Research Square

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Altered DNA methylation and Dnmt expression in obese uterus may cause implantation failure

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Abstract

Obesity is defined by increased adipose tissue volume and has become a major risk factor for reproduction. Recent studies have revealed a substantial link between obesity and epigenetics. Epigenome is dynamically regulated mainly by DNA methylation. DNA methylation, which is controlled by DNA methyltransferases (Dnmts), has been widely investigated since it is essential for imprinting and regulation of gene expression. In our previous study we showed that level of Dnmt1, Dnmt3a and global DNA methylation was dramatically altered in testis and ovary of high-fat diet (HFD)-induced obese mice. However, it has not yet been demonstrated that effect of HFD on Dnmts and global DNA methylation in mice uterus. Therefore, in the present study, we aimed to evaluate the impact of HFD on the level of Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and global DNA methylation in uterus. Our results showed that HFD significantly altered levels of Dnmts and global DNA methylation in the uterus. Total expression of Dnmt1, Dnmt3a and Dnmt3b significantly upregulated while level of Dnmt3l and global DNA methylation dramatically decreased ($p < 0.05$). Furthermore, we observed that expression of Dnmt3b and Dnmt3l significantly increased in endometrium including gland and epithelium ($p < 0.05$). Although Dnmt3b was the only protein whose expression significantly increased, level of global DNA methylation and Dnmt3l remarkably decreased in stroma and myometrium ($p < 0.05$). In conclusion, for the first time, our results show that obesity dramatically changes global DNA methylation and expression of Dnmts, and decreased DNA methylation and Dnmt expression may cause abnormal gene expression, especially in the endometrium.

Introduction

Obesity, which is among the most important health problems of developed and developing countries in recent years, is a metabolic syndrome that causes excessive fat accumulation in the body (Samblas et al., 2019; Wang et al., 2019). Obesity can impair reproductive functions by affecting both the ovaries and the endometrium. Having more than normal fat in women can cause various reproductive disorders. Some of these effects can be summarized as menstrual irregularity, infertility, miscarriage and difficulty in performing assisted reproduction (Broughton & Moley, 2017). Currently, about half of all pregnancies appear to be overweight or obese (Kim et al., 2010; Yeh & Shelton, 2005). Evidence from experimental models suggests that maternal obesity, regardless of birth weight, may cause an increased predisposition for developmental abnormalities, insulin resistance, and metabolic disorders in the offspring (Shankar et al., 2011).

There is a strong association between a high prevalence of obesity and impaired reproductive function in women of reproductive age (Catalano & Shankar, 2017). Having a body mass index higher than normal in women has negative effects on reproduction such as menstrual regulation, infertility, miscarriage and difficulty in performing assisted reproduction. Disruptions to nutritional deprivation or excess during pregnancy can permanently program a sequence to end (McMillen & Robinson, 2005). However, only a few studies have examined the early events leading to fetal programming (Igosheva et al., 2010;

Jungheim et al., 2010; Mao et al., 2010; Sen & Simmons, 2010). Most importantly, there is almost no information on the effect of pre-pregnancy maternal obesity on the uterus during implantation.

Embryo implantation is a critical step in embryo development and pregnancy outcomes. To make implantation possible, the uterus undergoes changes that prepare it to receive the embryo. During implantation, the trophectoderm cells of blastocysts interact with the luminal epithelium of the uterus in a series of intricately defined events (Dey et al., 2004; Wang & Dey, 2006). The purpose of implantation is to attach the developing embryo to the endometrial stroma, which is regulated by ovarian hormones and locally produced signaling molecules, including cytokines and growth factors (Dey et al., 2004). However, in the early stages, especially during implantation, the uterine environment is likely to have a major impact on the development of the offspring (Shankar et al., 2011). Recent studies have suggested that DNA methylation may be involved in endometrial change during the preimplantation stages (Gao et al., 2012).

DNA methylation is one of the most well-studied epigenetic mechanisms leading to stable silencing of gene expression in mammals and is required for the normal development and cascading of essential biological processes, including X chromosome inactivation, genomic imprinting, repetitive element suppression, and carcinogenesis (Bird, 2002; Li, 2002). In mammals, DNA methylation meditates intergenerational gene silencing effects and is required for the generation and maintenance of cell type-specific expression changes during the progression of different biological processes not only in development but also in adult tissues (Futscher et al., 2002; Ko et al., 2010; Waterland et al., 2009). DNA methylation as the primary regulator of hereditary information is accomplished by the accumulation of 5-methylcytosine in the DNA chain via enzymatic transfer of a methyl group of CpG to the 5'-cytosine with the aid of DNA methyltransferases (Law & Jacobsen, 2010; Straussman et al., 2009). These CpG islands are usually between the promoter regions of genes, and methylation of CpG islands results in transcriptional repression. It is known that this mechanism is controlled in two ways: maintenance and *de novo* methylation. So far, six major DNA methyltransferase enzymes (Dnmts) have been characterized, including Dnmt1, Dnmt2, Dnmt3a, Dnmt3b, Dnmt3c, and Dnmt3l. However, abnormal DNA methylation has been found to lead to abnormal embryonic development, birth malformations, and other diseases such as human carcinoma (Okano et al., 1999; You et al., 2017).

Dnmt1 is primarily involved in methylating the hemi-methylated helix of DNA after it has been formed and constitutively expressed (Li et al., 1992). Dnmt3a and Dnmt3b, which play a key role in the de novo methylation of unmethylated DNA strands, cooperate (Chedin et al., 2002; Hata et al., 2002). Expression of Dnmt3a is ubiquitous in adult tissues, whereas abundance of Dnmt3b appears to be low in most tissues (Okano et al., 1999). Because Dnmt3l lacks Dnmt activity, it cooperates with Dnmt3a or Dnmt3b for de novo functions. Dysfunction of any of these DNMTs can have serious consequences for embryonic development and subsequent pregnancy, even embryonic lethality, as the epigenetic status of genes or their repeat sequences have a profound effect on cell physiology and can markedly alter embryonic and fetal development (Dion et al., 2008; Li et al., 1992; Okano et al., 1999). It shows that expression disorders of Dnmts are closely related to developmental abnormalities. Dnmt1 knockout ($Dnmt1^{-/-}$) mouse

embryos causes genome-wide demethylation, loss of imprinting, and embryonic lethality in the early stages of pregnancy (Hirasawa et al., 2008). Dnmt3a$^{-/-}$ and Dnmt3b$^{-/-}$ mice failed to initiate de novo methylation after implantation and also exhibited embryonic development arrest (Li, 2002; Li et al., 1992). In the case of Dnmt3l$^{-/-}$ it has been associated with male infertility, azoospermia and abnormal maternal pressure. However, it remains unclear whether dysfunction of Dnmts causes early pregnancy loss in humans (Deplus et al., 2002; Margot et al., 2003).

