often of the papillary type, with numerous projections of the anaplastic epithelium in to the lumina, which were filled with eosinophilic fluid. They also reported that the neoplastic cells were round to ovoid in shape, consisting of normochromatic to hyperchromatic nuclei with scanty amount of supporting stroma. Further Raskin (2001) also reported that sweat gland adenocarcinomas were uncommon skin tumour.

2.1.2.6 Squamous papilloma

Lenet *et al.,* (1997) and Raskin (2001) reported that cutaneous papilloma occurred more frequently on head and appeared as raised growths with keratin covered multiple projections.

Raskin (2001) observed in cytological preparations, presence of squamous epithelium of all stages of development, predominantly mature cells with benign appearing nuclei in squamous papilloma.

Histologically, squamous papilloma was reported to be characterized by proliferating neoplastic cells of various stages of development with keratin covered finger like projections and a core of dermal stroma that supported the proliferating epithelium by Weiss and Frese (1974), Kubo (1992), Raskin (2001) and Goldschmidt and Hendrick (2002). Further Sapierzynski and Sapierzynska (2005) reported that papillomas were common in dogs and associated with papilloma virus in young dogs.

2.1.2.7 Fibropapilloma

Maclachlan and Kennedy (2002) reported that fibropapillomas occurred as elevated fleshy multinodular proliferations.

Weiss and Frese (1974), Yager and willcock (1994), Maclachlan and Kennedy (2002), Mathur (2004) and Bolos and Baba (2005) reported that in fibropapilloma, proliferation of the fibrous tissue was greater than that of epithelial tissue and epithelial retepegs penetrated deep into the fibrous moiety.

2.1.2.8 Trichoepithelioma

Macroscopically Nielsen and cole (1960), Weiss and Frese (1974) and Raskin (2001) described trichoepitheliomas as firm, well circumscribed, round or ovoid masses with atrophic, hairless skin covering.

Raskin (2001) reported that cytologically trichoepitheliomas consisted of keratinaceous debris, keratinocytes and low number of germinal epithelium resembling basal cells.

Histopathologically, Nielsen and cole (1960), Weiss and Frese (1974), and Goldschimidt and Hendrick (2002) reported that trichoepitheliomas occurred as islands of compactly arranged proliferating neoplastic cells with keratinization at center. Further they reported that histological appearance of trichoepithelioma would vary depending upon the degree of differentiation of proliferating neoplastic cells.

2.1.2.9 Hepatoid gland adenoma

Isitor (1983), Goldschmidt and Shofer (1992), Anilkumar *et al.,* (1997) and Cammarata- Parodi *et al.,* (1998) observed that perianal adenomas were common in dogs and frequently occurred in intact males.

Macroscopically, hepatoid gland adenoma was reported to be characterized by smooth, raised, round, slowly growing nodular growths with thin alopecic skin covering and brown coloured cut surface by Bostock (1986), Goldschmidt and Shofer (1992) and Goldschmidt and Hendrick (2002).

In perianal gland tumour, Tvedten (1994) observed cytologically cells resembling hepatocytes with square or polyhedral shape with abundant granular cytoplasm, round single or multiple nuclei and one or more prominent nucleoli.

Tvedten (1994) and Alleman and Bain (2000) and Raskin (2001) stated that clusters of mature round hepatoid cells with abundant finely granular pinkish blue cytoplasm and round nuclei in more number along with a few smaller basophilic reserve cells with lack of cellular pleomorphism were suggestive of hepatoid gland adenoma.

Bostock and Dye (1980), Mukhopadhayay and Som (1992), Er and Sutton (1989), Williamson and Middleton (1998) recorded that fibrosarcoma was the most commonly encountered soft tissue tumour in their study.

Histopathologically, Weiss and Frese (1974), Isitor (1983), Bostock (1986), Tvedten (1994), Alleman and Bain (2000) and Goldschmidt and Hendrick (2002) observed island or cord like arrangement of neoplastic cells resembling hepatocytes with eosinophilic granular cytoplasm and ovoid nuclei surrounded by basaloid reserve cells characteristic of hepatoid gland adenoma.

2.1.2.10 Sebaceous gland adenoma

Nielson and Cole (1960), Raskin (2001) and Goldschmidt and Hendrick (2002) described sebaceous adenomas as solitary, raised, hairless, cauliflower like lesions or as an intradermal multilobulated nodular mass with pale yellow to white cut surface.

Cytologically, Raskin (2001) reported presence of mature sebocytes arranged in clusters with pale foamy cytoplasm and small dense centrally placed nucleus along with reserve cells having basophilic cytoplasm and higher nuclear to cytoplasmic ratio in sebaceous adenoma.

Histologically, sebaceous adenomas were observed to be featured by solid sheaths of sebaceous cells, fat filled mature cells and immature reserve cells with little or no pleomorphism by Nielson and Cole (1960), Bostock (1986), and Goldschmidt and Hendrick (2002).

2.1.2.11 Sweat gland adenoma

Goldschmidt and Hendrick (2002) described morphological appearance of apocrine adenomas as soft masses located in the dermis or subcutis and raised above from the surrounding skin.

Amorphous debris along with low number of inflammatory cells and ductular epithelium in clusters with absence of pleomorphism in adenoma of apocrine sweat gland were recorded by Raskin (2001)

Histopathologically, Weiss (1974), Knecht and Priester (1978), Yoxal (1978), Griffith *et al.,* (1984), Bostock (1986), Felizzola *et al.,* (1999) Raskin (2001) and Mathur (2004) recorded broad interlacing bundles of spindle cells with malignant features and presence of collagen.

2.1.3.2 Hemangiosarcoma

Culbertson (1982) and Vonbeust *et al.,* (1998) stated that hemangiosarcomas could occur at any site of the body involving skin.

Macroscopically Culbertson (1982), Goldschmidt and Hendrick (2002) described cutaneous hemangiosarcomas as solitary or multicentric growths, well defined, soft to firm, reddish brown to black coloured with exudation of blood from cut surface, occurring in dermis or subcutaneous tissue.

Cytological features of hemangiosarcomas such as pleomorphic, spindle to stellate shaped neoplastic cells with basophilic cytoplasm, high nuclear to cytoplasmic ratio, oval nuclei, multiple nucleoli along with low cellularity and numerous blood cells in the back ground have been described by Raskin (2001).

Highly cellular, pleomorphic, spindle to round shaped neoplastic cells with hyperchromatic nuclei arranged in the form of solid sheets or forming vascular sinusoids containing blood cells and thrombi along with frequent mitotic figures as characteristic histologic features of hemangiosarcoma were described by Weiss (1974), Culbertson (1982), Pletcher and Murphy (1984), Hargis *et al.,* (1992) and Goldschmidt and Hendrick (2002). Further Hargis *et al.,* (1992) stated that cytologic features like presence of pleomorphic, spindle shaped cells with vesicular, ovoid to elongated nuclei lining the blood containing channels of varying size helped in diagnosis of hemangiosarcoma.

2.1.3.3 Leiomyosarcoma

Cooper and Valentine (2002) stated that macroscopically leiomyosarcomas occurred as solitary, raised, solid, firm, nodular to multinodular masses in the dermis.

Leiomyosarcomas were reported to be characterized cytologically by typical round to spindle shaped neoplastic cells with punctuate cytoplasmic vacuolations which exfoliated as single cells or small aggregates by Borjesson (2001).

Histopathologically, Weiss (1974), Burnnert *et al.*, (1990), Cooper and Valentine (2002) have recorded leiomyosarcomas as nonencapsulated, invasive tumours with variable histological features consisting of densely packed relatively homogenous pleomorphic spindle, ovoid or round cells. Further they also reported that differentiation of leiomyosarcomas from other connective tissue tumours demanded electron microscopy and immunohistochemical techniques.

2.1.3.4 Fibroma

Raskin (2001) and Goldschmidt and Hendrick (2002) described macroscopically fibromas as firm, rubbery, round to oval intradermal or subcutaneous masses with grey or white cut surface which commonly occurred on the limbs and head of the dogs.

Cytologically, variable numbers of spindle or fusiform cells with small uniform dense oval nuclei, basophilic cytoplasm and poorly defined cell border forming cytoplasmic tail on either sides of the nucleus were described by Raskin (2001), Goldschmidt and Hendrick (2002) and Mathur (2004). They also reported that exfoliation was less and only a few cells were observed in cytological preparations.

Histologically, fibroma was reported to be characterized by mature uniform fibrocytes with spindle shaped nuclei and abundant collagenous material which were arranged in interwoven fascicles and rarely in whorls. (Weiss, 1974 and Goldschmidt and Hendrick, 2002).

2.1.3.5 Hemangioma

Raskin (2001) and Goldschmidt and Hendrick (2002) reported that grossly hemangiomas occurred as well demarcated, encapsulated, discrete soft nodules with cut surface revealing honey comb pattern of fibrous trabeculae separating blood filled cavities. Further Vonbeust (1998) stated that hemangiomas were most frequently observed in cutaneous tissue although they could occur any where in the body.

Raskin (2001) stated that cytologically, aspirates from hemangioma appeared bloody, resembled blood contamination and contained a few basophilic endothelial cells with large number of blood cells.

Histopathologically, Weiss (1974), Hargis *et al.,* (1992) and Goldschmidt and Hendrick (2002) recorded blood spaces of varying size and shape lined by monolayer of endothelium containing erythrocytes and thrombi separated by varying amount of stroma in hemangioma.

2.1.3.6 Lipoma

Macroscopically, Weiss (1974), Knecht and Preister (1978), Bostock (1986), Raskin (2001) and Mathur (2004) described lipoma as soft, circumscribed, dome shaped, movable masses with oily, whitish yellow coloured cut surface.

Tvedten (1994) and Dunn and Villiers (1998) and Mathur (2004) cytologically observed adipose tissue containing numerous adipocytes with pyknotic, eccentrically placed nuclei and free fat droplets in lipoma cases.

Raskin (2001) reported that fat in lipoma may be best demonstrated with a water soluble stain such as new methyelne blue or the fat stain oil red 'O'. He also indicated that when alcohol fixatives were used with Romanowsky stains, the lipid dissolved leaving slides often void of cells.

Histopathologically, lipomas were reported to be characterized by numerous adipocytes with eccentrically placed nucleus and thin connective tissue stroma by Weiss (1974), Knecht and Priester (1978), Yoxal (1978), Stockhaus and Teske (1999), Kaldrymidou *et al.,* (2002) and Mathur (2004).

2.1.3.7 Myxoma

Raskin (2001) described myxomas macroscopically as soft grayish white poorly defined raised masses with fluctuant texture and exudation of stringy clear mucoid fluid, occurring commonly in limbs, thorax and abdominal regions. Similar observations were also made by Grindem *et al.,* (1990) and Goldschmidt and Hendrick (2002).

Raskin (2001) reported that cytologically myxoma consisted of well differentiated fusiform and stellate cells in less number, with intercellular matrix in background appearing as granular eosinophilic amorphous material.

Histologically, myxomas were reported to be composed of an unencapsulated proliferation of stellate to spindle shaped fibroblasts loosely arranged in an abundant myxoid matrix, which stained blue with routine H&E stains (Goldschmidt and Hendrick, 2002)

2.2 AgNOR

Agyrophilic nucleolar organizer regions are silver stained proteins co-localized with chromosomal segments in which ribosomal RNA (r RNA)

is encoded. The two major AgNOR proteins are nucleolin and protein B23 which are involved in rRNA synthesis and processing. NORs are directly related to proliferative activity of the cell due to the transcription of rRNA needed for assembly of ribosomes. The quantity of AgNOR proteins are very strong prognostic indicators of neoplastic disease [Gerdes *et al.,* (1991), Preziosi *et al.,* (1995), Lohr *et al.,* (1997), Derenzini *et al.,* (2004)].

2.2.1 AgNOR counts in cutaneous and subcutaneous tissue tumours

Using silver staining technique nucleolar organizer regions (NOR's) were studied in mastocytomas by Roccabianca *et al.,* (1992) and they reported that this method was readily applicable and AgNOR were enumerable easily.

Martin (1994) reported that mitotic index, DNA cytometry, flow cytometry determination of bromodeoxy uridine (BrdU) labeling index and immunohistochemical analysis of proliferation associated antigens (Ki 67, Proliferating cell nuclear antigen), AgNOR analysis were used for assessment of proliferative activity of malignant tumours. Further he also reported that AgNOR analysis gave reproducible, exact results of proliferative activity of malignant tumours.

While evaluating prognostic value of histologic grading, mitotic index, AgNOR counts, PCNA counts in canine mast cell tumours, Simoes *et al.,* (1994) observed a significant difference between AgNOR counts of recurring (2.9±0.2) and non recurring tumours (2.2 ±0.1), also between tumours with metastasis (2.8±0.2) and without metastasis (2.3±0.1).

The mean number of AgNOR per 100 nuclei for leiomyomas was significantly less than that obtained for leiomyosarcamas and significantly greater than that obtained for normal smooth muscle was reported by Johnson *et al.,* (1995). They also stated that enumeration of AgNOR in 100 nuclei as mitotic index was efficient in correctly

categorizing histologically benign and malignant smooth muscle neoplasms in dogs.

By analyzing nucleolar organizing regions in different types of perianal gland tumours in dogs, Preziosi *et al.,* (1995) showed progressive increase in proliferation rate with an increased histological malignancy. They reported mean AgNOR count of 1.6, 1.88 and 2.4 in hyperplasia, adenoma and adenocarcinoma respectively.

Thirty two histologically confirmed canine mast cell tumours were studied to determine the AgNOR frequency by Kravis *et al.,* (1996) and they reported that frequency of AgNOR was significantly associated with histological grade of mast cell tumours.

To differentiate intracutaneous cornifying epithelioma from squamous cell carcinoma Karademir *et al .,* (1996) employed AgNOR staining, PCNA immunostaining method and mitotic index score and reported that AgNOR count was significant ($p< 0.01$) to differentiate these two tumours whereas PCNA index and mitotic index were not significant in Mann-Whitney 'U' test.

Krishnamurthy and Paliwal (1998) reported that the AgNOR counts in neoplastic tissue were significantly higher than that of healthy tissue; malignant tumours had higher counts than benign tumours. Myeloma and venereal sarcoma had higher AgNOR indices than squamous epithelial tumours indicating increased proliferative activity.

Karademir *et al.,* (1998 a) employed AgNOR staining methods to differentiate transmissible venereal tumours (TVT) from canine cutaneous histiocytomas (CCH) and reported a mean AgNOR value of 9.65 for TVT and 5.41 for CCH.

Karademir *et al.,* (1998 b) reported that AgNOR count and PCNA indices separate benign from malignant lesions in the evaluation of fibromas from fibrosarcomas.

AgNOR and PCNA staining techniques were employed by Hung *et al.,* (2000) to measure the proliferative activity of canine mast cell tumours , perineal gland tumours, fibromas, fibrosarcomas using high resolution digital microscope camera and image analysis software. They reported that the mean AgNOR areas, relative AgNOR areas, PCNA positive rates of malignant and non malignant tissues (benign tumour and normal tissue) were significantly different. They also stated that mean AgNOR cut off points that discriminated grade II or III mast cell tumour from grade I, perianal gland carcinomas from adenomas, fibrosarcomas from non fibrosarcomas were found to be 6.0, 14.1, 9.4 and 8.8 respectively.

Della *et al.,* (2002) employed MIB-1 immunohistochemistry and AgNOR staining to evaluate cell proliferation in well differentiated squamous cell carcinoma and infundibular keratinizing acanthoma of dogs and reported that these neoplasms have different proliferative behavior.

Pich *et al.,* (2004) reported that AgNOR quantity is strictly related to rapidity of cell proliferation and used to evaluate cell doubling time in histological preparation.

Derenzini *et al.,* (2004) reported that a peculiar group of highly argyrophilic acidic proteins present in nucleolar organizer regions (NOR's) which allow them to be specifically stained by silver nitrates forming AgNORs were present abundantly in malignant neoplastic cells than benign ones. Further they reported that AgNORs were used to measure the rate of cell proliferation and assessment of the prognosis.

Mean AgNOR score of 1.56 in normal squamous epithelium, 3.29 in well differentiated squamous cell carcinoma, 4.29 in moderately differentiated squamous cell carcinoma and 5.21 in poorly differentiated squamous cell carcinoma was reported by Manu *et al.,* (2006). They also reported that AgNOR technique could be used for prognostic and therapeutic decision making in squamous cell carcinoma.

2.3 Ki 67 proliferation antigen

Ki 67 is a proliferation marker which is usually used to measure growth fraction of tumour. The expression of the Ki-67 protein is strictly associated with the cell proliferation. During interphase, the antigen can be exclusively detected within the nucleus, whereas in mitosis most of the protein is relocated to the surface of the chromosomes. The fact that the Ki-67 protein is present during all active phases of the cell cycle (G_1, S, G_2 and absent in resting cells (G_0), makes it an excellent marker for determining the tumour growth fraction and also the prognosis. Ki-67 protein expression is an absolute requirement for progression through the cell-division cycle (Gerdes *et al.,* 1991 and Lohr *et al.,* 1997)

2.3.1 Immunohistochemical detection of Ki 67 proliferation in cutaneous and subcutaneous tissues.

To compare leukocyte proliferation and papilloma cell proliferation in progressing and regressing papillomas, Okabayashi *et al.,* (1993) employed Ki 67 monoclonal antibody along with MAB – 019 [specific for DNA or bromodeoxy uridine (BrdU) complexes] and showed a homogenous distribution of positive cells from basal layer to the upper layer in progressing papilloma and more number of positive cells only in basal and lower layers in regressing papilloma.

Proliferative activity in canine testicular tumours was assessed by Sarli *et al.,* (1994) by making use of Ki 67 monoclonal antibodies along

with proliferation cell nuclear antigen with an objective to study the behaviour of these neoplasms.

In a study designed to correlate P^{53} expression with the expression of cellular proliferation marker Ki 67, Copete *et al.,* (1997) reported 26.7 ±14.4 percent of Ki 67 positive cells in basal layers of papilloma.

Rossen *et al.,* (1997) reported basal cell carcinomas as a slow growing tumour. Further they stated that basaloid proliferations, basal cell carcinomas and normal epidermis had similar Ki 67 index ranging from 11-15 percent.

Thirty seven cases of leiomyosarcomas were analyzed for cell proliferation factors (mitotic index and Ki 67 index) and P^{53} status with an objective of evaluating these factors as indicators in prognosis by Konomoto *et al.,* (1998) and they reported a high Ki 67 index in superficial layers of leiomyosarcomas. Further they concluded that abnormal P^{53} and high Ki 67 index were useful as prognostic indicators.

Proliferation rates in ninety six cutaneous melanomas were assessed by Miracco *et al.,* (1998) by employing MIB 1 labeling index who observed high MIB 1 positive nuclei of 16.7 percent in thin nodular melanomas.

Clinical behaviour of melanocytic tumours in dogs by quantitative computerized analysis of proliferation associated antigens (Ki 67 and PCNA) was carried out by Roels *et al.,* (1999). They reported a significant difference in Ki 67 positivity between benign and malignant melanomas and high Ki 67 positivity along with macroscopic invasive growth, associated with decreased survival time. They also suggested that Ki 67 was a potentially important prognostic factor in melanomas of dogs.

Hung *et al.,* (2000) used high resolution digital microscope cameras and image analysis software as an improved system for quantifying proliferative activity by measuring PCNA and reported a statistically significant difference between perianal gland carcinoma and adenomas. They also reported that the improved system was sensitive and precise for quantifying PCNA and PCNA measurement for differentiating benign from malignant tumours.

In an effort to validate the tissue array technique, Hoos *et al.,* (2001) conducted a study that defined the concordance of single duplicate and triplicate, 0.6 mm core biopsies on tissue arrays in comparison to full section analysis. They studied 59 fibroblastic tumours for expression of Ki 67, P^{53} and retinoblastic proteins and reported that more than 20 percent of Ki 67 positive tumour nuclei of full section was considered as a high proliferation index.

Growth fraction of sixty eight canine cutaneous melanomas was determined by immunostaining with MIB 1 monoclonal antibody by Laprie *et al.,* (2001) and they reported a Ki 67 proliferation index greater than 15 percent in 13 cases. They also stated that a high Ki 67 proliferation and histological malignancy were both associated with significantly poorer two year survival and evaluation of growth fraction by Ki 67 proliferation index was highly predictive of biological behavior of canine cutaneous melanoma.

Skin tumours were examined immunohistochemically to determine the expression of Ki 67 antigen by Sakai *et al.,* (2001). They observed a large number of basal cells of epidermis and hair follicles of skin positive for Ki 67 antigens. Further they also reported that Ki 67 proliferation index of squamous cell carcinomas was significantly higher than those of trichoepitheliomas and basal cell tumours.

To compare the accuracy of prognosis provided by MIB 1 proliferation index with classical histological criteria and location Millanta *et al.,* (2002) analyzed 62 melanocytic tumours immunohistochemically using MIB 1 monoclonal antibody. They reported a significant difference in MIB 1 proliferation index between melanocytoma and primary malignant melanoma and concluded that MIB-1 proliferation index had prognostic value in primary malignant melanomas.

Ki 67 positive index, mitotic index , bromodeoxy uridine labeling index were employed by Sakai *et al.,* (2002) to measure proliferative activity of 91 canine mast cell tumours and reported that Ki 67 proliferation index was found to be significantly different between histological tumour grade I and II and between grade II and III. They also stated that Ki 67 index, BrdU index were useful markers to determine proliferative fractions in canine mast cell tumours.

While studying malignant fibrous histiocytoma and atypical fibroxanthoma of skin, Rizzardi *et al.,* (2003) reported that malignant histiocytoma behaved aggressively. Further they stated that Ki 67 proliferation index, P^{53}, bcl-2 protein expression and DNA polyploidy were associated with high malignant potential.

Fifty cases of desmoplastic melanoma and 13 cases of desmoplastic nevi were analyzed for expression of Melan – A and Ki 67 antigen by Kucher *et al.,* (2004) and they reported 11 to 30 percent of Ki 67 positive cells in 10 percent of desmoplastic melanoma.

While evaluating Ki 67 labeling index as a marker of malignancy in ocular surface neoplasms Ohara *et al.,* (2004) reported that Ki 67 labeling index was significantly higher in sebaceous gland carcinoma when compared to squamous cell carcinoma.

Abe *et al.,* (2005) based on histological and immunohistochemical features (MIB-1 index, erythrocyte glucose transporter protein, vascular endothelial growth factor, glucocorticoid receptor) reported that both central nervous system capillary hemangioma and lobular capillary hemangioma of the skin were benign lesions.

Mikhail *et al.,* (2005) studied phosphatase and tensin homologue (PTEN) expression and its relationship with patient survival, bcl-2 expression and Ki 67 expression in 127 primary melanomas and reported a high proliferative index of more than or equal to 20 percent of Ki 67 immunoreactive cells in 53 cases and high proliferative index was correlated with increased tumour thickness and higher histological grade.

To determine association of AgNOR, Ki 67, PCNA scores with histologic grades and survival time, sixty dogs with soft tissue sarcomas were studied by Ettinger *et al.,* (2006) and they reported that Ki 67 scores greater than median Ki 67 scores and AgNOR scores greater than median AgNOR scores were prognostic factors for decreased survival time. They concluded that AgNOR and possibly Ki-67 should be routinely evaluated with histologic grading for soft tissue sarcomas in dogs.

In a retrospective study Scase *et al.,* (2006) performed immunohistochemical staining for Ki 67, proliferating cell nuclear antigen (PCNA), survivin expression and AgNOR staining on 121 canine cutaneous mast cell tumours and reported that Ki 67 score could be used to divide Patnaik grade II mast cell tumours into two groups with markedly different expected survival times. They also stated that mean AgNOR scores and Ki 67 scores could be used as prognostic markers for canine mast cell tumours.

Materials and Methods

3. MATERIALS AND METHODS

3.1 General

The study was carried out in the Department of Veterinary Pathology, Veterinary College, Bangalore during 2005-2006. A total of 122 cases of cutaneous and subcutaneous tissue tumours encountered while screening a total population of 16987 dogs presented to Department of Veterinary Surgery, Veterinary College, Bangalore, Private clinics and Government Veterinary Hospitals in Bangalore formed the source of material for the present study.

Particulars of animals like breed, age and sex were recorded. In addition, the history and clinical manifestations exhibited by the animals were documented. Further, gross appearance with reference to shape, size, color, consistency, ulceration, hemorrhage and inflammatory changes of the growths were also recorded.

The study was carried out in four parts.

1. Collection and preparation of tissues for cytological and histopathological examination.
2. Characterization of the proliferation state in cutaneous and subcutaneous tissue tumours by AgNOR staining.
3. Immunohistochemical detection of Ki 67 proliferation antigen in tissue sections of cutaneous and subcutaneous tissue tumours.
4. Correlation of histological type, proliferation index and AgNOR index in canine cutaneous and subcutaneous neoplasms.

3.2 Cytology

Tissue samples for cytological examination in the form of scarping smears were collected from surgically excised neoplastic growths.

3.2.1 Methods of collection and preparation of smears

The cut surface of the tumour mass was first cleaned and blotted dry with blotting paper to make it free of blood and body fluids. Subsequently, it was firmly held with forceps and scraped with the help of scalpel. The scrapings thus obtained were made as smears on the glass slides. Air dried smears were fixed in methanol for three minutes for Giemsa staining.

3.2.2 Staining technique

For cytological studies Giemsa staining technique was adopted to stain the smears as indicated here under.

3.2.2.1 Giemsa staining

I. The smears prepared by scraping were air dried and placed on the staining rack.

ii. The smears were fixed with methanol for three minutes,

iii. Phosphate buffer with a pH of 7.2 was poured over the surface of fixed smear and four drops of concentrated Giemsa solution were added.

iv. The mixture was uniformly and thoroughly mixed by blowing with the help of rubber bulb attached to the pipette.

v. Then the smears were allowed to stain for 40 minutes.

vi. The slides were washed in tap water and air dried immediately.

vii. The stained smears were mounted with DPX before microscopic examination (Coles, 1986).

3.3 Interpretation of cytological smears

The smears prepared for cytological examination were first checked for the cellularity and adequate staining quality and were classified into discrete round cell tumours, mesenchymal and epithelial tumours based

on the predominant cell type. Further, they were differentiated as benign or malignant based on the cytologic criteria of malignancy.

3.3.1 Criteria for malignancy

The criteria for malignancy were adopted as per Dunn and Villers (1998) and Alleman and Bain (2000).

3.3.1.1 Cellular criteria

- Cellularity of specimen – Low/ high
- Type of cells present – monomorphic/polymorphic (round, round to caudate/ spindle)
- Degree of cellular pleomorphism (anisocytosis) – low/ high.
- Cellular arrangement – individual/ clusters/ sheets.

3.3.1.2 Cytoplasmic criteria

- Cell margins – regular/ irregular
- Cytoplasmsic content – vacuolation / secretary product/ granules
- Basophilia –moderate /high

3.3.1.3 Nuclear criteria

- Anisokaryosis – variable
- Nuclear to cytoplasmsic (N:C) ratio –variable /high
- Mitotic activity – low / moderate/ high with abnormal mitotic figures
- Nucleoli – indistinct/ single / multiple
- Nucleolar pleomorphism – moderate /high
- Chromatin pattern – hypochromatic / hyperchromatic /coarse /clumped /uneven margination at the nuclear membrane
- Nuclear molding – present / absent
- Multinucleation – present / absent

3.4 Histopathology

Tissue samples collected either by biopsy or excision from cutaneous and subcutaneous growths of dogs were fixed immediately in chilled 10% neutral buffered formalin. Representative samples from the growths were processed by routine paraffin embedding technique. Sections of four to five micron thickness were cut using Leitz rotary microtome with disposable blades. These sections were then stained with routine Haematoxylin and Eosin method (Luna, 1968).

The following special stains were used as and when required as per Luna (1968) to identify the type of neoplasms

- Masson's trichrome for connective tissue
- Masson Fontana for melanin pigments,
- Toluidine blue for mast cell granules
- Oil red O for fat

3.5 AgNOR Staining

Tissue sections from samples diagnosed as cutaneous and subcutaneous tissue tumours by histopathology were subjected for AgNOR staining to demonstrate agyrophilic nucleolar organizer regions in proliferating neoplastic cells.

3.5.1 Materials

- **Stock solutions**

I. Formic acid – gelatin solution

a) One per cent formic acid solution was prepared by dissolving 1 ml of 88 per cent formic acid in 99 ml of distilled water.

b) Two gram gelatin (Sigma G-6650; gelatin type B from bovine skin, 75 bloom) was dissolved in 1 per cent of formic acid solution.

c) The solution was left at room temperature till the gelatin dissolved. This solution was freshly prepared and filtered before use.

II. 50 % Silver nitrate solution

a) Fifty per cent silver nitrate solution was prepared by dissolving 25g of silver nitrate in 50 ml of water in a dark glass bottle.

- **Working solution**

  Working solution was prepared just before use by mixing 1 part of gelatin - formic acid solution with 2 parts of 50 per cent silver nitrate solution.

3.5.2 Staining procedure

- The tissue sections were deparaffinized and rehydrated to distilled water and washed in three changes of ultra pure water.
- Excess of water was drained off and slides were placed horizontally on staining rack.
- Working solution was mixed and was poured on section so as to cover the section.
- For ideal staining slides were kept in dark place for 45 minutes.
- Then the slides were washed thoroughly in three changes of ultra pure water.
- Sections were then dehydrated in graded alcohols, cleared in xlyene and cover slipped with DPX.

3.5.3 AgNOR Index

The number of clearly defined silver stained black dots in 100 neoplastic cells per specimen was counted under oil immersion objective and expressed as mean value per cell. According to the recommendations for AgNOR enumeration by Crocker *et al.,* (1989), total AgNOR's dots, both intra and extra- nucleolar were counted. Areas with necrosis pronounced inflammation and artificial damage were avoided for counting the NORs.

3.6 Immunohistochemical detection of Ki 67 proliferation antigen

Tissue sections from samples diagnosed as cutaneous and subcutaneous tissue tumours by histopathology were subjected for immunohistochemistry to demonstrate proliferation associated nuclear antigen Ki 67 using monoclonal antibody raised against Ki 67 antigen (clone MIB-1).

