Improvement in sperm quality and spermatogenesis following faecal microbiota transplantation from alginate oligosaccharide dosed mice

Very recent publications in *Gut* and elsewhere[1 2] suggest that gut microbiota affects fertility. The application of faecal microbiota transplantation (FMT) to modify fertility is an emerging novel area of interest.[3] FMT from women with polycystic ovary syndrome (PCOS) leads to the disruption of ovarian function and a decrease in fertility which indicates that modification of gut microbiota may be a valuable approach in the management of PCOS.[2] FMT of gut microbes, that developed under a high-fat diet, into mice on a normal diet leads to the disruption of spermatogenesis and a reduction of sperm motility,[1] which highlights that restoring gut microbiota may be a means of improving disturbed male infertility caused by environmental factors.[1] However, to date, there are no reports that address improvements of fertility following FMT. In a recent study,[4] we found that busulfan damages spermatogenesis and sperm quality, and disturbs gut microbiota as found in many other studies.[5 6] Alginate oligosaccharides (AOS), a natural product with many benefits, rescues busulfan disrupted spermatogenesis by supporting gut microbiota through an increase in 'beneficial' bacteria[4] such as *Bacteroidales* and *Lactobacillaceae* and a decrease in 'harmful' bacteria, such as

Desulfovibrionaceae.[7] Gut microbiota from AOS dosed animals may improve spermatogenesis through benefit to the recipients gut microbes.

To test this hypothesis, we set out to explore the beneficial improvement of sperm quality and spermatogenesis following FMT from AOS dosed animals to busulfan treated mice (online supplementary file 1, online supplementary figure 1). A10-FMT (busulfan plus gut microbiota from AOS 10 mg/kg mice) significantly increased sperm concentration (twofold) and sperm motility (twentyfold) (figure 1A,B). Spermatogenesis was significantly improved by A10-FMT as shown by the germ cell marker VASA (figure 1C) in murine testicular samples. The protein level of the anti-oxidant enzyme GPX1 was higher in the A10-FMT group (online supplementary figure 2A). Moreover, A10-FMT improved gene expression related to spermatogenesis in testes (figure 2A–D; online supplementary figure 2), and increased the protein levels of the most important genes for spermatogenesis (figure 2E; online supplementary table 1).[8] A10-FMT improved busulfan stimulated dysbiosis of gut microbiota through an increase in the 'beneficial' bacteria *Bacteroidales* and *Bifidobacteriales* (online supplementary figures 3 and 4; online supplementary table 2).[7] Furthermore, there was good correlation between gut microbiota and sperm quality (online supplementary figure 3F). A10-FMT ameliorated the blood metabolome through recovery of blood metabolites (online supplementary figures 5 and 6; online supplementary data file 1; online supplementary table 3).[9] Most blood metabolites were positively correlated with some of the gut microbes. The data suggested that A10-FMT may improve small intestine function and gut microbiota, which assists in digestion and absorption.[9] A10-FMT improved the testicular metabolome (online supplementary figures 7 and 8; online supplementary data file 2; online supplementary tables 4 and 5) to help the recovery of spermatogenesis since unsaturated fatty acids and sphingolipids are protective for biological systems.[10] Testicular metabolites and sperm quality were well correlated (online supplementary figure 7H,I). It was most interesting that gut microbiota, blood metabolites and testicular metabolites were well correlated, respectively, between the A10-FMT dosed and AOS dosed studies (online supplementary figure 9; online supplementary tables 6 and 7). Worldwide, 10% to 15% of couples are infertile and many of them have abnormal spermatogenesis.[8]

