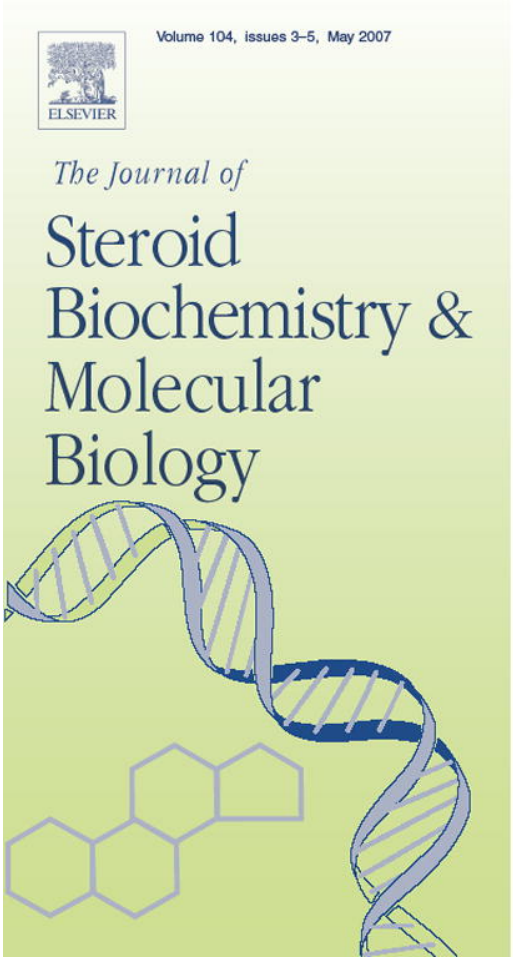

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Steroid pathway and oestrone sulphate production in canine inflammatory mammary carcinoma☆

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Abstract

Spontaneous canine mammary inflammatory carcinoma (IMC) shares epidemiologic, histopathologic and clinical characteristics with the inflammatory breast carcinoma (IBC) disease in humans. We have analysed the steroids levels in serum and in tissue homogenates of IMC, the expression of two of their receptors (androgen and β-estrogen) and of three enzymes included in the steroidogenesis pathway (aromatase (CYP19A1), steroid sulphatase (STS) and estrogen sulfotransferase (EST)) trying to explain the specific accumulation of steroids in IMC tissues generating deposits in the form of lipid droplets whose presence can be attributed to steroids secreted by IMC cells. According to our working hypothesis, oestrone sulphate would be the main component of these lipid droplets. The presence of these steroid deposits would contribute to the intense proliferation and invasive behaviour of IMC and IBC, although their involvement in angiogenesis is yet to be demonstrated.

Keywords: Steroid hormones; Steroid receptors; Oestrone sulphate pathway; Canine inflammatory mammary carcinoma

1. Introduction

Inflammatory breast carcinoma (IBC) is a rare form of human mammary carcinoma considered the most malignant type of breast cancer. IBC has a poor survival rate [1–3] and accounts for 6% of diagnosed breast cancers. Given that nodules are often absent and that chemotherapy is usually rapidly started before surgery, it is difficult to obtain IBC specimens for research purposes. Thus, several IBC cell lines have been established [4]. The use of animal models is another option, and spontaneous inflammatory mammary carcinoma (IMC) in the dog shares epidemiology, histopathology and clinical characteristics with the disease in humans [5–7].

The clinical presentation of IBC or IMC resembles that of an inflammatory process such as dermatitis or mastitis [8]. The only distinctive feature for a histological diagnosis is massive invasion of dermal lymph nodes by neoplastic cells [7], which block lymph drainage provoking the characteristic oedema [8,9]. Highly angiogenic, invasive and metastatic [10] characteristics are common and exclusive to both IBC and canine IMC. In the latter there is also histopathological evidence of lipid droplets, whose formation mechanism is still unclear but whose presence could be attributed to steroids secreted by IMC cells [7].

Two genes are differentially expressed in IBC [4], namely RhoC GTPase, a Ras-related guanosine triphosphatase that is overexpressed in IBC and seems to act as an oncogene, and loss of Wisp3, a tumour suppressor belonging to the IGFBP-rP family [4]. These two specific markers of IBC and other known genes, such as HMG-CoA reductase, H-Nuc/cdc27 and deoxyhypusine synthase, may be involved in breast cancer progression. Although these and other genes probably contribute to the mode of invasion of IBC, steroid formation is crucial for neoplastic growth and progression of inflammatory breast carcinoma.