3.6.1 Materials

3.6.1.1 Immunochemicals

- Primary antibody: Ready to use monoclonal mouse anti–human Ki-67, clone MIB-1 (Anti-Ki-67, MIB-1, code N1633) shown to react with Ki-67 nuclear antigen (345 and 395 kD double band in immunoblotting of protein extracted from proliferating cells) associated with cell proliferation was procured from Dako Cytomation, California, USA and was used at a dose of 100µl per slide as prescribed. It was stored at 2^0 to 8^0 C until used.

- Anti mice IgG raised in goat conjugated with HRPO (Horse Raddish Peroxidase) was obtained from Dako Cytomation, USA and was used at a dilution of 1:100 as prescribed. It was stored at 2^0 to 8^0 C until end.

3.6.1.2 Section adhesive 3-aminopropyltriethoxy-silane (APES). Sigma Chemicals, USA.

3.6.1.3 Hydrogen peroxide (H_2O_2) in methanol (3%)

Three per cent H_2O_2 in methanol was prepared by adding one ml of 30 per cent H_2O_2 in 9 ml of methanol (Sigma Chemicals, USA).

3.6.1.4 0.01M Citrate buffer (pH - 6)

I. Five hundred ml of 0.1M citric acid solution was prepared by dissolving 10.51g of citric acid monohydrate in 500 ml of distilled water.

II. Five hundred ml of 0.1M sodium citrate solution was prepared by dissolving 15.71g of sodium citrate in 500ml of distilled water.

III. Five hundred ml of 0.01M citrate buffer was prepared just before use by adding 9 ml of 0.1M citric acid solution and 41 ml of 0.1M sodium citrate solution to 450 ml of distilled water. The pH was adjusted to 6.0 with 1N NaOH just before use.

3.6.1.5 Substrate

3,3-diamine benzidine tetrahydrochloride substrate was prepared freshly at the time of use by addition of 1 mg of 3, 3-diamine benzidine tetrahydrochloride in 1 ml of 0.01 M PBS to which 12 µl of 3 per cent H_2O_2 was added.

3.6.1.6 0.01 M Phosphate buffer saline (pH – 7.4)

3.6.1.7 Harris haematoxylin for nuclear staining (Luna, 1968)

3.6.2 Preparation of Organosilane (APES) treated slides for IHC

- The slides were washed thoroughly in soap water, rinsed in tap water and finally rinsed in distilled water and dried completely.
- A 2 per cent solution of 3-aminopropyltriethoxy-silane (APES) in acetone in a dry staining dish was prepared.

- The slides were immersed in the APES solution for 5 - 15 minutes.
- The slides were rinsed in acetone and then rinsed in two changes of distilled water.
- The slides were allowed to dry at 37^{0}C for two hours and then stored at room temperature until used.

3.6.3 Method

- Tissue sections were mounted on 3-aminopropyltriethoxy-silane (APES) coated slides and dried at 37^{0}C for three hours.
- The tissue sections were deparaffinized and rehydrated.
- Endogenous peroxidase was blocked by covering the whole section with 3 per cent of H_2O_2 in methanol (100µl). Incubated at room temperature for fifteen minutes. Washed thrice in 0.01M PBS.
- Heat induced epitope retrieval (HIER) of tissue section was carried out by immersing tissue section in a cooker containing citrate buffer, pH 6.0 and was cooked for 2 minutes after maximum pressure was attained. Sections were allowed to cool down to room temperature for approximately 20 to 30 minutes.
- Addition of primary antibody: - Mouse anti–human Ki-67, clone MIB-1, ready to use was added to cover the section. Subsequently the section was incubated at 37^{o}C in humidified chamber for one hour and washed with PBS as mentioned earlier.
- Addition of secondary antibody (Anti goat IgG conjugated with HRPO):- The whole section was covered with secondary antibody and incubated at 37^{o}C in humidified chamber for one hour. After incubation, it was washed with PBS as mentioned earlier.
- Addition of substrate:- Freshly prepared 3, 3-diamine benzidine tetrahydrochloride (DAB) with 3 per cent H_2O_2 was poured to cover the sections. Incubated for five to ten minutes or until desired

color developed at room temperature. The sections were washed again with PBS as mentioned earlier.

- Nuclear staining with Harris hematoxylin for three minutes was done. The sections were washed in distilled water, dehydrated, cleared, and cover slipped with DPX.
- Lymphnode (germinal centre) was used as positive control for standardization of the technique.

3.6.4 Ki 67 proliferation index

The positive reactivity for Ki 67 proliferation antigen was observed as dark brown coloured granular material restricted to the nucleus of proliferating cell.

Ki 67 expression was mainly assessed at the periphery of the tumour where cell proliferation was likely to be higher than in other tumour areas. To determine Ki 67 index per tumour section, approximately 1000 neoplastic cells were counted in 10 representative fields of vision at high magnification. The number of positive cells per 1000 cells was expressed as percentage.

3.7 Statistical analysis

Statistical analysis was performed using the statistical software Graph pad Prism, version 4.01. Mean Values and standard error of mean were calculated and all values were expressed as mean ± standard error. The data were analyzed by using unpaired *t* test and correlation analysis where ever appropriate.

Results

4. RESULTS

A total of 122 neoplastic growths from cutaneous and subcutaneous tissue of dogs were studied during the present investigation over a period of one year. Results pertaining to frequency of occurrence of cutaneous and subcutaneous tissue tumours in relation to population, age, breed, site, gross appearance, cytological appearance, histopathological findings, AgNOR staining and immunoreactivity to Ki 67 proliferation antigen have been presented as here under.

A total of 16987 dogs presented to Veterinary College Hospital, Bangalore, Private and Government Veterinary Hospitals and Animal Shelters were examined for cutaneous and subcutaneous tissue tumours from October 2005 to September 2006. Out of 16987 dogs, 122 were found to be affected with cutaneous and subcutaneous tissue tumours accounting for 0.72 per cent of incidence in relation to general population.

The age of susceptibility of dogs to cutaneous and subcutaneous tissue tumours in the present study varied from 5 months to 18 years with an average age of 7.73 years. The highest incidence of 36.88 per cent of cutaneous and subcutaneous tissue tumours was observed in dogs aged between 6 to 8 years, followed by 20.49 per cent in 8 to 10 years, 13.11 percent in 4 to 6 years and 10.65 per cent in 10-12 years. The incidence was low in dogs aged less than 4 years and above 14 years (Table 1).

Out of 122 dogs, the highest frequency of occurrence of cutaneous and subcutaneous tissue tumours was observed in nondescript (34.42%) followed by German Shepherd (16.41), Labrador Retriever (10.65%), Pomeranian (9.00%) and Boxer (5.73). The other

breeds which were affected at a lower grade were Cocker Spaniel, Great Dane, Golden Retriever, Beagle, Doberman, Lhasa Apso, Dachshund, Pug, Dalmatian and Mudhol (Table 2).

In the present investigation the cutaneous and subcutaneous tissue tumours were recorded in 72.95% of male dogs and 27.05% in female dogs.

4.1 Classification of cutaneous and subcutaneous tissue tumours.

The classification of 122 cutaneous and subcutaneous tissue tumours has been presented in Table 3.

The tumours were classified based on the predominant cell type and histological characteristics as (a) round cell tumours, (b) epithelial tumours-malignant and benign types and (c) mesenchymal tumours-malignant and benign types.

The round cell tumours were observed in 23 cases and included mast cell tumour (15), cutaneous histiocytoma (1) and malignant melanoma (7).

The epithelial tumours were observed in 71 cases which comprised 44 cases of malignant tumours and 27 cases of benign tumours. Malignant epithelial tumours included squamous cell carcinoma (17), basal cell carcinoma (15), hepatoid gland carcinoma (6), sebaceous gland carcinoma (3) and sweat gland carcinoma (3). The benign epithelial tumours encountered were squamous papilloma (5), fibropapilloma (4), trichoepithelioma (2), hepatoid gland adenoma (11), sebaceous gland adenoma (4) and sweat gland adenoma (1).

In 28 cases, mesenchymal tumours were observed which comprised 16 cases of malignant tumours and 12 cases of benign tumours. The various malignant mesenchymal tumour types

encountered were fibrosarcoma (10), hemangiosarcoma (5) and leiomyosarcoma (1)

The benign mesenchymal tumours included fibroma (2), hemangioma (6), lipoma (3) and myxoma (1).

In the present study malignant neoplasms (83) predominated over benign type (39). Among all the tumours, epithelial tumours predominated with 71 cases in which 44 were malignant type and 27 were benign type followed by mesenchymal tumours (28) and round cell tumours (23). Among the round cell tumours mast cell tumour was the most common type. In the group of epithelial malignant type squamous cell carcinoma was the most presented type followed by basal cell tumour and others and among benign types it was hepatoid gland adenoma. In the group of malignant mesenchymal tumours, fibrosarcoma was the most common type and among benign types it was hemangioma (Table 3).

4.2 Gross, cytological and histopathological details of cutaneous and subcutaneous tissue tumours.

4.2.1 Round cell tumours

4.2.1.1 Mast cell tumour: Fifteen out of 122 neoplasms recorded in this study were mast cell tumours.

The tumour occurred at various locations such as leg, head, neck, back, abdomen and scrotum.

Grossly, the size of the tumours varied from 2.0 to 8.0 cm at their greatest diameter. The growths were raised, firm and well circumscribed. They occurred as either single (13 cases) or multiple growths (2 cases) and the skin covering the tumour mass appeared smooth and alopecic. Ulceration was observed in three cases. In one

case the cut surface was dirty brown in colour and honey comb like structures surrounding the creamy white coloured nodules were observed where as in rest of the cases the cut surface of the tumours was creamy to yellowish coloured (Plate 1).

Cytologically, the smears revealed high cellularity. The cells were pleomorphic with round or oval shaped nuclei containing multiple clumps of chromatin and indistinct nucleolus. The nuclei were weakly stained in Giemsa. The cells showed variable nuclear to cytoplasmic ratio and the cytoplasm of a few cells contained variable number of pinkish coloured distinct granules. The eosinophilic granules were also observed scattered throughout the smear. In addition, a few eosinophils were also observed among neoplastic cells (Plate 2).

Microscopically, the occurrence of mast cell tumour was observed mainly in the dermis with extension in to subcutaneous tissue and underlying musculatures. The cells were compactly as well as loosely arranged which were spherical to oval shaped with pale eosinophilic granular cytoplasm. The nuclei were roughly spherical to oval in shape and eccentrically placed with coarse chromatin. In addition, areas of collagenolysis, oedema, remnants of atropied muscle fibres and infiltration of varying number of eosinophils were also observed (Plate 3). The granules of neoplastic cells varied in number according to the degree of differentiation with large number of cytoplasmic granules in the well differentiated and occasional cells with granules in poorly differentiated tumours. The granules appeared purplish in toluidine blue stained sections (Plate 4).

4.2.1.2 Histiocytoma

A case of cutaneous histiocytoma was recorded out of 122 neoplasms. The tumour mass was located at the opening of right ear canal.

Grossly, the tumour mass measured about 2.0 cm at its greater diameter, which was well circumscribed, smooth surfaced, alopecic and pinkish red in colour (Plate 5).

Cytologically, the smears revealed a high cellularity. The cells were round or oval shaped and the nuclei were eccentrically placed which showed minimal anisokaryosis. The chromatin was fine and dispersed and the nucleoli were indistinct. The cytoplasm was lightly basophilic.

Histopathologically, the section of cutaneous histiocytoma revealed a compact arrangement of solid sheets of round neoplastic cells consisting of nuclei with fine chromatin, indistinct nucleoli and lightly eosinophilic cytoplasm. The fibrovascular stroma was sparse and found separating sheets of proliferating neoplastic cells. Numerous mitotic figures were also observed, along with infiltration of inflammatory cells (Plate 6).

4.2.1.3 Melanoma

Melanoma was encountered in seven out of 122 neoplasms. The various sites of occurrence of melanoma were eyelid (3), digit of fore limb (1), tail region (1), groin (1) and perineal region (1).

Grossly, the size of the melanomas ranged from 0.7 to 4.0 cm at their greater diameter. They were firm and moderately hard in consistency. The growths were round, ovoid or cauliflower like. Both surface and cut surface of growths were brownish black to jet black in colour. One of the seven cases showed multiple lobulated growths of varying sizes with a tendency to bleed from the ulcerated surface (Plate 7).

Cytologically, the smears contained a large number of cells arranged individually or in clusters. The cells showed a high degree of anaplasia with pleomorphism and anisokaryosis. The nuclei revealed coarse chromatin and prominent nucleoli. The nucleus to cytoplasmic ratio was high and occasional cells revealed presence of varying number of brownish to blackish coloured granules which varied in their size. A few mitotic figures and binucleate cells were also observed (Plate 8).

Microscopically, all the melanomas were confirmed as malignant with varying grades of malignancy and were characterized by high cellularity, high cellular anaplasia and numerous mitotic figures. All cases were diagnosed as melanotic melanomas as the cytoplasm showed brown to black coloured melanin pigment, at times obscuring the nuclear details. The histological diagnosis in all the seven cases was confirmed by silver staining (Masson Fontana), which revealed granular black coloured melanin pigments in proliferating neoplastic cells.

The seven cases of melonomas were further sub-classified based on the predominant cellular type as epitheloid (2 cases), spindle and dendritic (1 case) and mixed type (4 cases) histopathologically.

Epitheloid type of malignant melanoma was characterized by round cells with vesicular nuclei and the growth appeared as a carcinoma. There were cellular pleomorphism and numerous mitotic figures. The tumour cells were closely packed having large nuclei with prominent nucleoli and varying amount of chromatin. Occasional cells contained scanty amount of brownish black cytoplasmic granular pigments (Plate 9).

Malignant melanoma which was located over the dorsal surface of skin of tail showed spindle and dendritc cellular pattern. It was

characterized by densely packed, heavily pigmented spindle or dendritic cells arranged in band like pattern with high cellular pleomorphism and presence of occasional giant cells (Plate 10).

Mixed types of malignant melanomas were characterized by presence of both epitheloid and spindle shaped cells. The cells contained moderate to high amount of cytoplasmic pigment granules. Cellular anaplasia and pleomorphism were also observed (Plate 12). In all the cases the melanin pigments stained brownish black in H&E stained section. In silver stained sections, the cytoplasmic melanin pigments stained black in colour (Plate 13).

4.2.2 Epithelial tumours

4.2.2.1 Malignant epithelial tumours

4.2.2.1.1 Squamous cell carcinoma

Seventeen out of 122 neoplasms recorded in this study were squamous cell carcinoma. They were located on head (2), neck (3), forelimb (3), hind limb (3), abdomen (2), back, inguinal, groin and perianal regions (1 each).

Grossly, the size of the tumour growths ranged from 0.5 to 5.0 cm in diameter and appeared as lobulated pinkish growths. In three cases the growths were irregular in shape with raised multiple small growths. Nine cases showed superficial necrosis and ulcerations. Two cases were cystic in nature (Plate 14).

Squamous cell carcinoma cytologically presented a large number of malignant squamous cells occurring either individually or in clusters along with keratinized anucleated cells. The cells were pleomorphic, round to caudate in shape with prominent anisokaryosis. The nuclei varied from small and pyknotic to large, round and immature type with prominent nucleoli in accordance with degree of differentiation. The

nuclear to cytoplasmic ratio was variable and also revealed binucleation and multinucleation. The cytoplasm in nonkeratinized cells was moderately to deeply basophilic, whereas in keratinized cells it was bluish green in Giemsa stained smears. Numerous polymorphonuclear cells were also observed (Plate 15). In spindle cell type of squamous cell carcinoma a large number of spindle shaped cells with variable N:C ratio was observed cytologically. In addition, extracellular amorphous pinkish coloured keratin material was also observed.

Microscopically, both well differentiated (12) and moderately to poorly differentiated (5) types were observed. Well differentiated squamous cell carcinomas revealed proliferating neoplastic squamous epithelial cells arranged in compact cords or nests with keratinized centers, often in the form of well lamellated keratin pearls. Prominent individual cell keratinization was also observed (Plate 16). The degree of cellular pleomorphism and the mitotic activity varied from moderate to high. Prominent intercellular bridges and brick red coloured lamellated keratin pearls were demonstrable in Masson's trichrome stained sections. The amount of connective tissue stroma varied from minimal to high in these cases. Infiltrated neoplastic cells in thick connective tissue stroma were also observed (Plate 19). Focal to multifocal areas of necrosis and inflammatory changes with lymphoid cell infiltration, apoptotic bodies with fragmented crescent shaped nuclei were also noticed (Plate 21).

Squamous cell carcinoma that occurred in the abdominal region showed cholesterol crystals in addition to other features (Plate 18).

Five cases of squamous cell carcinomas with moderate to poor differentiation, revealed minimal or absence of keratinization and highly proliferative cell nests consisting of pleomorphic cells showing cellular anaplasia and high mitotic activity (Plate 17).

An undifferentiated squamous cell carcinoma spindle cell type that originated from the left oral commissure was characterized by elongated spindle shaped cells with oval nucleus and vacuolated cytoplasm. The neoplastic cells were arranged in palisade and occasionally in whorled pattern. The degree of cellular anaplasia, pleomorphism and mitotic activity was moderate to high. In addition, focal areas of necrosis with lymphoid cell infiltration were also noticed (Plate 22).

4.2.2.1.2 Basal cell carcinoma (Trichoblastoma)

Fifteen out of 122 neoplasms recorded in this study were basal cell carcinoma and were found located in head (4), neck (3), limbs (5), thorax (2) and tail region (1).

Grossly, the tumours were well demarcated, firm, round to oval shaped and firmly attached to the skin. The over lying epithelium was smooth and partially haired. Ulceration was seen in two cases. The size varied between 1.5 to 4.0 cm at its highest diameter (Plate 23).

Cytologically, the smears revealed moderate cellularity and the cells were arranged in clusters, rows or ribbons. The cells were small, uniform sized, round with monomorphic nuclei, high nuclear to cytoplasmic ratios and basophilic cytoplasm (Plate 24).

Microscopically, the section of basal cell carcinoma showed different histological patterns such as garland or ribbon (4), trabecular (4), solid (5), medusoid (1) and rosette (1) types. In ribbon pattern the cells occurred as long cords of one or more layers cells which were branching and anastamosing type. The cells were palisadally arranged and consisted of prominent nuclei with little cytoplasm. The amount of stroma varied which occurred between the cords. The number of mitotic figures also varied between the locations as well as the

tumours (Plate 25). In medusoid type the cords of cells streamed outward from the central aggregation of cells (Plate 28). In trabecular type, multiple lobules of neoplastic cells surrounded by thin connective tissue stroma were observed (Plate 26). The cells at the periphery of the lobules revealed typical palisade arrangement. In solid pattern compact arrangement of neoplastic cells in the form of large cords was noticed (Plate 27). In rosette type islands of neoplastic cells with nuclei arranged in the periphery giving flower like appearance (Plate 29).

4.2.2.1.3 Hepatoid gland carcinoma

Six cases of hepatoid gland adenocarcinoma were recorded out of 122 neoplasms and were found located at perianal region including ventral aspect of base of tail (5) and left lateral abdomen (1).

Grossly, the size of tumour growths ranged from 2.0 to 4.5 cm in diameter. The growths were spherical to irregular in shape, single or multiple in numbers, lobulated, ulcerated with moderately firm consistency and pale brown or pink coloured cut surface. Areas of hemorrhages were also observed (Plate 30).

The cytological smears revealed moderate number of cells showing pleomorphism. Occasional cells resembled hepatocytes and were round, ovoid or polygonal in shape. The nucleus was spherical to oval shaped, eccentrically placed with coarse chromatin and prominent nucleolus. The cytoplasm was faint to intensely basophilic in Giemsa stained smears. Many binucleated cells were also observed. The nuclear to cytoplasmic ratio was variable. In addition a large number of small sized oval shaped cells with condensed nuclei and scanty cytoplasm which resembled reserve cells of hepatoid gland were also observed (Plate 31).

Microscopically, the tumour was characterized by compactly arranged sheets of highly cellular masses consisting of occasional polygonal to spindle shaped cells, interspersed with a large number of small sized undifferentiated cells. Also revealed connective tissue stroma separating islands of proliferating masses comprising numerous small blood vessels and inflammatory cells. The proliferating cells were pleomorphic with nuclear basophilia and varying amount of eosinophilic cytoplasm. Presence of small amount of keratin like structure at the centre of islands of glandular cells was also observed (Plate 32).

4.2.2.1.4 Sebaceous gland carcinoma

Three cases of sebaceous gland adenocarcinoma were recorded out of 122 neoplasms which were located at ventral abdomen (2) and lateral thorax (1) region.

Macroscopically, the size of sebaceous gland carcinoma varied between 1.0 and 5.0 cm. The tumours were smooth surfaced and varied in shape. The tumour growths were round or oval shaped, lobulated or irregular cystic like. The cut surface of tumour was oily, pale yellow to whitish in colour (Plate 34).

Cytologically, sebaceous carcinomas were characterized by pleomorphic cells displaying malignant nuclear features such as anisokaryosis, prominent nucleoli and frequent mitotic figures along with vacuolated cytoplasm.

Histopathologically, the sebaceous carcinoma showed lobules of varying sizes, surrounded by connective tissue stroma. The lobules contained compactly arranged neoplastic cells with occasional differentiated sebaceous glandular epithelial cells with intracytoplasmic lipid vacuoles. Some of the nodules revealed central necrosis with infiltration of inflammatory cells. In addition, invasion of

neoplastic cells to the connective tissue stroma was also observed (Plates 35& 36).

4.2.2.1.5 Sweat gland adenocarcinoma

Sweat gland adenocarcinoma was encountered in three out of 122 neoplasms which were observed at lateral lumbar region (1), gluteal region (1) and in the tail (1).

Grossly, the size of the tumour mass was 4.0 to 5.0 in diameter. They were nodular and dome shaped. One of the tumours was cystic and contained whitish small nodules. In another case tumour mass was ulcerated with lobulated appearance in the cut surface (Plate 37).

Cytology of apocrine sweat gland adenocarcinoma included presence of large number of neoplastic epithelial cells in clusters of basophilic cells with cellular and nuclear pleomorphism, a high nuclear to cytoplasmic ratio and vacuolated cytoplasm. Spindle shaped fibroblasts with elongated nuclei were also observed (Plate 38).

Histologically, sweat gland adenocarcinoma was characterized by papillary projections lined by double layer of neoplastic cells. Proliferating neoplastic cells were arranged palisadally and were cuboidal with eosinophilic cytoplasm. The nuclei were large vesicular and spherical or oval with one or two nucleoli. Eosinophilic material was observed with in the cystic lumen. Stromal connective tissue was moderate in amount and there was invasion of stroma by the neoplastic cells (Plate 39).

4.2.2.2 Benign epithelial tumours

4.2.2.2.1 Squamous papilloma

Five cases of squamous papilloma were encountered out of 122 neoplasms all of which were located on head.

Grossly, the growths appeared papillary with finger like projections. The surface was rough and thick and measured 0.5 to 1.0 cm in diameter (Plate 40).

Cytologically, the squamous papilloma smears revealed moderate cellularity and consisted of keratinocytes which stained deeply basophilic with out nuclei and matured epithelial cells which were round or oval shaped with benign appearing nuclei (Plate 41).

Histopathologically, sections of papilloma revealed long papillary projections covered by thick eosinophilic keratin, over a core of connective tissue stroma and lined by multiple layers of proliferating epithelial cells. The cells were in the various stages of development from keratinized cells to immature cells (Plate 42).

4.2.2.2.2 Fibropapilloma

Four cases of fibropapilloma were encountered out of 122 neoplasms which were observed in fore limb (1), hind limb (1), ventral thorax (1), and below the eye in head region (1).

Grossly, fibropapillomas occurred as fleshy, elevated, irregular and nodular growths covered by alopecic smooth skin. The cut surface was whitish to pinkish coloured and measured about 1.0 to 2.5 cm in diameter (Plate 43).

Cytologically, the fibropapillomas revealed a low cellularity with fibrocytes and fibroblasts having elongated nucleus and few squamous epithelial cells with benign characteristics (Plate 44).

Histologically, fibropapillomas revealed abundant proliferating fibrous tissue covered by epithelium of various thicknesses with retepegs of the epithelium extending into the fibrous tissue (Plate 45).

4.2.2.2.3 Trichoepithelioma

Two cases of trichoepitheliomas were recorded out of 122 neoplasms which were observed at tail (1) and thigh region (1).

Grossly, trichoepitheliomas appeared as hard, firm, raised, well circumscribed, masses measuring 2.0 to 3.0 cm in diameter.

Cytologically, trichoepithelioma was consisted of keratinocytes, keratin debris and moderate number of epithelial cells resembling basal cells.

Histopathologically, trichoepitheliomas revealed proliferating neoplastic cells which were compactly arranged in multiple concentric layers around the cornified hair follicle which consisted of keratin material and melanin pigments. The keratinization was abrupt and the amount of keratinization varied between the groups of proliferating cells (Plate 46).

4.2.2.2.4 Hepatoid gland adenoma

Hepatoid gland adenoma was encountered in 11 cases out of 122 neoplasms which were located at perianal region (6), base of tail (4) and thigh region (1).

Grossly, the size of the tumour mass varied between 1.0 to 5.0 cm diameters. The growths were roughly spherical, oval or irregular with or with out ulcerations. The skin over nonulcerated tumours was thin and alopecic and the cut surface appeared pale brown in colour (Plate 47).

Cytologically, the smears contained a large number of mature round hepatoid cells occurring in clusters or individually which resembled hepatocytes in morphology. The cells were round or polygonal with single or double nucleus placed eccentrically. In

addition small sized cells which resembled reserve cells with a high nuclear to cytoplasmic ratio were also observed occasionally (Plate 48).

Microscopically, hepatoid gland adenoma revealed hepatoid cells arranged as cords or islands surrounded by anastamosing connective tissue stroma. The cells were polyhedral with large ovoid vesicular normochromatic nuclei with a central small nucleolus and abundant eosinophilic granular cytoplasm. The cell borders were distinct. In addition basaloid reserve cells in a single layer were found arranged at periphery of each lobule, which possessed small hyperchromatic nuclei and scanty cytoplasm. In some cases secondary vascularization characterized by vascular space with RBCs surrounded by a line of reserve cells and keratin pearl formation were also observed (Plate 49).

4.2.2.2.5 Sebaceous gland adenoma

Four cases of sebaceous gland adenocarcinoma were recorded out of 122 neoplasms which were found at various locations such as ear (2) and eye lid (2).

Grossly, the size of the tumour growth varied from 0.5 to 3.0 cm in diameter. The tumours were smooth cauliflower like raised lesions with alopecic skin covering. The cut surface of the tumour mass was oily and pale yellow to white in colour (Plate 50).

Cytologically, sebaceous adenoma revealed mature sebocytes in clusters or as individual cells characterized by pale foamy cytoplasm and small dense centrally placed nucleus.

Microscopically, sebaceous adenomas revealed multiple lobules consisting of mature sebocytes separated by connective tissue stroma. At the periphery of the lobule a rim of small basophilic reserve cells consisting of hyperchromatic nuclei and scanty cytoplasm with little or

no pleomorphism was observed. The sebocytes contained abundant pale eosinophilic, vacuolated cytoplasm and a small centrally placed dense nucleus (Plate 51).

4.2.2.2.6 Sweat gland adenoma

A case of sweat gland adenoma was recorded out of 122 neoplasms which was located at dorsal thoracolumbar region.

Grossly, the size of the tumour mass was 3.0 cm in diameter, which was soft, raised above the surrounding skin with alopecic pigmented skin covering.

Cytology of sweat gland adenoma included presence of large number of almost uniform sized basophilic neoplastic cells with a high nuclear to cytoplasmic ratio.

Histopathologically, the sections of sweat gland adenoma revealed glandular structures with narrow cleft like lumina of variable sizes consisting of one or two layers of lining neoplastic cells. The proliferating neoplastic cells were cylindrical, well defined and had dense eosinophilic cytoplasm. The nucleus was vesicular and situated at the base of cylindrical neoplastic cells (Plate 52).

4.2.3 Mesenchymal tumours

4.2.3.1 Malignant mesenchymal tumours

4.2.3.1.1 Fibrosarcoma: Out of 122 neoplasms 10 were fibrosarcomas in the present study.

The various locations at which the tumours occurred included fore limb around elbow joint (3), hind limb around stifle joint (5), abdomen (1) and chest (1).

Grossly, fibrosarcomas were firm and hard in consistency and measured 3.0 to 10.0 cm at their highest diameter. The shape varied from round to discoid in eight cases and occurred as flat diffused mass in two cases (Plate 53).

Out of ten cases, four were superficially ulcerated with oozing of gelatinous fluid. In all the cases the growths were hard to cut and had grayish white coloured cut surface.

The cytological smears showed a moderate number of pleomorphic plumpy cells occurring either individually or in aggregates in the smears. The cells were round to oval shaped with cytoplasmic extensions from both the ends. The nuclei showed marked anisokaryosis and contained coarse chromatin with multiple aggregations (Plate 54). Occasional binucleated and multinucleated cells were also observed. Pinkish collagenous material in the intercellular spaces was an additional feature.

Microscopically, the fibrosarcomas revealed interlacing bundles of spindle to stellate shaped cells with elongated plumpy nuclei. Cytoplasmic processes were observed trailing away from the nucleus. High degree of cellular anaplasia, pleomorphism and infiltration of plasma cells with varying degree of mitotic activity were also noticed (Plate 55). In Massons trichrome stained sections the collagen appeared blue in colour and varied in its amount with respect to degree of differentiation (Plate 56).

4.2.3.1.2 Hemangiosarcoma

Out of 122 neoplastic growths 5 were hemangiosarcomas. The sites of occurrence were base of ear (1), abdomen (1), inter digital space of fore limb (1), lateral thorax (1) and hind limb (1).

Grossly, hemangiosarcomas occurred as solitary (3) or multiple (2) growths in dermis and subcutaneous tissue. They measured about 2.0 to 6.0 cm at their greater diameter. The growths were soft to firm in consistency, red or black in colour with oozing of blood from the cut surface. Larger growths revealed ulceration (Plate 57).

