

Seminal fluid metabolome and epididymal changes after antibiotic treatment in mice

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Abstract

Paternal environment can induce detrimental developmental origins of health and disease (DOHaD) effects in resulting offspring and even future descendants. Such paternal-induced DOHaD effects might originate from alterations in a possible seminal fluid microbiome (SFM) and composite metabolome. Seminal vesicles secrete a slightly basic product enriched with fructose and other carbohydrates, providing an ideal habitat for microorganisms. Past studies confirm the existence of a SFM that is influenced by genetic and nutritional status. Herein, we sought to determine whether treatment of male mice with a combination of antibiotics designed to target SFM induces metabolic alterations in seminal vesicle gland secretions (seminal fluid) and histopathological changes in testes and epididymides. Adult (10- to 12-week-old) National Institutes of Health (NIH) Swiss males ($n=10$ per group) were treated with Clindamycin 0.06 mg/kg day, Unasyn (ampicillin/sulbactam) 40 mg/kg day and Baytril (enrofloxacin) 50 mg/kg day designed to target the primary bacteria within the SFM or saline vehicle alone. Fourteen-day antibiotic treatment of males induced metabolomic changes in seminal vesicles with inosine, xanthine and L-glutamic acid decreased but D-fructose increased in glandular secretions. While spermatogenesis was not affected in treated males, increased number of epididymal tubules showed cribriform growth in this group (7 antibiotic-treated males; 3 saline control males; $P=0.01$). Antibiotic-treated males showed more severe cribriform cysts. Current findings suggest antibiotic treatment of male mice results in seminal fluid metabolome and epididymal histopathological alterations. It remains to be determined whether such changes compromise male reproductive function or lead to DOHaD effects in resulting offspring.

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Introduction

The pre- and post-natal environment can permanently shape future health, for better or worse, of an individual (Chow & Lee 1964, Roeder & Chow 1972, Dorner 1977, Gram *et al.* 1995). The late Sir David Barker was the first to formulate this concept of ‘fetal origin of adult disease’ (Barker 1990). This paradigm later morphed into the final term of ‘developmental origins of adult health and disease (DOHaD)’ to reflect the premise that extrinsic factors encountered by the developing fetus/neonate might also affect later health (Gillman *et al.* 2007, Hanson *et al.* 2011, Barouki *et al.* 2012, Hanson & Gluckman 2014, Hanson 2015). The original DOHaD concept assumed that only the *in utero* or maternal

environmental condition could influence health or disease risk of resulting offspring. However, it is increasingly becoming apparent that paternal state prior to fertilization may be equally if not more important.

Paternal condition can lead to DOHaD-based diseases in his offspring and even potential transgenerational effects (Binder *et al.* 2012a,b, Rando 2012, Rodgers *et al.* 2013, Bromfield 2014, Bromfield *et al.* 2014, Gapp *et al.* 2014, Sharma & Rando 2014, Binder *et al.* 2015, Faure *et al.* 2015). Paternal obesity in mice has been linked with F₁ embryo and offspring disruptions, including delayed development, carbohydrate utilization, mitochondrial disturbances, metabolic disorders and reduced sperm motility (Binder *et al.* 2012a,b, Fullston *et al.* 2015). Offspring of male mice fed a low-protein diet show DNA

methylation and gene expression changes associated with cholesterol and lipid metabolism and leads to cardio-metabolic disorders (Carone *et al.* 2010, Watkins & Sinclair 2014). Paternal exposure to environmental chemicals results in transcriptomic and miRNA changes in resulting embryos (Brevik *et al.* 2012a,b).

The well-documented birth records kept by the parish of Överkalix in northern Sweden from the late 1800s to the 1900s has allowed for assessments on how grandparents' nutritional state affects their grandoffspring. The combined studies suggest that disease predilection in granddaughters was primarily associated with overall health of their grandmothers. In contrast, physical condition of grandfathers correlated with risk of cardio-metabolic disorders in their grandsons (Bygren *et al.* 2001, Kaati *et al.* 2002, Pembrey *et al.* 2006).

Paternal-induced DOHaD effects may also originate due to fluctuations in seminal vesicle fluid contents, in particular, metabolites and cytokines, and/or female immune response to these compounds (Bromfield 2014, Bromfield *et al.* 2014, Binder *et al.* 2015). We have discovered that a novel microbiome resides in the seminal vesicles, and the microorganism inhabitants are influenced by genetic status and nutritional status of the male (Javurek *et al.* 2016, 2017). It is yet to be determined whether changes in the seminal fluid microbiome (SFM) affect the conceptus environment or programming. These findings though raise the concern as to how antibiotic treatment prior to fertilization might affect the microbiota within the seminal vesicles.