In mammals, it has been shown that Dnmts are regulated during the endometrial cycle (Ghabreau et al., 2004; Vincent et al., 2011a), and this regulation correlates with the expression of ovarian steroid receptors (Ghabreau et al., 2004). Expressions of Dnmt1, Dnmt3a, and Dnmt3b are seen at relatively high levels in the proliferative phase of the human endometrium, but at lower expression levels in the second secretory phase. These dynamic processes are essential for human endometrial changes throughout the reproductive cycle (Vincent et al., 2011b; Yamagata et al., 2009). It is also known that the mRNA levels of Dnmt1, Dnmt3a and Dnmt3b vary according to the phases of the menstrual cycle (Vincent et al., 2011b; Yamagata et al., 2009). A previous study demonstrated the localization of Dnmt1, Dnmt3a and Dnmt3b in the uterus of pregnant mice (Ding et al., 2012) .

In our previous study, we showed that a high-fat diet differentially alters Dnmt1 and Dnmt3a expression and global DNA methylation levels in the ovaries and testis (Sukur et al., 2023). In this study, we aimed to evaluate the effects of high-fat diet-induced obesity on Dnmt1, Dnmt3a, Dnmt3b and Dnmt3l expression and global DNA methylation in the uterus in estrus phase of mice. Briefly, the results showed that the high-fat diet-induced obesity differentially altered Dnmts and global DNA methylation levels in the uterus.

Materials and Methods

Animals

The experimental protocol was approved by the Animal Care and Usage Committee of Ankara University (protocol no. 2020-17-145). C57BL6/J mice (4 weeks old) were housed in cages under a 12-h/12-h light/dark cycle with an ambient temperature of 22–25°C. After the female (n = 8) and male (n = 8) mice were randomly allocated to the appropriate cages, they were fed either a normal diet (Research Diets, D12450J) consisting of 20% protein, 10% fat and 70% carbohydrate or an HFD (Research Diets, D12492) consisting of 20% protein, 60% fat and 20% carbohydrate for 12 weeks. Body weights were measured weekly. At the end of 12 weeks, animals were euthanized, following ablation of the uterus, they were transferred to buffered solutions. Establishment of a HFD-induced obese mouse model was revealed in our previous article (Sukur et al., 2023).

Immunohistochemistry

The level of the Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and global DNA methylation in the uterus was evaluated by using immunohistochemistry. The paraffin sections of the tissues at 5 µm thickness were deparaffinized in fresh xylene and then rehydrated in a series of decreasing ethanol concentrations. After

deparaffinization and rehydration processes, citrate buffer (pH 6.0) was used for antigen retrieval using microwave (7 min at 750 W). The endogenous peroxidase activity in the sections was blocked by 3% hydrogen peroxide for 25 min at room temperature (RT). Following three times washes with 1× phosphate-buffered saline (PBS, Sigma-Aldrich, P44417), sections were blocked with blocking buffer (Thermo Scientific, UK) at RT for 7 min to prevent non-specific binding. Then, sections were incubated with the following primary antibodies specific for 5-methylcytosine (5mC) (1:100; catalog no. ab214727, Abcam), Dnmt1 (1:400; catalog no. ab87654, Abcam), Dnmt3a (1:100; catalog no. ab23565, Abcam), Dnmt3b (1:400; catalog no. 48488S, Cell Signalling), Dnmt3l (1:300; catalog no. ab194094, Abcam) at + 4°C overnight. For negative control, slides were incubated in PBS without primary antibody for each group. After that, sections were washed three times in PBS for 5 min each and subsequently incubated with anti-rabbit IgG secondary antibody (1:200; catalog no. BA-1000, Vector) at RT for 1 h. Finally, sections were incubated with streptavidin–horseradish peroxidase (HRP) complex (catalogue no. TS-125, Thermo Scientifc) for 20 min at RT. The immune reactions were visualized using 3,3'-diaminobenzidine (DAB) chromogen (catalogue no. D4168, Sigma-Aldrich) under a light microscope. Sections were washed in tap water and counterstained with Mayer's haematoxylin for 1 min. Slides were examined and images were obtained with a light microscope (ZEISS Scope.A1, Zeiss, Germany) at 20× (Zeiss, A-Plan 20×/0.45 Working Distance (W.D.) = 0.46 M27) and 40× (Zeiss, N-Achroplan 40×/0.65 W.D.=0.60 M27) magnifications. Images were analyzed and captured with AxioCam camera (Zeiss, AxioCam MRc5) and AxioVision image software (Zeiss AxioVision v. 4.8). Their dimensions were at 1292×968 (width×height) pixels, and each of them has a depth of 24 bits. Also, all micrographs were evaluated for the related expression using the software and presented a resolution of 300 dpi in the panels. We applied H-score analysis for specific regions, cell type evaluations, total protein expressions and global DNA methylation level. H-score analysis were performed by two independent observers. Uterine glands, epithelium, stroma, and myometrium were evaluated by H-score. Briefly, sections were evaluated using a numerical score based on the number of positive cells in the section (ten different fields in each section). H-score was calculated using the following equation: H-score = $\sum$Pi (i); where i is the intensity of staining with a value of 0, 1, 2, 3 (no signal, weak signal, moderate signal, high signal, respectively), Pi is the percentage of stained related cells for each intensity varying from 0–100%.

