ORIGINAL ARTICLE

Effects Of Probiotics on Sperm Quality, Spermatogenesis, and Germinal Cell Count in Depression-Induced Male Mice

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ABSTRACT

Introduction: Male infertility is a major reproductive problem, with depression recognised as a contributing factor. Fluoxetine, a commonly prescribed antidepressant, is well known for having spermicidal properties. **Objective:** This study aims to investigate the impact of Bifidobacterium spp. supplementation on sperm quality and spermatogenesis in depression-induced mouse model of male infertility. **Methodology:** Fifty Male Mus musculus mice were randomly divided into five groups for seven weeks experiments. Depression was induced in four groups using corticosterone injection for four weeks, followed by a two-weeks intervention with either fluoxetine of Bifidobacterium spp. The groups included: negative control (CTR), positive control (DEP), fluoxetine-treated (FLX), Bifidobacterium spp.-treated (BFD), and Bifidobacterium spp.-prevention (pBFD), which received probiotic before corticosterone exposure. Sperm quality parameters (concentration, motility, morphology) and spermatogenesis markers (Johnsen's score and germinal cell count) were analysed. **Results:** The preventive Bifidobacterium spp. group showed significantly superior sperm quality, with higher concentration, motility, morphology, Johnsen's score, and spermatid cell count compared to other groups. **Conclusion:** Bifidobacterium spp. Supplementation demonstrates potential as a probiotic intervention to improve sperm quality and spermatogenesis in depression-related male infertility, suggesting its potential role in infertility management.

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Keywords: Probiotics, Bifidobacterium spp., sperm quality, spermatogenesis, depression-related infertility.

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INTRODUCTION

One of the major contributing factors to the recently increasing male infertility is the stress, and infertility could induce psychological stress even to the depressive state (1,2). Factors affecting male fertility such as sperm morphology, motility, concentration, and total count was founded decreased in depressed and stressed people (3,4). This poor sperm quality together with low spermatogenesis is the result of disruption of hormone and gut homeostasis affected by chronic stress (5,6). Whereas stress medication from SSRI group worsens the sperm quality and spermatogenesis. One of the potential therapies is Probiotic which could enhance gut membrane integrity and stimulate serotonin release (7). Therefore, the level of serotonin could increase near to the therapeutic effect of SSRI without impairing male fertility (8). Previous study had revealed that the

administration of probiotic rehabilitated the stress-related reproductive disturbance in male mice (9).

Compared with previous studies focused on specific effects of probiotics, this study aims to examine the impact comprehensively. Besides sperm motility, morphology, and concentration assessment, this study also examined the spermatogenesis through simple and practical germinal cell counts and the established, applicable Johnsen scoring (10–12). These assessments intended to provide insights into testicular changes in stressed mice treated with probiotics and also to evaluate how probiotics impact spermatogenesis in a model of stress-induced infertility.

MATERIALS AND METHODS

Animal Model

Fifty male mice (Mus musculus, 28-30 g 8-10 weeks old) were divided into five groups (n=10 per group) for seven weeks study. Depression was induced in four groups using corticosterone injections (20 mg/kgBW/day) for five weeks (13), followed by a two weeks intervention with either fluoxetine (20 mg/kgBW/day or Bifidobacterium spp. (30 mg/kgBW/day). The groups were negative control (CTR, no treatment), positive control (DEP, corticosterone injection only), fluoxetine-treated (FLX, corticosterone injection followed fluoxetine administration), probiotic-treated (BFD, corticosterone injection followed Bifidobacterium spp. administration), probiotic prevention (pBFD, Bifidobacterium spp. before corticosterone injection to assess its protective effect). Corticosterone was injected intraperitoneally and both of probiotic and fluoxetine were administered orally. After seven weeks of experiment, their testes and epididymis were collected. Sperm quality parameters (concentration, motility, morphology) and spermatogenesis markers (Johnsen's score, germinal cell count) were assessed. Data were analysed using One Way ANOVA with the significance level at $p < 0.05$ continued with post-hoc Tukey HSD. All experimental procedures were approved by the Health Research Ethics Committee Faculty of Medicine Brawijaya University and conducted following established ethical guidelines for animal research.