Cytologically, hemangiosarcomas showed low cellularity with numerous blood cells. The cells were pleomorphic and were spindle to stellate shaped. Cytoplasm was basophilic with distinct cell borders and occasional punctate vacuolations. Cells showed high nuclear to cytoplasmic ratio, oval nuclei with coarse chromatin and prominent multiple nucleoli (Plate 58).

Microscopically, hemangiosarcomas were characterized by compactly arranged neoplastic cells which ranged from spindle to polygonal in shape with occasional vascular spaces consisting of blood cells. The nuclei were hyperchromatic and mitotic cells were frequent. In some cases, areas of haemorrhages were observed adjacent to tumour mass. Invasion of stromal tissue with proliferating neoplastic cells was an additional feature (Plate 59).

4.2.3.1.3 Leiomyosarcoma

A case of leiomyosarcoma was recorded out of 122 tumours screened and was located in ventral thorax involving the dermis.

Grossly, the tumour mass measured about 3.0 cm at its greater diameter. Tumour mass was nodular, roughly spherical in shape and firm in consistency.

Cytologically, leiomyosarcoma comprised moderate number of pleomorphic cells that occurred individually or in small aggregates. The cell were round, oval, spindle shaped with moderate degree of

anisokaryosis and anisocytosis. The nuclei showed dispersed chromatin clumps.

Histopathologically, leiomyosarcoma was composed of bundles of spindle shaped cells with elongated nuclei, granular chromatin and abundant eosinophilic cytoplasm forming interlacing fascicular pattern of arrangement separated by thin collagenous stroma. In less well differentiated areas densely packed, large number of cells with less cytoplasm and round to elongated nuclei consisting of granular chromatin were observed. In addition cells under mitosis and apoptosis were also observed (Plate 60).

4.2.3.2 Benign mesenchymal tumours

4.2.3.2.1 Fibroma

Two cases of fibroma were recorded out of 122 neoplasms. The sites of occurrence were hind limb (1) and fore limb (1).

Grossly, the fibromas were well circumscribed, round to oval in shape, firm in consistency with grayish white coloured cut surface in the deep dermal region, measuring about 4.0 to 5.0 cm in diameter.

Cytologically, fibromas comprised spindle shaped cells with elongated nuclei. The cytoplasm was found trailing from both the ends which was lightly basophilic. In addition, eosinophilic material indicative of collagen was also observed.

Microscopically, fibromas revealed matured uniform fibrocytes arranged in wavy interwoven fascicles and whorls with abundant amount of collagen. The nuclei were elongated with dispersed chromatin (Plate 62).

4.2.3.2.2 Hemangioma

Hemangioma was encountered in six out of 122 neoplasms. The sites of occurrence were ventral thorax (1), lateral thorax (1), ventral abdomen (1), tail region (2) and hip region (1).

Hemangiomas were grossly well demarcated and encapsulated. They measured about 2.5 to 5.0 cm in diameter. The cut surface appeared bright red to dark brown in colour and revealed honeycomb structures filled with blood (Plate 63).

Cytologically, the smears showed low cellularity with numerous blood cells in the background. The neoplastic cells were elongated with oval nuclei and basophilic cytoplasm (Plate 64).

Microscopically, cavernous type of hemangioma was observed in all six cases which were characterized by variable sized vascular spaces lined by single layer of endothelial cells and filled with RBCs (Plate 65). The vascular spaces were separated by fibrous connective tissue stroma. In addition several vascular spaces showed microthrombi filling up the vascular space, composed of eosinophilic fibrinous material (Plate 66).

4.2.3.2.3 Lipoma

Three cases of lipoma were recorded out of 122 neoplasms. The location of occurrence was thigh region (2) and carpal joint region (1).

Grossly, lipomas were soft palpable and movable masses measuring 3.0 to 7.0 cm at their highest diameter with oily, whitish yellow coloured cut surface.

Cytologically, the smears revealed adipocytes with abundant clear cytoplasm and a small condensed basophilic nucleus placed

eccentrically. The cells were well appreciated in Oil O Red stained smears in which the fat appeared red in colour (Plates 67 and 68).

Microscopically, the lipomas were characterized by presence of matured, almost uniform sized fat cells with flattened eccentrically placed nuclei. Bands of dense connective tissue were also observed between the clusters of cells (Plate 69).

4.2.3.2.4 Myxoma

A case of myxoma was recorded out of 122 neoplasms which occurred in thoracic region.

Macroscopically, the tumour appeared as raised mass with soft fluctuant texture measuring about 4.0 cm in diameter. The cut surface was grayish white in colour and showed oozing of clear mucoid fluid.

Cytologically, the smear revealed low cellularity. The cells were fusiform to stellate shaped with granular eosinophilic material in the background.

Histopathologically, myxoma was composed of proliferation of almost uniform sized stellate to spindle shaped cells which were loosely arranged in abundant myxoid matrix. The cellularity was low (Plate 71).

4.3 AgNOR Staining

In the present study, 122 cases of cutaneous and subcutaneous tissue tumours were subjected for AgNOR silver staining to characterize the proliferation fraction of the tumours. The NORs in stained sections appeared as dark brown or black coloured dots or specks distributed within nucleolus or dispersed in the nucleoplasm. Based on morphological and distribution pattern with in the nucleus, NORs were classified into 3 types. Round and solitary NORs, varying

in their size, found any were in the nucleus were termed type I; NORs restricted to nucleolus with dispersion of two or more NORs within nucleolus contributing to an enlarged and irregular morphological appearance were considered as type II and those NORs which were dispersed as small dots throughout the nucleoplasm as type III. The type I NORs were observed in the resting cells in the tumours as well as in small lymphocytes. Type II was observed in proliferating cells irrespective of being neoplastic or hyperplasic and type III, in highly immature malignant cells. The details regarding AgNOR count are given in Tables 4 and 5.

The AgNOR count per cell in the present investigation for all the tumours ranged from 2.05 to 6.10 per nucleus. Malignant tumours had an average of 4.21±0.08 AgNOR per cell while benign tumours had a mean of 2.71±0.08 AgNOR per cell (Table 5).

Statistical analysis of AgNOR count per cell in cutaneous and subcutaneous tissue tumours in the present study using Student's *t*-test revealed statistically significant ($P<0.05$) difference in the average number of AgNOR's per cell between malignant and benign tumours.

Among all benign cutaneous and subcutaneous tissue tumours, irrespective of tissue of origin, squamous papilloma had the highest AgNOR count per cell of 3.76±0.09 followed by trichoepithelioma, sebaceous adenoma, hepatoid gland adenoma, sweat gland adenoma, fibropapilloma, hemangioma, fibroma, lipoma, and myxoma with mean AgNOR counts of 3.37±0.12, 2.75±0.10, 2.62±0.07, 2.53, 2.40±0.03, 2.40±0.11, 2.33±0.07, 2.28 and 2.27 respectively (Table 4).

Among all the cases of malignant tumours irrespective of tissue of origin subjected for AgNOR staining, highest mean AgNOR count was observed in histiocytoma (5.60) followed by squamous cell

carcinoma (5.36±0.12), melanoma (4.72±0.13), sebaceous carcinoma (4.64±0.07), hepatoid gland carcinoma (4.44±0.15), sweat gland carcinoma (4.23±0.10), mast cell tumour (3.78±0.07), hemangiosarcoma (3.73±0.07), fibrosarcoma (3.62±0.12) and leiomyosarcoma (3.42).

Among all the round cell tumours, the highest mean AgNOR ount per cell was observed in histiocytoma (5.60) followed by malignant melanoma (4.72±0.13) and mast cell tumour (3.78±0.07)

In the group of malignant epithelial tumours squamous cell carcinoma had highest mean AgNOR count per cell (5.36±0.12) followed by sebaceous gland carcinoma (4.64±0.07), hepatiod gland carcinoma (4.44±0.15), sweat gland carcinoma (4.23±0.10) and basal cell tumour (3.45±0.04).

Among the malignant mesenchymal tumours the mean AgNOR count per cell was highest in hemangiosarcoma (3.73±0.07) followed by fibrosarcoma (3.62±0.12) and leiomyosarcoma (3.42).

Among benign epithelial tumours the mean AgNOR count per cell was highest in squamous papilloma (3.76±0.09) followed by trichoepithelioma (3.37±0.12), sebaceous adenoma (2.75±0.10), hepatoid gland adenoma (2.62±0.07), sweat gland adenoma (2.53) and fibropapilloma (2.40±0.05).

In the group of benign mesenchymal tumours the mean AgNOR count per cell was highest in hemangioma (2.40±0.11) followed by fibroma, lipoma, myxoma, with a count of 2.33±0.07, 2.28±0.13 and 2.27 respectively (Table 4).

The type of NOR distribution in benign neoplasms was predominantly type I and type II in most of the types except in

squamous papilloma and trichoepithelioma in which the cells also revealed type III NOR distribution in addition (Plates 85 to 91).

Among the malignant neoplasms the type of NOR distribution was mainly type II and type III along with occasional cells showing type I NORs.

In squamous cell carcinoma, the central area of cell nests revealed type I NORs characterized by presence of one centrally placed relatively large nucleolus with large NOR, while peripherally placed immature proliferating cells showed type II and III NORs. In spindle type of squamous cell carcinoma the spindle shaped cells with multiple AgNOR of type III predominated over cells with single AgNOR (Plates 75, 76 and 77).

In cutaneous histiocytoma dispersed type III NORs were observed. In malignant melanoma small sized multiple AgNORs as well as large undispersed AgNORs were observed (Plate 73).

In adenocarcinoma of hepatoid gland (Plate 79) and sebaceous gland (Plate 80) the reserve cells showed dispersed, multiple, type II and type III NORs, where as the mature cells showed type I NORs. In sweat gland adenocarcinoma cells lining the papillary structures showed multiple AgNORs compared to the mature cells with one or two NORs (Plate 81). The size of the NORs in mesenchymal neoplastic cells was larger compared to epithelial neoplastic cells

4.4 Immunohistochemistry of cutaneous and subcutaneous tissue tumours.

All cases of cutaneous and subcutaneous tissue tumours were subjected for immunohistochemistry to measure the growth fraction of tumours using anti–human Ki 67 antibodies, clone MIB-1 raised

against Ki 67 nuclear proliferation antigen which is specific for proliferating cells. The positive reactivity in the present study for Ki 67 proliferation antigen (clone MIB-1) was observed as dark brown coloured granular material restricted to nucleus. The immunostaining gave mild to strong nuclear labeling which occurred as diffuse, granular, nucleolar or a mixture of all types with mitotic figures always strongly labeled. The cells that were not proliferating showed no immunoreactivity. The details regarding Ki 67 index have been shown in Tables 6 and 7.

Ki 67 index in the present investigation ranged from 2.03 to 51.02 for all tumours. Malignant tumours had a mean Ki 67 index of 23.26±1.33 while benign tumours had an index of 8.75±0.97 (Table 7).

Statistical analysis of Ki 67 index in cutaneous and subcutaneous tissue tumours in the present study using Student's *t*-test revealed statistically significant ($P<0.05$) difference in the mean Ki 67 index between malignant and benign canine cutaneous and subcutaneous tissue tumours.

Among the round cell tumours the Ki 67 index was highest in histiocytoma (25.34) followed by malignant melanoma (20.21±0.20) and mast cell tumour (18.52±0.76) respectively (Table 7).

In the group of malignant epithelial tumours squamous cell carcinoma had peak mean Ki 67 index of 41.89±1.47 followed by sebaceous gland carcinoma (40.89±0.36), hepatoid gland carcinoma (29.83±0.21), sweat gland carcinoma (25.59±0.28) and basal cell carcinoma (12.66±0.30).

Among the malignant mesenchymal tumours the mean Ki 67 index was highest in hemangiosarcoma (20.49±0.37) followed by fibrosarcoma (9.59±0.13) and leiomyosarcoma (7.52).

The mean Ki 67 index in the group of benign epithelial tumours, was maximum in squamous papilloma (22.58±0.69) followed by trichoepithelioma (12.44±0.31), sebaceous adenoma (10.10±0.12), sweat gland adenoma (8.74), hepatoid gland adenoma (8.01±0.11) and fibropapilloma (7.21±0.14).

Among the benign mesenchymal tumours the highest mean Ki 67 index was observed in fibroma (4.10±0.01) followed by hemangioma (3.39±0.13) lipoma (2.24±0.12) and myxoma (2.24).

Among all benign tumours irrespective of type Ki 67 index was highest in squamous papilloma, followed by trichoepithelioma, sebaceous gland adenoma, sweat gland adenoma, hepatoid gland adenoma, fibropapilloma, fibroma, hemangioma, lipoma and myxoma.

Among 83 cases of malignant tumours of cutaneous and subcutaneous tissue, highest mean Ki 67 index was observed in followed squamous cell carcinoma, sebaceous gland carcinoma, hepatoid gland carcinoma, sweat gland carcinoma, histiocytoma, malignant melanoma, hemangiosarcoma, mast cell tumour, basal cell carcinoma, fibrosarcoma and leiomyosarcoma.

The proliferative cells with positive immunoreactivity were occasional and were found distributed through out the tumour mass in benign neoplasms. The location of distribution of Ki 67 antigen positive cells varied between the tumours. In squamous papilloma the immunoreactive cells were found all along the core of papilla with absence of immunereactivity in the peripheral mature cells (Plate 104). In fibropapilloma the lining cells of the squamous component showed more immunopositivity compared to fibrous component (Plate 105). In hepatoid gland adenoma occasional Ki 67 positive cells were observed only among the reserve cells which were found peripherally around mature cells (Plate 106). In sebaceous adenoma Ki 67 positive cells were observed at the periphery of the island of neoplastic cells where

reserve cells were observed (Plate 107). In sweat gland adenoma occasional Ki-67 positive cells were observed among the lining cells of glandular structure. The benign mesenchymal neoplasms such as fibroma, hemangioma, lipoma and myxoma revealed a few Ki 67 positive cells.

In sebaceous gland adenocarcinoma islands of compactly arranged neoplastic cells showed more immunopositive cells at the periphery with absence of immunopositive cells at central necrotic areas (Plate 101).

In squamous cell carcinoma cells at periphery of cell nest revealed more number of Ki 67 positive cells. The cells under mitoses were intensely stained and cell layers immediately next to keratin pearls were negative for Ki 67. In cell nests where keratin pearls were not formed Ki 67 positive cells were found distributed throughout the nests (Plates 97, 98 and 99).

In hepatoid gland adenocarcinoma more number of Ki 67 positivity was observed among proliferative reserve cells which were at the periphery of the cell nest (Plate 100). Immunopositive cells were also observed just below the capsule of neoplastic mass. The immunopositive cells were pleomorphic and large in size with fine granularity in the nucleus.

In sweat gland adenocarcinoma Ki 67 positive cells were pleomorphic with more intensely stained and highly granular nuclei. More number of immunopositive cells were present at the periphery of the cell nest lining all along the connective tissue capsule (Plate102 and 103).

Histiocytoma showed very darkly stained pleomorphic Ki 67 positive cells with absence of immunoreactivity in the surrounding connective tissue (Plate 93 and 94). Hemangiosarcoma also showed

very darkly stained pleomorphic immunopositive cells (Plate 110 and 111).

In malignant melanoma the nuclei of positive cells showed homogenous staining with very less granularity. More number of immunopositive cells were seen in the stromal connective tissue indicating infiltration of neoplastic cells (Plate 95 and 96).

In basal cell carcinoma slight immunopositive reaction was observed in the form of pale yellow colouration in the nucleus of proliferating neoplastic cells.

Fibrosarcoma showed more immunopositive cells at the periphery of the neoplastic growth with intensely stained mitotic figures (Plate 108 and 109). Leiomyosarcoma and mast cell tumour showed occasional immunopositive cells. (Plate 112)

4.5 Correlation of histological type, Ki 67 index and AgNOR index

The Ki 67 index and AgNOR index of benign and malignant histological type of cutaneous and subcutaneous tissue tumours were positively correlated with each other and the correlation was statistically significant at alpha =0.05.

The Ki 67 index and AgNOR index of benign histological types were positively correlated to each other at alpha=0.05 and the correlation coefficient (Pearson r value) between these two indices was 0.93.

Similarly, The Ki 67 index and AgNOR index of malignant histological types were positively correlated to each other at alpha=0.05 and the correlation coefficient (Pearson r value) between these indices was 0.87.

Table 1 : Age-wise occurrence of cutaneous and subcutaneous tissue tumours in dogs

Age in years	No. of dogs with tumours	Percentage of occurrence
0 - 2	10	8.20
2 - 4	7	5.75
4 - 6	16	13.11
6 - 8	45	36.88
8 - 10	25	20.50
10 - 12	13	10.65
12 - 14	5	4.1
14-18	1	0.81
Total	**122**	**100**

Table 2 : Breed-wise occurrence of cutaneous and subcutaneous tissue tumours in dogs

Breed	No. of dogs affected	Percentage
Non descript	42	34.42
German Shepherd	16	16.41
Labrador Retriever	13	10.65
Pomeranian	11	9.00
Boxer	7	5.73
Cocker Spaniel	5	4.10
Great Dane	5	4.10
Golden Retriever	4	3.30
Beagle	4	3.30
Doberman	4	3.30
Lhasa Apso	3	2.45
Dachs hund	1	0.81
Pug	1	0.81
Dalmatian	1	0.81
Mudhol	1	0.81
Total	**122**	**100**

Table 3: Histological classification of cutaneous and subcutaneous tissue tumours in dogs.

	Histological type	Number	Percentage
Round cell tumours(n=23)			
Malignant type(23)			
	Mast cell tumour	15	12.30
	Histiocytoma	1	0.81
	Malignant melanoma	7	5.75
Epithelial tumours(n=71)			
Malignant type(n=44)			
	Squamous cell carcinoma	17	13.93
	Basal cell carcinoma	15	12.30
	Hepatoid gland carcinoma	6	4.91
	Sebaceous gland carcinoma	3	2.45
	Sweat gland carcinoma	3	2.45
Benign type(n=27)			
	Squamous papilloma	5	4.10
	Fibropapilloma	4	3.30
	Trichoepithelioma	2	1.65
	Hepatoid gland adenoma	11	9.00
	Sebaceous gland adenoma	4	3.30
	Sweat gland adenoma	1	0.81
Mesenchymal tumours(n=28)			
Malignant type(n=16)			
	Fibrosarcoma	10	8.20
	Hemangiosarcoma	5	4.10
	Leiomyosarcoma	1	0.81
Benign type(n=12)			
	Fibroma	2	1.65
	Hemangioma	6	4.91
	Lipoma	3	2.45
	Myxoma	1	0.81
	Total	**122**	**100**

Table 4: Mean and range of AgNOR count in cutaneous and subcutaneous tissue tumours of dogs.

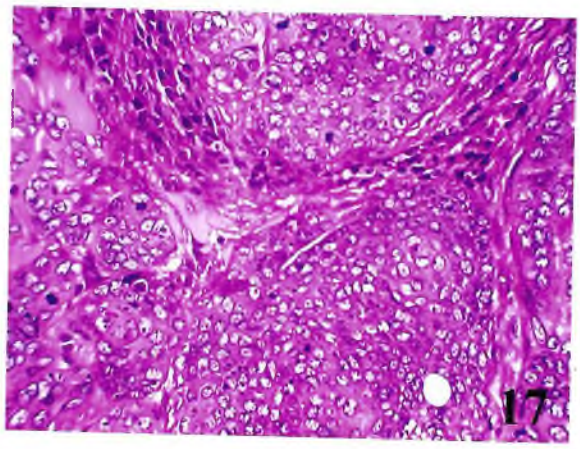
Type of tumour	n	Mean ± SE AgNOR's /cell	Range of AgNOR /cell
Round cell tumours(n=23)			
Malignant type			
Mast cell tumour	15	3.78±0.07	3.05-4.20
Histiocytoma	1	5.60	
Malignant melanoma	7	4.72±0.13	4.16-5.12
Epithelial tumours(n=71)			
Malignant type(n=44)			
Squamous cell carcinoma	17	5.36±0.12	4.40-6.01
Basal cell tumour	15	3.45±0.04	3.05-3.82
Hepatoid gland carcinoma	6	4.44±0.15	4.02-4.95
Sebaceous gland carcinoma	3	4.64±0.07	4.50-4.75
Sweat gland carcinoma	3	4.23±0.10	4.00-4.44
Benign type(n=27)			
Squamous papilloma	5	3.76±0.09	3.50-4.02
Fibropapilloma	4	2.40±0.03	2.30-2.47
Trichoepithelioma	2	3.37±0.12	3.25-3.49
Hepatoid gland adenoma	11	2.62±0.07	2.25-3.18
Sebaceous adenoma	4	2.75±0.10	2.53-3.04
Sweat gland adenoma	1	2.53	
Mesenchymal tumours(n=28)			
Malignant type(n=16)			
Fibrosarcoma	10	3.62±0.12	3.20-4.23
Hemangiosarcoma	5	3.73±0.07	3.54-3.95
Leiomyosarcoma	1	3.42	
Benign type(n=12)			
Fibroma	2	2.33±0.07	2.26-2.40
Hemangioma	6	2.40±0.11	2.05-2.82
Lipoma	3	2.28±0.13	2.08-2.53
Myxoma	1	2.27	

Table 5: Mean and range of AgNOR count in benign and malignant cutaneous and subcutaneous tissue tumours of dogs.

Type of tumour	n	Mean ± SE AgNOR's / cell	Range of AgNOR /cell
Malignant tumours	83	4.21±0.08	3.02-6.10
Benign tumours	39	2.71±0.08	2.05-4.02

Table 6: Mean and range of Ki 67 count in cutaneous and subcutaneous tumours of dogs.

Type of tumour	n	Mean ± SE Ki 67	Range of ki67
Round cell tumours(n=23)			
Malignant type			
Mast cell tumours	15	18.52±0.76	13.47-23.08
Histiocytoma	1	25.34	
Malignant melanoma	7	20.21±0.20	19.27-21.09
Epithelial tumours(n=71)			
Malignant type(n=44)			
Squamous cell carcinoma	17	41.89±1.47	31.03-51.62
Basal cell tumour	15	12.66±0.30	11.05-14.79
Hepatoid gland carcinoma	6	29.83±0.21	29.18-30.49
Sebaceous gland carcinoma	3	40.89±0.36	40.19-41.42
Sweat gland carcinoma	3	25.59±0.28	25.14-26.13
Benign type(n=27)			
Squamous papilloma	5	22.58±0.69	20.68-24.5
Fibropapilloma	4	7.21±0.14	7.02-7.64
Trichoepithelioma	2	12.44±0.31	12.12-12.75
Hepatoid gland adenoma	11	8.01±0.11	7.38-8.52
Sebaceous adenoma	4	10.10±0.12	9.79-10.34
Sweat gland adenoma	1	8.74	
Mesenchymal tumours(n=28)			
Malignant type(n=16)			
Fibrosarcoma	10	9.59±0.13	9.17-10.33
Hemangiosarcoma	5	20.49±0.37	19.51-21.53
Leiomyosarcoma	1	7.52	
Benign type(n=12)			
Fibroma	2	4.10±0.01	4.09-4.12
Hemangioma	6	3.39±0.13	3.09-3.96
Lipoma	3	2.24±0.12	2.03-2.47
Myxoma	1	2.24	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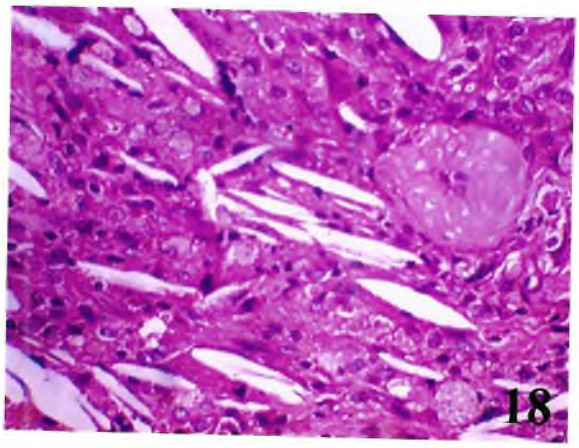
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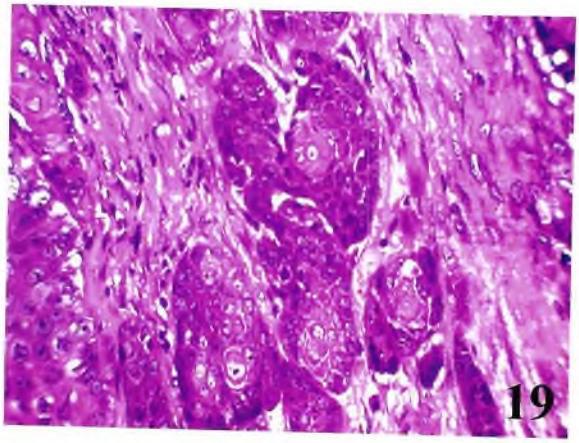
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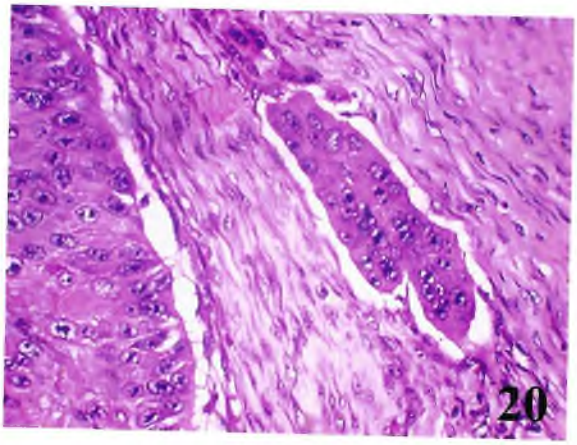
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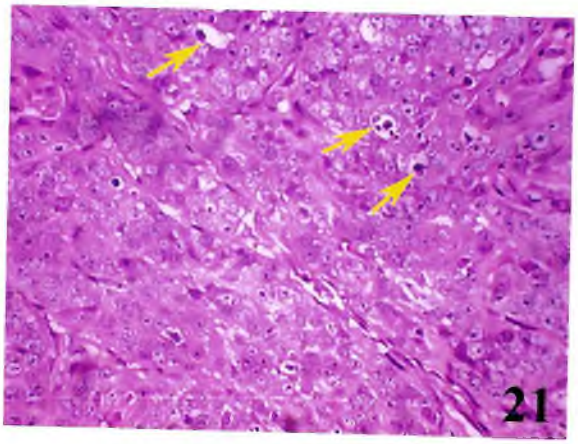
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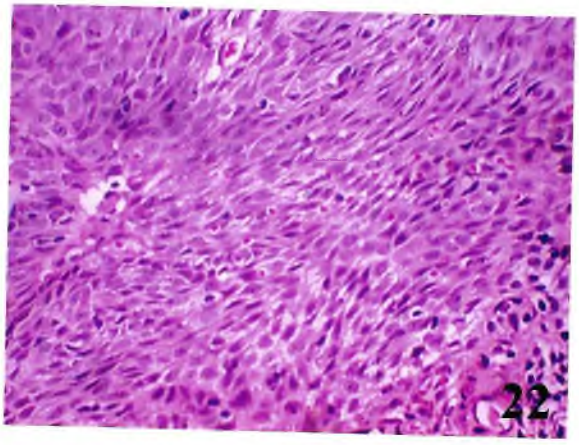
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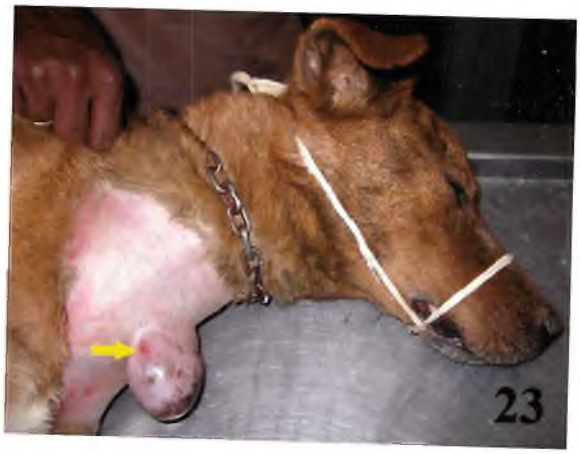
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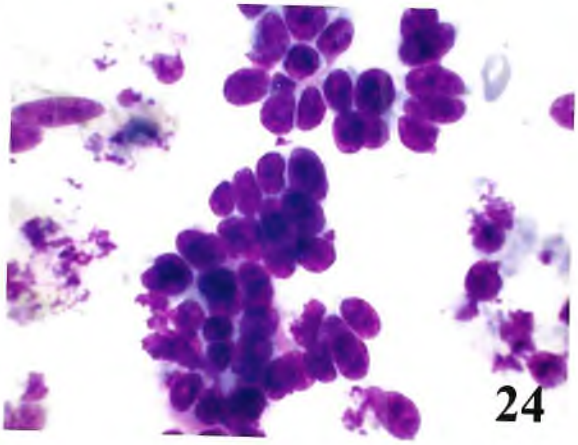
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Plate 1 Gross picture of mast cell tumour after excision appearing well circumscribed, smooth and alopecic.

Plate 2 Cytological picture of mast cell tumour showing mast cells with purplish pink coloured distinct granules in the cytoplasm and also in extracellular space.

Giemsa x 1250

Plate 3 Section of mast cell tumour showing compactly arranged spherical to oval shaped neoplastic cells with massive infiltration of eosinophils.

H&E x500

Plate 4 Section of well differentiated mast cell tumour showing varying amount of purplish coloured intracytoplasmic granules.

Toluidine blue x 1250

Plate 5 Gross picture of histiocytoma located in the ear canal showing smooth, pinkishred coloured irregular growth.