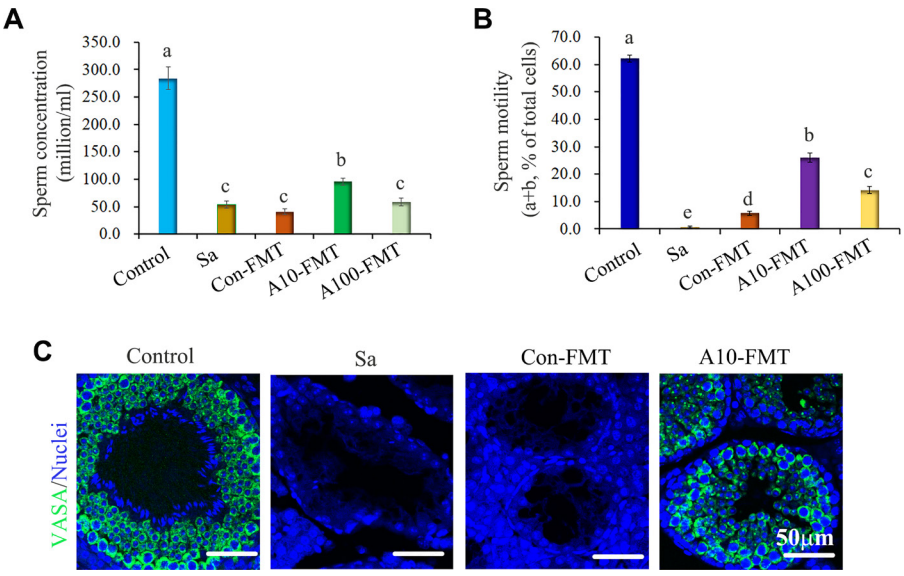

Figure 1 Mouse sperm motility, concentration, VASA staining and apoptosis status. (A) Mouse sperm concentration. The y-axis represents the concentration. The x-axis represents the treatment (n=30/group). ^{a,b,c} means not sharing a common superscript are different ($p<0.05$). (B) Mouse sperm motility. The y-axis represents the percentage of cells. The x-axis represents the treatment (n=30/group). ^{a,b,c} means not sharing a common superscript are different ($p<0.05$). (C) Germ cell marker VASA staining for mouse testicular samples. (1) Control (dosed with saline); (2) Sa (busulfan (a single injection 40 mg/kg BW of busulfan)[4] plus saline); (3) Con-FMT (busulfan plus gut microbiota from regular mice); (4) A10-FMT (busulfan plus gut microbiota from AOS 10 mg/kg dosed mice); (5) A100-FMT (busulfan plus gut microbiota from AOS 100 mg/kg dosed mice). See more detailed information in online supplementary file 1. FMT, faecalmicrobiota transplantation.

Many studies have tried to improve spermatogenesis using different approaches, however, there has been little progress. This investigation found a mechanistic dimension linking an improved gut microbiota with the rescue of spermatogenesis and sperm quality. Because gut microbiota and host interact in very complex ways, more work is needed to clarify the deep mechanisms through which FMT improves spermatogenesis. The current data, for the first time, highlighted that gut microbiota could be used to treat male infertility through the improvement of spermatogenesis.

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Contributors PZ, YF, LL, WG, SYu, YH, WS, XH, DM, SYi, YT and LM performed the experiments and analysed the data. HZ and YZ designed and supervised the study. ZS, QS, HZ and YZ wrote the manuscript. All the authors edited the manuscript and approved the final manuscript.