While past studies have not considered the potential effects of antibiotic treatment on the resident seminal vesicle microbiome, the effects of several bacteriostatic and bactericidal antibiotics on the testes and epididymides have been examined. Short-term antibiotic treatment of rodent models and amphibians with antibiotics, including tetracycline, doxycycline, gentamycin, ciprofloxacin, enrofloxacin, ofloxacin, salinomycin, streptomycin, ceftazidime and cefmetazole, results in impaired spermatogenesis, decreased sperm motility, suppression of androgen production by Leydig cells and generation of oxygen-free radicals within the male reproductive tract (Moe *et al.* 1989, Crotty *et al.* 1995, Demir *et al.* 2007, Aral *et al.* 2008, Farombi *et al.* 2008, El-Harouny *et al.* 2010, Antohi *et al.* 2011, Alp *et al.* 2012, Elzeinova *et al.* 2013, Ojo *et al.* 2013, Silla *et al.* 2015). Some of these changes are reversible after cessation of the antibiotic treatment (Moe *et al.* 1989, Ojo *et al.* 2013). It is not clear though if these pathologies are due to direct antibiotic-induced testicular toxicity or secondary to dysbiosis and associated bacterial metabolic imbalances in the seminal vesicles. The underlying hypothesis thus tested in the current studies was that short-term administration of antibiotics designed to target resident microorganisms in the seminal vesicles results in metabolome imbalances in this organ and histopathological changes in the testes

and epididymides. To test this possibility, NIH Swiss male mice were treated for 14 days with the 'CUB' antibiotic protocol – Clindamycin, Unasyn (ampicillin/sulbactam) and Baytril (enrofloxacin) or vehicle saline solution. This combination of antibiotics is designed to target the primary bacteria identified previously in the SFM (Javurek *et al.* 2016, 2017). Metabolomic assessments were then performed on the seminal vesicle gland secretions (seminal fluid), and testes and epididymides were histologically analyzed.

Materials and methods

Animals and treatments

The animal experiments were approved by the University of Missouri Animal Care and Use Committee (Protocol #8693) and performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Ten-to-twelve-week-old, NIH Swiss male mice were purchased from Envigo (Madison, WI, USA) and shipped to the University of Missouri Animal Sciences Research Center (ASRC). They were then placed on a control diet (7.2% fat, TD.140790, Envigo) and provided access to water *ad libitum*. Mice were maintained on a 12-h light:12-h darkness cycle with the lights on at 7:30h and off at 19:30h. Mice were habituated to the ASRC animal facility for 1 week. Males were then randomly assigned to one of two daily treatment groups: combination antibiotic group (CUB antibiotic protocol – Clindamycin (0.06 mg/kg day; Hospira, Inc. – acquired by Pfizer), Unasyn (ampicillin/sulbactam, 40 mg/kg day, Pfizer, Inc.) and Baytril (enrofloxacin, 50 mg/kg day; Bayer Animal Health, Whippany, NJ, USA) or saline vehicle alone ($n=10$ per group). Mice were injected intraperitoneally daily for 2 weeks with a volume of 0.2 mL for the CUB or saline treatment and monitored daily for any evidence of infection, pain or distress. No such signs were observed. After 2 weeks of the respective treatments, males were killed and whole seminal fluid collected from the seminal vesicles in a sterile aseptic manner, as described previously (Javurek *et al.* 2016, 2017). Testes with attached epididymides were fixed in Bouin's fixative for 24 h and then changed over daily for 3 days to 70% ethanol solution.

Metabolome analyses

Seminal fluid samples were first partitioned into two immiscible extracts by adding chloroform (1.5 mL) and 1.5 mL of HPLC-grade water containing 25 µg/mL ribitol (as internal standard). The mixture was vortexed and centrifuged to facilitate phase partitioning. The upper aqueous phase was recovered and analyzed, as described previously (Broeckling *et al.* 2005). Briefly, the aqueous phase was dried under gaseous nitrogen stream, methoximated in pyridine with 120 µL of 15 mg/mL methoxyamine hydrochloride, and then trimethylsilylated with 120 µL MSTFA (N-methyl-N-(trimethylsilyl)trifluoroacetamide) + 1% TMCS (chlorotrimethylsilane) reagent. Metabolic profiling was performed using an Agilent 6890N GC coupled to a 5973N single quadrupole