Neoplastic mammary gland is an endocrine tissue by the local biosynthesis of estrogens [11,12]. Two principal

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pathways are implicated in the last steps of 17β-estradiol formation in breast cancer tissues: the 'aromatase pathway' [13,14] which transforms androgens into estrogens, and the 'sulfatase pathway' which converts estrone sulphate into estrone by the STS [15–17].

The significant role played by the oestrone sulphatase (STS) pathway in human breast cancers has long been suggested by several authors, who demonstrated oestrone sulphate concentrations to be higher in cancerous than in non-cancerous breast tissue [18,19].

Further studies have shown that oestrogens that are locally produced in human breast cancer tissue may contribute to tumour progression [11,12] leading to metastasis, to the extent that STS mRNA expression correlates well with disease-free survival and is an independent prognostic factor for human breast cancer.

Little is known, however, about the mechanisms responsible for steroid hormone production in IBC. Thus, to improve current knowledge on this particular type of breast cancer and using IMC as a natural model for the study of IBC [7] here we compare canine inflammatory mammary carcinoma (IMC) with canine malignant non-IMC mammary tumours (MMT) and with normal mammary tissue, in terms of the specific steroidogenesis characteristics of IMC. For this purpose, we examined androgen and oestrogen receptor expression levels, three enzymes catalysing steroid changes (aromatase (CYP19A1), steroid sulphatase (STS) and estrogen sulfotransferase (EST)) and several steroid hormones (progesterone (P4), dehydroepiandrosterone (DHEA), androstenedione (A4), testosterone (T), 17β-oestradiol (E2) and oestrone-sulphate (SO4E1)). Our findings reveal the atypical behaviour of IMC characterised by the production of lipid droplets.

In non-inflammatory breast carcinomas, high levels of oestrogens formed through the action of oestrone sulphatase on oestrone sulphate have been described, leading to the build up of non-conjugated oestrogens [14]. In IMC, there is also an accumulation of steroids but these, however, seem to be mainly oestrone sulphate. According to our working hypothesis, oestrone sulphate would build in this type of inflammatory carcinoma to generate deposits up because of the low expression of STS in the form of lipid droplets. The presence of these deposits would in turn contribute to the intense proliferation and invasive behaviour of IMC and IBC, although their involvement in angiogenesis is yet to be demonstrated.

2. Materials and methods

2.1. Animals and sampling procedure

Mammary tissue specimens were surgically obtained from 23 bitches recruited over a 2-year period: 6 with spontaneous malignant (histological malignant grade III) non-IMC mammary tumours (MMT), 11 with spontaneous IMC, and 6 control animals with no history of any disease. The stage in the oestrous cycle at the time of sampling was established by taking vaginal smears and only animals at anoestrus were included in the study. Three tissue fragments were separated from each specimen and fixed in buffered formalin (to be processed for histopathology and immunohistochemistry), frozen (for enzyme immuno-assay), or preserved in RNA *later* (SIGMA), for expression analysis by real time PCR (RT-PCR).

2.2. Histopathology and immunohistochemistry

Histopathological diagnoses and immunohistochemical staining for the oestrogen β receptor (ERβ) and androgen receptor (AR) were performed according to previously described procedures [20]. Briefly, tissue samples were fixed in formalin, embedded in paraffin and cut into 4-μm thick sections. Deparaffined sections were then immunolabelled for ERβ and AR by the streptavidin–biotin complex peroxidase method. The primary antibodies used were the rabbit polyclonal antibodies antiERβ (dilution 1:50) (Upstate Biotechnology) and antiAR (dilution 1:15) (Neomarkers), corresponding to the N-terminal domain of both receptors (manufacturer's information). Labelled cells were counted with the help of a computer-assisted image analyser (Olympus Microimage TM image analysis, software v4.0) and a tumour was considered positive when more than 10% of the cells were receptor positive in five microscopy fields (20×) selected at random. The intensity of immunostaining was also scored simultaneously by two observers as low, moderate or intense depending on the staining shown by most of the nuclei.

