Geographic Variation of Environmental Chemicals and Testicular Pathology in Dogs

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Abstract

Geographical differences in the prevalence of human testicular cancer and poor semen quality may reflect differential exposures to environmental contaminants. Here we report on pathology, histology and chemical content of dog testes from three locations in the UK [West Midlands (WM), East Midlands (EM), South East (SE)] and two in Scandinavia (Copenhagen, Denmark and Vantaa, Finland). Compared to the UK, Vantaa testes had fewer pathologies (p≤0.0001), fewer Sertoli cells and reduced germ cell staining for ‘depleted in azoospermia-like’ (DAZL), and proliferation cell nuclear antigen (PCNA) [p≤0.0001: Sertoli, DAZL, PCNA]. Scandinavian testes had relatively higher PBDE concentrations [Vantaa vs SE and EM (p≤0.01), Copenhagen vs EM (p≤0.05)], lower DEHP concentrations (Vantaa vs UK: WM: p≤0.01; SE p≤0.0001; EM: p≤0.05, Copenhagen vs UK: SE: p≤0.05) and lower PCB concentrations (Vantaa vs UK: WM: p≤0.001, Copenhagen vs UK: WM; p<0.01). Compared to Copenhagen, Vantaa had a lower pathological score (p≤0.05) and reduced DAZL staining (p≤0.05). UK PCB concentrations were greater in WM vs SE (p≤0.01). Both negative and positive correlations were observed between specific chemical types and all three cell markers. Disparities in testicular pathology and morphology across geographical locations may reflect differences in the profile of chemicals detectable in testicular tissue.

Introduction

For more than seven decades, the presence of anthropogenic chemicals in the environment and their potential effects on human male fertility has been a significant area of concern. A series of widely cited meta-analyses have reported a temporal decline in human male semen quality and, over the same period, increased incidences of testicular germ cell cancer in younger men and malformations of male babies at birth (cryptorchidism, hypospadias) have been reported. This has led to the suggestion that these abnormalities are linked to exposure to chemical pollutants and that these developmental and functional abnormalities have the same environmental aetiology. Despite the many studies suggesting that temporal changes in male reproductive function reflect environmental change, true cause and effect remains to be demonstrated. Nevertheless, one may predict that if temporal changes in environmental factors are responsible for the time-based changes in male reproductive function, it is likely that geographical differences in contaminating chemicals may similarly be linked to regional differences in fertility and reproductive function.

In support of this contention, temporal declines in semen quality appear specific to the Western industrialised parts of the world such as North America, Europe and Asia. This is exemplified by regional differences in sperm quality across China, and by reports of younger men in Denmark having poorer sperm quality and a higher incidence of testicular cancer than similar populations in Finland. Although attributed to environmental contaminants, few studies have investigated geographical influences on pollutants, particularly those in biological tissues and fluids. Chemical contaminants have however been detected in urine, serum and, to a lesser extent, in seminal plasma. With respect to the latter, some studies report that seminal chemical concentrations negatively correlate with semen quality whereas others indicate that due to the low concentrations present, establishing such a relationship is difficult.

The linkage of post-natal reproductive problems with earlier developmental events, some of which occur in utero, suggests that identified testicular pathologies may be a presage of later functional perturbations. For example, male fetuses from pregnant ewes exposed to mixtures of chemicals in a commonly used fertiliser (biosolids), exhibit reduced numbers of Sertoli and Leydig cells. In addition, a subset of male offspring exposed pre and post-natally exhibit pathologies in their testes such as Sertoli cell-only tubules. Although the weight of evidence suggests that
such early pathologies are linked to perturbed reproductive function in the adult, further work is required to consolidate this theory. Some studies suggest that type two testicular germ cell tumours arise from germ cell neoplasia in situ cells (GCNIS) and recent work suggests that these arise from perturbed differentiation of “very small embryonic like stem cells (VSEL)” \(^{11}\). To our knowledge, no studies have looked at the histology and/or pathology of testes linked to variable degrees of exposure as a result of environmental differences.

We have previously shown that a population of stud dogs, in an assistance dog breeding programme, exhibit similar temporal trends in declining semen quality and increasing incidences of cryptorchidism in the offspring, as reported in the human \(^{12}\). Furthermore, the detection of chemicals in testicular tissue and seminal plasma, at concentrations that inhibit sperm motility \textit{in vitro}, indicate that these trends are environmentally linked \(^{12,13}\). Given the availability of testes from dogs undergoing routine castration for veterinary purposes, the present study was designed to use the dog as an index (sentinel) species of human exposure and to determine if dog testes from different geographical locations exhibit differences in chemical profiles and pathology.