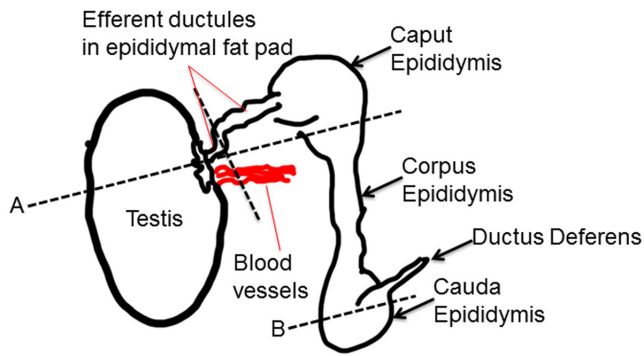


Figure 1 Diagram of where the testes and epididymis were grossly sectioned. Section (A) included the testes, efferent ductules and caput epididymis. Section (B) permitted examination of the cauda epididymis.

mass spectrometer with a scan range from m/z 50 to 650 (Agilent Technologies, Inc.). Separation was achieved with a temperature program of 80°C for 2 min, then ramped at 5°C/min to 315°C and held at 315°C for 12 min, a 30 m DB-5MS column (J&W Scientific, 0.25 mm ID, 0.25 μ m film thickness) and a constant flow of 1.0 mL/min. A standard alkane mix was used for gas chromatography coupled to mass spectrometry (GCMS) quality control and retention index calculations. The data were deconvoluted using Automated Mass Spectral Deconvolution and Identification Software (AMDIS) and annotated through mass spectral and retention index matching to an in-house constructed spectra library. The unidentified components were then identified using spectral matching to a commercial NIST17 mass spectral library. The combined identifications were in .ELU file format, and the abundance of the ions were extracted using custom MET-IDEA software (Lei 2012). The abundances were then normalized to the internal standard, ribitol, and the normalized values were used for statistics. Statistics such as Partial Least Squares Discriminant Analysis (PLS-DA) and volcano plots were performed with the MetaboAnalyst 3.0 program, after the data were normalized

to sum, log transformation and Pareto scaling. (<http://www.metaboanalyst.ca/>, Date Accessed March 21, 2018).

Testes and epididymides histopathology

The testes and epididymides were sectioned under a Nikon SMZ2B stereomicroscope (Nikon Instruments Inc., Melville, NY, USA) and placed in a cassette with foam sponges for histological analysis. The areas sampled for the testes and epididymides are depicted in Fig. 1. From each animal, five to ten 3–5 μ m sections at 50 μ m apart were cut and stained with periodic acid-Schiff (PAS)/hematoxylin to visualize the acrosome formation in spermatids. Histopathology of testes involved an analysis of all stages of spermatogenesis (Hess & de Franca 2008) and an examination of acrosome formation in the spermatids. In the epididymides, portions of caput, corpus and cauda regions were examined for potential differences. The only major pathological change observed in the epididymides was the formation of cribriform cysts (Butterworth & Bisset 1992, Kempinas & Klinefelter 2014); therefore, this observation received further investigation. We defined mature cribriform cysts as those having epithelial changes that included at least three major features: intra-epithelial lumens surrounded by epithelial cells forming a bridge over the apical aspect of the cyst, secretory material within the lumen and microvilli having grown into the cystic lumen (Nistal *et al.* 1990, Butterworth & Bisset 1992). Immature cysts were defined as those having only one of the three major identifying characteristics or showing only a large vacuole along the basement membrane.

Statistical analyses

Histomorphological changes in the epididymides antibiotic-treated vs saline control males were analyzed with the chi-square function in Graphpad Prism 7 Program (GraphPad Software, Inc.).

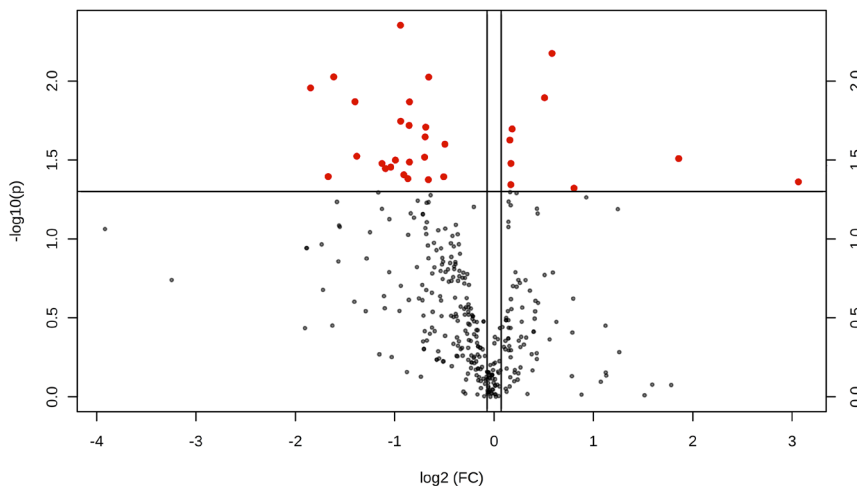


Figure 2 Volcano plot after exclusion of the five outlier samples that includes the remaining seven replicates in the CUB antibiotic group and eight replicates in the saline vehicle control group. The red circles indicate that the metabolites are significantly different between two groups ($P < 0.05$) while the black ones are not statistically different.