2.3. Steroid concentrations in serum and tissue homogenates

Frozen tissue specimens were homogenized in 4 ml of PBS (pH 7.2) and centrifuged at 3500 rpm, for 20 min at 4 °C. Progesterone, dehydroepiandrosterone, androstenedione, testosterone, oestrone sulphate and 17β-oestradiol were determined by enzyme-immunoassay in the tissue homogenates and also in serum samples.

2.4. RNA extraction and cDNA synthesis

Total RNA was extracted using a commercial kit (RNeasy® Midi Kit, QIAGEN) from breast tissue samples, preserved in RNA *later* (SIGMA) and frozen at −80 °C. After checking its integrity, the RNA was aliquoted and preserved in RNA secure™ Reagent (AMBION) at −80 °C. The isolated RNA was reverse transcribed using the iScriptTM cDNA Synthesis Kit (BIORAD) in a standard mixture containing 2 μl of total RNA in a 20 μl reaction volume. The heat conditions for reverse transcription were 25 °C for 5 min, 42 °C for 30 min, 85 °C for 5 min and finally, rapid cooling to 4 °C.

Table 1
Primers used for the RT-PCR analysis

Primer	GenBank no.	Sequence	T^a melting (°)
ERβ-N	AF197950	Forward: 5′-GCTACCTGGCTCTGGATGAG-3′; reverse: 5′-AGTCATTCTCGTCCGGTGGT-3′	67.2
ERβ-Z	AF197950	Forward: 5′-CCAAGTTCAAAGAGGGATGC-3′; reverse: 5′-CTTCACACGACCAGACTCCA-3′	65.2
ERβ-C	AF197950	Forward: 5′-GGATGGAGGTGCTAATGGTG-3′; reverse: 5′-GAACGAGGTCTGGAGCAAAG-3′	62
AR-N	AJ313196	Forward: 5′-GCTACCTGGCTCTGGATGAG-3′; reverse: 5′-AGTCATTCTCGTCCGGTGGT-3′	61.6
AR-Z	AJ313196	Forward: 5′-GTCTGATCTGCGGTGATGAA-3′; reverse: 5′-CTTCACACGACCAGACTCCA-3′	60
AR-C	AJ313196	Forward: 5′-GACCAGATGGCAGTCATTC-3′; reverse: 5′-AGGTCAGGGGCGAAGTAGAG-3′	61.6
Aromatase	XM540989	Forward: 5′-CCTCGTGCGTATGGTAACAG-3′; reverse: 5′-ACCCAGTTCGTTGCTGACTT-3′	61.6
STS	XM548847	Forward: 5′-ATGACCTGATGCCGCTACTT-3′; reverse: 5′-GAACTTGGGCGTGAAGAAGA-3′	61.6
EST	XM845548.1	Forward: 5′-GTGAGCGAGATCCTGGACAT-3′: reverse: 5′-AATCCCTGGAGCCTTGAACT-3′	61.7
GAPDH	NM001003142	Forward: 5′-TGCACCACCAACTGCTTGGC-3′; reverse: 5′-GGCATGGACGGTGGTCATGAG-3′	62.7
β-Actin	XM536888	Forward: 5′-CTGGAACGGAGAAGGTGACA-3′; reverse: 5′-AAGGACTTCCTGTAACAATGCA-3′	62
18S	AY262732	Forward: 5′-CGGCTACCACATCCTATGAA-3′; reverse: 5′-TGGAGCTGGAATTACCGCGG-3′	60

Indicated are the primer name, GenBank Accession Number, primer sequence and melting temperature used.

2.5. Primer design

Primers were designed based on GenBank sequences for AR, ERβ, CYP19A1, STS, EST, glyceraldehyde-3-phosphate deshydrogenase (GAPDH), 18S rRNA and β-actin using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3-www.cgi) and the Oligo Analysis and Plotting Tool (QIAGEN; http://www.operon/oligos/toolkit.php). The sequences for these primers are provided in Table 1. We examined all three domains (N-terminal, DNA binding and steroid binding) of the AR and ESRβ receptors.