**Results**

**Relative quantification of tissue pathologies.**

Examination of all haematoxylin & eosin (H & E) stained testis sections revealed a range of pathologies across geographic locations including; the presence of luminal cellular debris, Sertoli cell-only tubules, interstitial fibrous hyper-cellularity, vacuolated germ cells and multinucleated cells (Figure 1, Table 1). The histopathological scoring of H & E stained testes from five geographical locations (UK: West Midlands, South East, East Midlands; Finland: Vantaa, Denmark: Copenhagen) revealed a significant difference in the incidence of testicular abnormalities (Figure 2; \(p \leq 0.0001\)). Testes from Finland (Vantaa) had a significantly lower pathology score than all three UK regions; West Midlands \([p \leq 0.001]\), South East \([p \leq 0.001]\) and East Midlands \([p \leq 0.01]\). Testes from Copenhagen (Denmark) were not significantly different to those collected in the UK but exhibited a trend towards reduced testicular health (Figure 2).

Quantification of specific testis pathologies revealed further geographical differences between those originating from the UK and Scandinavia (Table 1). Testes from Vantaa, Finland had lower pathology scores for most pathologies: ‘gross appearance’ (overall histological abnormality), \(p \leq 0.05\) than all UK areas combined; fewer tubular multinucleated cells \((p \leq 0.05)\); fewer atrophic tubules than the South East, UK \((p \leq 0.05)\); less luminal debris than each of the three UK regions (West Midlands: \(p \leq 0.001\), East Midlands and South East: \(p \leq 0.01\)) and less vacuolation (West Midlands: \(p \leq 0.001\), East Midlands: \(p \leq 0.01\)). Testes from Copenhagen, Denmark also generally had lower pathology scores than UK areas: South East (gross appearance: \(p \leq 0.05\); degeneration: \(p \leq 0.001\)), East Midlands (degeneration: \(p \leq 0.05\)) & West Midlands (luminal debris: \(p \leq 0.001\)). Vantaa testes also had fewer vacuolated cells than those from Denmark \((p \leq 0.05)\). No other differences were noted between the two Scandinavia locations (Table 1).

**Table 1:** Overview of testicular pathologies by geographical region.*
| Region | Gross appearance | Tubular MNC | Interstitial MNC | Atrophic tubules | Degeneration | Luminal debris | Vacuolation | SCO tubules |
|--------|------------------|-------------|------------------|-----------------|--------------|---------------|-------------|-------------|
| WM     | 1.88 ± 0.43<sup>ac</sup> | 1.16 ± 0.34<sup>a</sup> | 0.44 ± 0.35 | 1.40 ± 0.37<sup>ab</sup> | 1.76 ± 0.45<sup>abc</sup> | 2.20 ± 0.35<sup>a</sup> | 1.48 ± 0.43<sup>a</sup> | 0.64 ± 0.34 |
| EM     | 2.25 ± 0.58<sup>ac</sup> | 0.67 ± 0.31<sup>ab</sup> | 0.25 ± 0.22 | 1.75 ± 0.58<sup>a</sup> | 2.17 ± 0.45<sup>abc</sup> | 1.08 ± 0.32<sup>ac</sup> | 1.25 ± 0.36<sup>a</sup> | 0.92 ± 0.72 |
| SE     | 2.50 ± 0.59<sup>a</sup> | 0.79 ± 0.43<sup>ab</sup> | 0.43 ± 0.25 | 1.50 ± 0.56<sup>ab</sup> | 2.64 ± 0.64<sup>a</sup> | 2.14 ± 0.70<sup>ac</sup> | 0.71 ± 0.29<sup>ab</sup> | 0.79 ± 0.69 |
| V      | 0.70 ± 0.23<sup>b</sup> | 0.30 ± 0.23<sup>b</sup> | 0.30 ± 0.23 | 0.60 ± 0.24<sup>b</sup> | 1.20 ± 0.30<sup>bc</sup> | 0.80 ± 0.20<sup>b</sup> | 0.30 ± 0.23 | 0.30 ± 0.23 |
| C      | 1.41 ± 0.39<sup>bc</sup> | 0.71 ± 0.23<sup>ab</sup> | 0.59 ± 0.25 | 1.00 ± 0.42<sup>ab</sup> | 1.06 ± 0.40<sup>c</sup> | 1.35 ± 0.24<sup>bc</sup> | 1.24 ± 0.27<sup>ac</sup> | 0.41 ± 0.39 |

*Data denotes the mean pathology count ± 1 S.D; SCO = Sertoli Cell Only; MNC = Multinucleated cells. Differences between superscripts depict significant differences. Bold depicts Vantaa Finland region significantly different to all three UK areas. See text for differences between UK areas.