Sperm Cell Quality

Removal, isolation, and submersion of cauda epididymis in phosphate buffer solution were purposed to prepare sperm suspensions for sperm concentration, motility, and morphology analysis using a light microscope.

Germinal Cell Count

Testes were fixed in 10% formalin, sectioned, and stained with H&E. Germinal cells were counted manually in three random seminiferous tubules from five animals per group (14).

Johnsen Score

Spermatogenic cells and the seminiferous tubular epithelium were evaluated from H&E-stained slides, and the Johnsen score was assigned on a 1-10 scale, where 10 indicates full spermatogenesis and 1 indicates the absence of seminiferous epithelium (15).

ETHICAL CLEARANCE

The ethical approval of this research was issued by the Health Research Ethics Committee, Faculty of Medicine, Brawijaya University (No. 118/EC/KEPK-S1PD/06/2020).

RESULTS

Sperm Cell Quality

The sperm cell concentration and motility significantly reduced in DEP and FLX group ($p < 0.001$, Fig. 1A – 1B), while the sperm morphology was less severe in DEP group ($p < 0.05$) rather than FLX group ($p < 0.001$, Fig 1C). The sperm cell quality was increased in BFD and pBFD group compared to DEP and FLX group ($p < 0.001$, Fig. 1), where pBFD group showing more significant sperm concentration increasement rather than BFD group ($p < 0.05$, Fig. 1A), although these groups neither restored it to control levels.

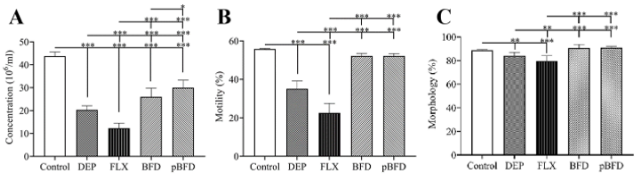

Figure 1: Effect of Bifidobacterium spp. and Fluoxetine on Sperm Quality Parameters in Depression-Induced Mice. Sperm quality parameters were analysed across experimental groups. (a) The graph represents sperm concentration (x10⁶/mL), (b) The graph shows the percentage of normal motile sperm (%), and (c) The graph illustrates the percentage of morphologically normal sperm (%). Experimental groups: Control (CTR): negative control, DEP: corticosterone-induced depression model, FLX: fluoxetine-treated, BFD: Bifidobacterium spp.-treated, pBFD: probiotic-prevention group. Data are presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 indicate statistically significant differences between groups.

Germinal Cell Count

The number of germinal cells decreased in DEP group compared to the Control ($p < 0.05$), and FLX treatment more significantly reduce the number ($p < 0.001$). Contrary, both BFD and pBFD group shown significant increase in germinal cells compared to FLX group (respectively $p < 0.05$ and $p < 0.001$, Fig. 2)

Johnsen Score

The testicular histopathological examination and Johnsen score analysis revealed the lowest quality of

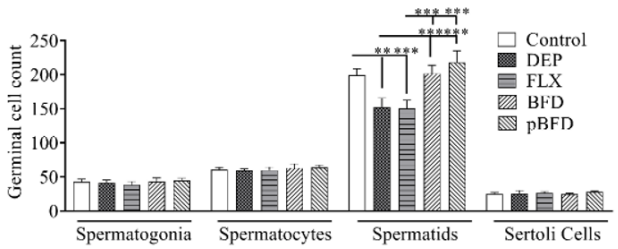

Figure 2: Effect of Bifidobacterium spp. and Fluoxetine on Spermatogenesis Markers in Depression-Induced Mice. Spermatogenesis markers were analysed across experimental groups, including spermatogonia, spermatocytes, spermatids, and Sertoli cells. The spermatid count showed significant differences between groups. Experimental groups: Control (CTR): negative control, DEP: corticosterone-induced depression model, FLX: fluoxetine-treated, BFD: Bifidobacterium spp.-treated, pBFD: probiotic-prevention group. Data are presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 indicate statistically significant differences between groups.