2.6. Analysis of mRNA expression by RT-PCR

Messenger RNA expression reactions were performed in an iCycler TM Thermal Cycler (BIORAD) using the fluorescent dye SYBR Green I (Molecular probes). Two microliters of each reverse transcription reaction diluted 1/10 were amplified using AmpliTaq Gold DNA polymerase (Applied Biosystems). The following concentrations were used for PCR: 1× PCR Buffer II, 2 mM $MgCl_2$ solution, 0.8 mM deoxynucleotide triphosphate, 0.3 μM each of forward and reverse primers, SYBR Green diluted 1:10,000 in sterile water from a 10,000× stock solution and 0.2 U/reaction of polymerase. Buffer blanks contained all the reaction components without cDNA. All samples were run in duplicate. The cycling conditions were: (stage 1) 95 °C for 8 min and (stage 2), 45 cycles at 95 °C for 30 s, 61.6 °C for AR, CYP19A1 and STS, 67.2 °C for ESRβ and 61.7 for EST, for 15 s and 72 °C for 40 s. In the final cycle (stage 3), the products were heated slowly to 95 °C to allow fluorescence measurements to be used to generate a dissociation curve from which the degree of product purity was assessed. This was accomplished by confirming the presence of a single peak at the known product melting temperature and the absence of any primer dimers that may generate a peak at a lower temperature [21].

Standard curves were produced from serial dilutions of samples and used to evaluate the consistency of the enzyme reactions for each gene. The house-keeping genes used to check for variations due to RNA isolation in samples and PCR efficiency were 18S rRNA, GAPDH and β-actin.

2.7. Statistical analysis

Data obtained by real time RT-PCR were log transformed using the qBase system [22]. All dependent variables (either expression and immunohistochemistry data) were adjusted through a linear model with a single independent variable which was the canine tissue type with three levels (normal, MMT and IMC). Independent ANOVAs were carried out for each variable to test significant differences among the three canine tissue groups. All data were processed using the PROC GLM with SAS system. Immunohistochemical data were expressed as low, moderate and high staining intensities. These categorical variables were analysed by Pearson and Yates χ^2-tests. The grade of statistical concordance between AR and ERβ positive/negative immunostaining was analysed by the κ-test. For all statistical comparisons, the level of significance was set at $p < 0.05$.

3. Results

Mean values for hormones, hormone receptors and enzymes measured by enzyme-immunoassay, immunohistochemistry and quantitative PCR in different mammary tissue samples corresponding to normal, canine malignant non-IMC mammary tumours (MMT) and inflammatory mammary carcinoma (IMC) are shown in Table 2.

Serum levels of the hormones dehydroepiandrosterone (DHEA), androstenedione (A4), testosterone (T), and oestrone sulphate (SO4E1) were significantly higher in the IMC tissue specimens than in the non-IMC tumours. 17β-Oestradiol concentrations were significantly lower in IMC specimens compared to non-IMC specimens (MMT). No significant differences were observed in P4 levels. In tissue homogenates, the levels of all the steroid hormones (P4,

Table 2
Means and standard deviations results of the individual ANOVA analysis performed to find significant differences for dehydroepiandrosterone (DHEA), androstenedione (A4), testosterone (T4), progesterone (P4), 17β-oestradiol (E2) and oestrone sulphate (SO4E1), immunohistochemistry (IHC) and RT-PCR data for ERβ-IHC, ERβ-N (N-terminal domain), ERβ-Z (DNA binding domain), ERβ-C (steroid binding domain), AR-IHC, AR-N (N-terminal domain), AR-Z (DNA binding domain), and AR-C (steroid binding domain), and RT-PCR data for CYP19A1, steroid sulphatase (STS) and estrogen sulfotransferase (EST) variables recorded on normal, canine malignant non-IMC mammary tumours (MMT) and inflammatory mammary carcinoma (IMC) tissue specimens