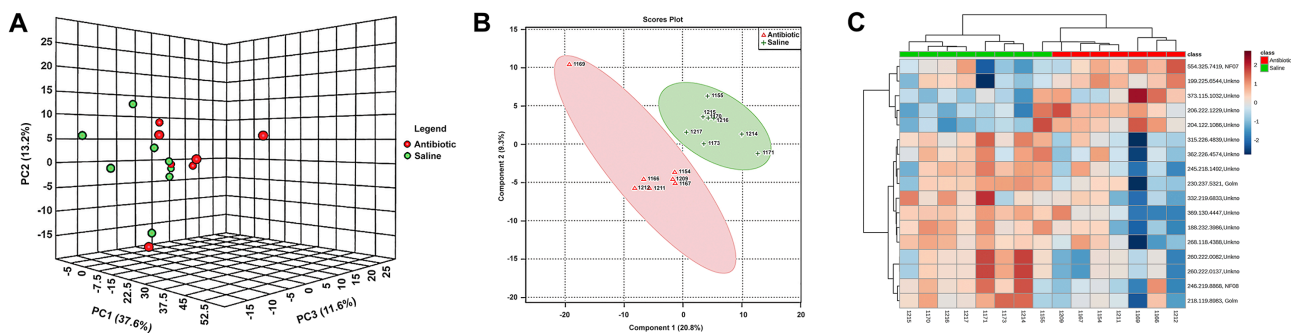


Figure 3 Metabolome data from CUB antibiotic-treated (red) and saline control (green) males. (A) 3D PCA score plot for metabolome data from CUB antibiotic treated (red) and saline control (green) males that show overall metabolomics profile of the two groups separated especially on the PC2 axis. (B) Partial least squares discriminant analysis (PLS-DA) plot of the samples with outliers removed. The red triangle represents samples treated with antibiotics while the green cross represents saline controls. (C) Heat map based on the top differentially expressed metabolites between CUB antibiotic and saline-treated males.

Results

Seminal fluid metabolome

The metabolomics data preprocessing were performed using the R programming language (<https://www.r-project.org/>, Date Accessed March 21, 2018). Initial preprocessing revealed that several samples had abnormal variables as determined by three times its first and third quartiles (first quartiles $-3 \times \text{IQR}$, third quartiles $+3 \times \text{IQR}$, IQR: interquartile range). The samples were then analyzed using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Three samples from the antibiotic group and two samples from the saline control group were clearly segregated from the rest of the samples on PCA (PC1: 85.3%, PC2: 3.6%) and orthogonal partial least squares discriminant analysis (oPLS-DA; T score: 68.7%, orthogonal T score: 7.5%, $P < 0.001$ from 1000 permutation tests in oPLS-DA). These samples were considered as outliers and excluded from the data analyses. Data in a .Raw file format, which shows the instrument response of ions including the internal standard, ribitol, are listed in [Supplementary data 1](#) (see section on [supplementary data](#) given at the end of this article). [Figure 2](#) illustrates the volcano plot after exclusion of the above described five outlier samples. Metabolites showing statistical differences ($P < 0.05$) are provided in [Supplementary data 3](#) (Volcano Plot Results). Visualization of the metabolomics data with a 3D PCA score plot reveals that the CUB antibiotic-treated mice samples show distinct separation, in particular, on the PC2 axis ([Fig. 3A](#)). PLS-DA was also used to visualize the data ([Fig. 3B](#)). Similarly, heat map analyses based on the top differentially expressed metabolites reveal distinct separation between CUB antibiotic- and saline-treated males ([Fig. 3C](#)). The primary metabolites that were significantly different in the seminal fluid in CUB antibiotic-treated males versus saline control males are shown in [Fig. 4](#). Inosine, xanthine and L-glutamic acid were decreased in CUB-treated males, whereas,

D-fructose was elevated in this group. There were also several unidentified metabolites that differed between the two groups. The raw metabolomics data are included in [Supplementary data 1](#). Pathway analyses of the metabolomics data indicate that the metabolites that differ between the two groups are primarily involved in