Statistical analysis

Signal intensities of immunohistochemistry images were digitalized with ImageJ software. Data were transferred to SigmaStat software (version 3.5, Jandel Scientifc Corp) for statistical analysis. The Shapiro–Wilk test for normality was used to test whether the data were normally distributed. Logarithmic transformation was applied to non-normally distributed data, when applicable. Normally distributed data were presented as mean ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) to examine the differences between the groups, and then a post hoc Tukey's test was applied. The significance level was set at $p < 0.05$.

Results

Changes in the overall expression of Dnmts and level of global DNA methylation in the uterus induced by the high-fat diet (HFD)

Relative expressions of Dnmts and global DNA methylation in uterus sections from both HFD and control group were evaluated (Fig. 1, $p < 0.05$). Expression of Dnmt1, Dnmt3a and Dnmt3b significantly increased in HFD group compared to control (Fig. 1b, $p < 0.05$). On the other hand, expression of Dnmt3l and level of global DNA methylation dramatically decreased in HFD group compared to control (Fig. 1b, $p < 0.05$).

Relative level of Dnmts and global DNA methylation in uterine glands

Relative expressions of Dnmts and global DNA methylation in uterine glands from both HFD and control group were evaluated (Fig. 2, $p < 0.05$). Expression of Dnmt3b and Dnmt3l remarkebly induced in HFD compared to control (Fig. 2b, $p < 0.05$). On the other hand, expression of Dnmt1 and Dnmt3a, and level of global DNA methylation exhibited similar level in both groups (Fig. 2b, $p < 0.05$). When subcellular localizations of Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC were considered in glandular cells, Dnmts were found in both nucleus and cytoplasm in both control and HFD groups. However, 5mC staining was revealed in nucleus of control group while it was observed in both nucleus and cytoplasm, but mainly in nucleus in HFD group.

Relative level of Dnmts and global DNA methylation in uterine stroma

Relative expressions of Dnmts and global DNA methylation in uterine glands from both HFD and control group were evaluated (Fig. 3, $p < 0.05$). Expression of Dnmt1 and Dnmt3a remained compareble in HFD compared to control (Fig. 3b, $p < 0.05$). Although, expression of Dnmt3b significantly increased in HFD compared to control, level of Dnmt3l and global DNA methylation remarkebly decreased in HFD compared to control (Fig. 3b, $p < 0.05$). Aditionally, subcellular localizations of Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC were examined in stromal cells, Dnmts were present in both nucleus and cytoplasm in both control and HFD groups. On the other hand, 5mC staining was revealed in nucleus of control group while it was observed in both nucleus and cytoplasm, but predominantly in nucleus in HFD group.

Relative level of Dnmts and global DNA methylation in uterine myometrium

Relative expressions of Dnmts and global DNA methylation in uterine glands from both HFD and control group were evaluated (Fig. 4, $p < 0.05$). Expression of Dnmt3b significantly increased in HFD compared to control (Fig. 4b, $p < 0.05$). On the other hand, level of Dnmt1, Dnmt3a, Dnmt3l and global DNA methylation dramatically decreased in HFD compared to control (Fig. 4b, $p < 0.05$). Moreover, subcellular localizations of Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC were investigated in myometrial cells, Dnmts

were located in both nucleus and cytoplasm in both control and HFD groups. Interestingly, 5mC staining was revealed in nucleus of control group while it was observed in both nucleus and cytoplasm, but mostly in nucleus in HFD group.

Relative level of Dnmts and global DNA methylation in uterine epithelium

Relative expressions of Dnmts and global DNA methylation in uterine glands from both HFD and control group were evaluated (Fig. 5, $p < 0.05$). Expression of Dnmt3b and Dnmt3l significantly increased while expression of Dnmt3a dramatically decreased in HFD group compared to control (Fig. 5b, $p < 0.05$). However, expression of Dnmt1 and level of global DNA methylation remained compareble in HFD compared to control (Fig. 5b, $p < 0.05$). Also, subcellular localizations of Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC were evaluated in epithelial cells, Dnmts were observed in both nucleus and cytoplasm in both control and HFD groups. However, 5mC staining was revealed in nucleus of control group while it was observed in both nucleus and cytoplasm, but notably in nucleus in HFD group.

Discussion

Obesity, which is a metabolic disease resulted from increased body mass index, is found to alter negatively endometrium receptivity (Bellver et al., 2021). It is known that obesity leads to alteration on epigenome (Ou et al., 2019), and in our previous study we found that significant alteration of global DNA methylation, expression of Dnmt1 and Dnmt3a in ovary and testis of high-fat diet induced-obese mice (Sukur et al., 2023). Additionally, it was demonstrated that there is a strong relationship between obesity and endometrial cancer (McDonald & Bender, 2019). Moreover, recent study revealed that Hand2, transcription factor expressed in endometrial stroma, is strongly associated with endometrial cancer. It was shown that *Hand2* was hypermethylated in the uterus of patients with endometrial cancer and *Hand2* conditional knockout mice exhibited precancerous lesion development in uterus and loss of Pten expression in endometrial epithelium (Jones et al., 2013). However, it was not shown the link between obesity, global DNA methylation and Dnmts in uterus. For the first time in this study, we revealed the alteration of Dnmts and global DNA methylation levels in uterus of HFD-induced obese mice (Fig. 6).

In the current study, we found that Dnmt1, Dnmt3a and Dnmt3b expression significantly increased while global DNA methylation and Dnmt3l levels dramatically decreased in uterus of HFD-induced obese group. Also, we revealed that even though global DNA methlyation level was not altered, Dnmt3b expression was significantly induced in uterine stroma of HFD group. We think that hypermethylation of *Hand2* can be achieved by increased level of Dnmt3b. Furthermore, we found that Dnmt3b and Dnmt3l expressions dramatically increased in uterine epithelium of HFD group. It was shown that uterine repairment of epithelium after menstruation is critical for excessive bleeding and HFD group showed impaired proliferation of uterine epithelium and eventually impaired repairment (Reavey et al., 2021), so we think that increased expression of Dnmt3b and Dnmt3l in epithelium may lead to repression of gene expression related with proliferation and repairment of epithelium. Additionally, we showed that

expression of Dnmt3b significantly increased while rest of the enzymes and global DNA methylation level decreased in myometrium of HFD group. It is known that dysfunctionality of myometrium observed in obese women leads to increased rate of delivery by caesarean section and it was shown that decreased expression of connexin 43 in myometrium of obese women (Carvajal & Oporto, 2021). Also, it is known that expression of connexin 43 is regulated by nitric oxide (Roh et al., 2002), thus we think that methylation status of *GJA1* and *NOS1* which encode connexin 43 and nitric oxide synthase respectively needs further investigation in case of obesity. Furthermore, uterine glands secrete several components which are necessary for uterine receptivity, implantation of blastocyst and stromal cell decidualization (Kelleher et al., 2019). We demonstrated that Dnmt3b and Dnmt3l expressions significantly induced in uterine glands of HFD group.