	Normal		MMT		IMC		R^2	p-value
	Means	S.D.	Means	S.D.	Means	S.D.		
Androgens								
DHEA serum	4.25[a]	1.55	7.08[a]	0.44	13.17[b]	3.36	0.67	<0.0001
A4 serum	1.48[a]	0.70	2.23[a,b]	0.41	3.92[b]	1.59	0.40	0.0113
T4 serum	8.51[a]	3.59	20.45[b]	0.63	37.30[c]	10.76	0.66	<0.0001
DHEA tissue	72.07[a]	44.03	245.54[a]	24.98	676.79[b]	173.73	0.79	<0.0001
T4 tissue	0.61[a]	8.06	48.90[b]	0.66	274.79[c]	32.07	0.96	<0.0001
A4 tissue	41.6[a]	15.77	136.7[a]	24.91	1027.3[a]	1221.68	0.28	0.1246
Progestagens								
P4 serum	0.22[a]	0.12	0.23[a]	0.28	0.26[a]	0.09	0.01	0.85
P4 tissue	1.23[a]	1.72	4.79[b]	0.15	15.5[c]	3.01	0.87	<0.0001
Estrogens								
E2 serum	60.73[a]	39.78	202.06[b]	11.95	153.39[c]	25.80	0.72	<0.0001
SO4E1 serum	1.37[a]	0.74	3.01[b]	0.25	6.20[c]	1.18	0.81	<0.0001
E2 tissue	150.65[a]	29.73	278.31[b]	34.853	627.01[c]	97.09	0.88	<0.0001
SO4E1 tissue	405.10[a]	84.07	1023.30[a]	45.09	2.385.30[b]	950.21	0.58	0.0005
ERβ								
ERβ-IHC	Low 0/6, moderate 3/6, intense 3/6	1.14	Low 3/5, moderate 1/5, intense 1/5		Low 2/9, moderate 4/9, intense 3/9	0.78	0.13	0.18
ERβ-N	0.57[a]	0.58	0.20[a]	0.19	1.37[a]	1.60	0.21	0.14
ERβ-Z	0.84[a]	1.26	2.03[a]	2.75	1.14[a]	2.48	0.05	0.67
ERβ-C	0.48[a]	0.60	1.30[a]	1.73	0.34[a]	0.47	0.15	0.28
AR								
AR-IHC	Low 3/6, moderate 3/6, intense 0/6	0.83	Low 4/6, moderate 2/6, intense 0/6		Low 2/10, moderate 3/10, intense 5/10	0.82	0.31	0.03
AR-N	105.97[a]	85.82	20.36[a]	16.47	10.59[b]	65.74	0.47	0.0029
AR-Z	947.80[a]	757.47	121.7[b]	42.45	25.10[b]	20.74	0.54	0.0006
AR-C	0.86[a]	0.35	0.67[a]	0.56	0.12[b]	0.14	0.49	0.0015
Enzymes								
CYP19A1	6.00[a]	45.84	42.49[a]	3.75	79.57[a]	190.07	0.05	0.59
STS	121.73[a]	106.40	57.93[a,b]	121.21	8.00[b]	7.41	0.27	0.049
EST	49.39[a]	65.65	12.22[a]	7.53	13.58[a]	25.46	0.17	0.18

The R^2 (variability explained by the model) and the p-values for each variable are also shown. Tissue steroid levels are expressed in μg/g (except for E2 and T which are expressed in ng/mg) and serum steroid levels in ng/ml (except for E2 and T which are expressed in pg/ml). Immunohistochemical data are expressed as labelling intensity scores as low, moderate and intense. RT-PCR data are expressed as values normalised to three house-keeping genes (18s, GAPDH, β-Actin). Means with different letters (a–c) in the same line denote statistical differences ($p < 0.05$).

DHEA, A4, T4, SO4E1 and E2) for each IMC specimen were roughly three-fold the mean values recorded for the MMT and control specimens.

It should be noted that we did not determine the oestrogen α receptor in our specimens since in a previous study in which 33 cases of canine IMC were examined [7,20], no tumour specimen was ERα-positive, and for Q-PCR there was no signal of growing curve. No significant differences among specimens were observed in oestrogen receptor β levels, as determined by immunohistochemistry, nor in the expression of the receptor determined by quantitative PCR. Using this last procedure, the three domains, N-terminal, DNA binding and steroid binding, were independently analysed and all three were coincident in the lack of significant differences between the three groups of samples.

In contrast, when we compared the expression of the androgen receptor among the tissue specimens, significant differences emerged for all the domains analysed. Despite lower expression levels of all the domains detected in the IMC tumour specimens, these differences were especially significant for the steroid-binding domain ($p < 0.0015$) compared to the MMT and control tissue specimens. This lower expression of the androgen receptor was, nevertheless, not confirmed by our immunohistochemical findings, in that significantly higher labelling intensities were observed in the IMC compared to the MMT specimens.