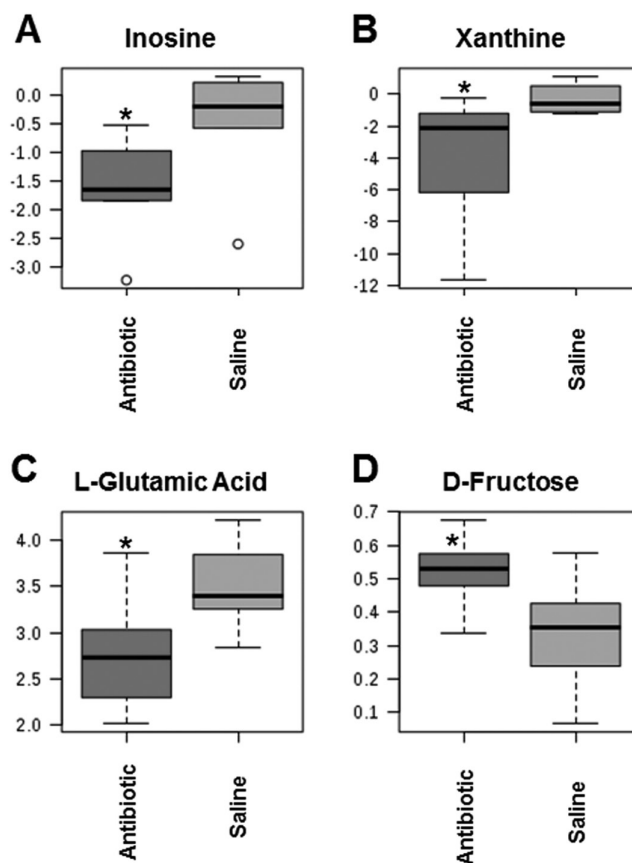


Figure 4 Select metabolites (A-D) that were differentially expressed in the seminal fluid in CUB antibiotic-treated males versus saline control males. $*P \leq 0.05$.

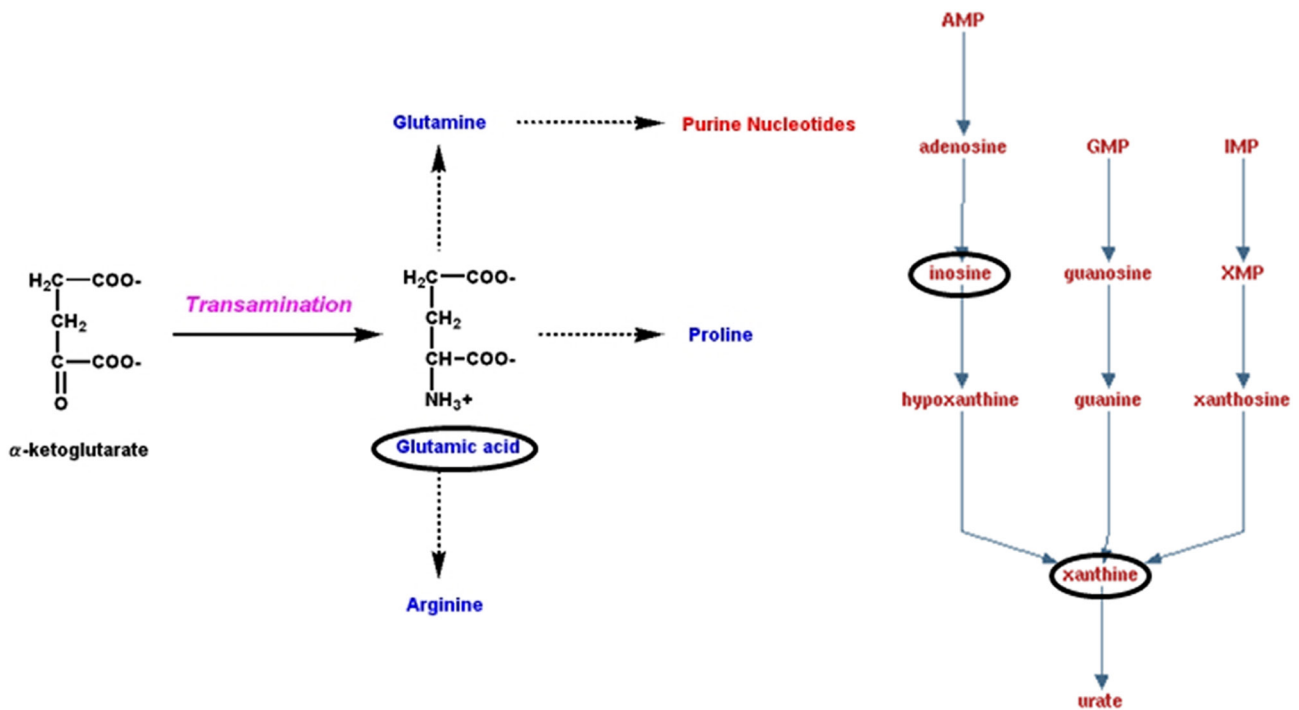


Figure 5 Purine degradation and urate metabolism pathway. The circled metabolites were decreased in the seminal fluid of males treated with the CUB antibiotic protocol regimen. Purines require glutamic acid for their original synthesis.