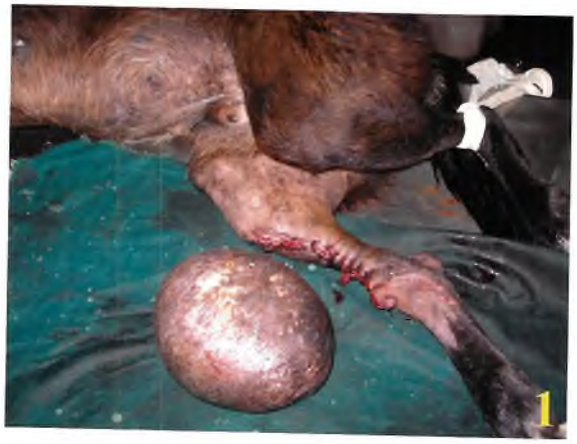
Plate 6 Section of histiocytoma showing compact arrangement of neoplastic cells with eosinophilic cytoplasm and condensed nucleus with numerous mitotic figures.

H&Ex500

Plate 7 Gross picture of malignant melanoma located near medial canthus of lower eyelid showing black coloured round dry growth.

Plate 8 Smear of malignant melanoma showing pleomorphic cells, anisokaryosis, nuclei with coarse chromatin and differentiated cells with black coloured cytoplasmic granules.

Giemsa x1250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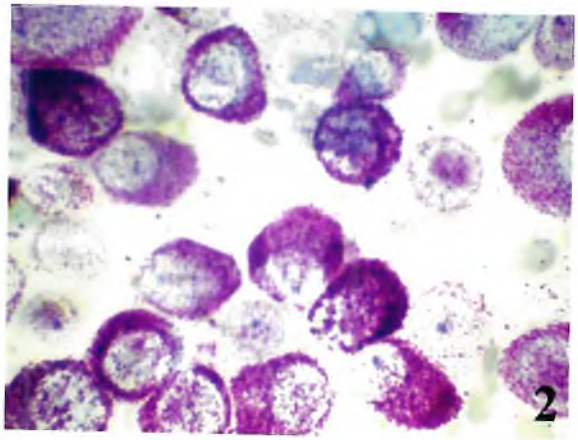
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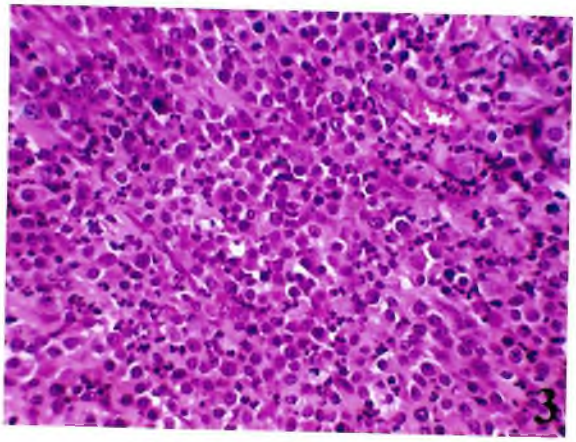
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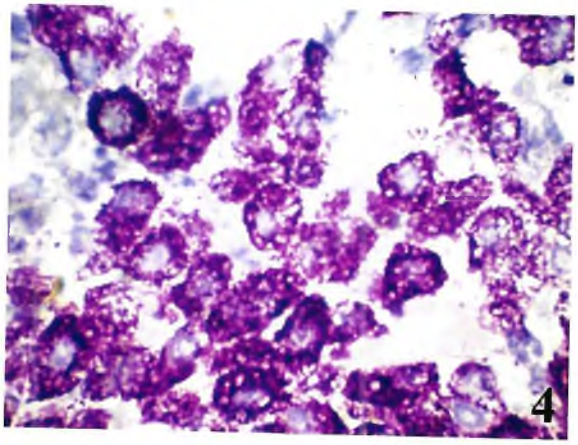
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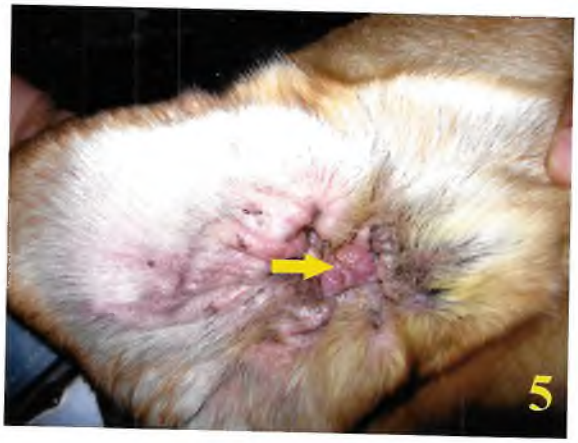
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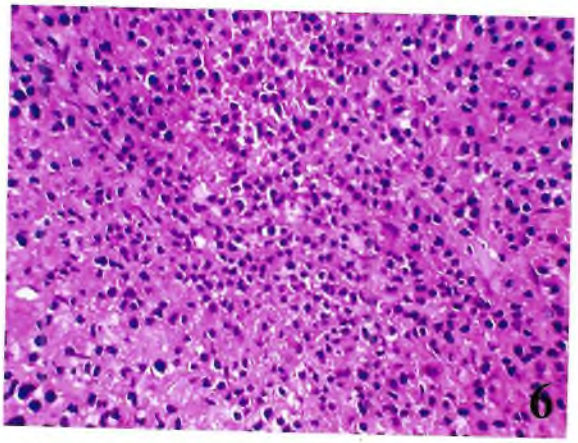
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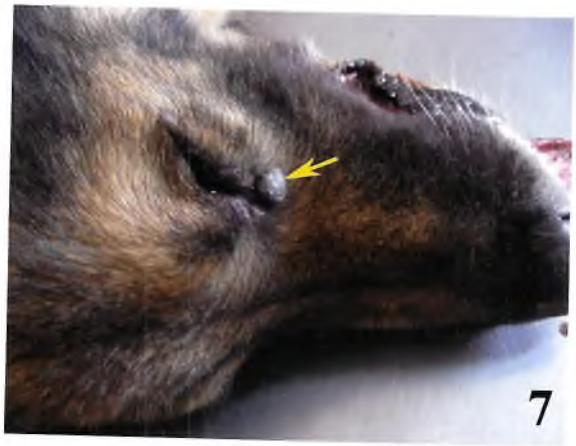
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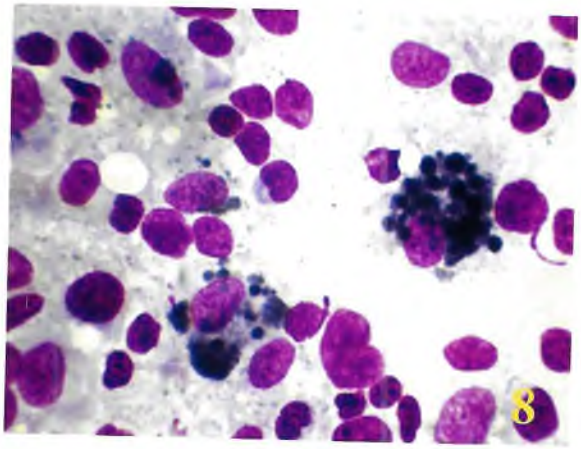
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Plate 9 Section of malignant melanoma - epitheloid type, showing round to oval shaped cells with vesicular nuclei and brownish granular cytoplasmic pigments.

H&Ex500

Plate 10 Section of malignant melanoma- spindle cell type, showing many differentiated spindle shaped cells with brown pigments.

H&E x 500

Plate 11 Section of malignant melanoma showing infiltrative neoplastic melanocytes in the stromal connective tissue.

H&E x 500

Plate 12 Section of malignant melanoma showing highly anaplastic cells with few differentiated cells.

H&E x 500

Plate 13 Section of malignant melanoma- epitheloid type, showing black coloured melanin pigment in the cytoplasm.

Massons Fontana x 500

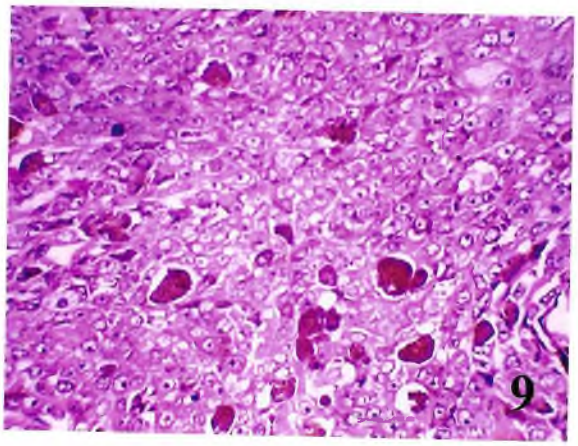
Plate 14 Gross picture of squamous cell carcinoma located in the digit showing superficial ulceration with necrosis.

Plate 15 Cytological smear of squamous cell carcinoma showing binucleated and multinuclieated cells with basophilic cytoplasm.

Giemsa x 1250

Plate 16 Section of well differentiated squamous cell carcinoma showing proliferating neoplastic squamous cells arranged in the form of nest with keratin pearl.

H&E x 500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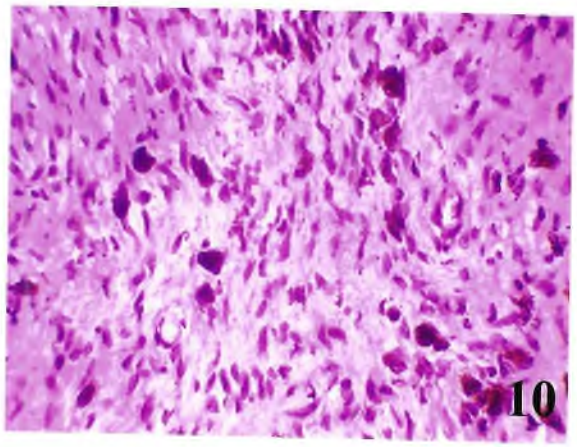
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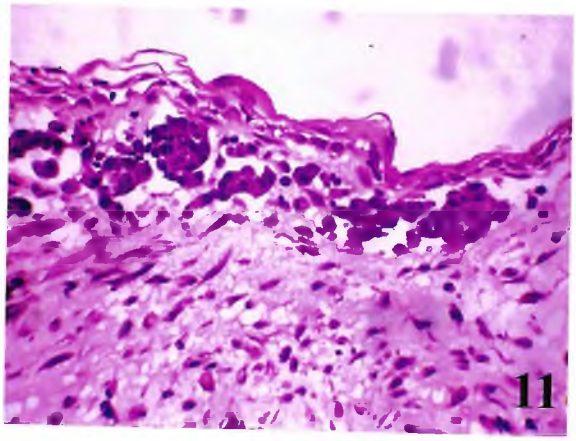
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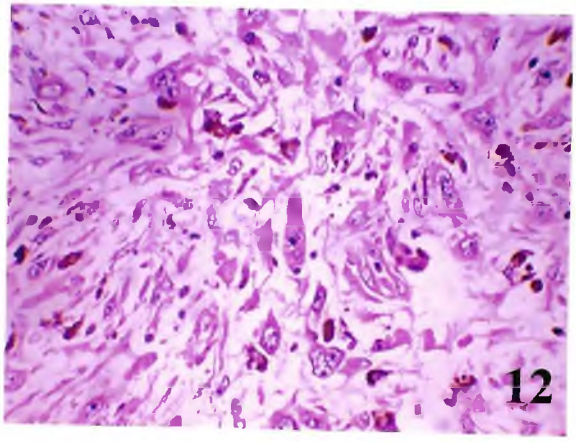
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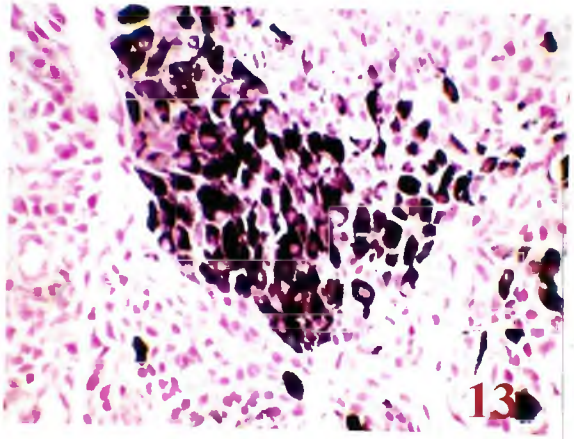
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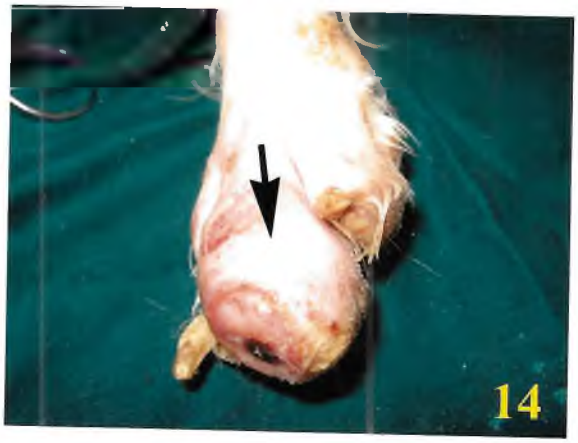
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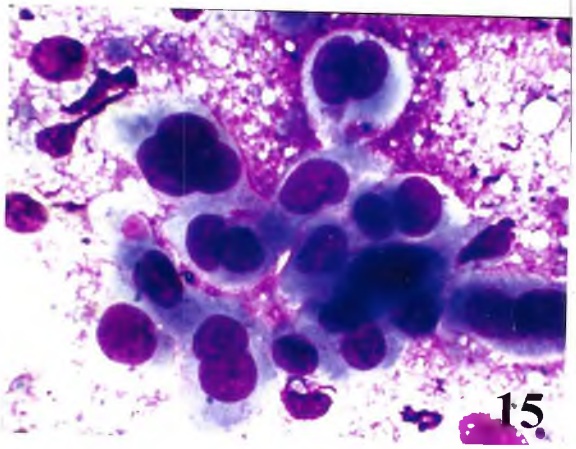
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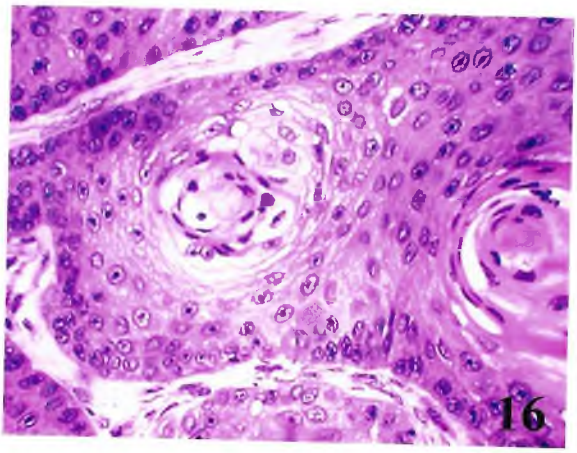
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Table 7: Mean and range of KI 67 index in benign and malignant cutaneous and subcutaneous tissue tumours of dogs.

Type of tumour	n	Mean ± SE Ki 67 Index	Range of Ki 67 Index
Malignant tumours	83	23.26±1.33	11.05-51.02
Benign tumours	39	8.75±0.97	2.03-24.50

Plate 17 Section of moderately differentiated squamous cell carcinoma showing proliferative cell nests with pleomorphic cells and more number of mitotic figures.

H&E x 500

Plate 18 Section of squamous cell carcinoma showing proliferating neoplastic cells and entrapped cholesterol clefts.

H&E x 500

Plate 19 Section of squamous cell carcinoma showing neoplastic squamous cells in the form of cell nests infiltrated in to surrounding stromal tissue.

H&E x 500

Plate 20 Section of squamous cell carcinoma showing infiltration of neoplastic cells consisting of prominent nucleoli in to the lymphatics.

H&E x 500

Plate 21 Section of poorly differentiated squamous cell carcinoma Showing apoptotic cells.

H&E x 500

Plate 22 Section of squamos cell carcinoma - spindle cell type, showing spindle shaped proliferating neoplastic cells.

H&E x 500

Plate 23 Gross picture of basal cell carcinoma located in the neck region showing oval shaped growth which is firmly attached to the Skin.

Plate 24 Cytological picture of basal cell carcinoma showing palisade arrangement of oval shaped neoplastic cells with prominent nucleus and scanty cytoplasm.

Giemsa x1250

Plate 25 Section of basal cell carcinoma- ribbon type, showing palisade arrangement of neoplastic cells with granular cytoplasm and round to oval shaped nuclei.

H&E x 500

Plate 26 Section of basal cell carcinoma- trabecular type, showing small islands of neolplastic cells separated by thin connective tissue stroma.

H&E x 500

Plate 27 Section of basal cell carcinoma- solid type, showing compact arrangement of neoplastic cells.

H&E x 500

Plate 28 Section of basal cell carcinoma- medusoid type showing cords of neoplastic cells streaming outwards from the central aggregation.

H&E x 500

Plate 29 Section of basal cell carcinoma- rosette type showing islands of neoplastic cells with nuclei arranged in the periphery giving flower like appearance. H&E x 500

Plate 30 Gross picture of hepatoid gland carcinoma located at the base of tail showing spherical growth with ulceration.

Plate 31 Cytological picture of hepatoid gland adenocarcinoma showing group of cells with few matured hepatoid cells with abundent basophilic cytoplasm and numerous reserve cells.

Giemsa x 1250

Plate 32 Section of hepatoid gland adenocarcinoma showing more number of proliferating reserve cells along with few mature differentiated cells and concentrically arranged keratin material.

H&E x 500

Plate 33 Section of hepatoid gland adenocarcinoma showing vascular spaces filled with eosinophilic fluid and RBCs surrounded by reserve cells.

H&E x 500

Plate 34 Gross picture of sebaceous gland carcinoma located at ventral abdomen showing smooth surfaced irregular growth.

Plate 35 Section of sebaceous gland carcinoma showing islands of compactly arranged neoplastic cells with occasional differentiated sebaceous glandular epithelial cells(arrow).

H&E x 500

Plate 36 Section of sebaceous gland carcinoma showing islands of compactly arranged neoplastic cells with occasional differentiated sebaceous glandular epithelial cells with infiltrated mononuclear cells.

H&E x 500

Plate 37 Gross picture of sweat gland adenocarcinoma located on the dorsal aspect at the base of the tail showing dome shaped growth.

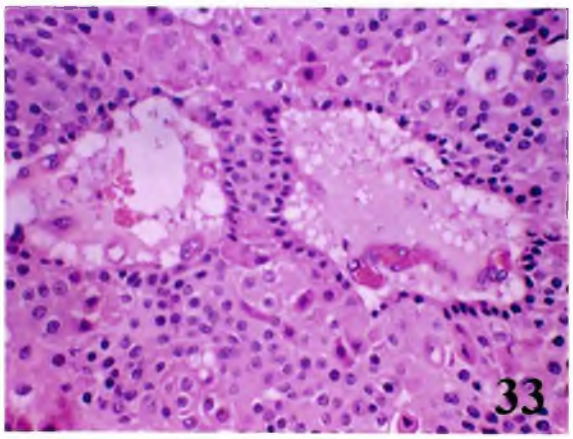
Plate 38 Cytoogical picture of sweat gland adenocarcinoma showing group of neoplastic cells with pleomorphism, anisokaryosis and hyperchromism.

Giemsa x1250

Plate 39 Section of sweat gland adenocarcinoma showing papillary projections lined by double layer of cuboidal type of neoplastic cells all along the fibrous connective tissue stroma on either side.

H&E x 500

Plate 40 Gross picture of squamous papilloma appearing raised, irregular and pinkish in colour.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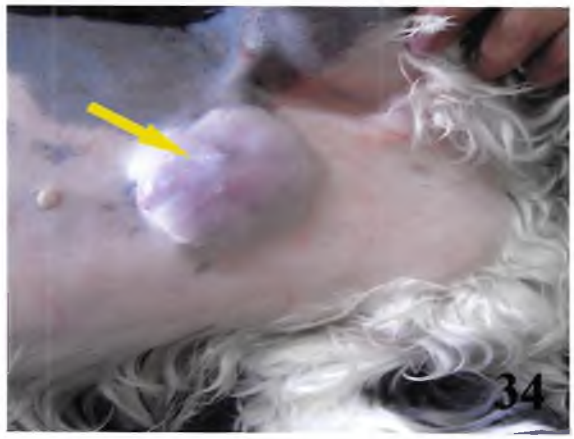
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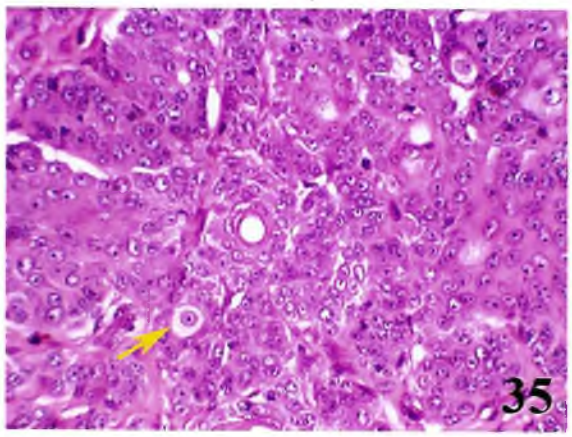
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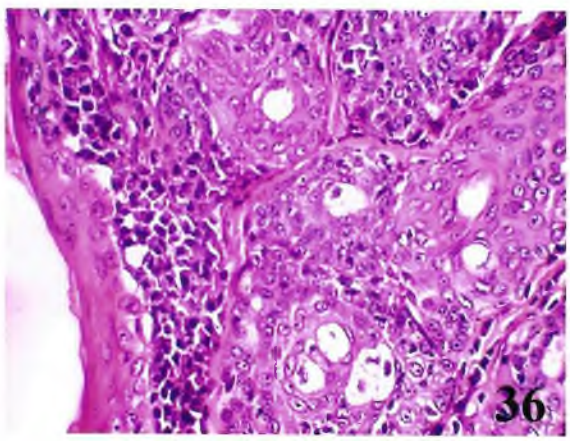
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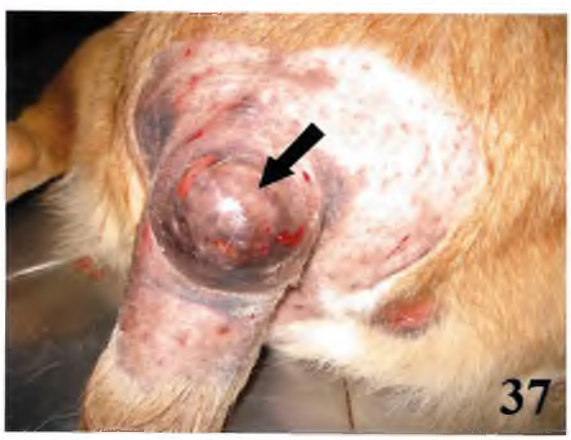
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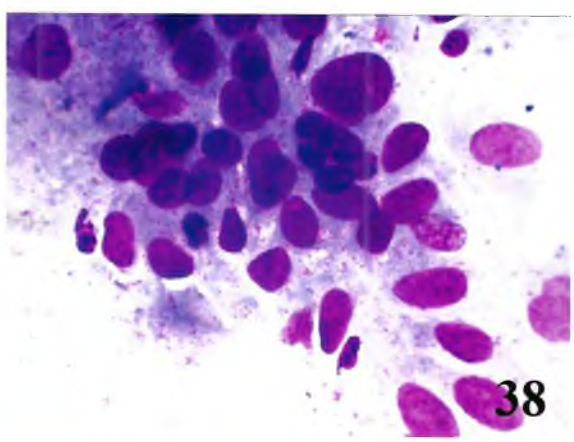
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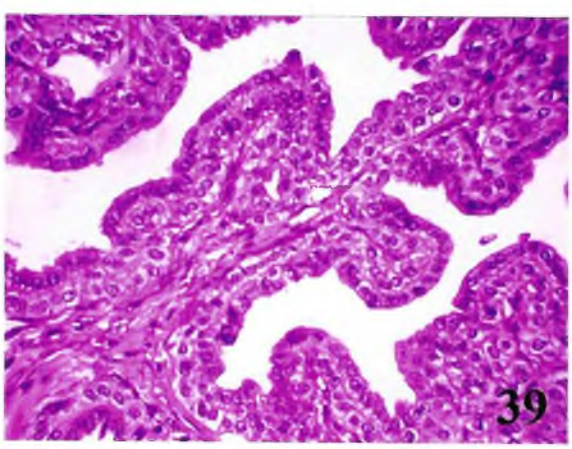
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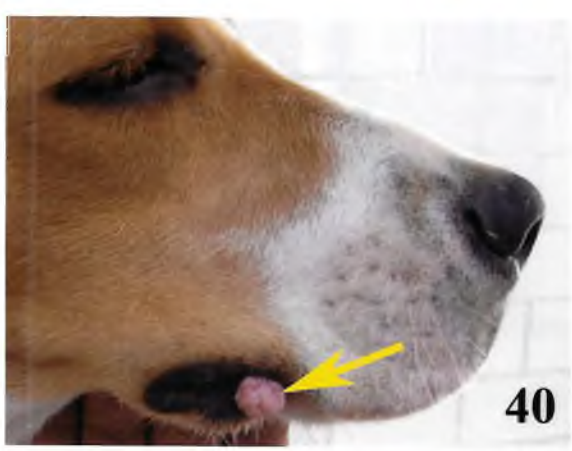
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Plate 41 Cytological picture of squamous papilloma showing clump of almost uniform sized round to oval mature squamous epithelial cells.

Giemsa x 500

Plate 42 Section of squamous papilloma showing a papillary projection covered by thick eosinophilic keratin material and proliferating epithelial cells over a core of connective tissue stroma.

H&E x 500

Plate 43 Gross picture of fibropapilloma showing raised multiple growths covered by alopecic pigmented skin.

Plate 44 Cytological picture of fibropapilloma showing fibrocytes with elongated nucleus and basophilic cytoplasm extending from both the ends.

Giemsa x1250

Plate 45 Section of fibropapilloma with proliferating fibrous tissue covered by epithelium with retipegs extending in to the fibrous tissue.

H&E x 500

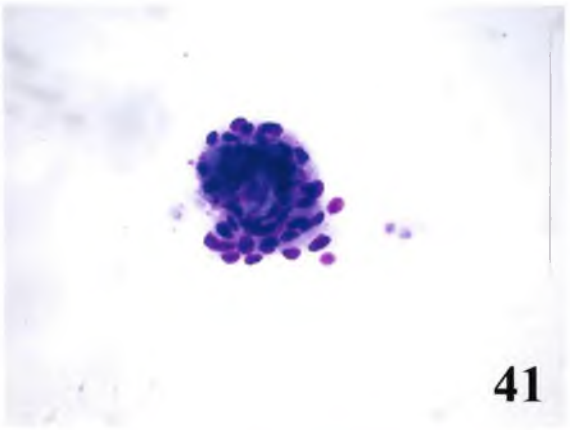
Plate 46 Section of trichoepitheloma showing proliferation of epithelial cells around cornified hair follicle.

H&E x 500

Plate 47 Gross picture of hepatoid gland adenoma located at the base of tail showing oval shaped growth covered by alopecic skin.

Plate 48 Cytological picture of hepatoid gland adenoma showing mature hepatoid cell with abundant cytoplasm and also small sized reserve cell.

Gimsa x1250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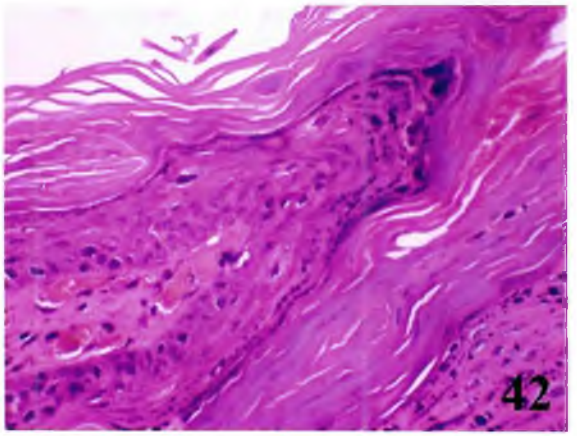
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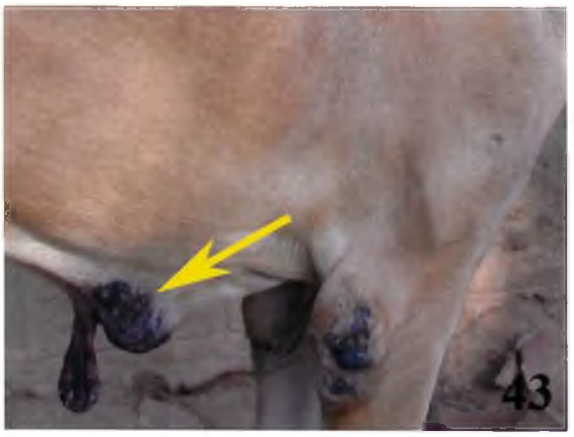
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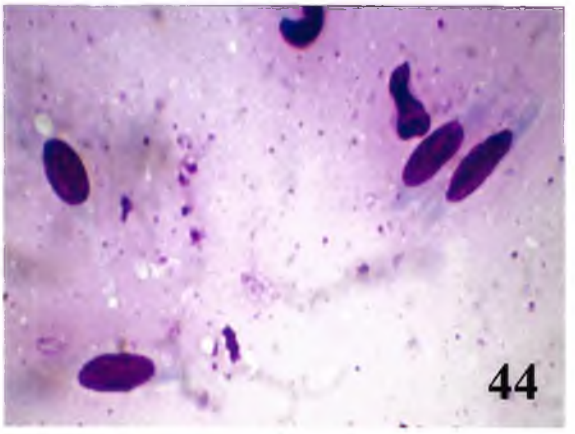
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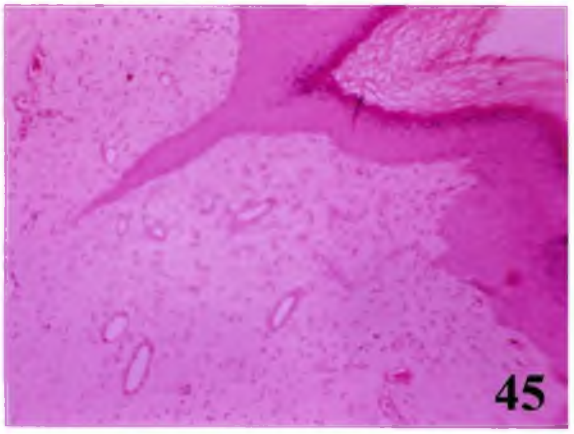
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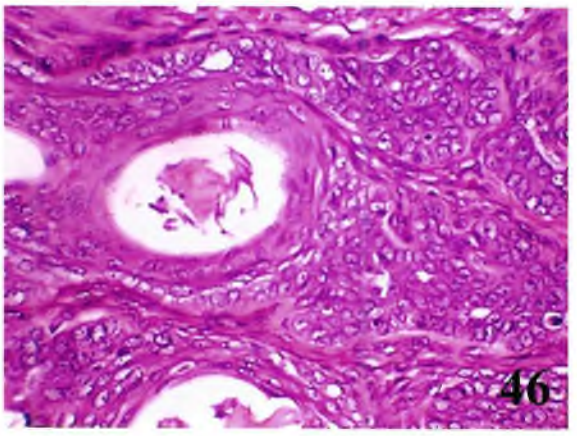
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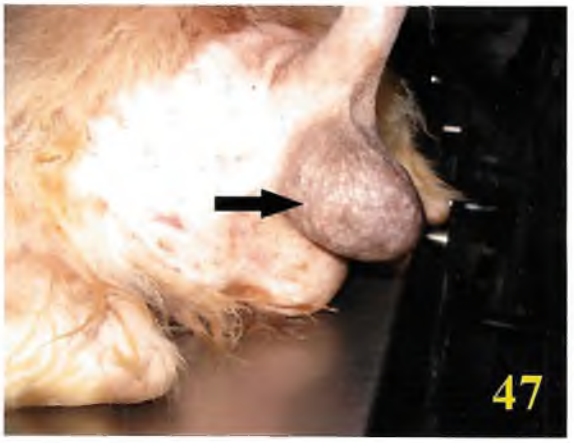
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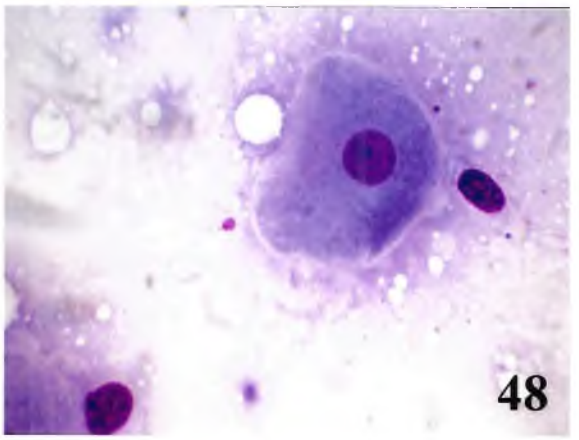
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Plate 49 Section of hepatoid gland adenoma with secondary vascularisation showing vascular spaces surrounded by a layer of reserve cells.