We also examined the expression in the tissue samples of the enzymes catalysing the different reactions involved in converting androgens to oestrogens (aromatase or CYP19A1), oestrone sulphate to oestradiol or oestrone

(steroid sulphatase or STS) and oestrone to oestrone sulphate (estrogen sulfotransferase or EST). The results shown in Table 2 indicate significantly reduced STS expression in IMC specimens compared to control tissue but not to the MMT specimens, despite lower mean levels recorded for IMC. CYP19A1 expression was higher in the IMC samples compared to the remaining specimens, although differences lacked significance probably as a consequence of the high intra-group variability (coefficient of variation = 207.56) shown by these data. Results for EST did not show significant differences among specimens ($p > 0.05$) indicating a similar behaviour of this enzyme regardless of the sample tested.

4. Discussion

Human inflammatory breast carcinoma (IBC) and canine inflammatory mammary carcinoma (IMC) are the most malignant breast cancers; their prognosis is extremely poor, yet their prevalence is low [1–3]. Nonetheless, the current multimodal treatment regimen for IBC, with or without surgery, has improved the disease-free survival and overall survival of patients [10,23]. Fortunately, this type of breast cancer is rare both in humans and dogs, although the prevalence of IMC seems to have increased in the last few decades [7,24], as did the incidence of IBC in women in the 1990s [25]. Clinical presentation resembles that of an inflammatory process such as dermatitis or mastitis [8]. The only distinctive feature for a histological diagnosis is massive invasion of dermal lymph nodes by neoplastic cells [7], which block the drainage of lymph leading to the characteristic oedema [8,9]. Highly angiogenic, invasive and metastatic [10] features are common to both inflammatory breast carcinomas, although in the dog there is also some histopathological evidence of lipid secretion by IMC tumour cells, possibly steroids [7], not described in humans probably due to the unavailability of non-treated tumour specimens. Because of these features common to IBC and IMC, canine inflammatory mammary carcinoma has been proposed as a natural model of IBC [7,20]. This model has the advantage that it provides spontaneously affected non-treated tissue samples not available from humans.

The hormone dependency of breast cancer has been the subject of intense investigation [26]. Both normal and neoplastic breast tissue contains and produces several forms of androgens [27]. Androgens may influence breast cancer risk directly by binding to the AR or indirectly through their conversion to oestradiol [28]. Our results point to the activation of this latter pathway in IMC for two reasons: (a) high androgen production, attributed elsewhere to local synthesis [26,29], was reflected in our experiments by the significantly higher tissue and serum levels of DHEA, A4 and T in IMC compared to MMT and control breast tissues and (b) interestingly all three AR domains analysed showed significantly lower expression in the IMC specimens. These two findings indicate that the high amounts of androgens found here in canine mammary tissue, are proportionally superior to that of androgen receptor, suggesting that either the androgens not used in the tissue would be redirected to the blood, or can be used in the tissue through another mechanism not implicating the androgen receptor, the latter being the hypothesis we would support. Thus, the androgen receptor does not seem to mediate alone the effect of this high production of androgens. This hypothesis points to the conversion of androgens to oestrogens through the action of the enzyme CYP19A1, which tends to be expressed (although non-significantly) in greater amounts in the IMC tissue samples. According to the findings of Zhou et al. [30], who reported anti-proliferative testosterone effects on the mammary epithelium directly mediated by the AR, this high androgen production in IMC not mediated by the AR would render the proliferative effects observed in cases of IMC probably through the conversion of androgens into oestrogens.

Immunohistochemistry data show, however, a labelling intensity in all IMC samples. This apparent contradiction has been detected for other steroid receptors [31,32] and according to Murphy and Watson [33] can be due to the kind of antibody used.

It is well known that oestrogen modulates cell growth and differentiation in the mammary gland and numerous animal studies have shown that this hormone can induce breast cancer [34]. The levels of oestrogens detected in our canine tissues were significantly higher (three-fold) in the IMC compared to the MMT specimens. This notable increase in all the oestrogens measured in IMC tissue can be obtained by a high uptake of steroids from the plasma, or confirms the fact that the neoplastic mammary gland is an endocrine tissue by virtue of its oestrogen and androgen production [11,12,27,28,35]; it can then be hypothesized that these hormones, particularly high in inflammatory mammary carcinoma, can be accumulated and/or used *in situ*.