**Localisation of Sertoli cells, germ cells and testicular cells undergoing proliferation.** Figure 3 depicts examples of immunohistochemical staining of mature dog testes for vimentin, depleted in azoospermia-like protein (DAZL) and proliferating cell nuclear antigen (PCNA). Vimentin positive cells were localised across the seminiferous epithelium with intensity being primarily located to the basal region populated by Sertoli cells [Figure 3A]. DAZL was localised to primary spermatocytes and spermatogonia [Figure 3B] whilst proliferating cell nuclear antigen (PCNA), a nuclear protein localised to sites of on-going DNA replication, was localised primarily to spermatogonia, lining the basement membrane [Figure 3C]. No staining was observed on negative control testis sections incubated with appropriate non-specific IgG antibodies [Figure 3D, E and F: IgG controls for Vimentin, DAZL and PCNA respectively].

**Regional variations in Sertoli cell numbers**

Sertoli cell numbers, adjusted for tubular area, were calculated for each of the UK and Scandinavian locations. Figure 4 illustrates that testes from both Scandinavian locations and those from the West Midlands UK, had fewer Sertoli cells than those collected from the East Midlands [Vantaa: \( p \leq 0.0001 \), Copenhagen: \( p \leq 0.01 \), West Midlands; \( P \leq 0.01 \), South East NS]. Eight of nine South East testes also had fewer Sertoli cells than the mean of the East Midlands cohort.

**Regional variation in testicular immunostaining for vimentin (Sertoli cells), deleted in azoospermia-like-protein (DAZL: germ cells) and proliferating cell nuclear antigen (PCNA).**

Figure 5 depicts the percentage cellular testicular area immunostained with antibodies against vimentin, PCNA and DAZL. For Sertoli cell staining [Figure 5A], Vantaa was found to have significantly less vimentin staining than the UK regions, South East [\( p \leq 0.05 \)] and East Midlands [\( p \leq 0.0001 \)], and the Scandinavian Region, Copenhagen [\( p \leq 0.05 \)]. Testes from Vantaa also expressed a significantly lower percentage area stained for DAZL [Figure 5B] than all other regions [\( p \leq 0.05 \)]. For cellular proliferation [Figure 5C], PCNA immunostaining of germ cells [Figure 3] was significantly lower in testes from Vantaa, Finland compared to all three UK regions; West Midlands (\( p \leq 0.05 \)), South
Regional variation in testicular chemical profiles

The chemical concentrations of diethyl hexyl phthalate (DEHP), sum of polychlorinated biphenyl congeners (PCB: 28, 52, 101, 118, 138, 153, 180) and sum of polybrominated diethyl ether congeners (PBDE: 28, 47, 99, 100, 153, 154, 183) found in the testes of dogs living in five different geographical regions are illustrated in figure 6. A significant difference in chemical profile by region was observed (p ≤ 0.01). Testicular concentrations of DEHP were lower in Vantaa, Finland than in all three UK regions (Figure 6A: West Midlands: p ≤ 0.01; South East p ≤ 0.0001; East Midlands: p ≤ 0.05). Testicular concentrations of DEHP were also lower in Copenhagen than in the South East (p ≤ 0.05). Concentrations of PCB congeners [Figure 6B] were greatest in the West Midlands, with significant differences between this region and; the South East (p ≤ 0.05), Vantaa (p ≤ 0.001) and Copenhagen (p ≤ 0.01). Concentrations of PBDE congeners [Figure 6C] were greatest in Vantaa, significantly higher than in testes from the South East and the East Midlands of the UK (p ≤ 0.01). Dog testes from Copenhagen also had significantly higher concentrations of PBDE congeners compared to the East Midlands, UK (p ≤ 0.05).