H&E x 500

Plate 50 Gross picture of sebaceous gland adenoma located near upper eyelid showing cauliflower like raised growth.

Plate 51 Section of sebaceous gland adenoma showing proliferating neoplastic cell with a large number of differentiated cells.

H&E x 500

Plate 52 Section of sweat gland adenoma showing proliferating cylindrical shaped neoplastic cells with abundant eosinophilic cytoplasm.

H&E x 500

Plate 53 Gross picture of fibrosarcoma showing large round firm growth.

Plate 54 Cytological picture of fibrosarcoma showing plumpy fibroblasts with basophilic cytoplasm prominent nucleus, multiple nucleoli and anisokaryosis.

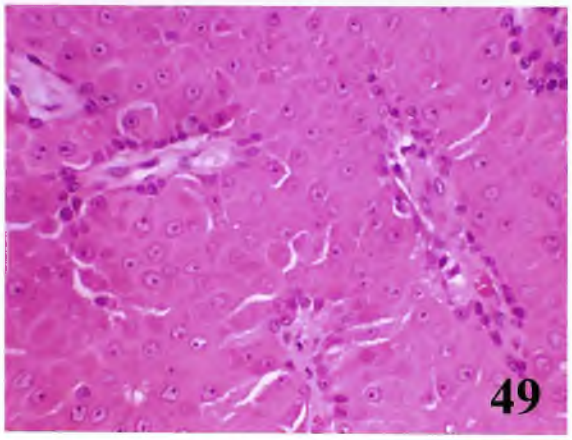
Giemsa x 1250

Plate 55 Section of fibrosarcoma showing interlacing bundles of spindle shaped proliferating neoplastic cells with elongated nuclei.

H&E x 500

Plate 56 Section of fibrosarcoma showing blue coloured collagen material and purplish coloured elongated neoplastic cells

Massons trichrome x 500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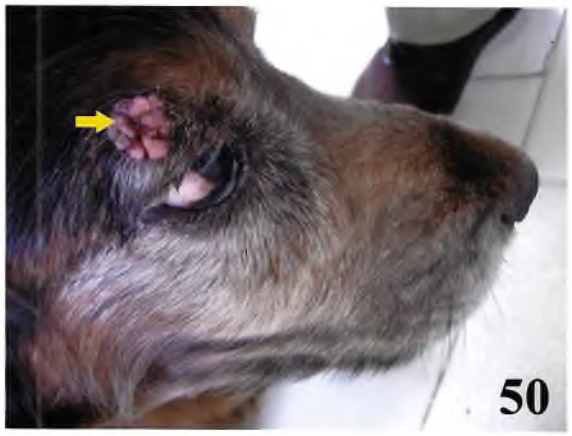
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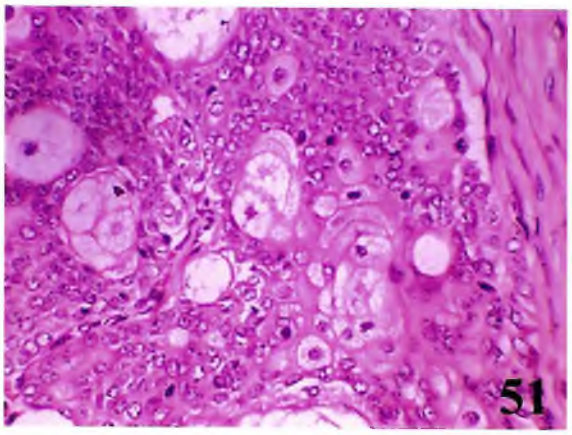
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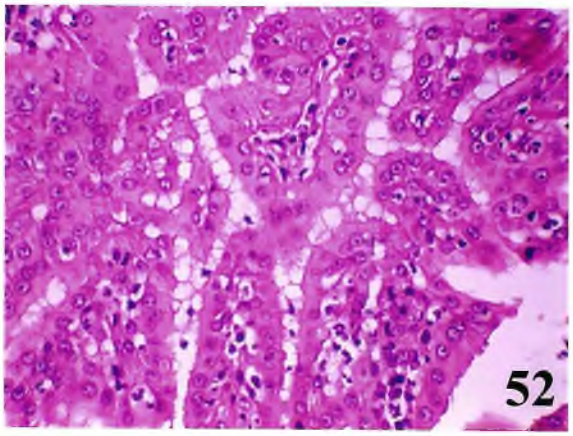
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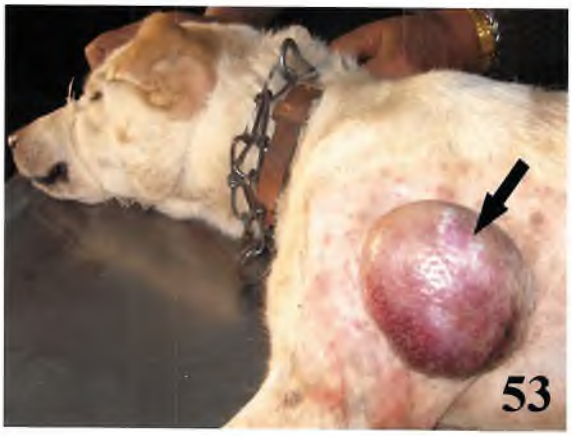
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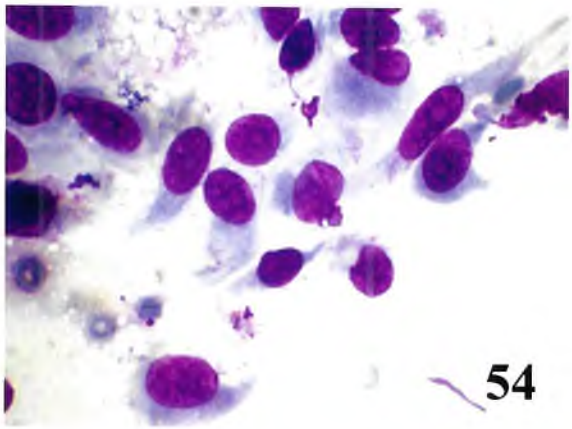
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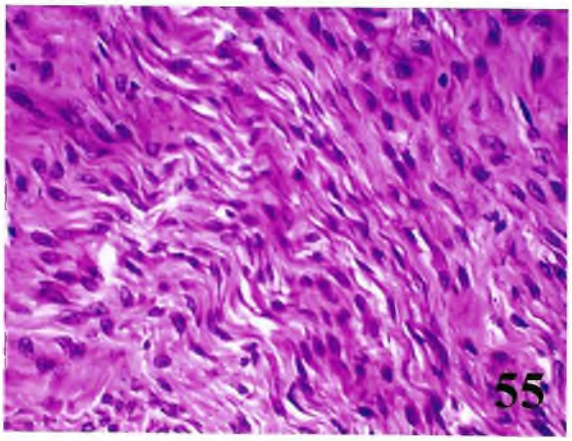
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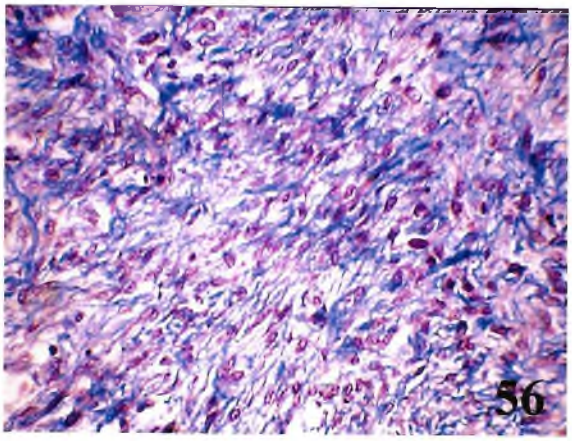
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Plate 57 Gross picture of hemangiosarcoma showing ulceration and hemorrhage.

Plate 58 Cytological picture of hemangiosarcoma showing group of neoplastic cells consisting of elongated plumpy nuclei, basophillic tapering cytoplasm and prominent nucleoli with blood cells.

Giemsa x 1250

Plate 59 Section of hemangiosarcoma showing proliferating spindle shaped cells with formation of small blood vessels consisting of RBCs.

H&E x 500

Plate 60 Section of leiomyosarcoma showing spindle shaped cells with eosinophillic cytoplasm and elongated nuclei separated by thin collagenous stroma

H&E x 500

Plate 61 Section of leiomyosarcoma showing purplish coloured proliferating neoplastic cells with minimum amount of bluish coloured collagen.

Massons trichrome x 500

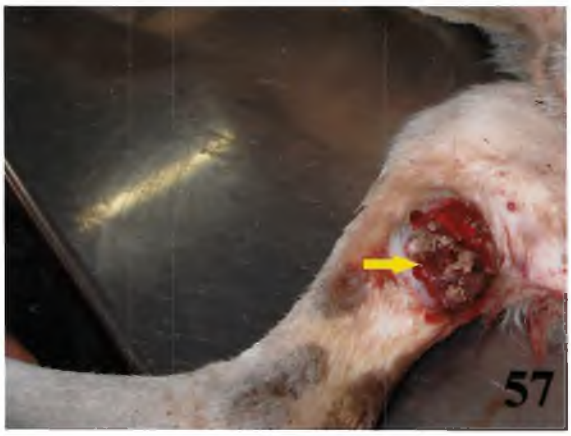
Plate 62 Section of fibroma showing sparse cellularity and abundant amount of collagen.

H&E x 500

Plate 63 Gross picture of hemangioma showing multiple cysts appearing dark in colour.

Plate 64 Cytological picture of hemangioma showing spindle shaped cells with RBCs in the background.

Giemsa x 1250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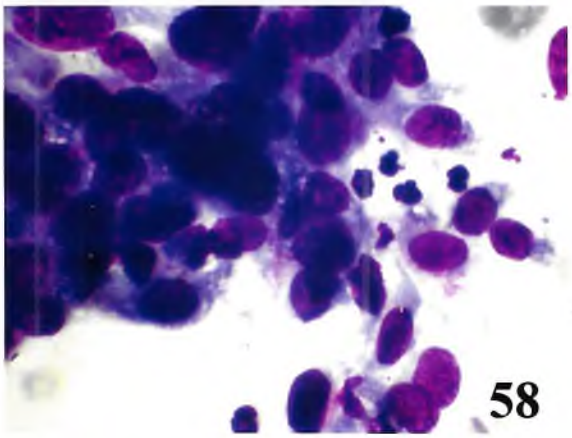
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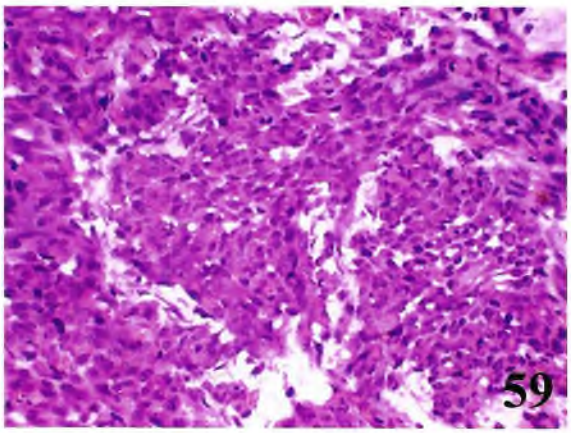
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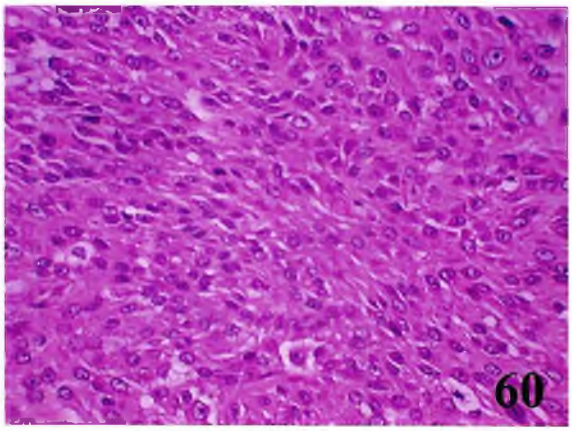
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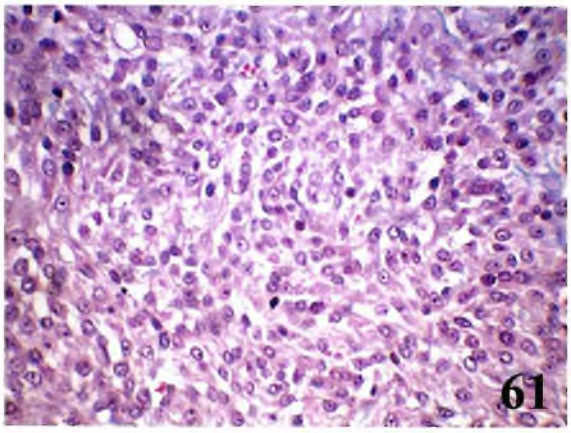
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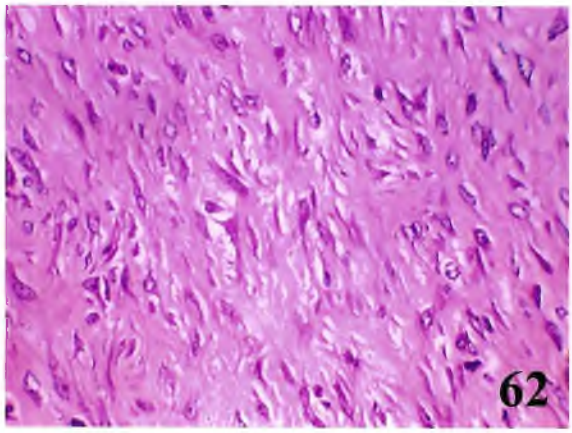
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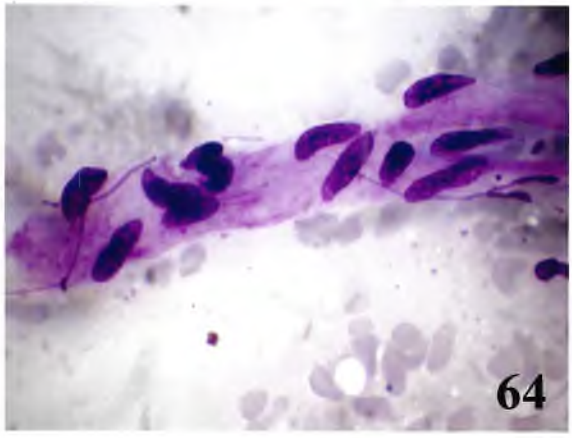
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Plate 65 Section of cavernous hemangioma showing vascular spaces containing RBCs, lined by endothelial cells.

H&E x 500

Plate 66 Section of cavernous hemangioma showing multiple thrombi within the blood spaces.

H&E X 500

Plate 67 Cytological picture of lipoma showing collapsed adipose tissue with liberated fat droplets aronud the mass.

Giemsa x 500

Plate 68 Cytological picture of lipoma showing red coloured collapsed adipose tissue with liberated fat droplets around the mass.

Oil red O x 500

Plate 69 Section of lipoma showing uniform sized fat cells with eccentrically placed nuclei.

H&E X 500

Plate 70 Section of lipoma showing orange red coloured fat globules.

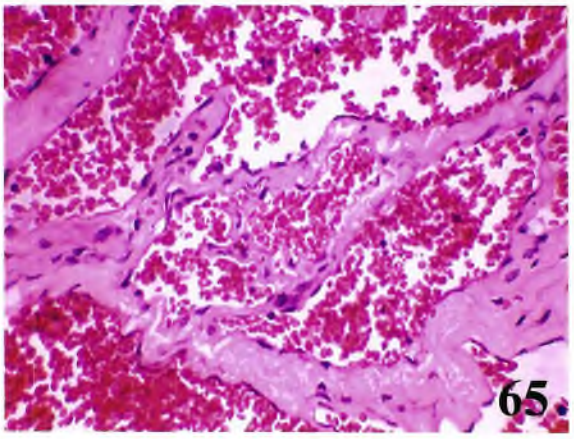
Oil red O x 500

Plate 71 Section of myxoma showing spindle shaped cells with eosinophilic material in the back ground.

H&E X 500

Plate 72 Section of mast cell tumour showing dispersed as well as undispersed NORs within the neoplastic cells.

AgNOR x 1250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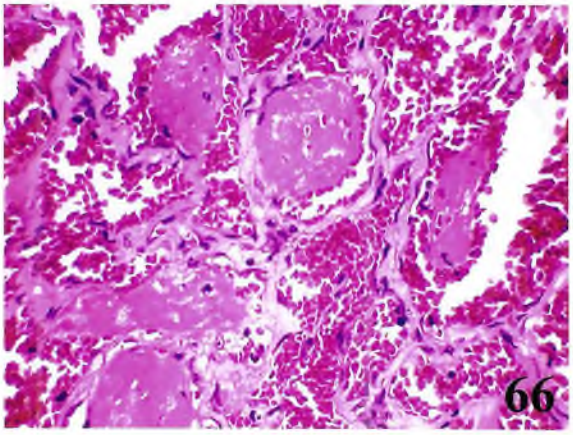
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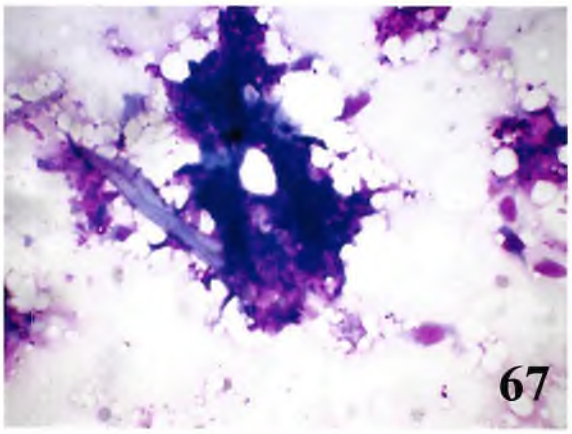
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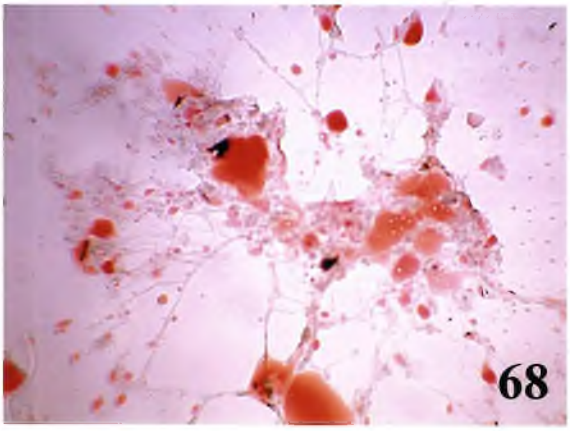
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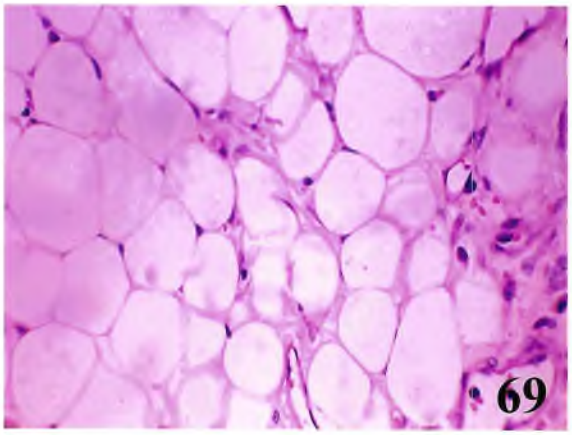
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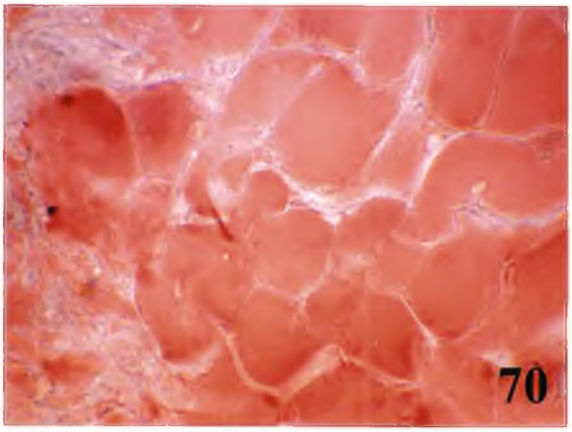
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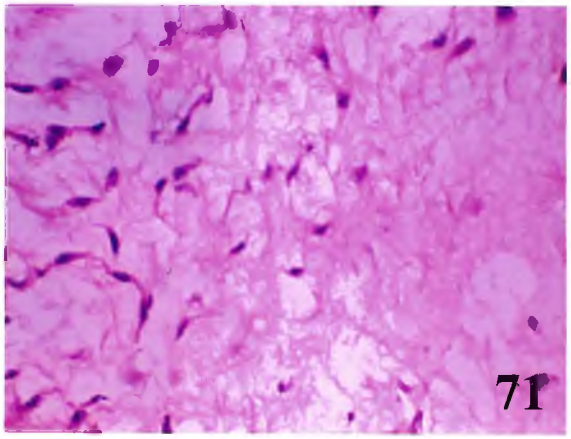
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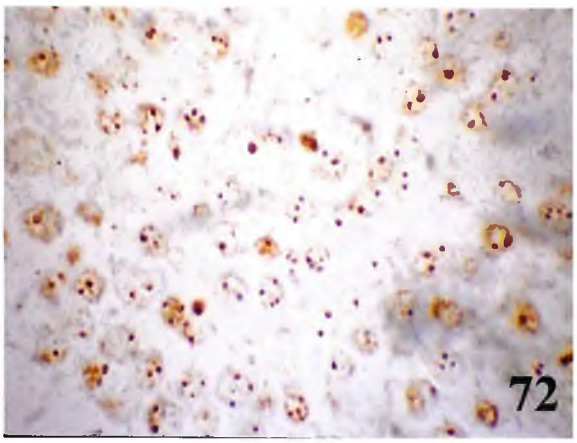
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Plate 73 Section of histiocytoma showing dispersed type III NORs.
AgNOR x1250

Plate 74 Section of malignant melanoma showing small multiple Dispersed NORs. Note black coloured melanin pigments in a few cells.
AgNOR x1250

Plate 75 Section of squamous cell carcinoma showing dark brown coloured type II and type III NORs in proliferating cells.
AgNOR x1250

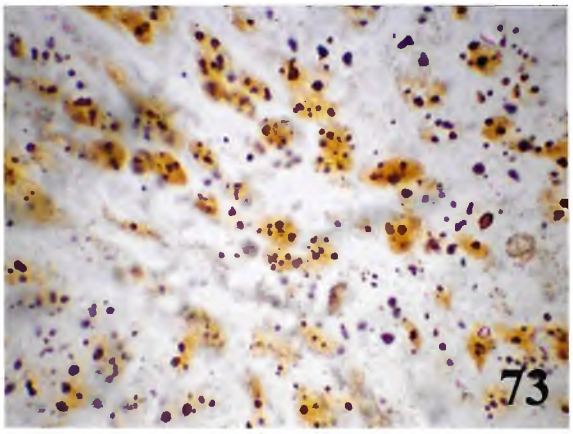
Plate 76 Section of squamous cell carcinoma showing centrally placed large solitary NOR in well differanciated cells.
AgNOR x1250

Plate 77 Section of squamous cell carcinoma- spindle cell type showing multiple NORs of type III in spindle shaped cells.
AgNOR x1250

Plate 78 Section of basal cell carcinoma showing dispersed NORs in the neoplastic cells.
AgNOR x1250

Plate 79 Section of hepatoid gland carcinoma showing multiple dispersed NORs in reserve cells and occasional large sized NORs in differentiated hepatoid cells.
AgNOR x1250

Plate 80 Section of sebaceous gland carcinoma showing multiple as well as single NOR.
AgNOR x1250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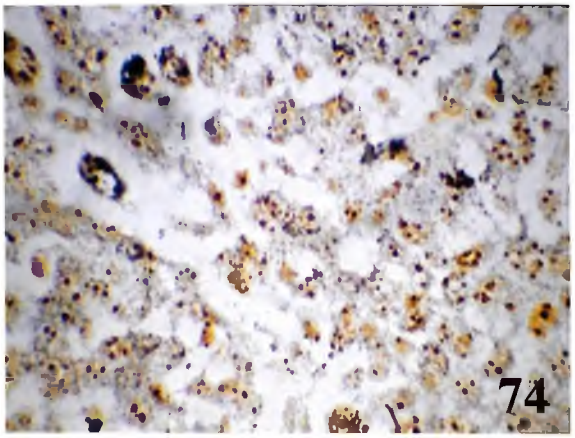
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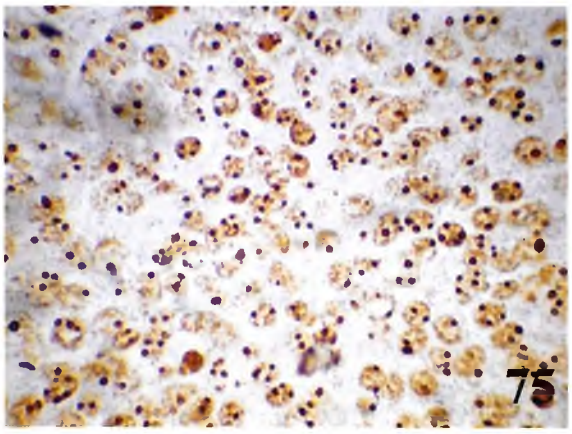
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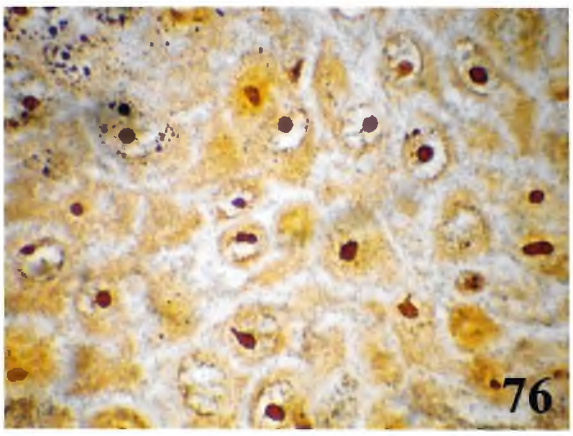
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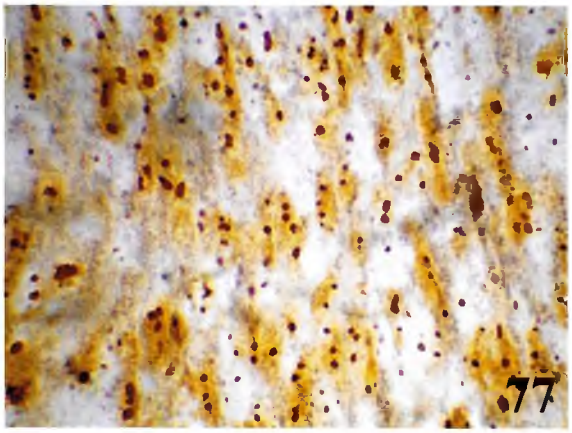
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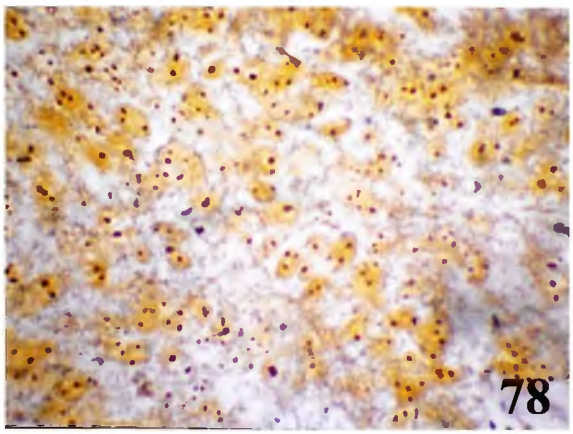
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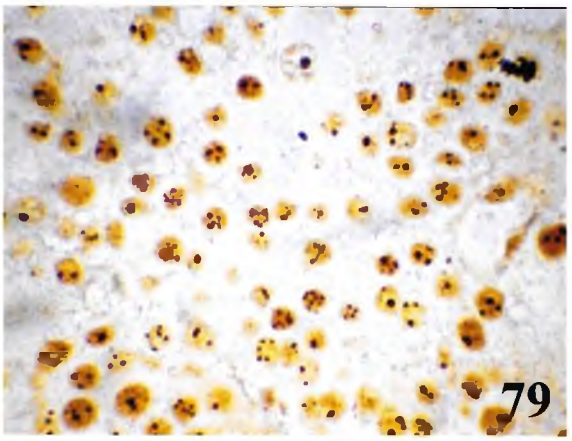
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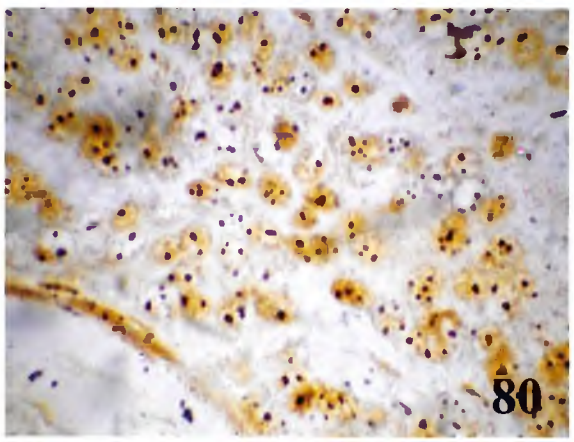
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Plate 81 Section of sweat gland adenocarcinoma showing type III and type II NORs.