This finding should correlate with the high expression levels of the oestrogen receptor, which nevertheless failed to differ in the three types of specimen examined. Neither did our immunohistochemical results indicate significant differences between IMC and MMT specimens. Thus, if androgens and oestrogens, whose levels were increased in the IMC tissue samples, are products of local steroid synthesis, the normal expression of the oestrogen receptor beta and diminished expression of the androgen receptor observed would indicate that steroid production follows another pathway. Indeed, the expression patterns shown by the enzymes aromatase, steroid sulphatase and estrogen sulfotransferase, together with the high oestrone sulphate levels detected, suggest the transformation of DHEA, androstenedione and testosterone to oestrone and oestradiol and, in turn, the conversion of oestrone and oestradiol into oestrone sulphate, which appeared in highest amounts in the IMC tissue samples. Since STS expression was, in turn, significantly lower in these specimens, this would suggest no reversal of oestrone sulphate to oestrone or oestradiol, which was confirmed by the lack of substantial amounts of the oestrogen receptor.

Oestrone sulphate could thus be deposited as lipid droplets to act as a reservoir, as has been reported previously [37]. In effect, a high proportion of lipid-rich carcinomas has been described among cases of IMC, but equivalent observations have not been possible in human IBC due to a lack tumour specimens [5].

There have been reports of higher concentrations of SO4E1 in cancerous than non-cancerous breast tissue [18] and this steroid is thought to play an essential role in regulating several key physiological and pathological processes [38]. In addition, good inverse correlation has been observed between intratumoral steroid sulphatase mRNA expression and relapse-free survival in breast cancers [39]. Further, significantly higher concentrations of this steroid have been reported in cancerous tissues than in plasma in postmenopausal patients [19]. The higher concentration [40] and longer half-life of SO4E1 [41] compared to oestradiol [17] suggests a reservoir for the formation of biologically active oestrogens in breast tissue [16]. However, inconsistent with our findings, quantitative determinations made in breast cancer tissue indicate that the conversion of oestrone sulphate into oestradiol through the oestrone sulphatase pathway is 40- to 500-fold that of the conversion of androgens into oestradiol mediated by the aromatase pathway [19]. Despite reports of good correlation between low STS mRNA expression and relapse-free survival in breast cancers [36], our results would suggest a poor prognosis related to low STS expression levels in IMC. Also, our study and previous [20] reflect that a majority of canine carcinomas are ERα−, ERβ+ which would give if compared to human breast cancer and according to Murphy and Watson [33] a specific function which relation with pronostic is difficult to establish.

Our findings clearly indicate that oestrone sulphate levels are significantly elevated in IMC with respect to non-malignant control tissue. This gives rise to a reservoir in the form of lipid droplets, rendering the typical neoplastic appearance of IMC (Fig. 1). In turn, this biologically active oestrogen pool could enhance vascular endothelial growth factor (VEGF) [42], explaining the highly angiogenic and angio-invasive nature of the aggressive behaviour of IMC and IBC. These hypothesis can be also supported by the different expression of the caveolin1 (CAV1) gene detected in IMC tissue specimens (non-published results), a gene encoding a scaffold protein that is the main component of the plasma membrane of caveolae found in most cell types and that promotes cell cycle progression and by the significant correlations between this gene and both receptors and the aromatase. These last events are, nevertheless, yet to be demonstrated.

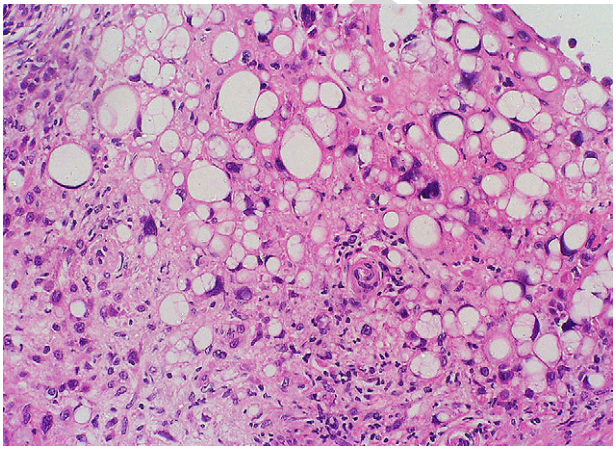

Fig. 1. Canine inflammatory mammary carcinoma. Lipid-rich carcinoma. H–E 40×. Abundant enlarged lipid droplets in the neoplastic cells.

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