In conclusion, the current work, for the first time, demonstrates that significant alteration level of global DNA methylation and Dnmts in uterus of high-fat diet induced-obese mice. Our results suggest new insights for literature regarding the effect of obesity on uterus through global DNA methylation and Dnmts. Further investigation is required to reveal different DNA methylation patterns and differentially expressed genes in uterus of obese mice. Even though this could be a limitation for this study, our results are fundamental for the possible therapeutic agent for infertile obese women and will lead to further studies.

Declarations

Competing Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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Author Contributions

All authors qualify for authorship by contributing substantially to this article. F.U. and N.B. developed the original concept of this study and wrote the manuscript. N.B, T.K and G.S collected data and F.U. and O.C performed statistical analysis. F.U and O.C have contributed to critical discussion. All authors reviewed the final version of the article, and approved it for publication.

Ethics Approval

The experimental protocol was approved by the Animal Care and Usage Committee of Ankara University (Protocol no: 2020-17-145).

References

1. Bellver, J., Marín, C., Lathi, R. B., Murugappan, G., Labarta, E., Vidal, C., Giles, J., Cabanillas, S., Marzal, A., Galliano, D., Ruiz-Alonso, M., Simón, C., & Valbuena, D. (2021). Obesity Affects Endometrial Receptivity by Displacing the Window of Implantation. *Reprod Sci*, *28*(11), 3171-3180. https://doi.org/10.1007/s43032-021-00631-1
2. Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev*, *16*(1), 6-21. https://doi.org/10.1101/gad.947102
3. Broughton, D. E., & Moley, K. H. (2017). Obesity and female infertility: potential mediators of obesity's impact. *Fertil Steril*, *107*(4), 840-847. https://doi.org/10.1016/j.fertnstert.2017.01.017
4. Carvajal, J. A., & Oporto, J. I. (2021). The Myometrium in Pregnant Women with Obesity. *Curr Vasc Pharmacol*, *19*(2), 193-200. https://doi.org/10.2174/1570161118666200525133530
5. Catalano, P. M., & Shankar, K. (2017). Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. *BMJ*, *356*, j1. https://doi.org/10.1136/bmj.j1
6. Chedin, F., Lieber, M. R., & Hsieh, C. L. (2002). The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. *Proc Natl Acad Sci U S A*, *99*(26), 16916-16921. https://doi.org/10.1073/pnas.262443999
7. Deplus, R., Brenner, C., Burgers, W. A., Putmans, P., Kouzarides, T., de Launoit, Y., & Fuks, F. (2002). Dnmt3L is a transcriptional repressor that recruits histone deacetylase. *Nucleic Acids Res*, *30*(17), 3831-3838. https://doi.org/10.1093/nar/gkf509
8. Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., & Wang, H. (2004). Molecular cues to implantation. *Endocr Rev*, *25*(3), 341-373. https://doi.org/10.1210/er.2003-0020
9. Ding, Y. B., He, J. L., Liu, X. Q., Chen, X. M., Long, C. L., & Wang, Y. X. (2012). Expression of DNA methyltransferases in the mouse uterus during early pregnancy and susceptibility to dietary folate deficiency. *Reproduction*, *144*(1), 91-100. https://doi.org/10.1530/REP-12-0006
10. Dion, V., Lin, Y., Hubert, L., Jr., Waterland, R. A., & Wilson, J. H. (2008). Dnmt1 deficiency promotes CAG repeat expansion in the mouse germline. *Hum Mol Genet*, *17*(9), 1306-1317. https://doi.org/10.1093/hmg/ddn019
11. Futscher, B. W., Oshiro, M. M., Wozniak, R. J., Holtan, N., Hanigan, C. L., Duan, H., & Domann, F. E. (2002). Role for DNA methylation in the control of cell type specific maspin expression. *Nat Genet*, *31*(2), 175-179. https://doi.org/10.1038/ng886
12. Gao, F., Ma, X., Rusie, A., Hemingway, J., Ostmann, A. B., Chung, D., & Das, S. K. (2012). Epigenetic Changes Through DNA Methylation Contribute to Uterine Stromal Cell Decidualization. *Endocrinology*, *153*(12), 6078-6090. https://doi.org/10.1210/en.2012-1457
13. Ghabreau, L., Roux, J. P., Niveleau, A., Fontanière, B., Mahe, C., Mokni, M., & Frappart, L. (2004). Correlation between the DNA global methylation status and progesterone receptor expression in normal endometrium, endometrioid adenocarcinoma and precursors. *Virchows Arch*, *445*(2), 129-134. https://doi.org/10.1007/s00428-004-1059-4