AgNOR x1250

Plate 82 Section of fibrosarcoma showing neoplastic cells multiple NORs.

AgNOR x1250

Plate 83 Section of leiomyosarcoma showing cells with dispersed NORs as well as undispersed NORs with in the large nucleoli.

AgNOR x1250

Plate 84 Section of hemangiosarcoma showing multiple small sized dispersed type III NORs in the elongated nucleus of the neoplastic cells.

AgNOR x1250

Plate 85 Section of squamous papilloma showing dispersed NORs.

AgNOR x1250

Plate 86 Section of fibropapilloma showing dispersed as well as large sized NORs.

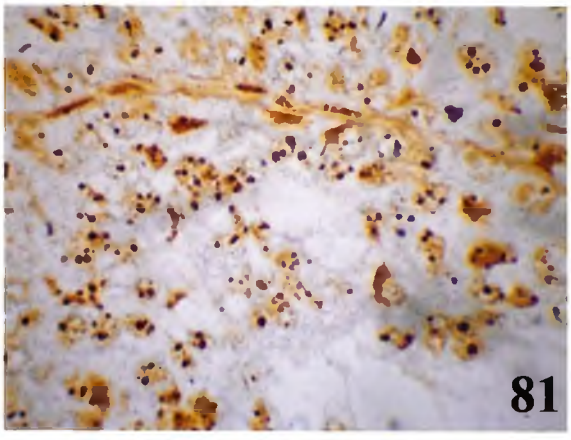
AgNOR x1250

Plate 87 Section of hepatoid gland adenoma showing more number of mature cells with large sized NORs and reserve cells with dispersed NORs.

AgNOR x1250

Plate 88 Section of sweat gland adenoma showing dispersed as well as undispersed NORs in the nucleolus.

AgNOR x1250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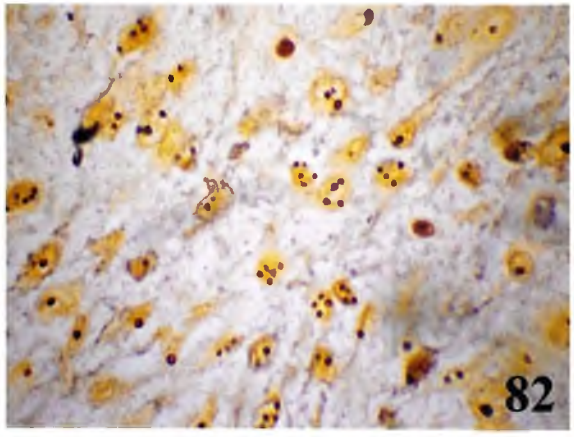
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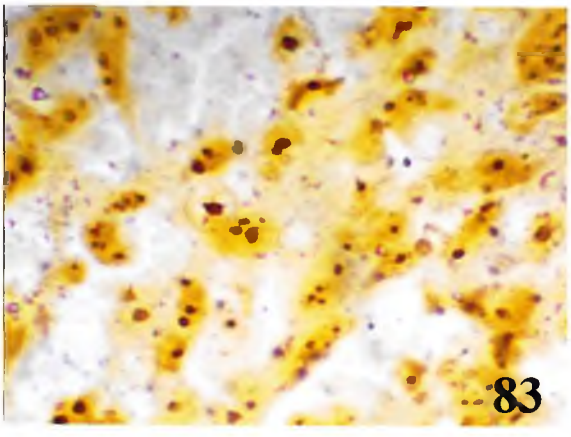
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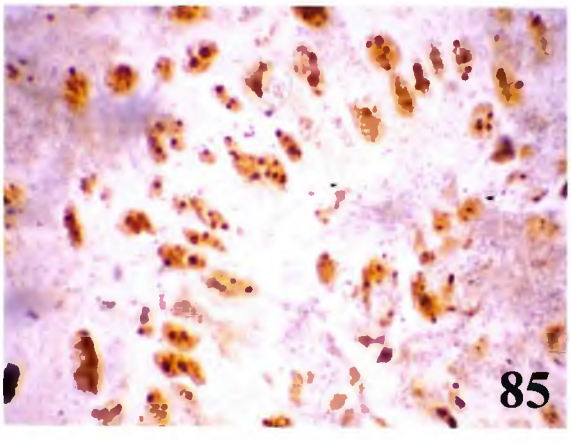
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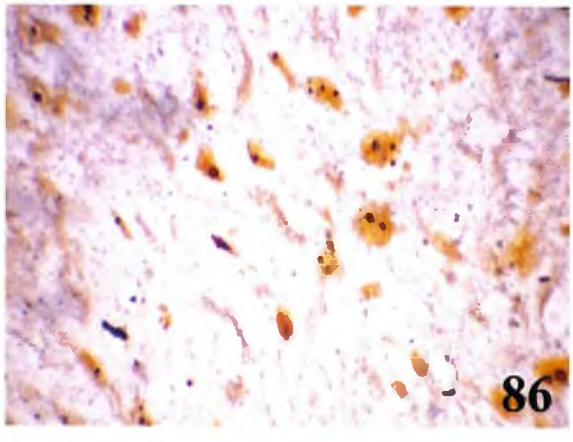
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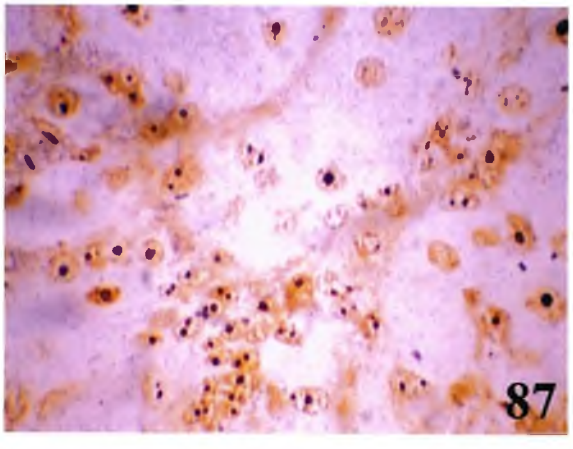
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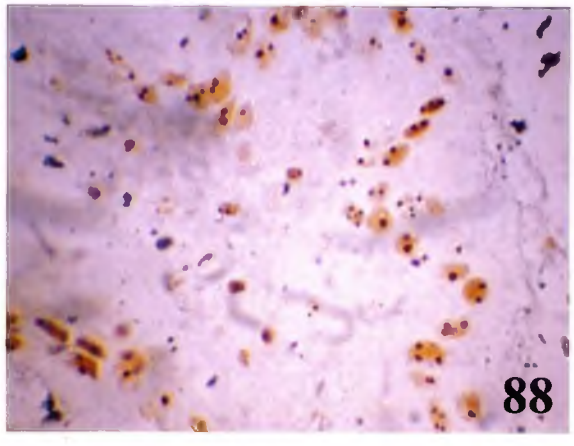

88

Plate 89 Section of fibroma showing proliferating cells with only a few NORs in the nucleus.

AgNOR x1250

Plate 90 Section of hemangioma showing both dispersed and undispersed NORs in the lining cells of the cavernous spaces.

AgNOR x1250

Plate 91 Section of myxoma showing both dispersed and undispersed NORs.

AgNOR x1250

Plate 92 Section of mast cell tumour showing positive immunostaining for Ki 67 antigen.

IHC x 1250

Plate 93 Section of histiocytoma showing darkly stained pleomorphic immuno positive cells for Ki 67 proliferation antigen with absence of immunoreactivity in surrounding connective tissue.

IHC x 500

Plate 94 Section of histiocytoma showing darkly stained immune positive cells.

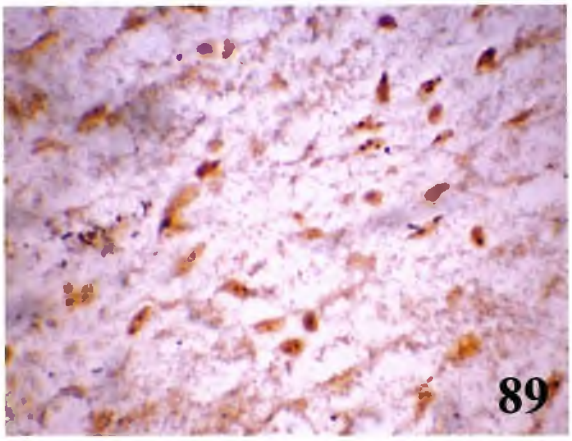
IHC x 1250

Plate 95 Section of malignant melanoma showing Ki 67 positive cells in a group of cells that are showing invasion into capsule.

IHCx500

Plate 96 Section of malignant melanoma with multiple positive cells showing varying intensity of colouration with very less granularity.

IHCx1250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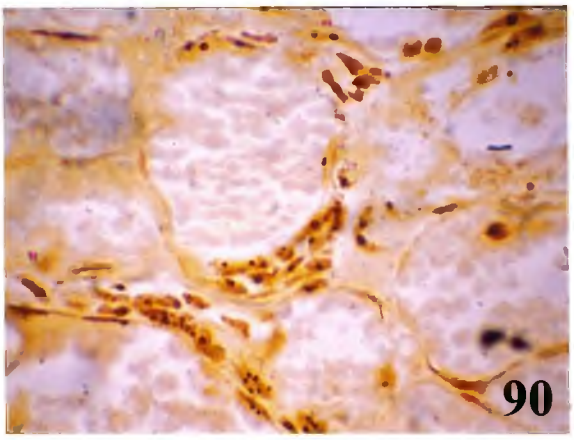
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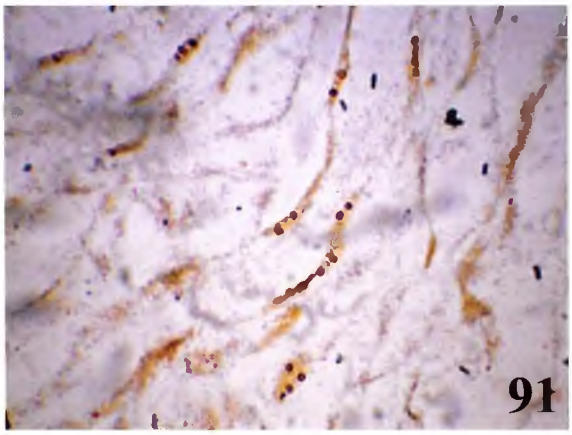
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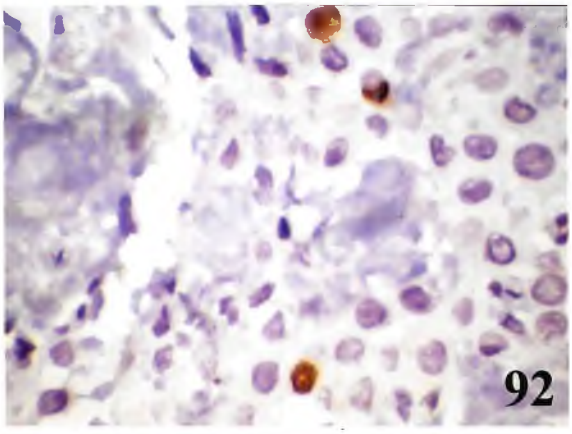
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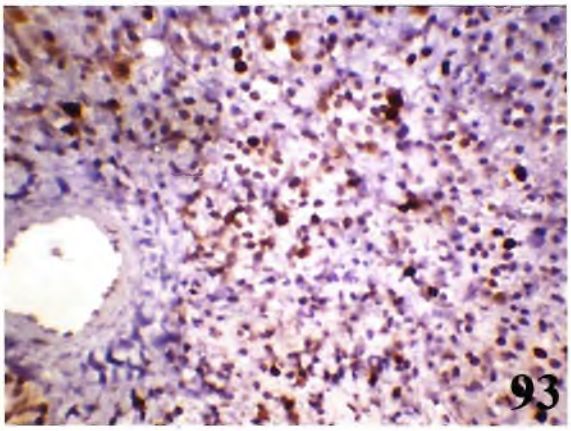
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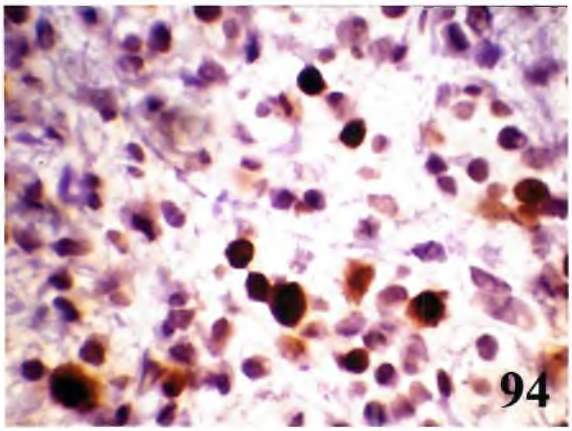
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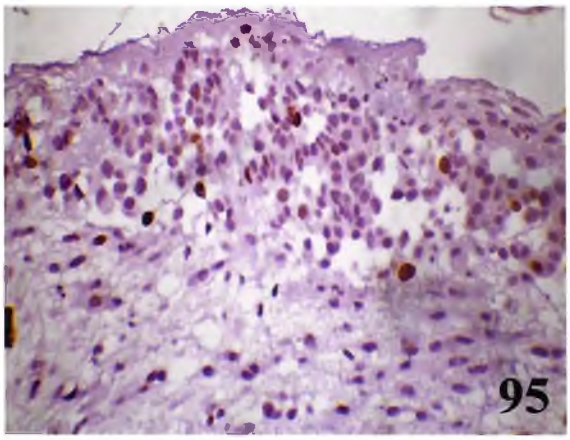
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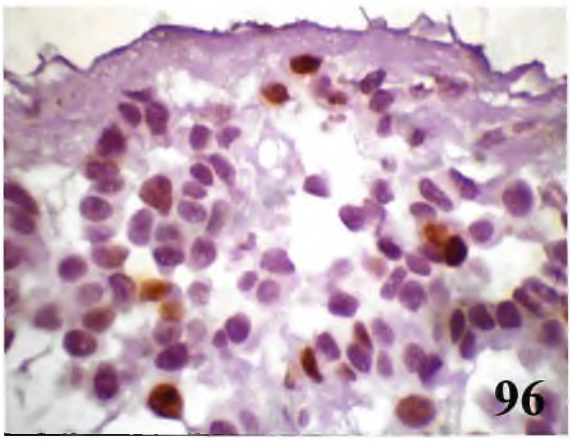
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Plate 97 Section of squamous cell carcinoma showing positive immune reactivity in the peripherally placed neoplastic cells of the cell nest with intensely stained mitotic cells.

IHC x 1250

Plate 98 Section of squamous cell carcinoma - spindle cell type showing pleomorphic cells with anisokaryosis and brown coloured granular material in the immunopositive cells.

IHC x 500

Plate 99 Section of spindle cell type of squamous cell carcinoma showing immunoreactive cells with darkly stained nuclei.

IHC x 1250

Plate 100 Section of hepatoid gland carcinoma showing pleomorphic Ki 67 positive cells.

IHC x 1250

Plate 101 Islands of compactly arranged sebaceous gland carcinoma cells showing more number of Ki 67 positivity in the cells that are at the periphery with nucleoli being stained more intensely.

IHC x 500

Plate 102 Islands of proliferating cells of sweat gland carcinoma showing more number of positive cells at the periphery.

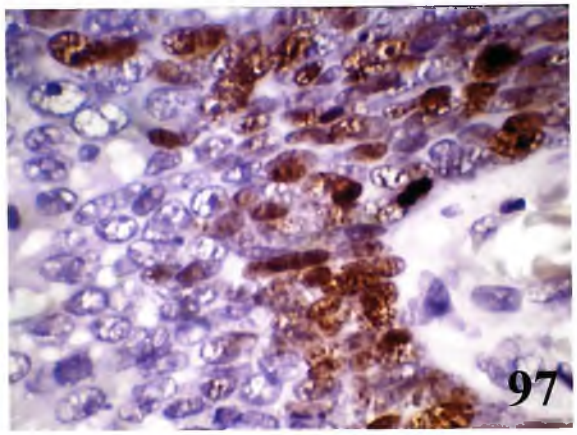
IHC x 500

Plate 103 Section of sweat gland carcinoma showing very large sized pleomorphic Ki 67 positive cells.

IHC x 1250

Plate 104 Section of squamous papilloma showing immuno positive cells all along the core with absence of immune reactivity in mature cells.

IHC x 1250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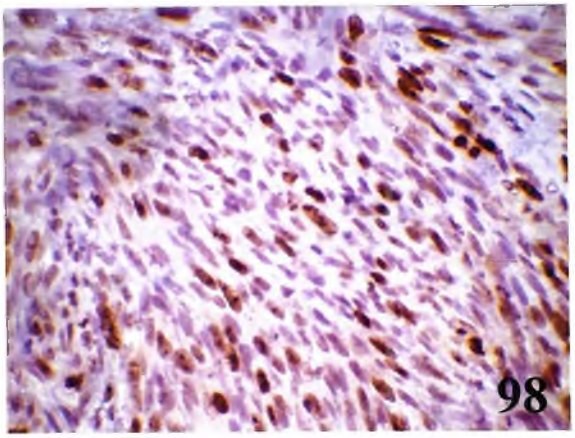
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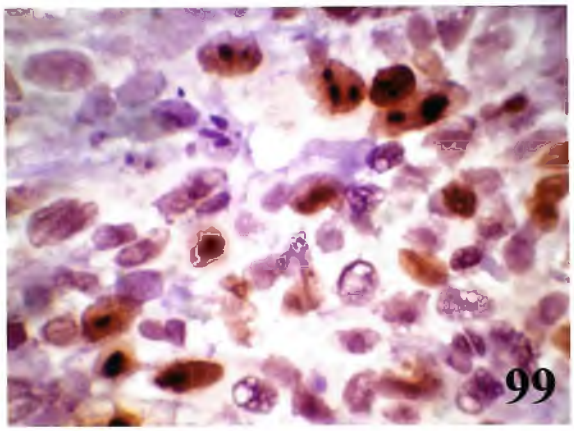
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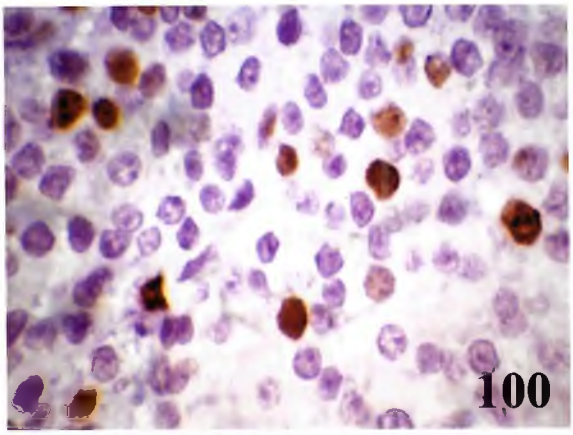
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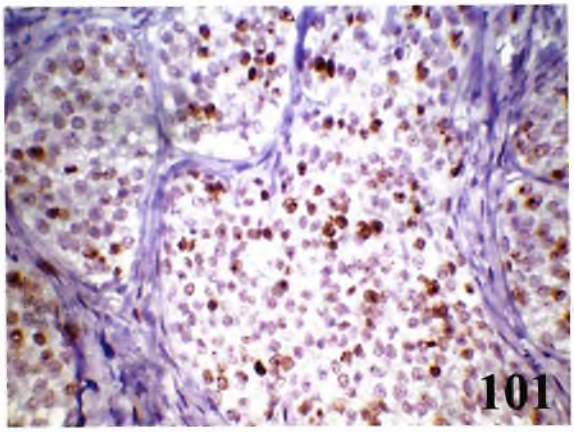
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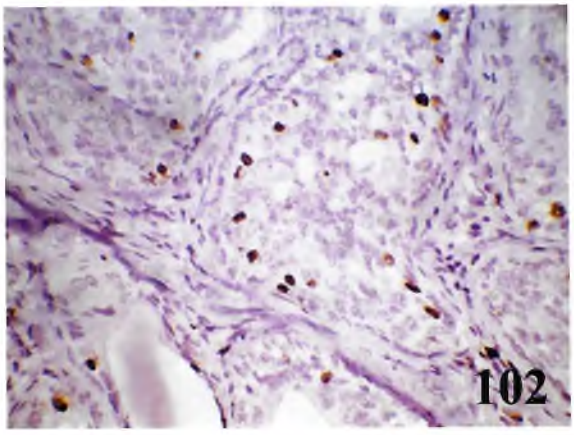
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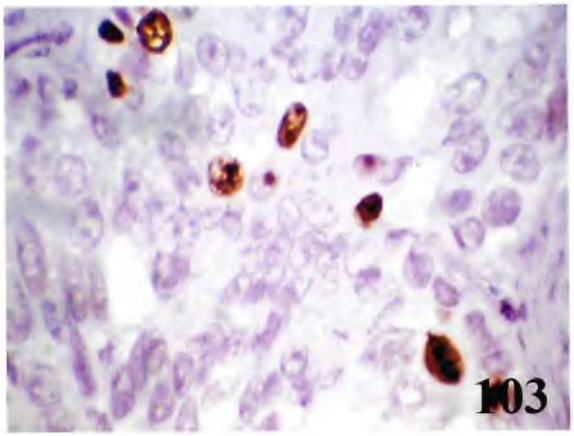
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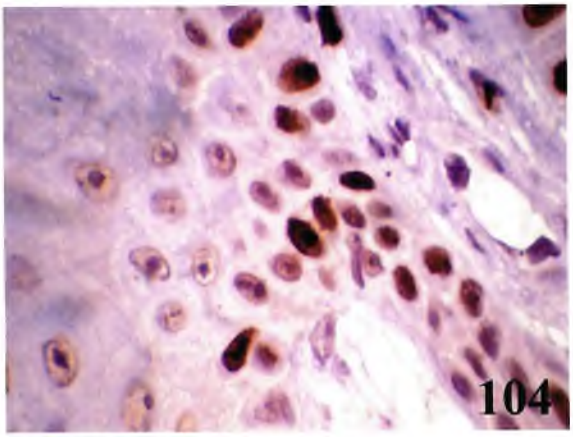
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Plate 105 Section of fibropapilloma showing immuno positive reaction in the lining cells of the squamous component.

IHC x 500

Plate 106 Section of hepatoid gland adenoma showing occasional immuno positive cells among reserve cells which are found peripherally around mature hepatoid cells.

IHCx500

Plate 107 Section of sebaceous gland adenoma showing immuno positive cells at the periphery of islands of neoplastic cells withabsence in the mature cells.

IHCx500

Plate 108 Section of fibrosarcoma showing immuno positive cells with intensly stained mitotic figures.

IHC x 1250

Plate 109 Section of fibrosarcoma showing immuno positive cells

IHC x1250

Plate 110 Section of hemangiosarcoma showing pleomorphic Ki 67 immuno positive cells.

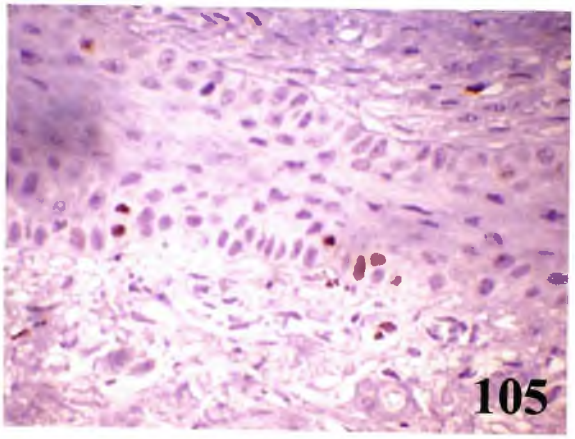
IHCx500

Plate 111 Section of hemangiosarcoma showing very darkly stained pleomorphic immune positive cells.

IHC x 1250

Plate 112 Section of leiomyosarcoma showing occasional immune positive cells.

IHC x 1250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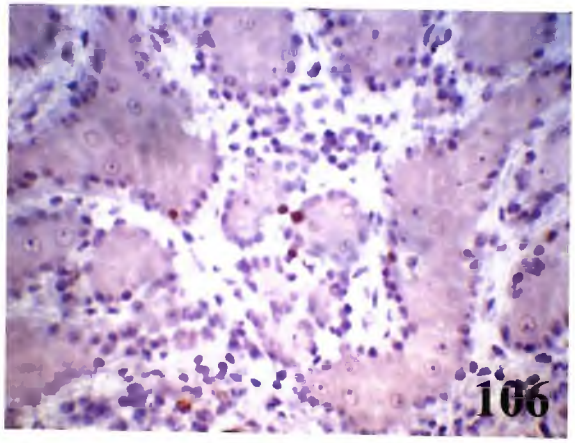
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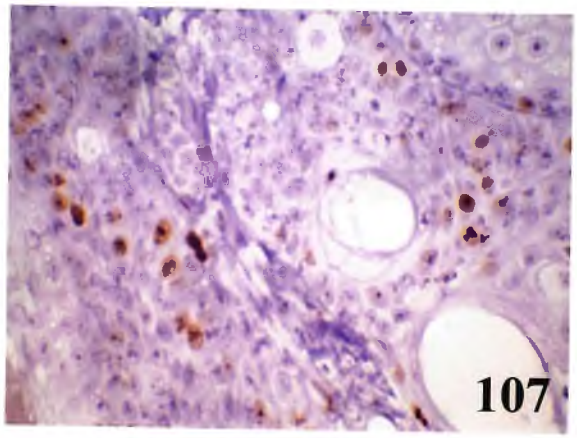
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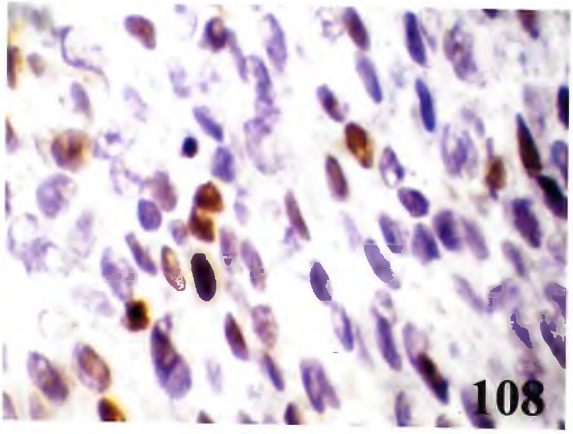
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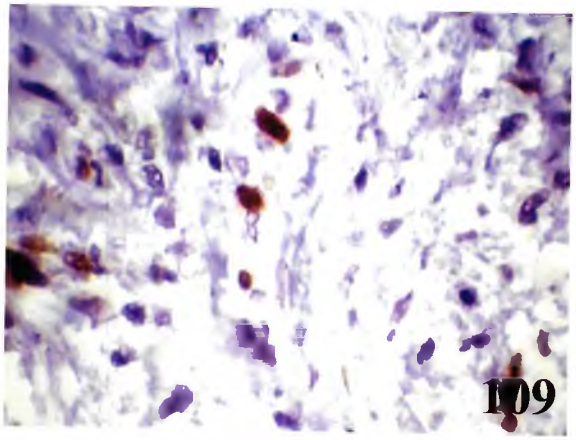
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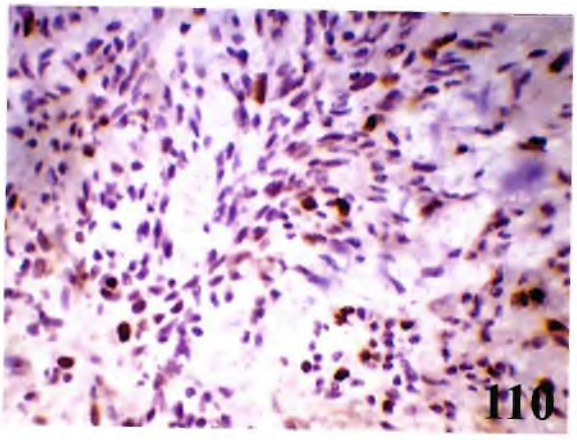
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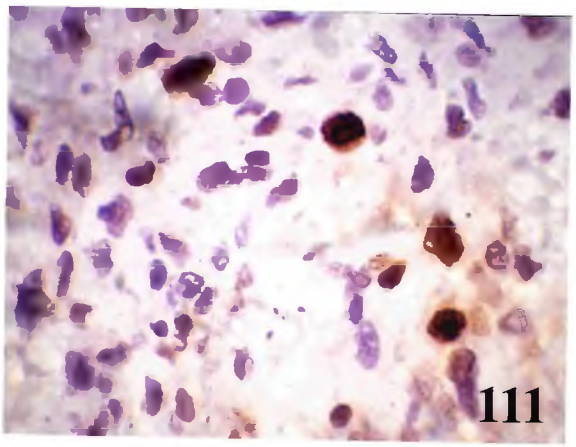
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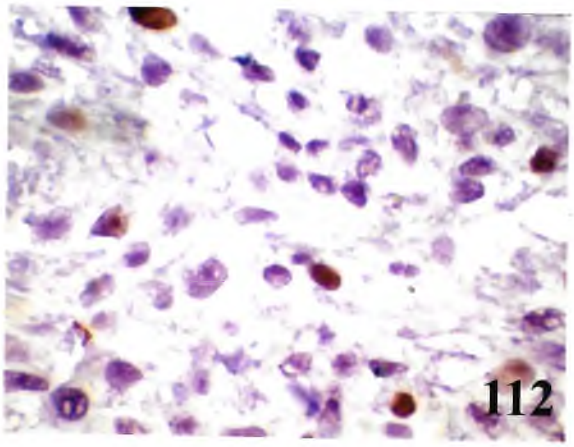
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Discussion

5. DISCUSSION

Neoplastic growths from cutaneous and subcutaneous tissue of dogs were studied during the present investigation. The results of the present study are discussed under the subheadings of macroscopic appearance, cytological and histopathological examination, AgNOR staining and Ki 67 immunohistochemistry of round cell tumours, epithelial tumours and mesenchymal tumours of cutaneous and subcutaneous tissue.