purine degradation and potentially urate metabolism (Fig. 5).

Testes and epididymides histopathology

The 2-week CUB antibiotic protocol did not affect spermatogenesis, as the seminiferous epithelium was normal in both treated and control testes (Fig. 6A and B). Sperm structure and acrosome formation were normal, as indicated by PAS and a normal number of spermatozoa were observed in the epididymal lumen (Fig. 6A and B). However, in antibiotic-treated males the epididymides had an increased incidence of cribriform, cyst-like growths of the epithelium (Fig. 6C and D), which were always located in the caput/corpus and/or the corpus/cauda epididymides junctions. In the antibiotic-treated group, 70% (7/10) of the males had cribriform changes in the epithelium, while in the control group, only 30% (3/10) of the males had these changes and they appeared to be less severe ($P=0.01$). To delineate further this pathological change between the groups, the sections were reanalyzed to determine the number of males in each group that showed well-defined and mature cribriform cysts with 50% (5/10) of the antibiotic-treated males (Fig. 6D and F), but only one control male (10%) possessed this change (Fig. 6E, $P=0.02$). Mitotic figures were also observed in the epididymal epithelium of three antibiotic-treated males (Fig. 6D), which is very rare in adult mice, and none were observed in epididymides of saline control males ($P=0.03$). Table 1 provides a

summary of the quantitative histopathological changes observed in the epididymides.

Discussion

The primary goal of this study was to determine whether short-term antibiotic treatment for 2 weeks might disrupt the normal balance of resident microflora within the seminal vesicles leading to metabolome changes in this organ. An ancillary goal was to determine whether this combined antibiotic regimen would affect spermatogenesis and result in histopathological changes in the testes and epididymides. Several characterized and uncharacterized metabolites differed in CUB antibiotic-treated males compared to controls. Of the characterized ones, inosine, xanthine and L-glutamic acid were reduced in CUB-treated males, but D-fructose was increased in this group.

Pathway analysis demonstrates that the three known metabolites decreased in CUB antibiotic-treated males are involved in purine degradation and urate pathway metabolism. *Propionibacterium acnes* has been previously identified to be a resident bacterium within the seminal fluid (Javurek et al. 2016, 2017). This bacterium can metabolize purines. Both *P. acnes* and an increase in urate production, i.e. gout, are associated with chronic prostatitis and prostate cancer (Shannon et al. 2006, Fassi Fehri et al. 2011, Perry & Lambert 2011, Kuo et al. 2012, Olsson et al. 2012, Shinohara et al. 2013, Bae et al. 2014, Chen et al. 2014).