14. Hata, K., Okano, M., Lei, H., & Li, E. (2002). Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. *Development*, *129*(8), 1983-1993. https://doi.org/10.1242/dev.129.8.1983
15. Hirasawa, R., Chiba, H., Kaneda, M., Tajima, S., Li, E., Jaenisch, R., & Sasaki, H. (2008). Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. *Genes Dev*, *22*(12), 1607-1616. https://doi.org/10.1101/gad.1667008
16. Igosheva, N., Abramov, A. Y., Poston, L., Eckert, J. J., Fleming, T. P., Duchen, M. R., & McConnell, J. (2010). Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One*, *5*(4), e10074. https://doi.org/10.1371/journal.pone.0010074
17. Jones, A., Teschendorff, A. E., Li, Q., Hayward, J. D., Kannan, A., Mould, T., West, J., Zikan, M., Cibula, D., Fiegl, H., Lee, S. H., Wik, E., Hadwin, R., Arora, R., Lemech, C., Turunen, H., Pakarinen, P., Jacobs, I. J., Salvesen, H. B., . . . Widschwendter, M. (2013). Role of DNA methylation and epigenetic silencing of HAND2 in endometrial cancer development. *PLoS Med*, *10*(11), e1001551. https://doi.org/10.1371/journal.pmed.1001551
18. Jungheim, E. S., Schoeller, E. L., Marquard, K. L., Louden, E. D., Schaffer, J. E., & Moley, K. H. (2010). Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology*, *151*(8), 4039-4046. https://doi.org/10.1210/en.2010-0098
19. Kelleher, A. M., DeMayo, F. J., & Spencer, T. E. (2019). Uterine Glands: Developmental Biology and Functional Roles in Pregnancy. *Endocr Rev*, *40*(5), 1424-1445. https://doi.org/10.1210/er.2018-00281
20. Kim, S. Y., England, L., Wilson, H. G., Bish, C., Satten, G. A., & Dietz, P. (2010). Percentage of gestational diabetes mellitus attributable to overweight and obesity. *Am J Public Health*, *100*(6), 1047-1052. https://doi.org/10.2105/AJPH.2009.172890
21. Ko, Y. G., Park, H. J., Yun, J., Koh, P. O., Min, W., Cho, K. W., Won, C. K., Seong, H. H., Kim, G. S., & Cho, J. H. (2010). DNA methylation contributes to the tissue-specific expression of the rPL-lv gene. *Placenta*, *31*(11), 969-975. https://doi.org/10.1016/j.placenta.2010.08.010
22. Law, J. A., & Jacobsen, S. E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat Rev Genet*, *11*(3), 204-220. https://doi.org/10.1038/nrg2719
23. Li, E. (2002). Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet*, *3*(9), 662-673. https://doi.org/10.1038/nrg887
24. Li, E., Bestor, T. H., & Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell*, *69*(6), 915-926. https://doi.org/10.1016/0092-8674(92)90611-f
25. Mao, J., Zhang, X., Sieli, P. T., Falduto, M. T., Torres, K. E., & Rosenfeld, C. S. (2010). Contrasting effects of different maternal diets on sexually dimorphic gene expression in the murine placenta. *Proc Natl Acad Sci U S A*, *107*(12), 5557-5562. https://doi.org/10.1073/pnas.1000440107
26. Margot, J. B., Ehrenhofer-Murray, A. E., & Leonhardt, H. (2003). Interactions within the mammalian DNA methyltransferase family. *BMC Mol Biol*, *4*, 7. https://doi.org/10.1186/1471-2199-4-7
27. McDonald, M. E., & Bender, D. P. (2019). Endometrial Cancer: Obesity, Genetics, and Targeted Agents. *Obstet Gynecol Clin North Am*, *46*(1), 89-105. https://doi.org/10.1016/j.ogc.2018.09.006

28. McMillen, I. C., & Robinson, J. S. (2005). Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev*, *85*(2), 571-633. https://doi.org/10.1152/physrev.00053.2003
29. Okano, M., Bell, D. W., Haber, D. A., & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell*, *99*(3), 247-257. https://doi.org/10.1016/s0092-8674(00)81656-6
30. Ou, X. H., Zhu, C. C., & Sun, S. C. (2019). Effects of obesity and diabetes on the epigenetic modification of mammalian gametes. *J Cell Physiol*, *234*(6), 7847-7855. https://doi.org/10.1002/jcp.27847
31. Reavey, J. J., Walker, C., Murray, A. A., Brito-Mutunayagam, S., Sweeney, S., Nicol, M., Cambursano, A., Critchley, H. O. D., & Maybin, J. A. (2021). Obesity is associated with heavy menstruation that may be due to delayed endometrial repair. *J Endocrinol*, *249*(2), 71-82. https://doi.org/10.1530/joe-20-0446
32. Roh, C. R., Heo, J. H., Yang, S. H., & Bae, D. S. (2002). Regulation of connexin 43 by nitric oxide in primary uterine myocytes from term pregnant women. *Am J Obstet Gynecol*, *187*(2), 434-440. https://doi.org/10.1067/mob.2002.123600
33. Samblas, M., Milagro, F. I., & Martinez, A. (2019). DNA methylation markers in obesity, metabolic syndrome, and weight loss. *Epigenetics*, *14*(5), 421-444. https://doi.org/10.1080/15592294.2019.1595297
34. Sen, S., & Simmons, R. A. (2010). Maternal antioxidant supplementation prevents adiposity in the offspring of Western diet-fed rats. *Diabetes*, *59*(12), 3058-3065. https://doi.org/10.2337/db10-0301
35. Shankar, K., Zhong, Y., Kang, P., Lau, F., Blackburn, M. L., Chen, J. R., Borengasser, S. J., Ronis, M. J., & Badger, T. M. (2011). Maternal obesity promotes a proinflammatory signature in rat uterus and blastocyst. *Endocrinology*, *152*(11), 4158-4170. https://doi.org/10.1210/en.2010-1078
36. Straussman, R., Nejman, D., Roberts, D., Steinfeld, I., Blum, B., Benvenisty, N., Simon, I., Yakhini, Z., & Cedar, H. (2009). Developmental programming of CpG island methylation profiles in the human genome. *Nat Struct Mol Biol*, *16*(5), 564-571. https://doi.org/10.1038/nsmb.1594
37. Sukur, G., Uysal, F., & Cinar, O. (2023). High-fat diet induced obesity alters Dnmt1 and Dnmt3a levels and global DNA methylation in mouse ovary and testis. *Histochem Cell Biol*, *159*(4), 339-352. https://doi.org/10.1007/s00418-022-02173-2
38. Vincent, Z. L., Farquhar, C. M., Mitchell, M. D., & Ponnampalam, A. P. (2011a). Expression and regulation of DNA methyltransferases in human endometrium. *Fertil Steril*, *95*(4), 1522-1525.e1521. https://doi.org/10.1016/j.fertnstert.2010.09.030
39. Vincent, Z. L., Farquhar, C. M., Mitchell, M. D., & Ponnampalam, A. P. (2011b). Expression and regulation of DNA methyltransferases in human endometrium. *Fertil Steril*, *95*(4), 1522-1525 e1521. https://doi.org/10.1016/j.fertnstert.2010.09.030
40. Wang, H., & Dey, S. K. (2006). Roadmap to embryo implantation: clues from mouse models. *Nat Rev Genet*, *7*(3), 185-199. https://doi.org/10.1038/nrg1808