Figure 7 depicts the relationship between the immuno-expression of Vimentin, PCNA and DAZL, with the testicular concentrations of ΣPCB congeners (µg/kg), ΣPBDE congeners (µg/kg) and DEHP (µg/g). DAZL (germ cells) positively correlated with DEHP [Fig7A; p ≤ 0.001; r = 0.6216; n=25] and ΣPCB congeners [Fig7B; p ≤ 0.01; r = 0.4676; n=33] but showed no significant correlation with ΣPBDE congeners [Fig7C]. PCNA positively correlated with DEHP [Fig7D; p ≤ 0.01; r = 0.5592; n=25] and ΣPCB congeners [Fig7E; p ≤ 0.01; r = 0.4628; n=33], but negatively correlated with the ΣPBDE congeners [Fig7F; p ≤ 0.01; r = -0.4952; n=33]. Vimentin positively correlated with DEHP [Fig7G; p ≤ 0.01; r = 0.622; n=19], negatively correlated with the ΣPBDE congeners [Fig7I; p ≤ 0.05; r = -0.4574; n=28] but showed no significant correlation with ΣPCB congeners [Fig 7H].

Discussion

Data presented in this paper are significant since they illustrate for the first time that dog testes collected from different geographical locations (UK and Scandinavia) exhibit differences in (1) indices of testicular pathology, (2) Sertoli cell numbers, (3) indices of spermatogenesis and cellular proliferation and (4) testicular chemical profiles. Furthermore, our data indicate that the ΣPBDE congeners negatively correlate with Sertoli cell numbers and proliferative activity primarily in germ cells. Intriguingly, some chemical types positively correlated with DAZL (DEHP, ΣPCB), Vimentin (DEHP) and PCNA (DEHP; ΣPCB). These data suggest that the chemicals detected in testicular tissue may reflect chemicals detected in the immediate environment and that this may have negative implications for male reproductive development and function.

The current study builds on our previous work in which we reported that temporal changes in male reproductive function in a population of stud dogs from a controlled breeding programme, paralleled that reported in the human. Specifically, this was manifest by a decline in semen quality over a 26-year period and male pups from the same population showed an increased incidence of cryptorchidism. We further demonstrated that testes collected from dogs in the same area contain environmental contaminants and that testicular concentrations of these chemicals can adversely affect sperm function in short term cultures. These data suggest that the dog may be a sentinel species for human exposure to contaminants and that reported geographical differences in human male reproductive function may be reciprocated in the dog. Here we have extended our work by assessing chemical
profiles and morphology in dog testes collected from different UK and Scandinavian locations, including Denmark and Finland where differences in human male reproductive health have been reported.

In the human, geographical differences have been reported for three major indices of male reproductive function: reduced sperm counts, increased incidence of testicular germ cell cancer (TGCC) and malformations of male infants at birth (hypospadias and cryptorchidism). This has been the topic of many independent studies and many extensive review articles. Of note is that Denmark has been reported to have a 300% higher rate of testicular cancer compared to Finland, reduced semen quality and a higher rate of reproductive abnormalities.

In the current study, we have used testicular morphology and chemical profiles as a possible index of altered male reproductive function and/or health in the dog. Notably, we report that dog testes from Vantaa, Finland were different to those from Copenhagen, Denmark and the UK in terms of reduced pathology. Although this appears to support human studies indicating a lower relative prevalence of testicular cancer in Finland (vs Denmark), reduced Sertoli and germ cell staining in the same sample set was unexpected. That is, if reduced Sertoli cell numbers are linked to reduced sperm counts, as reported elsewhere, this would be at odds with reports of higher human sperm counts in Finland compared to Denmark. Although sperm counts were not measured in the current study, it is interesting that testicular PBDE concentrations were highest in Scandinavia and that concentrations negatively correlated with staining for both Sertoli and germ cells. One possible explanation for this is that our recent measurements in the dog may reflect different exposures more reflective of the present day. Evidence is emerging that the incidence of human TGCC in Finland, Norway and Sweden is increasing whereas that in Denmark and Iceland has not changed since the 1990s.

In the human, studies suggestive of a linkage between chemical exposure and perturbed male reproductive function have been largely epidemiological. Chemical concentrations in blood, breast milk, urine and to a lesser extent, semen, have been used as an index of environmental exposure. For example, human serum PBDE concentrations have been associated with reduced sperm motility and this was linked to congener PBDE-47: the predominant PBDE congener in dog testes. Furthermore, geographic differences in human blood and breast milk PBDEs have been attributed to differences in diet and exposure within dust. In the current study, dog testes used as an index of environmental exposure have shown higher concentrations of PBDE congeners in dogs from Vantaa and a negative correlation with proliferating germ cell numbers. Although cause and effect is not conclusive, the difference between Scandinavian and UK testicular PBDE concentrations are striking and add to the weight of evidence linking environment to reproductive health. Differences in testicular DEHP and PCB concentrations across regions were similarly most evident in samples from Finland.