A total of 122 cases of cutaneous and subcutaneous tissue tumours were encountered in the present investigation while screening a total population of 16987 dogs. The percentage incidence of dogs affected with cutaneous and subcutaneous tissue tumours in relation to population was 0.72, which was in acceptance with the findings of the Dorn *et al.,* (1968), Bostock (1986), Chu *et al.,* (1992), Rostami *et al.,* (1994), and Bolos and Baba (2005) who reported that the cutaneous neoplasms were the most common spontaneously occurring neoplasms in canines.

The age of susceptibility of dogs to cutaneous and subcutaneous tissue tumours in the present study varied from 5 months to 18 years with an average age of 7.72 years. The highest incidence of 36.88 per cent of cutaneous and subcutaneous tissue tumours was observed in dogs aged between 6 to 8 years, followed by 20.49 per cent in 8 to 10 years, 13.11 per cent in 4 to 6 years and 10.65 per cent in 10-12 years. The incidence was low in dogs aged less than 4 years and above 14 years. These observations were adequately supported by the findings of Strafuss (1976), Strande (1987), Er and Sutton (1989), Chu *et al.,* (1992), Chiti and Amber (1992) and Bolos and Baba (2005) who observed that prevalence of skin tumours in dogs increased with age and most occurred in dogs above six years. However the study indicated that skin

tumours can occur at any age in canines as it was observed in all age groups.

It was observed in the present study that maximum incidence of cutaneous and subcutaneous tissue tumours occurred in nondescript dogs followed by German shepherd, Labrador Retriever, Pomeranian and Boxer. The other breeds which were affected at a lower grade were Cocker Spaniel, Great Dane, Golden Retriever, Beagle, Doberman, Lhasa Apso, Dachshund, Pug, Dalmatian and Mudhol. The probable reason for high incidence in nondescript dogs could be due to their dense population and higher presentation to various hospitals in the recent years, a finding well supported by the reports of Mathur (2004) and Girish (2004). In addition, occurrences of skin tumours were also reported in different breeds with no predilection to any particular breed by many earlier workers. [Marchevsky *et al.,* (1980), Shakir and sundararaj (1994), Strande (1987), Er and Sutton (1989), Chiti and Amber (1992)]. Based on these observations it could be inferred that incidence of skin tumours probably depends upon popularity of these breeds and their relative number in different geographical area.

In the present study male dogs were found to be highly susceptible to cutaneous and subcutaneous tissue tumours than females. Similar observations have also been made by Shakir and sundararaj (1994), Strande (1987) and Chiti and Amber (1992).

In the present study the cutaneous and subcutaneous tissue tumours were classified based on cytology and histopathology as round cell tumours, epithelial tumours – malignant and benign types and mesenchymal tumours – malignant and benign types. The malignant type of tumours predominated over benign types. Several earlier workers have also encountered in their study on canine cutaneous neoplasms, occurrence of malignant tumours at higher frequency than benign.

(Glavits 1977, Gomes 1987, Swak *et al.,* 1993, Solcan *et al.,* 1998). However, in contrast Frese *et al.,* (1982), Rostami, *et al.,* (1994), Kolodzieyski *et al.,* (1998), Sevcik *et al.,* (2000) and Bolos and Baba (2005), observed more commonly benign cutaneous neoplasms than malignant types. This clearly indicated that there was no preference in the occurrence of the type of cutaneous neoplasms and varied from one geographical area to other.

Among the cutaneous and subcutaneous neoplasms in the present study the epithelial tumours predominated with 71 cases followed by mesenchymal tumours with 28 cases and round cell tumours with 23 cases. Predominance in occurrence of epithelial tumours in skin has also been reported by Marchevsky (1980), Frese *et al.,* (1982), Giesel *et al.,* (1987), Mukhopadhayay and Som (1992), Kolodzieyski *et al.,* (1998) and Mukaratirwa (2005).

Among round cell tumours mast cell tumour was the most common type as also reported by Er and Sutton (1989), Finamor (2004), Mukaratirwa (2005) and Souza (2006) who observed the mast cell tumour as an important and most common cutaneous neoplasm in canine. In the group of malignant epithelial tumours squamous cell carcinoma was the most presented type followed by basal cell tumour in the present study which was also an observation of Mukhopadhayay and Som 1992, Finamor *et al.,* 2004 and Jakab *et al.,* 2006. Among the benign types it was hepatoid gland adenoma as also reported by Frese *et al.,* 1982, Giesel *et al.,* 1987, Shakir and sundararaj 1994 and Anilkumar *et al.,* 1997. In the mesenchymal malignant tumours fibrosarcoma was the most common and among the benign types, it was the hemangioma (Bostock and Dye 1980, Er and Sutton 1989, Mukhopadhayay and Som 1992, Williamson and Middleton 1998).

5.1 Gross, cytology and histopathology of cutaneous and subcutaneous tissue tumours.

5.1.1 Round cell tumours

5.1.1.1 Mast cell tumour

Fifteen mast cell tumours were recorded in the present study. The various locations of occurrence of mast cell tumours were leg, head, neck, back, abdomen and scrotum. The mast cell tumours, which appeared as firm, raised, well circumscribed, single or multiple growths with smooth, alopecic and ulcerated skin coverings and creamy cut surface were similar to those descriptions recorded by Dean (1988), Simoes *et al.,* (1994) and Mathur (1999).

The cytological findings such as high cellularity, pleomorphism with oval or round shaped nucleus consisting of chromatin aggregates, indistinct nucleolus and intracytoplasmic and extracellular pinkish coloured granules observed in this study have also been described by Duncan and Prasse (1979), Pelt *et al.,* (1986), Dean (1988), Tvedten (1994), Dunn and Villiers (1998) and Alleman Bain (2000) and Raskin (2001). Further, Duncan and Prasse (1979), Pelt *et al.,* (1986), Dean (1988) and Nesbit *et al.,* (2002) have also indicated that mast cell tumours could be diagnosed easily based on cytology alone, because of metachromatically stained granules in the cells.

In the present study, in addition a few eosinophils were observed among neoplastic cells. Pelt *et al.,* (1986), Dorn (1968), Dean (1998), Tvedten (1994) and Nesbit *et al.,* (2002) also have observed the presence of eosinophils in cytological preparation and indicated that the eosinophils in cytological preparation were a clue for diagnosis of mast cell tumours.

Microscopically the occurrence of mast cell tumours in the dermis and subcutaneous tissue in compact or loose arrangement consisting of spherical to oval shaped cells, eosinophilic granular cytoplasm, oval to spherical nuclei, dust like purplish coloured cytoplasmic granules in the toluidine blue stained sections, collagenolysis, oedema, muscle atropy and infiltration of eosinophils were in accordance with those of Lund and Park (1978), Patnaik *et al.,* (1982), Gross *et al.,* (1992), Simoes *et al.,* (1994), Yager and Wilcock (1994) and Chenier and Dore (1998). They have been graded as type 1, type 2, and type 3, based on degree of differentiation and correlated with the survival time of dogs by earlier workers (Patnaik *et al.,* 1984, Jaffe *et al.,* 2000, Strefezzi *et al.,* 2003 and Simoes *et al.,* (1994). However such grading of mast cell tumours and it's correlation with survival time were not carried out in the present study as post surgical follow up could not be taken up.

The number of eosinophils that infiltrated the tumour varied in the present study which was in accordance with the findings of Chenier and Dore (1998) who reported that it was dependent upon the expression of P-selectin an adhesion molecule which plays a role in leukocyte recruitment.

5.1.1.2 Histiocytoma

A solitary case of histiocytoma was recorded in the present investigation which appeared grossly well circumscribed, smooth surfaced, alopecic, pinkish red coloured growth was in acceptance with those reported by Goldschmidt and Bevier (1981), Raskin (2001) and Goldschmidt and Hendrick (2002).

Cytological findings such as high cellularity, round or oval shaped cells and eccentrically placed nuclei with minimal ansiokaryosis, indistinct nucleoli, lightly basophilic cytoplasm observed in this study

were in accordance with those described by Duncan and Prasse (1979) and Raskin (2001).

Histopathologically, the section of cutaneous histiocytoma revealed a compact arrangement of solid sheets of round neoplastic cells consisting of nuclei with fine chromatin, indistinct nucleoli and lightly eosinophilic cytoplasm. The fibrovascular stroma was sparse and found separating sheets of proliferating neoplastic cells. Numerous mitotic figures were also observed along with infiltration of inflammatory cells. These findings were in agreement with those reported by Kelly (1970), Weiss (1974), Glick *et al.,* (1976), Bostock (1986), Raskin (2001) and Goldschmidt and Hendrick (2002).

5.1.1.3 Malignant melanoma

In the present study, seven cases of melanomas were recorded. The growths of melanoma were located on eyelid, digit of fore limb, tail region, groin and perineal region. Grossly, the melanomas were firm, moderately hard, round to ovoid blackish in colour with ulcerated surface. [Bostock (1986), Bolon *et al.,* (1990), Goldschmidt (1994), Monteros *et al.,* (2000), Raskin (2001), Smith *et al.,* (2002), Goldschmidt and Hendrick (2002), Mathur (2004), Sapierzynski (2005).

Cytologically, melanomas revealed large number of round to oval shaped cells either occurring individually or in clusters, with cellular pleomorphism and anisokaryosis. The nuclei were round, central to eccentrically placed with occasional prominent nucleoli. In occasional cells, the cytoplasmic pigments were observed which obscured the nucleus. Similar cytologic findings were reported by Alleman and Bain (2000), Smith *et al.,* (2002) and Mathur (2004).

Histopathologically, all the cases were melanotic and malignant showing high cellularity, anaplasia, numerous mitotic figures and varying amount of pigments in the cells.

The types of malignant melanoma included epitheloid, spindle, dendritic and mixed types histologically as also classified by Weiss and Frese (1974) and Smith *et al.,* (2002). In addition the silver stained sections of all the cases of melanomas showed brown to jet black cytoplasmic pigments confirming the tumours as melanotic type which was also reported by Weiss and Frese (1974), Turk and Leathers (1981) and Mathur (2004). Turk and Leathers (1981) in addition, concluded from the findings of their study that the histopathological types of melanoma had no established correlation with the prognosis.

5.1.2 Epithelial tumours

5.1.2.1 Malignant type

5.1.2.1.1 Squamous cell carcinoma

A total of seventeen cases of squamous cell carcinomas were recorded in the present study, which occurred in head, neck, forelimb, hind limb, abdomen, inguinal, groin and perianal regions.

Squamous cell carcinomas occurred either as solitary or multiple proliferative and ulcerative masses (Nielsen and cole 1960, Bostock 1986, Barrie *et al.,* 1982, Yager and Wilcock 1994, Mathur 2004, Girish 2004). These growths were firm, cauliflower like or nodular and pinkish grossly.

Cytologic smears revealed large number of malignant squamous cells, which were round to caudate shaped occurring either individually or in clusters. There was moderate to marked anisocytosis and anisokaryosis with small and pyknotic to large nuclei having prominent nucleoli. The cytoplasm of nonkeratinized cells was moderately to

deeply basophilic, where as in keratinized cells it was bluish green in Giemsa stained smears. In addition cells with binucleation and multinucleation were also observed. Similar findings were also reported by Griffiths *et al.*, (1984), Felizzola *et al.*, (1999), Raskin (2001), Mathur (2004) and Girish (2004).

The predominant number of matured keratinized and anucleated squamous cells cytologically indicated the well differentiation of the tumour,a factor also opined by earlier workers (Burkhard *et al.,* 2001, Raskin 2001 and Girish 2004). Similarly, cells with pleomorphism, ansiokaryosis, binucleation, multinucleation, indicated poor differentiation of the tumour (Raskin, 2001; Mathur, 2004; Girish, 2004).

The presence of keratinized cells with bluish green cytoplasm and tadpole shaped malignant epithelial cells were regarded as helpful criteria in determining the origin of cells by Garma- Avina (1994) and Girish (2004).

The extracellular amorphous pinkish coloured keratin material observed in the present study cytologically was also reported by earlier workers (Burkhard *et al.,* 2001 and Girish 2004).

The cytological smears also revealed a large number of polymorphonuclear cells as most of the tumours were ulcerated with necrotic areas (Anderson *et al.*, 2001, Mathur 2004 and Girish 2004).

Histopathologically, well differentiated squamous cell carcinomas were composed of proliferating neoplastic epithelial cells forming infiltrative cell nests with keratinized centers often in the form of well lamellated keratin pearls and variable amount of connective tissue stroma. Similar observations were also made by Weiss and Frese (1974),

Yager and Scott (1985), Viswanath *et al.,* (1998), Shekar (1999), Mathur (2004) and Girish (2004).

The poorly differentiated squamous cell carcinomas revealed minimal or absence of keratinization with proliferative cell nests consisting of pleomorphic cells showing anaplasia and high mitotic activity. These findings were in acceptance with those reported by Yager and Scott (1985), Raskin (2001), Mathur (2004) and Girish (2004).

The squamous cell carcinoma from the left oral commeasure was spindle cell type, in which the cytology revealed malignant, elongated, spindle shaped cells with oval nucleus and vacuolated cytoplasm with absence of keratinized cells obviously necessitated the histopathological identification of the tumour (Mathur 2004).

5.1.2.1.2 Basal cell carcinoma (Trichoblastoma)

A total of fifteen cases of basal cell carcinoma were recorded in the present study involving head, neck, limbs, thorax, and tail region. Basal cell carcinoma occurred as well demarcated, firm, round to oval shaped growth firmly attached to the skin. The over laying epithelium was smooth and partially haired. (Nielson and Cole, 1960, Seiler 1981, Raskin 2001, Goldschmidt and Hendrick, 2002, Helan *et al.,* 2003).

The cytological smears revealed a moderate number of cells which were arranged in clusters, rows or ribbons. The cells were small, uniform sized, round with monomorphic nuclei, high nuclear to cytoplasmic ratio and basophilic cytoplasm. These findings were in accordance with those reported by Raskin (2001).

Histopathologically, basal cell carcinoma showed different histological patterns such as garland or ribbon, trabecular, solid, medusoid and rosette types. In ribbon pattern the cells occurred as long

cords of one or more cell thickness. In medusoid type the cords of cells streamed outward from the central aggregation of cells. In trabacular type, multiple layers of neoplastic cells surrounded by thin connective tissue stroma were observed. In solid pattern compact arrangement of neoplastic cells in the form of large cords was noticed. These findings were in tune with the observations of earlier workers (Nielson and Cole, 1960, Weiss and Frese 1974, Diters and Walsh 1984, Bostock 1986, Enjung *et al.*, 1995 and Goldschmidt and Hendrick 2002). However, rosette pattern with arrangement of proliferating cells at the periphery giving a flower like arrangement which was encountered in the present study has not been reported by any earlier workers.

However, Seiler (1981) and Goldschmidt and Hendrick (2002) reported granular basal cell tumours characterized by classical basal cells as well as granular cells with numerous cytoplasmic granules, which was not encountered in the present study.

5.1.2.1.3 Adenocarcinoma of hepatoid gland

Six cases of hepatoid gland adenocarcinomas were recorded in the present study. They were roughly spherical and moderately firm in consistency with pink coloured cut surface, which were located at perianal region, base of tail and left lateral abdomen. (Yager and Scott, 1985; Withrow 2001, Anilkumar *et al.,* 1997).

Cytological smears revealed moderate number of cells showing pleomoprhism. Occasional cells resembled hepatocytes and were round, ovoid or caudate in shape. Nucleus contained coarse chromatin and prominent nucleolus. In addition a large number of small sized cells with oval shape and condensed nuclei which resembled reserve cells were also noticed. These findings were in accordance with those reported by Raskin (2001).

Presence of cells resembling hepatocytes, reserve cells cytologically and the location of neoplasm facilitated the diagnosis of hepatoid gland tumours, as also opined by Raskin (2001).

Histopathologically, hepatoid gland adenocarcinoma revealed compactly arranged sheets of highly cellular mass consisting of polygonal to spindle shaped cells, interspersed with a large number of small sized undifferentiated cells which resembled reserve cells. Connective tissue bands comprising numerous small blood vessels and inflammatory cells separated islands of proliferating mass. The proliferating cells were pleomorphic with basophilic nuclei and varying amount of eosinophilic cytoplasm. Similar histological observations have also been made by many earlier workers (Weiss and Frese, 1974; Yager and Scott, 1985; Anilkumar *et al.,* 1997; Villalobos, 2002 and Mathur, 2004).

5.1.2.1.4 Sebaceous gland carcinoma

Three cases of sebaceous gland adenocarcinomas were recorded in the present study which were located at ventral abdomen and lateral thorax region. The tumours were smooth surfaced and were round or oval shaped, lobulated or irregular cystic in appearance. The cut surface of tumour was oily and pale yellow to whitish in colour (Goldschmidt and Hendrick, 2002).

Cytologically, sebaceous carcinoma revealed pleomorphic cells displaying malignant nuclear features such as ansiokaryosis, prominent nucleoli and frequent mitotic figures along with vacuolated cytoplasm. These descriptions were similar to those described by Raskin (2001).

Histopathologically, the sebaceous carcinoma was composed of lobules of varying sizes, surrounded by connective tissue stroma. The lobules contained compactly arranged neoplastic cells with occasional

differentiated sebaceous glandular epithelial cells with intracytoplasmic lipid vacuoles. The number of matured glandular epithelial cells with lipid vacuoles varied with the degree of differentiation. These descriptions tallied well with those of Weiss and Frese (1974) and Goldschmidt and Hendrick (2002).

5.1.2.1.5 Sweat gland adenocarcinoma

Three cases of sweat gland adenocarcinoma were recorded in the present study which were observed at lateral lumbar region, gluteal region and tail. Grossly, they were nodular and dome shaped with lobulated and cystic appearance in the cut surface (Raskin 2001, Goldschmidt and Hendrick, 2002).

The cytological picture of apocrine sweat gland adenocarcinoma showed presence of clusters of basophilic cells with cellular and nuclear pleomorphism, a high nuclear to cytoplasmic ratio and vacuolated cytoplasm. Occasional fibroblasts along with ductular epithelium were also observed. These cytological findings were in accordance with those described by Raskin (2001).

Histologically, sweat gland adenocarcinoma was characterized by numerous papillary projections lined by layers of neoplastic cells which were large, cuboidal, palisadally arranged with eosinophilic cytoplasm. The nuclei were large, vesicular and spherical or oval with one or two nucleoli. Eosinophilic material was observed with in the lumen. The stroma was moderate and showed invasion by proliferating cells. Similar descriptions were also been reported by Nielsen and Cole (1960), Goldschmidt and Hendrick (2002). However Raskin (2001) reported that sweat gland adenocarcinomas were uncommon compared to other cutaneous neoplasms.

5.1.2.2 Benign epithelial tumours

5.1.2.2.1 Squamous papilloma

In the present study five cases of squamous papilloma were recorded which were located in the head region. Grossly, the tumours appeared as papillary growths. The surface was rough and thick (Lenet *et al.*, 1997 and Raskin 2001).

Cytologically, the squamous papillomas revealed moderate cellularity and consisted of keratinocytes which stained deeply basophilic with out nuclei and matured epithelial cells which were round or oval shaped with benign appearing nuclei. Similar findings were also reported by Raskin (2001).

Histopathologically, papillomas revealed papillary projections covered by thick eosinophilic keratin and multiple layers of proliferating epithelial cells over a core of connective tissue stroma. The cells were in the various stages of development from keratinized cells to immature cells. These findings were in tune with those of Kubo (1992), Raskin (2001) and Goldschmidt and Hendrick (2002).

Sapierzynski and Sapierzynska (2005) observed that papilloma was common in dogs and also indicated that papilloma that occurred in young dogs were associated with papilloma viruses. However in the present study, papillomas were not over presented.

5.1.2.2.2 Fibropapilloma

In the present study four cases of fibropapilloma were encountered which were located in fore limb, hind limb, ventral abdomen, and below the eye. They were fleshy, elevated, irregular and nodular growths covered by alopecic smooth skin. The cut surface was whitish to pinkish coloured (Maclachlan and Kennedy, 2002).

Cytologically, the fibropapillomas revealed a low cellularity with fibrocytes and fibroblasts having elongated nucleus and few squamous epithelial cells with benign characteristics.

Histologically, fibropapillomas revealed abundant proliferating fibrous tissue covered by epithelium of various thicknesses with retepegs of the epithelium extending into the fibrous tissue. These observations were in tune with those described by Weiss and Frese (1974), Maclachlan and Kennedy (2002), Bolos and Baba (2005) and Mathur (2004).

5.1.2.2.3 Trichoepithelioma

Two cases of trichoepitheliomas were recorded in the present study which were observed at tail and thigh regions. They appeared as hard, firm, raised and well circumscribed masses (Nielsen and cole, 1960, Raskin 2001).

Cytologic smears revealed keratinocytes, keratin debris and moderate number of epithelial cells resembling basal cells. Similar findings were also reported by Raskin (2001).

Histopathologically, trichoepitheliomas revealed proliferating neoplastic cells which were compactly arranged in multiple concentric layers around the cornified hair follicles. The keratinization was abrupt and the amount of keratinization varied between the proliferating groups of cells. These observations were in accordance with those described by

Nielsen and cole (1960), Weiss and Frese (1974) and Goldschmidt and Hendrick (2002).

5.1.2.2.4 Hepatoid gland adenoma

Hepatoid gland adenoma was the most common benign neoplastic type encountered in the present study as also was the finding of Isitor (1983), Goldschmidt and Schofer (1992), Anilkumar *et al.,* (1997) and Cammarata-Parodi *et al.,* (1998). The tumour growths were roughly spherical, oval or irregular with or with out ulcerations. The skin over nonulcerated tumour was thin and alopecic with pale brown cut surface. The growths were located at perianal region, base of tail and one in thigh region (Bostock, 1986, Goldschimdt and Shofer, 1992 and Goldschmidt and Hendrick, 2002).

Cytological smears revealed a large number of mature round hepatoid cells occurring in clusters or individually which resembled hepatocytes in morphology. The cells were round or polygonal with single or double nuclei placed eccentrically. These findings were in accordance with those described by Tvedten (1994), Raskin (2001) and Alleman and Bain (2002).

Histopathologically, hepatoid gland adenomas revealed neoplastic cells arranged as cords or islands surrounded by anastamosing connective tissue stroma. The cells were polyhedral with large ovoid vesicular normochromatic nuclei with a central small nucleolus and abundant eosinophilic granular cytoplasm. In addition basaloid reserve cells were found arranged at periphery of each lobule which had small hyperchromatic nucleus and scanty cytoplasm. In some cases keratin pearl formation and secondary vascularization were also observed. Similar histological observations have also been made by many earlier workers (Isitor, 1983; Bostock, 1986; Tvedten, 1994; Alleman and Bain, 2000 and Goldschimdt and Hendrick, 2002).

5.1.2.2.5 Sebaceous gland adenoma

Four cases of sebaceous gland adenoma were recorded in the present study and were found located in ear and eye lids. Grossly, the tumours were smooth or cauliflower like raised lesions with alopecic skin covering. The cut surface of the tumour mass was oily and pale yellow to white in colour (Nielson and Cole 1960, Raskin 2001 and Goldschmidt and Hendrick 2002).

Presence of mature sebocytes in clusters or as individual cells characterised by pale foamy cytoplasm and small dense centrally placed nucleus constituted the cytological picture and was in accordance with those described by Raskin (2001).

Microscopically, sebaceous adenomas were characterized by multiple lobules consisting of mature sebocytes separated by connective tissue stroma. At the periphery of the lobule a layer of small basophilic reserve cells consisting of hyperchromatic nuclei and scanty cytoplasm with little or no pleomorphism was observed. The sebocytes contained abundant pale eosinophilic, vacuolated cytoplasm and a small centrally placed dense nucleus. These descriptions well tallied with those of Bostock (1986), Nielson and Cole (1960) and Goldschmidt and Hendrick (2002).

5.1.2.2.6 Sweat gland adenoma

A case of sweat gland adenoma was recorded in the present study which was located at dorsal thoracolumbar region. Grossly, the tumour was soft, raised above the surrounding skin with nonulcerated, alopecic, pigmented skin covering (Goldschmidt and Hendrick, 2002).

Cytological picture of sweat gland adenoma included presence of large number of epithelial cells in clusters with minimum or no cellular and nuclear pleomorphism and other benign characteristics. Cells with

vacuolated cytoplasm and fibroblasts along with ductular epithelium were also observed Raskin (2001).

Histopathologically, the sections of sweat gland adenoma were characterized by glandular structures with narrow cleft like lumina of variable size consisting of one or two layers of lining neoplastic cells which were cylindrical, well defined and had dense eosinophilic cytoplasm and vesicular nucleus placed at the base. These findings tallied well with the observations of Nielsen and Cole (1960), Weiss and Frese (1974), Goldschmidt and Hendrick (2002).

5.1.3 Mesenchymal tumours

5.1.3.1 Malignant type

5.1.3.1.1 Fibrosarcoma

In the present study fibrosarcoma was the most common malignant mesenchymal tumour encountered with 10 cases which was also a finding of many earlier workers (Bostock and Dye 1980, Er and Sutton, 1989, Mukhopadhayay and Som, 1992, Williamson and Middleton, 1998).

Grossly, the fibrosarcoma growths appeared as firm, hard and well circumscribed masses with grayish white cut surface and found located at elobow joint, stifle joint, abdomen and chest region. (Weiss 1974, Bostock, 1986, Raskin, 2001, Goldschmidt and Hendrick, 2002 and Mathur, 2004).

Cytologically, fibrosarcomas yielded a few round or elongated mesenchymal cells occurring either individually or in small aggregates, showing anisocytosis and ansiokaryosis, binucleation, multinucleation prominent nucleolus and intercellular pinkish coloured collagenous material. These observations were in accordance with those described by Felizzola *et al.,* (1999) and Raskin (2001). Raskin (2001), Mathur (2004)

and Tammena *et al.,* (2004) indicated that the plumpy elongated cells with cytoplasmic extensions and pink collagen material intercellularly contribute to identification of fibrosarcomas.

Histopathologically, Weiss (1974), Felizzola (1999) Raskin (2001) and Mathur (2004) recorded broad interlacing bundles of spindle cells with malignant features and variable amount of collagen depending upon the differentiation which were also noticed in the present study.

5.1.3.1.2 Hemangiosarcoma

Hemangiosarcoma has been reported to occur at any site in the body including cutaneous location Culbertson (1982) and Vonbeust *et al.,* (1988). In the present study five cases of hemangiosarcomas were recorded which appeared as soft to firm and red or black in colour with oozing of blood from the cut surface. They occurred at the base of the ear, abdomen, inter digital space of fore limb and the hind limb. Similar location of occurrence were also reported by Culbertson (1982) and Goldschmidt and Hendrick (2002).

The cytological smears showed low cellularity with numerous blood cells, spindle to round shaped pleomorphic neoplastic cells, basophilic cytoplasm with distinct cell borders and occasional vacuolations. Cells with high nuclear to cytoplasmic ratio, oval nuclei with coarse chromatin and prominent multiple nucleoli were also observed. These findings were in conformity with those of Raskin (2001).

Hargis *et al.,*(1992) indicated that hemangiosarcoma could be diagnosed based on major cytologic features such as pleomorphic spindle shaped cells with vesicular ovoid to elongated nuclei lining blood containing channels of various sizes. Also reported that the size of the vascular lumen, presence of thrombi and necrosis also could be considered which are of less significance.

Histopathologically, hemangiosarcomas were characterized by compactly arranged neoplastic cells which ranged from spindle to polygonal to ovoid in shape with occasional vascular spaces consisting of blood cells. The nuclei were hyperchromatic and mitotic cells were frequent. These findings tallied well with observations of Culbertson (1982), Pletcher and Murphy (1984) and Goldschmidt and Hendrick (2002).