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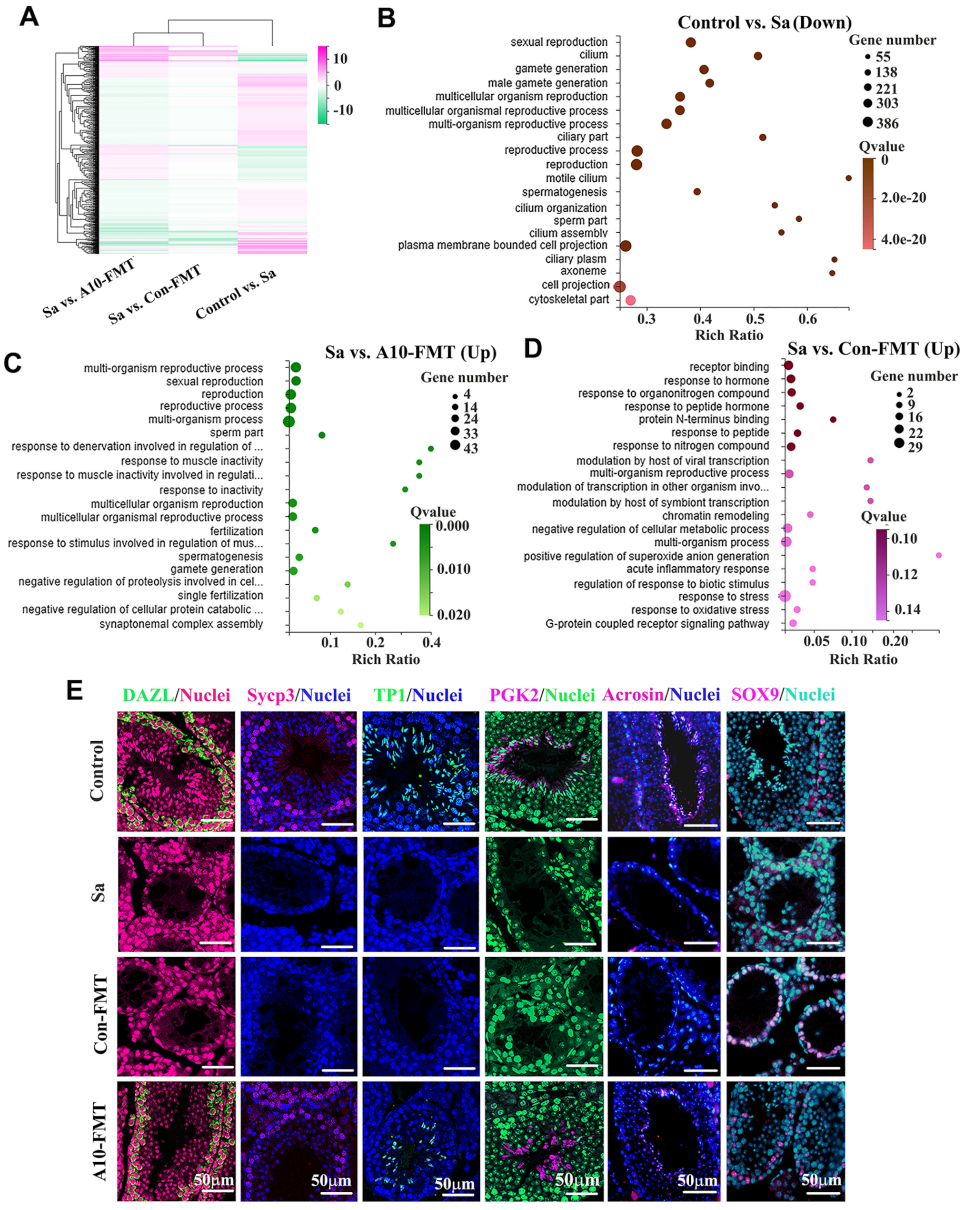

Figure 2 RNA-seq data for mouse testicular samples. (A) Heatmap summary of the differentially expressed genes in the three comparisons: control versus SA; SA versus Con-FMT; SA versus A10-FMT. (B) GO enrichment of downregulated genes in control versus SA. (C) GO enrichment of upregulated genes in SA versus A10-FMT. (D) GO enrichment of upregulated genes in SA versus Con-FMT. (E) Immunofluorescence staining (IHF) for some of the spermatogenesis related marker genes in mouse testes. (1) Control (dosed with saline); (2) Sa (busulfan (a single injection 40 mg/kg BW of busulfan)[4] plus saline); (3) Con-FMT (busulfan plus gut microbiota from regular mice); (4) A10-FMT (busulfan plus gut microbiota from AOS 10 mg/kg dosed mice); see more detailed information in online supplementary file 1. AOS, alginate oligosaccharides; FMT,faecal microbiota transplantation.

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