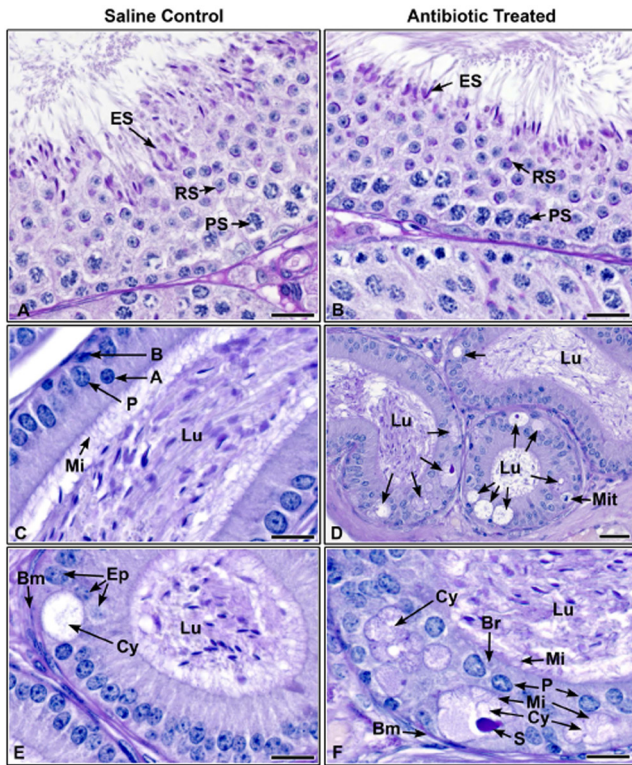


Figure 6 Histological section of testes and epididymides from saline control and CUB antibiotic-treated males. (A) Testis from saline control male showing normal spermatogenesis, Stage VII. Elongated spermatids (ES) line the epithelium with tails extending into the lumen. Round spermatids (RS) with normal acrosomal formations, as seen with periodic acid-Schiff's staining (PAS) are found in the central region of the epithelium (PS, pachytene spermatocytes). (B) Testis from CUB antibiotic-treated male, late Stage VII. Normal elongated spermatids (ES) line the epithelium as in the control. Round spermatids (RS) with normal acrosomal formations are present, as well as pachytene spermatocytes (PS). (C) Proximal corpus epididymis from a control male. Note the concentration of sperm in the lumen (Lu) and a columnar epithelium consisting of principal (P), apical (A) and basal (B) cells. Long microvilli (Mi) extend into the lumen. (D) Proximal corpus epididymis from a CUB antibiotic-treated male showing cribriform cystic changes (arrows) in the epithelium. These abnormal formations appear as large vacuoles in the basal region that develops into a cystic luminal space, surrounded by shortened epithelial cells. Sperm are found in the epididymal lumen (Lu). A mitotic figure (Mit) is seen in the basal region. (E) Proximal corpus epididymis from a control male showing a rare cribriform cyst in the epithelium. This cyst (Cy) appears less developed than in the treated samples, because there is a sparse amount of secretory material and microvilli are lacking within the cystic lumen (Bm, basement membrane; Lu, lumen of the epididymis). (F) CUB antibiotic-treated proximal corpus epididymis. This is a higher magnification of an area from D, showing more well-developed cribriform cysts (Cy), with some showing epithelial cells underneath on the basement membrane (Bm), as well as a well-developed 'bridge' of epithelial cells (Br). These cysts have well-defined secretory material (S) in the cystic lumen and the lining epithelial cells show microvilli (Mi) extending into both the cyst as well as the lumen (Lu) of the epididymis. Bars = 20 μ m.

Chronic treatment with allopurinol reduces the risk of prostate cancer in gout-afflicted patients (Shih *et al.* 2017). The current findings suggest that at least some of the urate production might originate within the seminal vesicles. Moreover, urate might influence antioxidant capacity that could affect oxidative stress in the epididymides or semen (Potts *et al.* 1999, Rhemrev *et al.* 2000, Guz *et al.* 2013). However, the current metabolomics analyses did not reveal any direct changes in urate levels, as this metabolite was below the level of detection and/or other signature reactive oxygen species molecules such as H_2O_2 . Future studies should measure mitochondrial function and potential oxidative stress in the epididymides and sperm with the various techniques currently available to examine for such perturbations (Brand & Nicholls 2011).

The increase in D-fructose in the CUB antibiotic-treated males suggests that there might be fewer commensal bacteria present in this organ to utilize this energy source resulting in the accumulation of this nutrient within the seminal fluid. To date, there has been one other study examining how paternal state, in this case, diet-induced obesity (DiO) affects the metabolome profiles in seminal fluid (Binder *et al.* 2015). DiO males had elevations in fructose and taurine within the seminal vesicle fluid. However, myo-inositol, glycerol phosphate, glycine, isoleucine, glutamic acid, unmethoxymated hexose and threonine were decreased in this group. Thus, this paternal environmental change resulted in opposing effects on fructose within the seminal fluid relative to antibiotic treatment, which is likely because DiO males were provisioned with greater nutrient substrates. It is interesting to note that both DiO and CUB antibiotic-treated males had lower glutamic acid within the seminal fluid, but the significance of this finding is uncertain.

In contrast to previous studies that tested similar antibiotics and duration of treatment (Moe *et al.* 1989, Crotty *et al.* 1995, Demir *et al.* 2007, Aral *et al.* 2008, Farombi *et al.* 2008, El-Harouny *et al.* 2010, Antohi *et al.* 2011, Alp *et al.* 2012, Elzeinova *et al.* 2013, Ojo *et al.* 2013, Silla *et al.* 2015), no effects on spermatogenesis were evident in the current studies. Careful examination of prior reports of antibiotic-induced testicular effects (Aral *et al.* 2008, Farombi *et al.* 2008, Elzeinova *et al.* 2013, Ojo *et al.* 2013), however, revealed critical concerns in the histopathological interpretation that might be attributed to usage of methods that are sub-optimal for testicular toxicological studies. Such methods can lead to improper fixation resulting in significant artifact, namely sloughing of the seminiferous epithelium (Creasy 2003). In two of the aforementioned studies, epididymal effects were noted, but histopathological images were not provided (Farombi *et al.* 2008, Elzeinova *et al.* 2013); however, in both studies, there were additional effects

Table 1 Histomorphometric changes in the epididymis of antibiotic-treated and saline control male mice.