5.1.3.1.3 Leiomyosarcoma

One case of leiomyosarcoma located at ventral thorax involving the dermis was encountered in the present study. Grossly, the tumour mass was nodular, roughly spherical in shape and firm in consistency. Similar gross findings were also reported by Cooper and Valentine (2002).

Cytologically, the presence of moderate number of pleomorphic cells that occurred individually as well as in small aggregates, which were round, oval, spindle shaped were in accordance with those described by Borjesson (2001).

Histopathologically, leiomyosarcoma was composed of bundles of spindle shaped cells with elongated nuclei, granular chromatin and abundant eosinophilic cytoplasm forming interlacing fascicles separated by thin collagenous stroma (Burnnert *et al.,* 1990 and Cooper and Valentine, 2002). They also indicated that differentiation of leiomyosarcoma from other connective tissue tumours was difficult and required electron microscopical and immunohistichemical techniques for confirmation.

5.1.3.2 Benign mesenchymal tumours

5.1.3.2.1 Fibroma

In the present investigation two cases of fibroma were recorded which occurred as well circumscribed, round to oval shaped, firm

masses with grayish white coloured cut surface. (Raskin, 2001 and Goldschmidt and Hendrick, 2002).

The cytological smears from fibromas comprised spindle shaped cells with elongated nuclei. The cytoplasm was found trailing from both the ends which was lightly basophilic. In addition, eosinophilic material indicating collagen was also observed. These observations were in conformity with those described by Raskin (2001), Goldschmidt and Hendrick (2002) and Mathur (2004).

Histopathologically, Weiss (1974) and Goldschmidt and Hendrick, (2002) recorded mature uniform sized fibrocytes and abundant collagen which were arranged in repetitive interwoven fascicles and whorls, which were also noticed in the present study.

5.1.3.2.2 Hemangioma

In the present study six cases of hemangiomas were encountered, which appeared grossly as well demarcated, encapsulated masses with bright red to dark brown coloured cut surface, honeycomb appearance and filled with blood. They occurred at ventral thorax, lateral thorax, ventral abdomen, tail and hip regions. Similar findings were also reported by Raskin (2001) and Goldschmidt and Hendrick (2002). Although hemangioma can occur any where in the body, they are reported to occur most frequently in cutaneous tissue. (Vonbeust, 1988)

Cytologically, the presence of low cellularity with numerous blood cells in the background and elongated neoplastic cells with basophilic cytoplasm were in accordance with those described by Raskin (2001). Histopathologically, hemangiomas were characterized by variable sized vascular spaces lined by single layer of endothelial cells and filled with blood cells. The vascular spaces were separated by fibrous connective tissue stroma. In addition, several vascular spaces showed microthrombi

filling up the vascular spaces composed of eosinophilic fibrinous material. These findings tallied well with the observations of Hargis *et al.,* (1992) and Goldschmidt and Hendrick (2002).

5.1.3.2.3 Lipoma

Three cases of lipomas, recorded in the present study, were located at thigh and carpal joint regions. They occurred as soft, circumscribed, dome shaped movable masses with oily, whitish yellow coloured cut surface as also described by Weiss (1974), Knecht and Preister (1978), Raskin (2001) and Mathur (2004).

Cytologically, the smears revealed adipocytes with abundant clear cytoplasm and a small condensed basophilic nucleus placed eccentrically. The cells were well appreciated in Oil O Red stained smears in which the fat appeared red in colour. (Raskin, 2001 and Mathur, 2004).

Histopathologically, lipomas were composed of mature adipocytes with eccentrically placed nucleus and thin connective tissue stroma in between the cells. These findings were in tune with those of Weiss (1974), Knecht and Preister (1978), Stockhaus and Teske (1999) and Mathur (2004)

5.1.3.2.4 Myxoma

One case of myxoma was recorded in the present study which occurred at thoracic region. Grossly, the tumour appeared as raised, soft fluctuant mass with oozing of clear mucoid fluid from the grayish white cut surface. These findings were in conformity with those reported by Grindem *et al.,* (1990), Raskin (2001) and Goldschmidt and Hendrick (2002).

Cytologically, the smears revealed only a few number of fusiform to stellate shaped cells with granular eosinophilic material in the background. Similar cytologic findings were also reported by Raskin (2001).

Histopathologically, proliferation of uniform sized stellate to spindle shaped cells which were loosely arranged in abundant myxoid matrix with less cellularity was recorded, which was in acceptance with those reported by Goldschmidt and Hendrick (2002).

5.2 AgNOR Staining

The proliferative activity provides valuable information on cellular growth kinetics, tumour progression and prognosis (Rieger *et al.,* 1993).

Assessment of proliferative activity of malignant tumours can be done by several methods such as determination of mitotic index (MI), DNA cytometry, flow cytometry, determination of bromodeoxy uridine labeling index, immunohistochemical analysis of proliferation associated antigens such as Ki-67, PCNA and AgNOR index. The AgNOR index has been found to be valuable in determining proliferative activity in several malignant tumours with reproducible results. (Martin, 1994; Krishnamurti and Paliwal, 1998; Hung *et al.,* 2000 and Sarli *et al.,* 2002)

In the present study AgNOR staining was employed to asses the proliferation fraction of cutaneous and subcutaneous neoplasms and also to determine their significance in benign and malignant types.

Nucleolar organizer regions (NORs) are a group of nucleolar proteins necessary for ribose biosynthesis which could be selectively stained by silver staining methods. The nucleolar proteins (nucleolin and protein B23) are involved in rRNA synthesis and in its processing (Derenzini *et al.,* 1998, 2004 and Castagnaro *et al.*, 1998). The AgNOR

proteins progressively increase when resting cells enter the mitotic cycle from G1 to the end of S phase and the amount of AgNOR proteins is directly related to the rapidity of cell proliferation, thus represent a very strong prognostic indicator of neoplastic condition (Derenzini *et al.,* 1998, 2000, 2004).

In the present study 122 cases of cutaneous and subcutaneous tissue tumours were subjected for AgNOR staining to characterize the proliferative activity in the tumours. The NORs in the stained sections appeared as dark brown to black coloured dots or specks distributed within nucleolus or dispersed in the nucleoplasm. Three types of NOR distribution in the nucleus was noted; namely type 1 characterized by presence of a solitary, round argyrophilic structure in resting cells, type II NORs restricted to nucleolus with dispersion of two or more NORs within the nucleolus, observed frequently in proliferating cells and type III, with dispersed NORs as multiple small dots throughout the nucleoplasm found in highly malignant cells. These findings tallied well with the observations of Crocker *et al.,* (1989) and Jelesijevic *et al.,* (2003) who observed similar distribution of AgNORs within neoplastic cells.

The AgNOR count per cell in the present investigation for all the tumours ranged from 2.05 to 6.10. Malignant tumours had an average of 4.21±0.08 AgNOR per cell while benign tumours had a mean of 2.71±0.08 per cell. The statistical analysis of cutaneous and subcutaneous tissue tumours revealed statistically significant ($p<0.05$) difference between the benign and malignant neoplasms. These results were in accordance with the studies of earlier workers with reference to average AgNOR count in malignant and benign tumours. (Simoes *et al.,* 1994, Johnson *et al.,* 1995, Preziosi *et al.,* 1995, Karadimir *et al.,* 1998a, Krishnamurthi and Paliwal 1998, Hung *et al.,* 2000 and Manu *et al.,*

2006) also observed a high mean AgNOR count in various malignant tumours in comparison with benign tumours.

The AgNOR number, their size and heterogeneity have been reported to represent proliferative cellular activity and directly related to the rate of cell duplication. It was also observed that greater the number and quantity of AgNOR, more rapid the cell proliferation and neoplastic mass expansion by Pich *et al.,* (2004), Derenzini *et al.,* (2004) and Manu *et al.,* (2006). These observations of earlier workers coincided well with the findings of the present study and clearly indicated that malignant tumours had high proliferative rate with high AgNOR index in the cutaneous and subcutaneous tissue tumours.

Perusal of literature concerned to AgNOR counts in cutaneous and subcutaneous tissue tumours of canines did not reveal sufficient reports except for occasional ones as for mast cell tumour, fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, perianal gland carcinoma, squamous cell carcinoma, histiocytoma and melanoma. Hence the present observations could not be compared well with that of earlier reports.

In the present study, under the group of round cell tumours histiocytoma had highest mean AgNOR count per cell followed by malignant melanoma and mast cell tumour. In the present study only a single case of histiocytoma was observed hence accounting this tumour as one with high proliferative activity may debatable. However Karademir *et al.,* (1998a) observed an AgNOR index of 5.60 comparable with that of present study in histiocytoma and indicated that it should be differentiated from TVT of canines which would have a higher AgNOR counts.

Many earlier workers have observed AgNOR count to be a reliable factor in differentiating the grades of mast cell tumours in canines and also as a prognostic factor. (Roccabianca *et al.,* 1992 and Kravis *et al.,* 1996).

Among the epithelial malignant tumours, in the present study squamous cell carcinoma had a highest AgNOR count followed by basal cell carcinoma, hepatoid gland carcinoma and others.

Karademir *et al.,* (1996), Krishnamurthi and Paliwal (1998) and Della *et al.,* (2002), determined the AgNOR index in squamous cell carcinoma of canines which correlated well with the findings of present study. They reported that AgNOR count was reliable in determining the proliferative rate in tumours.

Hung *et al.,* (2000) and Preziosi *et al.,* (1995) adopted AgNOR staining for hepatoid gland tumours and indicated that it was a useful technique to measure proliferative activity of tumour cells in general.

Among the benign epithelial tumours squamous papilloma had highest mean AgNOR count. It could be probably due to high rate of turn over that exists normally in the squamous epithelial cells which was adequately supported by high Ki 67 index in squamous papilloma in the present study.

In the group of mesenchymal malignant tumours hemangiosarcoma showed a highest mean AgNOR count followed by fibrosarcoma. Karademir *et al.,* (1998b) and Hung *et al.,* (2000) in their studies on AgNOR counts in fibroma and fibrosarcoma observed a higher index for malignant than benign tumours and concluded that AgNOR index can differentiate benign from the malignant tumours. Similarly Johnson *et al.,* (1995) observed a higher AgNOR count in leiomyosarcoma compared to benign leiomyoma.

Among the benign mesenchymal tumours hemangioma showed a higher AgNOR count and least was observed in myxoma. Several earlier workers have also reported a lower AgNOR index for fibroma in comparison with the counter malignant type. (Karademir *et al.,* 1998b and Hung *et al.,* 2000)

The type of NOR distribution varied between the malignant tumours in the present study. However, most of the malignant types such as histiocytoma, squamous cell carcinoma, sebaceous gland carcinoma, hepatoid gland carcinoma, malignant melanoma, hemangiosarcoma, fibrosarcomas and revealed type III with multiple dispersed NORs in the nucleoplasm. Such cells predominated at the periphery of the tumour mass indicating proliferation and outward spread. The malignant neoplasms also revealed cells with type I and type II NORs which indicated differentiation in to mature types.

5.3 Immunohistochemistry of cutaneous and subcutaneous tissue tumours.

Ki 67 antigen is the most widely assessed proliferation antigen in the field of oncology to determine proliferative activity. It is a non histone highly protease sensitive antigen assembled by polypeptide chain with an molecular weight of 345 and 395 K Dalton nuclear antigen. It is associated with cell proliferation and detectable in the nuclei of the cycling cells which are in G1 S, G2 and M phase but absent in resting cells (G0) and early part of G1 (Gerdes *et al.,* 1991 and Lohr *et al.*, 1997).

The expression of Ki 67 indicates proliferation and there is a good correlation between the Ki 67 reactivity and biological behavior, where in biologically active tumours express high level of KI 67 nuclear antigen. Hence, it has been reported to be immensely valuable in tumour grading and establishing prognosis in a variety of malignancies. [Sarli *et al.,*

(1999), Geraldes *et al.,* (2000), Laprie *et al.,* (2001), Ohora *et al.,* (2004) and Sakai *et al.,* (2002)].

In the present investigation all the cases of cutaneous and subcutaneous tissue tumours were subjected for immunohistochemistry to asses the growth fraction of tumours using Ki 67 MIB 1 antibodies which are specific for proliferating cells. The positive reactivity was observed as dark brown coloured granular material restricted to nucleus with mitotic figures strongly labeled and absence of reactivity in non proliferative cells. The neoplastic cells at the periphery of the tumour showed more positive reaction than at the center as proliferative cells predominate at the periphery during their progressive growth. [Laprie *et al.,* 2001, Sakai *et al.,* 2002 and Ohara *et al.,* 2004).

Ki 67 index in the present study ranged from 2.03 to 51.02 percent for all tumours of cutaneous and subcutaneous tissue. Malignant tumours had a mean Ki 67 index of 23.26 ±1.33 while benign tumours had an index of 8.75±0.97. Statistical analysis of Ki-67 index by Student's t- test revealed a significant difference ($p<0.05$) between the malignant and benign types. The findings of the present study are in concurrence with those of several earlier workers who have also reported the applicability of Ki-67 proliferation index in the differentiating the benign from the malignant neoplasms in humans and animals. [Abadie *et al.,* (1999), Roels *et al.,* (1999), Millanta *et al.,* (2002), Sakai *et al.,* (2002)].

Although there are umpteen reports on the Ki 67 proliferation index of human cutaneous and subcutaneous tumours, perusal of literature revealed only sparse reports in canine tumours. Hence the present observations could not be compared well with that of earlier reports.

In the present study, among the round cell tumours, the Ki 67 index was highest in histiocytoma followed by malignant melanoma and mast cell tumour. An aggressive behavior with DNA polyploidy and increased expression of proliferation antigen has been observed by Rizzardi *et al.,* (2003) in histiocytoma in man, which supports adequately a high proliferative rate observed in the present study in histiocytoma.

Mast cell tumour a most common tumour type of dogs has been investigated by many earlier workers to determine the proliferation rate among different grades by assessment of Ki 67 antigen (Abadie *et al.,* (1999) Sakai *et al.,* 2002 and Scase *et al.,* 2006). They observed significant difference between grade I, II and III and indicated that Ki-67 proliferation index could be considered as a gold standard of endogenous proliferation cell marker and prognostic factor.

In the present study, the mean Ki 67 index for melanoma was 20.21±0.20 which was in acceptance with that reported by Laprie *et al.,* (2001). A high Ki 67 index indicated malignancy and was reported to be associated with poor prognosis and decreased survival time in melanoma. [Roels *et al.,* (1999), Millanta *et al.,* (2002) in canines and Hernberg *et al.,* (1998), Miracco *et al.,* (1998), Kucher *et al.,* (2004) and Mikhail *et al.,* (2005) in man].

Among the malignant epithelial tumours in the present study the Ki 67 index was highest in squamous cell carcinoma, followed by sebaceous gland carcinoma, hepatoid gland carcinoma, sweat gland carcinoma and basal cell carcinoma.

The Ki-67 index of squamous cell carcinoma observed in the present study was in accordance with the reports of Sakai *et al.,* (2001) and Della *et al.,* (2002), who also observed a high proliferation index in squamous cell carcinoma in comparison with other skin tumours such

as trichoepithelioma and basal cell tumour. An high proliferation index in squamous cell carcinoma clearly indicated that it was a potentially aggressive tumour compared to others.

In the present study sebaceous gland carcinoma also had a higher proliferation index a finding also observed by Ohara *et al.,* (2004) in man which was higher than that of squamous cell carcinoma.

In hepatoid carcinoma the proliferative status of neoplastic cells was measured by determining proliferation nuclear antigen (PCNA) by Hung *et al.,* (2000) who reported that the proliferation index was significantly high in hepatoid gland carcinoma in contrast to hepatoid adenoma which was also a finding of present study and indicated that PCNA proliferation index was useful in differentiating benign from malignant tumours.

Basal cell tumours have been reported to bear less Ki-67 proliferation index when compared to squamous cell carcinoma and trichoepithelioma (Sakai *et al.,* 2001). The proliferation index of basal cell carcinoma of present study was in accordance with those, that were observed in basal cell tumour in man by Rossen *et al.,* (1997) and Naeyaert *et al.,* (2001).

Among the benign epithelial tumours the Ki 67 index was highest in squamous papilloma followed by trichoepithelioma and others. The high index in squamous papilloma and trichoepithelioma could be probably due to a high rate of turn over observed in normal squamous epithelial cells of skin and other locations which are the cells of origin of squamous papilloma. Sakai *et al.,* (2001) also observed a large number of basal cells of epidermis and hair follicle in normal skin positive for Ki-67 suggesting a high proliferative activity, which supported the present findings.

In the group of malignant mesenchymal tumours, the mean Ki 67 index was highest in hemangiosarcoma, followed by fibrosarcoma and leiomyosarcoma. These findings were in accordance with those reported by Konomoto *et al.,* (1998), Hoos *et al.,* (2001) Ettinger *et al.,* (2006) in human soft tissue tumours.

Among the benign mesenchymal tumours highest mean Ki 67 index was observed in fibroma and least in myxoma. The low Ki 67 index of benign tumours in the present study was comparable with those reported by Abe *et al.,* (2005) in human hemangioma.

The distribution of Ki-67 positive cells varied between the tumours. In squamous papilloma the immunoreactive cells were observed from basal layer to upper layer all along the core, a finding also reported by Okabayashi *et al.,* (1993) and Copete *et al.,* (1997) in papilloma of man. In fibropapilloma the immunoreactive cells were distributed in the squamous component, in hepatoid gland adenoma and sebaceous gland adenoma, among the reserve cells which were found peripherally around neoplastic cords and among lining cells of glandular structures in sweat gland adenoma. In benign mesenchymal tumours occasional Ki 67 positive cells were observed distributed in the tumour mass.

Among the malignant tumours, the distribution of Ki 67 immunoreactive cells was observed at the peripheral layers of compactly arranged neoplastic nests or cords in sebaceous gland carcinoma, squamous cell carcinoma, hepatoid gland adenocarcinoma and sweat gland carcinoma with absence or a few positive cells at the center. In hemangiosarcoma, fibrosarcoma, malignant melanoma, histiocytoma, mast cell tumour, basal cell tumour, the immunopositive cells were found distributed through out the tumour mass. These findings were in accordance with those of earlier workers [Rossen *et al.,* (1997), Roels *et al.,* (1999), Hoos *et al.,* (2001), Naeyeart *et al.,* (2001), Sakai *et al.,*

(2001), Della *et al.,* (2002), Millanta *et al.,* (2002), Sakai *et al.,* (2002), Rizzardi *et al.,* (2003), Ohara *et al.,* (2004), Mikhail *et al.,* (2005)].

5.4 Correlation of histological type, Ki 67 index and AgNOR index

In the present study the Ki 67 index and AgNOR index of benign and malignant histological types of cutaneous and subcutaneous tissue tumours were positively correlated with each other. Though reports on similar studies by earlier workers is lacking in cutaneous and subcutaneous neoplasms Lohr *et al.,* (1997), Sarli *et al.,* (2002) and Burnetti *et al.,* (2005) in mammary gland tumours and Ettinger *et al.,* (2006) in soft tissue sarcomas observed a positive correlation between benign and malignant tumours.

CONCLUSION

The present study indicated that cytological examination of scraping smears was of value only in tentative diagnosis of cutaneous and subcutaneous tissue tumours of dogs and definitive diagnosis and classification required detailed histological examination.

AgNOR staining was valuable in determination of proliferative activity of cutaneous and subcutaneous tissue tumours. Similarly immunohistochemical detection of Ki 67 proliferation antigen was valuable in determination of tumour growth fraction.

AgNOR and Ki 67 indices could be used for differentiating the benign from malignant tumours, to predict malignant potential and prognosis.

However, further studies along with surgical follow up are necessary to determine prognostic value of Ki 67 index and AgNOR counts in cutaneous and subcutaneous tissue tumours of dogs.

Summary

6. SUMMARY

In the present investigation, a total of 122 tumours from cutaneous and subcutaneous tissue in canines were studied in relation to their occurrence in population, age, breed, site, sex, gross appearance, cytological appearance, histopathological findings, AgNOR staining findings and immunoreactivity to Ki 67 antigen.

The frequency of occurrence of cutaneous and subcutaneous tissue tumour in relation to population was 0.72 and occurred in the age group that ranged from five months to eighteen years with mean age being 7.73 years. The most commonly affected breed was nondescript. Neoplasms occurred more in male dogs (72.95%) compared to female dogs (27.04%).

The tumours were classified based on the cell type as round cell tumours (23), epithelial tumours (71) and mesenchymal tumours (28).

Round cell tumours included mast cell tumour, cutaneous histiocytoma and malignant melanoma. The cytological smears of round cell tumours were highly cellular. Cytology of mast cell tumours revealed round or oval shaped pleomorphic cells, nuclei with clumped chromatin and variable number of distinct pinkish coloured cytoplasmic granules. Histopathologically, mast cell tumours showed pleomorphic cells and metachromatically stained intracytoplasmic granules in toluidine blue stained section.

Cytologically, histiocytoma showed round or oval shaped cells having eccentrically placed nuclei with fine and dispersed chromatin, indistinct nucleoli and lightly basophilic cytoplasm. Histopathologically, cutaneous histocytoma showed solid sheets of round neoplastic cells with numerous mitotic figures. The neoplastic cells consisted of nuclei with fine chromatin, indistinct nucleoli and lightly eosinophilic cytoplasm.

Malignant melanoma was characterized by large number of pleomorphic cells with anisokaryosis and cytoplasmic melanin pigments cytologically and microscopically highly proliferative group of cells with brownish to black coloured melanin pigment in the cytoplasm.

Squamous cell carcinoma was characterized by presence of pleomorphic round to caudate shaped squamous cells with anisokaryosis and small and pyknotic to large, round and immature nuclei with prominent nucleoli and showed high N:C ratio cytologically and microscopically keratin pearls, individual keratinized cells and proliferating cell nests were observed.

Cytologically, basal cell carcinoma revealed small, uniform sized, round cells arranged in clusters, rows or ribbons with monomorphic nuclei, high nuclear to cytoplasmic ratios and basophilic cytoplasm. Histopathologically, neoplastic cells with garland or ribbon, trabecular, solid, medusoid and Rosette patterns of arrangement were observed.

Cytology, of hepatoid gland adenocarcinoma revealed pleomorphic cells resembling hepatocytes with coarse chromatin, variable N:C ratio and reserve cells. Microscopically, the tumour was characterized by compactly arranged sheets of highly cellular mass consisting of polygonal to spindle shaped cells, interspersed with numerous reserve cells.

Cytologically, sebaceous gland carcinoma revealed pleomorphic cells displaying malignant nuclear features, frequent atypical mitotic figures and vacuolated cytoplasm. Microscopically, the tumours were characterized by lobules of varying sizes containing compactly arranged neoplastic cells with occasional differentiated cells with intracytoplasmic lipid vacuoles.

Cytologically, sweat gland adenocarcinoma was characterized by large number of ductular epithelium which occurred as clusters of

basophilic cells with cellular and nuclear pleomorphism. Microscopically, it revealed numerous papillary projections containing double layers of proliferating neoplastic cells.

Cytologically, squamous papilloma revealed moderate number of keratinocytes which stained deeply basophilic with out nuclei and round or oval shaped matured epithelial cells with benign appearing nuclei. Microscopically, it revealed a core of dermal stroma covered by thick eosinophilic keratin and multiple layers of proliferating epithelial cells.

Cytology of fibropapilloma revealed less number of fibrocytes and fibroblasts having elongated nucleus with few squamous epithelial cells, displaying benign characteristics. Histology of fibropapillomas revealed abundant proliferating fibrous tissue covered by epithelium of various thickness with retepegs of the epithelium extending into the fibrous tissue.

Cytologically, trichoepithelioma was characterized by keratinocytes, keratin debris and moderate number of epithelial cells resembling basal cells. Microscopically trichoepitheliomas revealed proliferating neoplastic cells, arranged in multiple concentric layers around the cornified hair follicle which consisted of keratin material and melanin pigments.

Cytology of hepatoid gland adenoma revealed large number of mature round hepatoid cells in clusters or as individual cells occasionally small sized cells which resembled reserve cells with a high nuclear to cytoplasmic ratio. Microscopically hepatoid gland adenomas revealed hepatoid cells arranged as cords or islands surrounded by and anastamosing connective tissue stroma with secondary vascularization and keratin pearl formation.

Cytologically, sebaceous adenoma revealed mature sebocytes in clusters or as individual cells characterized by pale foamy cytoplasm and

small dense centrally placed nucleus. Microscopically sebaceous adenomas revealed multiple lobules consisting of mature sebocytes separated by connective tissue stroma and at the periphery of the lobule a rim of small basophilic reserve cells were observed.

Sweat gland adenoma showed presence of large number of basophilic ductular epithelial cells in clusters with minimum or no cellular and nuclear pleomorphism. Histopathologically, glandular structures with narrow cleft like lumina consisting of one or two layers of regularly arranged neoplastic cell with benign characteristics were observed.

Malignant mesenchymal tumours included fibrosarcoma, hemangiosarcoma, leiomyosarcoma.

Fibrosarcoma was characterized by presence of individual or cluster of plumpy cells with cytoplasmic extensions from ends, occasional multinucleated cells and pinkish material in the intercellular area cytologically, and interlacing bundles of spindle to stellate shaped cells with elongated plumpy nuclei histopathologically.

Cytologically, hemangiosarcomas showed low cellularity with numerous blood cells. Nepolastic cells were pleomorphic, spindle to stellate shaped with basophilic cytoplasm and oval nuclei with high nuclear to cytoplasmic ratio.

Histopathologically, hemangiosarcoma showed compactly arranged spindle, polygonal to ovoid shaped neoplastic cells with occasional vascular spaces consisting of blood cells with hyperchromatic nuclei and frequent mitotic figures.

Cytologically, leiomyosarcoma comprised moderate number of pleomorphic cells with nuclei showing dispersed chromatin clumps, occurring individually or in small aggregates and histopathologically, bundles of spindle shaped cells with elongated nuclei, granular

chromatin and abundant eosinophilic cytoplasm forming interlacing fascicular pattern.

Fibroma was comprised of spindle shaped cells containing elongated nuclei with trailing of lightly basophilic cytoplasm from both the ends cytologically and matured uniform fibrocytes arranged in wavy interwoven fascicles and whorls with abundant amount of collagen microscopically.

Cytologically, Hemangioma comprised less number of elongated neoplastic cells containing oval nuclei and basophilic cytoplasm with numerous blood cells in the background. Histopathologically, variable sized vascular spaces lined by single layer of endothelial cells, filled with blood cells and microthrombi.

Lipoma was composed of adipocytes with abundant clear cytoplasm and eccentrically placed basophilic nucleus cytologically and almost uniform sized adipocytes with clear fat vacuole and eccentrically placed nucleus histopathologically.

Cytologically, myxoma comprised of less number of fusiform to oval shaped cells with granular eosinophilic material in the background. Histopathologically, loosely arranged, almost uniform sized, stellate to spindle shaped cells in abundant myxoid matrix was observed.

In the present study, 122 cases of cutaneous and subcutaneous tissue tumours were subjected for AgNOR silver staining to characterize the proliferation fraction of the tumours. The NORs in stained sections appeared as dark brown or black coloured dots or specks distributed within nucleolus or dispersed in the nucleoplasm.

Three types of NORs distribution in the nucleus were noted; namely type I characterized by presence of a solitary, round argyrophilic structure in resting cells, type II in which NORs are restricted to nucleolus with dispersion of two or more NORs within nucleolus,

observed frequently in proliferating cells and type III, with dispersed NORs as multiple small dots throughout the nucleoplasm, found in highly malignant cells.

In the present study malignant tumours had an average of 4.21 AgNOR/cell while benign tumours had a mean of 2.71 AgNOR per cell. Statistical analysis of AgNOR per cell in cutaneous and subcutaneous tissue tumour in the present study revealed statistically significant difference between malignant and benign canine cutaneous and subcutaneous tissue tumours.

Among all the round cell tumours, the highest mean AgNOR count per cell was observed in histiocytoma followed by malignant melanoma and mast cell tumour.

In the group of malignant epithelial tumours squamous cell carcinoma had highest mean AgNOR count per cell followed by sebaceous gland carcinoma, hepatiod gland carcinoma, sweat gland carcinoma and basal cell carcinoma.

Among the malignant mesenchymal tumours the mean AgNOR count per cell was highest in hemangiosarcoma followed by fibrosarcoma and leiomyosarcoma.

Among benign epithelial tumours the mean AgNOR count per cell was highest in squamous papilloma followed by trichoepithelioma sebaceous adenoma, hepatoid gland adenoma, sweat gland adenoma and fibropapilloma.

In the group of benign mesenchymal tumours, the mean AgNOR count per cell was highest in hemangioma followed by fibroma, lipoma and myxoma.

In the present study, 122 cases of cutaneous and subcutaneous tissue tumours were subjected for Ki 67 immunohistochemistry to

measure growth fraction. The positive reactivity for Ki 67 was observed as dark brown coloured granular material restricted to nucleus.

In the present investigation malignant tumours had a mean Ki 67 index of 23.26 while benign tumours had a mean Ki 67 index of 8.75. Statistical analysis of Ki 67 index in cutaneous and subcutaneous tissue tumours in the present study revealed statistically significant difference between malignant and benign canine cutaneous and subcutaneous tumours.

Among the round cell tumours the Ki 67 index was highest in histiocytoma followed by malignant melanoma and mast cell tumour.

In the group of malignant epithelial tumours squamous cell carcinoma had highest mean Ki 67 index followed by sebaceous gland carcinoma, hepatoid gland carcinoma, sweat gland carcinoma and basal cell carcinoma.

Among the malignant mesenchymal tumours the mean Ki 67 index was highest in hemangiosarcoma followed by fibrosarcoma and leiomyosarcoma.

The mean Ki 67 index in the group of benign epithelial tumours was maximum in squamous papilloma followed by trichoepithelioma, sebaceous adenoma, sweat gland adenoma, hepatoid gland adenoma and fibropapilloma.

Among the benign mesenchymal tumours the highest mean Ki 67 index was observed in fibroma followed by hemangioma, lipoma and myxoma.

The Ki 67 index and AgNOR index of benign and malignant histological type of cutaneous and subcutaneous tissue tumours were positively correlated with each other and the correlation was statistically significant at alpha =0.05.

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