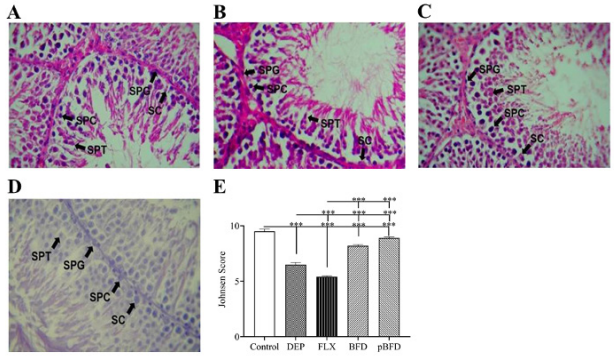

Figure 3: Histological Analysis of Seminiferous Tubules and Johnsen Score in Depression-Induced Mice (a–d): Hematoxylin and Eosin (H&E)-stained seminiferous tubules at Ч400 magnification across experimental groups: (a) Positive control (DEP), (b) Fluoxetine-treated (FLX), (c) Probiotic-treated (BFD), and (d) Preventive probiotic-treated (pBFD). (e): Johnsen Score comparison among groups (negative control (CTR), positive control (DEP), fluoxetine-treated (FLX), probiotic-treated (BFD), and preventive probiotic-treated (pBFD)). Data are presented as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 indicate statistical significance between respective groups. SPG = Spermatogonia, SPC = Spermatocyte, SPT = Spermatid, SC = Sertoli Cell.

spermatogenesis observed in FLX group (p < 0.001, Fig. 3B & 3E) and the highest is on the pBFD group (p < 0,001, Fig. 3D & 3E).

DISCUSSION

Stress-induced activation of the hypothalamus-pituitary-adrenal (HPA) axis leads to elevated cortisol levels, and suppresses the hypothalamus-pituitary-gonadal (HPG) axis, reducing Leydig cell activity and testosterone production, thus impairing sperm quality as observed in DEP groups (16). Elevated cortisol also inhibits Sertoli cells and increases oxidative stress, impairing spermatogenesis and reducing germinal cell numbers and Johnsen score by apoptosis induction (17,18). Additionally, Fluoxetine gonadotoxic effects observed in the greatest reduction of germinal cell count and Johnsen score in FLX group, as a result of Fluoxetine suppression on serotonin-HPG axis and its' cytotoxic effect on testicular cells (Fig. 2 & 3).

Oppositely, Bifidobacterium sp. probiotics administration significantly improved sperm quality in the BFD and pBFD group, since probiotics have anti-inflammatory, antioxidant, and gut-microbiome restoration abilities to restore HPA and HPG axis, ameliorating stress-induced damage (19–21). However, germinal cell restoration was not observed in BFD group, indicating irreversibility of stress-induced damage towards period-limited Sertoli cell proliferation (14). pBFD group shown opposite result with significantly high number of germinal cells, marking the protective property of the probiotic towards germinal cell, but not as far as epididymal transit, therefore pBFD group had insignificant difference in sperm motility and morphology (Fig. 1) (14).

Based on the findings, probiotic supplementation as stress-preventive therapy may provide testicular protection, and post-stress administration could repair sperm quality. Nevertheless, cell count method still inadequate to conclude the effect of the probiotic. Therefore, further research using molecular markers of apoptosis, oxidative stress indicators, and Sertoli cells proliferation markers is needed to gain greater understanding regarding the effect of the probiotic on spermatogenesis.

CONCLUSION

Bifidobacterium spp. supplementation demonstrated significant benefits in improving sperm quality and protecting spermatogenesis in a depression-induced mouse model of male infertility. While post-stress probiotic treatment enhanced sperm concentration, motility, and morphology, preventive supplementation effectively preserved germinal cell numbers, highlighting its protective potential. These findings suggest that probiotics may serve as a promising intervention for stress-related male infertility, offering an alternative to conventional treatments like fluoxetine.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DECLARATION OF FUNDING

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