41. Wang, Y., Liu, Q., Tang, F., Yan, L., & Qiao, J. (2019). Epigenetic Regulation and Risk Factors During the Development of Human Gametes and Early Embryos. *Annu Rev Genomics Hum Genet*, *20*, 21-40. https://doi.org/10.1146/annurev-genom-083118-015143

42. Waterland, R. A., Kellermayer, R., Rached, M. T., Tatevian, N., Gomes, M. V., Zhang, J., Zhang, L., Chakravarty, A., Zhu, W., Laritsky, E., Zhang, W., Wang, X., & Shen, L. (2009). Epigenomic profiling indicates a role for DNA methylation in early postnatal liver development. *Hum Mol Genet*, *18*(16), 3026-3038. https://doi.org/10.1093/hmg/ddp241

43. Yamagata, Y., Asada, H., Tamura, I., Lee, L., Maekawa, R., Taniguchi, K., Taketani, T., Matsuoka, A., Tamura, H., & Sugino, N. (2009). DNA methyltransferase expression in the human endometrium: down-regulation by progesterone and estrogen. *Hum Reprod*, *24*(5), 1126-1132. https://doi.org/10.1093/humrep/dep015

44. Yeh, J., & Shelton, J. A. (2005). Increasing prepregnancy body mass index: analysis of trends and contributing variables. *Am J Obstet Gynecol*, *193*(6), 1994-1998. https://doi.org/10.1016/j.ajog.2005.05.001

45. You, D., Nilsson, E., Tenen, D. E., Lyubetskaya, A., Lo, J. C., Jiang, R., Deng, J., Dawes, B. A., Vaag, A., Ling, C., Rosen, E. D., & Kang, S. (2017). Dnmt3a is an epigenetic mediator of adipose insulin resistance. *Elife*, *6*. https://doi.org/10.7554/eLife.30766

Figures

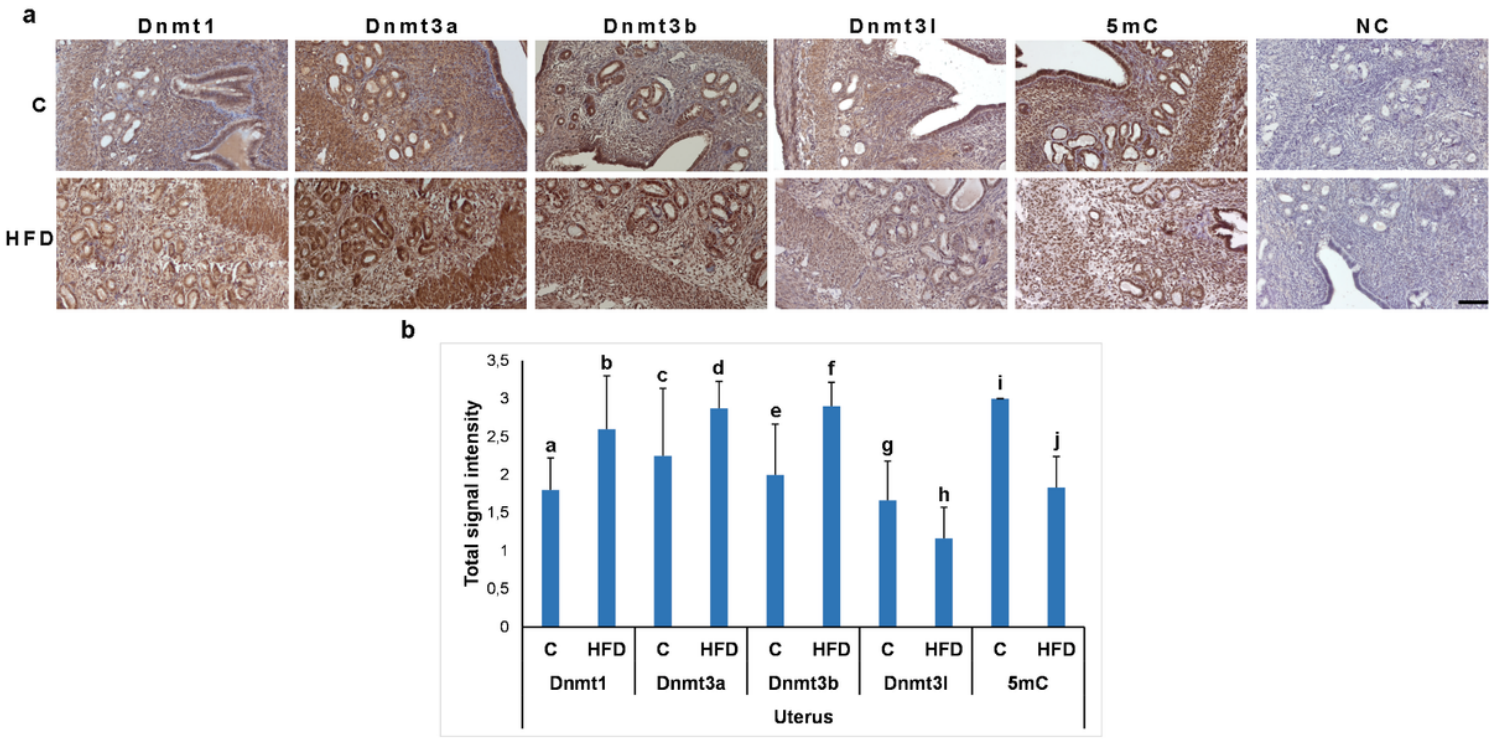


Figure 1

Changes in the overall expression of Dnmts and level of global DNA methylation in the uterus induced by the high-fat diet (HFD). **(a)** Demonstrative micrographs obtained from Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC stained sections **(b)** Total Dnmts and 5mC levels in immunohistochemistry (IHC) analysis. The statistical significance was determined by using one-way ANOVA followed by post hoc Tukey's test. The $p<0.05$ was considered statistically significant, shown different letters on the columns. Bars in graphs are represented as mean ± SD. Scale bar: 40 μm.