In the current study, our chemical analyses focussed on only three chemical types. This is not reflective of real-life exposure to chemical mixtures many of which interact and are influenced by metabolism and physico-chemical properties such as lipophilicity. Whilst many animal models have been used to demonstrate chemical effects, few are applicable to be used as real-life models, which approximate human exposure to chemical mixtures. One such model involves grazing sheep on pastures fertilised with processed human sewage sludge (biosolids) known to contain a wide range of anthropogenic chemicals at low levels. Notably, a cohort of male ram lambs from ewes exposed throughout pregnancy and then exposed post-weaning exhibited testicular abnormalities similar to those described in the current study: reduced germ cell numbers and Sertoli cell only tubules. Reduced Sertoli and germ cell numbers have also been reported in late gestation fetuses and this is viewed as a presage to altered development and reproductive health in adult life.
In conclusion, the dog has been used as a sentinel species to approximate human exposure to chemical mixtures present in the household. Regional influences on human male reproductive health have been linked to differential chemical exposures, therefore geographical differences in testicular chemical content in the dog may also impact on male reproductive health. Although we recognise that correlating testicular chemical content with morphology does not demonstrate cause and effect, we propose that the regional differences in testicular pathology and histology add further support for an environmental influence on male reproductive function.

**Methods**

**Processing of testis**

Whole dog testes, collected from specific UK and Scandinavian geographical locations, were obtained as surplus material from routine castrations performed at veterinary clinics. Testes fixation was undertaken by immersion in Bouin's fixative solution for six hours (Sigma-Aldrich) followed by storage in 70% ethanol until processing. Testes were processed through a 17hr cycle in an automated Leica tissue processor (Leica Microsystems). Five micrometre sections of Bouins-fixed, paraffin-embedded testes were cut using a fully automated rotary microtome (Leica RM2255; Leica Microsystems). Sections were transferred onto polysine slides (CAS: P4981; ThermoFisher Scientific Ltd) and dried overnight at 60°C.

**Ethics**

This research project was approved by the Committee for Animal Research and Ethics, University of Nottingham, School of Veterinary Medicine and Science [Refs: 208 101012, 513 120117 and 1097 140227]. All testes collections were performed in accordance with relevant guidelines and regulations.

**Pathology scoring**

Dog testes originated from five locations [n=77; West Midlands, UK = 25; South East, UK = 14; East Midlands, UK = 11; Copenhagen, Denmark = 17; Vantaa, Finland = 10]. Dog testis tissue sections were stained with haematoxylin and eosin and histopathological analysis was undertaken by light microscopic examination (x 20 objective). Testes were graded on a five-point scale for parameters indicative of abnormal pathology (depicted in Table 2). All testis scores were combined to calculate a median score and this was designated as the baseline against which abnormalities were defined. Since all normal heterogeneous tissues, in normal conditions, will exhibit some abnormal histopathological features, scores above this line were considered atypical.

**Table 2:** Histopathological features assessed on fixed dog testes. The parameters were scored by a five-point scale and grading scale percentages relate to proportion of entire testis. Asterix (*) denotes features assessed qualitatively and by general comment only.
| Classification                  | Distinguishing Feature                                                                 | Grading scale                                                                 | Magnification power |
|--------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|------------------|
| Sub-gross appearance           | What is the overall appearance of testis?                                              | Graded by 0-4 scale whereby 0 = typical and 4 = atypical                      | X 100            |
| Seminiferous tubules: Evidence of Atrophy or degeneration | Tubule size                                                                            | 0 = 0%                                                                        | X 400            |
|                                | Loss of tubular cells                                                                  | 1 = 1-25%                                                                     |                 |
|                                | Luminal diameter                                                                       | 2 = 26-50%                                                                    |                 |
| Cellular degeneration: Sertoli cells | Cytoplasmic Vaculation                                                                    | 3 = 51-75%                                                                    | X 400            |
| Germ cells                     | Multi-nucleation                                                                        | 4 = 76-100%                                                                   | Or              |
|                                | Cell swelling                                                                           |                                                                               | X 630           |
|                                | Spermatogenic arrest                                                                    |                                                                               |                 |
|                                | GCNIS cells                                                                             |                                                                               |                 |
|                                | Cell Sloughing                                                                          |                                                                               |                 |
|                                | Pyknosis                                                                                |                                                                               |                 |
|                                | Sertoli cell only                                                                       |                                                                               |                 |
| Interstitium Degeneration      | Multi-nucleation                                                                        |                                                                               | X 630           |
|                                | Fibrosis*                                                                               |                                                                               | Or              |
|                                | Hypo-cellularity*                                                                       |                                                                               | X 100           |
|                                | Hyper-cellularity*                                                                      |                                                                               |                 |
|                                | Haemorrhage*                                                                            |                                                                               |                 |
|                                | Oedema*                                                                                 |                                                                               |                 |