Group	# of males having cribriform changes in the epididymal epithelium	# of males with mature cribriform cysts in the epididymal epithelium	# of males with mitotic figures in the epididymal epithelium
Antibiotic treated	7/10*	5/10**	3/10***
Control	3/10	1/10	0/10

P* value comparison of antibiotic-treated to control males=0.01; *P* value comparison of antibiotic-treated to control males=0.02; ****P* value comparison of antibiotic-treated to control males=0.03.

on sperm motility. Although we did not determine sperm motility in the current study, histopathological changes were observed in the epididymides of antibiotic-treated males, which were more severe in antibiotic-treated males.

In the epididymides of CUB antibiotic-treated males, cribriform cysts were observed growing primarily in the caput-corporis and corpus-cauda epididymal junctions. Significantly more of these epithelial changes appeared in treated males than in the controls, and the cysts were dramatically more severe in the antibiotic-treated samples. Epididymal cribriform cysts are associated with glandular-like growths that form a lumen within the epithelium of the tubular system. Although there is no unified description for this type of cyst, some common features are reported. These include the formation of epithelial bridges over the cystic lumen within the epithelium, which forms a pitted pattern, secretions into the lumen and growth of microvilli into the cystic lumen from the surrounding epithelial cells (Nistal *et al.* 1990, Butterworth & Bisset 1992). This anomaly has been identified in various taxa, including humans, rodents, chimpanzees and dogs (James & Heywood 1979, Butterworth & Bisset 1992, Abbott 1993, Smithwick & Young 1997, Shah *et al.* 1998, Jones *et al.* 2000, La Perle *et al.* 2002). Although some investigators propose these cysts to be a variation of normal epithelium, others speculate they may represent pre-adenomatous growth.

It is uncertain though if this pathological change alters male reproductive function. However, an increased presence of cribriform cysts has been associated with several diseases and pathological conditions in men (Vazquez *et al.* 1986, Nistal *et al.* 1990, Glasker *et al.* 2006), in knockout and transgenic mice (Zhao *et al.* 1998, 2001, La Perle *et al.* 2002, Lupien *et al.* 2006, Krutskikh *et al.* 2011, Wild *et al.* 2012, Wang *et al.* 2017), as well as following chemical-induced male reproductive effects (Dunn & Green 1963, McIntyre *et al.* 2000, Sawamoto *et al.* 2003, Shin *et al.* 2010, Ramos-Ibeas *et al.* 2013, Aghaei *et al.* 2014, Miyaso *et al.* 2014, Lan *et al.* 2015). While we did not measure serum testosterone concentrations, no histological changes were evident in the Leydig cells. However, future studies should determine potential effects on testosterone and pituitary hormones. Postulated causes of inducing this formation include reduced testosterone concentrations (Itoh *et al.* 1999), sloughing of germ cells (Aruldas *et al.* 2004), occlusion of the efferent ductules

and epididymides (Aruldas *et al.* 2004) and necrosis of epididymal epithelium (Itoh *et al.* 1999). Nevertheless, much remains to be elucidated in the underlying mechanisms leading to this unusual cystic formation (Kempinas & Klinefelter 2014).

In conclusion, short-term treatment of male mice with antibiotics designed to target the primary microorganisms within the seminal vesicle fluid induced metabolomics changes with inosine, xanthine and L-glutamic acid reduced but D-fructose elevated in the glandular secretions. While spermatogenesis was apparently normal in antibiotic-treated males, greater number of epididymal tubules showed cribriform growth and mitotic figures in this group, suggesting a potential effect that could influence sperm maturation in the epididymides. Current findings reveal short-term CUB antibiotic treatment of male mice results in seminal fluid metabolome and histopathological changes in the epididymides. It remains to be determined whether such disturbances affect male reproductive function and lead to DOHaD effects in resulting offspring.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/REP-18-0072>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

All authors read and approved the final version of the manuscript. L W S, R A H and C S R conceived and designed

the experiments. S A J, A B J and Z L performed the experiments. Z L, R A H and C S R analyzed the data. Z L, L W S, R A H and C S R drafted the manuscript. S A J, A J B, Z L, L W S, R A H and C S R revised the manuscript.

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