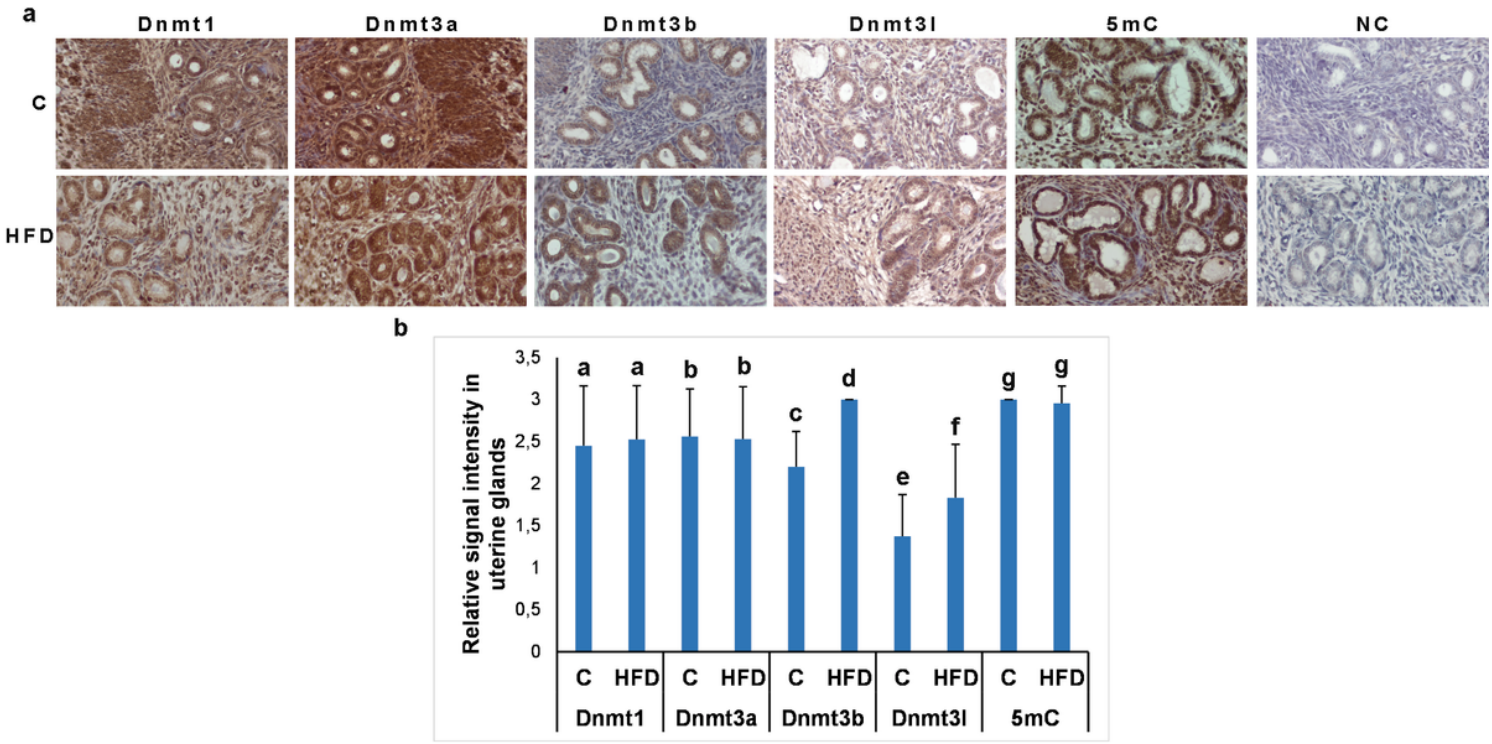


Figure 2

Relative level of Dnmts and global DNA methylation in uterine glands. **(a)** Demonstrative micrographs obtained from Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC stained sections. **(b)** Dnmts and 5mC levels in immunohistochemistry (IHC) analysis. The statistical significance was determined by using one-way ANOVA followed by post hoc Tukey's test. The $p<0.05$ was considered statistically significant, shown different letters on the columns. Bars in graphs are represented as mean ± SD. Scale bar: 20 μm.