**Immunohistochemistry**

Ten dog testes from each location (UK: West Midlands, South east, East midlands; Scandinavia: Vantaa, Finland and Copenhagen, Denmark) were immuno-stained for Proliferating Cell Nuclear Antigen (Abcam, [PCNA: ab18197]); Deleted in Azoospermia like factor (Santa Cruz Biotechnology Inc., [DAZL (C-20): sc-27333]) and Vimentin (DAKO, [M7020]). Briefly, prepared slides were rehydrated and incubated in PBS. Heat induced epitope retrieval (HIER) was carried out by immersing the slides in 0.1 M sodium citrate buffer (PCNA and DAZL, pH 6) or TRIS-EDTA buffer (Vimentin, pH 9). Slides were incubated in 3% hydrogen peroxide for five minutes to suppress endogenous peroxidase activity. Sections were briefly washed in PBS and incubated for 20 minutes in 1% normal blocking serum to block nonspecific binding of the biotinylated secondary antibody. Biotin blocking solution was applied for 15 minutes to block endogenous Avidin/ Biotin activity (CAS: SP-2001; Vector Laboratories Ltd). PCNA and Vimentin incubated tissues were labelled using the Vectastain Elite Universal avidin-biotin-peroxidase detection kit (Vectastain Elite ABC HRP Kit; CAS no: PK-6200; Vector Laboratories Ltd) and incubated with 3,3’– diaminobenzidine substrate (DAB; SK-4100, Vector Laboratories Ltd, Peterborough, UK). DAZL immunolocalisation required biotinylated rabbit
anti Goat IgG (Code BA-5000; Vector Laboratories Ltd) secondary antibody due to species specificity and therefore utilised the Vectastain Elite Goat IgG peroxidase kit [CAS no: PK-6105; Vector Laboratories Ltd].

**Cellular staining quantification**

Vimentin, PCNA and DAZL were quantified using the analytical computer package ‘Image pro plus 6.3’ (Media cybermatics). Following computer recognition of colour specific pixels, the percentage of DAB chromogen brown staining (antigen) was expressed as a percentage of haematoxylin stained nuclei. The percentage cellular area stained was then objectively calculated for each marker and values exported to Microsoft Excel for analysis.

**Sertoli cell number quantification**

Sertoli cell numbers, adjusted for tubule area, were calculated using ‘Image pro plus 6.3’ (Media cybermatics). Forty images (x630 magnification) were taken of each vimentin immunostained testis, 10 from each ‘compass’ pole. This was deemed representative of the testis section 28. For each image, the tubule area (pixels) and all Sertoli cells were counted. This area of the testicular tubules (pixels) was then transformed into a ratio based on the total pixel area of each image. This was multiplied by the Sertoli cell count to ensure consistency across samples: Sertoli cell count* (total area/tubule area).

**Chemical detection**

Adult dog testes (n = 77), were subjected to environmental chemical analysis in an ISO17025 accredited laboratory (James Hutton Institute, Aberdeen). The predominant contaminants routinely measured were diethylhexyl phthalate (DEHP), a range of polychlorinated biphenyl congeners (PCB: 28, 52, 101, 118, 138, 153, 180) and a range of polybrominated diethyl ether congeners (PBDE: 28, 47, 99, 100, 153, 154, 183) (Lea et al., 2016).

**Statistical Analysis**

Statistical analysis was undertaken by either; Ordinary one-way ANOVA incorporating Sidak’s multiple comparisons test for parametric data or analysis of variance incorporating Dunn’s multiple comparison tests for non-parametric data. A non-parametric Spearman’s rank correlation statistical test was used to investigate correlation coefficients and statistical dependence between two variables. Statistical significance was determined when $P \leq 0.05$.

** Declarations**

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**Author contribution:**

RGL and GCWE conceived of and designed the study. AB, GCWE and RGL managed the collection of dog testes for chemical and histological analyses. RNS and AB performed experiments and analysed data. ZZ carried out all
chemical analyses. RNS and RGL wrote the paper.

Additional information

The authors declare no competing financial interests.

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