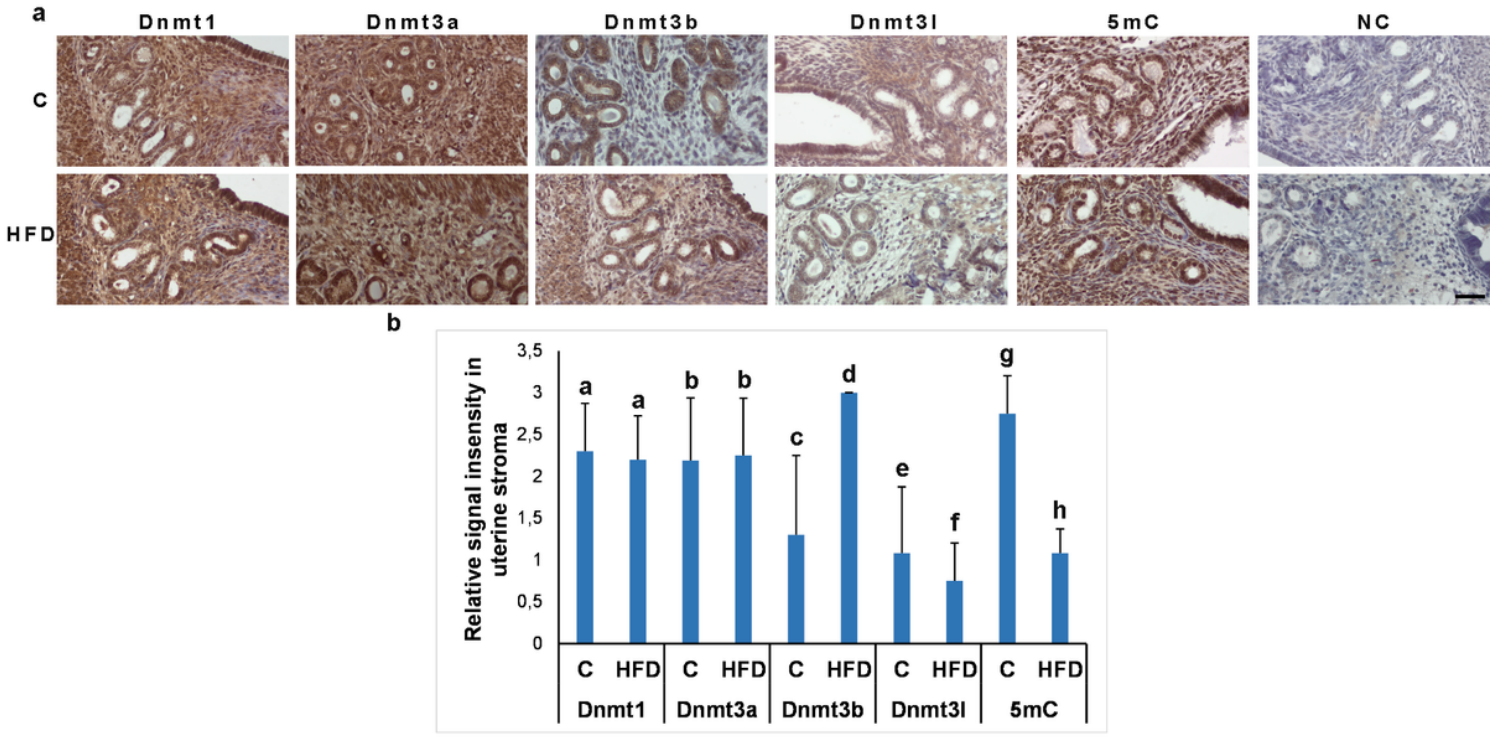


Figure 3

Relative level of Dnmts and global DNA methylation in uterine stroma. **(a)** Demonstrative micrographs obtained from Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC stained sections. **(b)** Dnmts and 5mC levels in immunohistochemistry (IHC) analysis. The statistical significance was determined by using one-way ANOVA followed by post hoc Tukey's test. The $p<0.05$ was considered statistically significant, shown different letters on the columns. Bars in graphs are represented as mean ± SD. Scale bar: 20 μm.

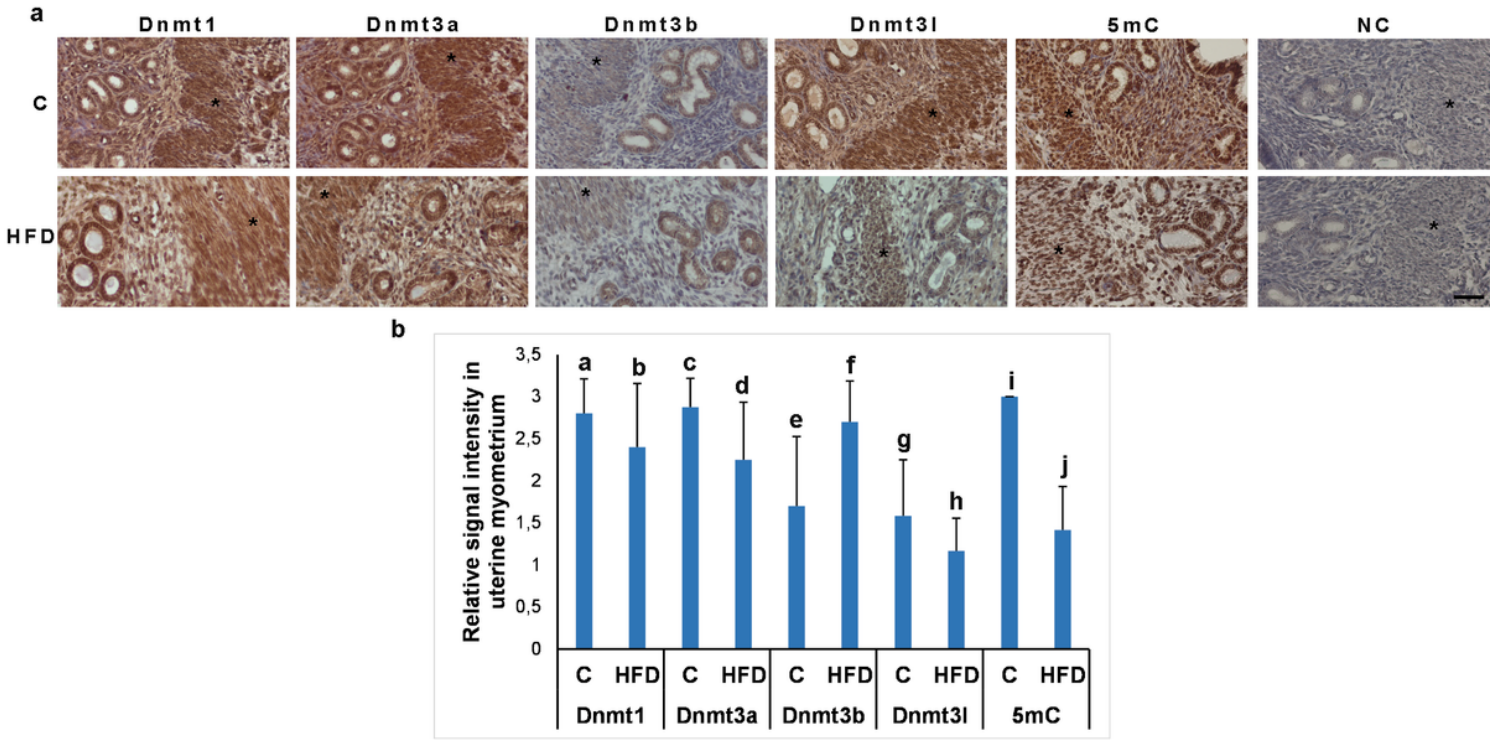


Figure 4

Relative levels of Dnmts and global DNA methylation in uterine myometrium. **(a)** Demonstrative micrographs obtained from Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC stained sections. **(b)** Dnmts and 5mC levels in immunohistochemistry (IHC) analysis. The statistical significance was determined by using one-way ANOVA followed by post hoc Tukey's test. The $p<0.05$ was considered statistically significant, shown different letters on the columns. Bars in graphs are represented as mean ± SD. Scale bar: 20 μm.

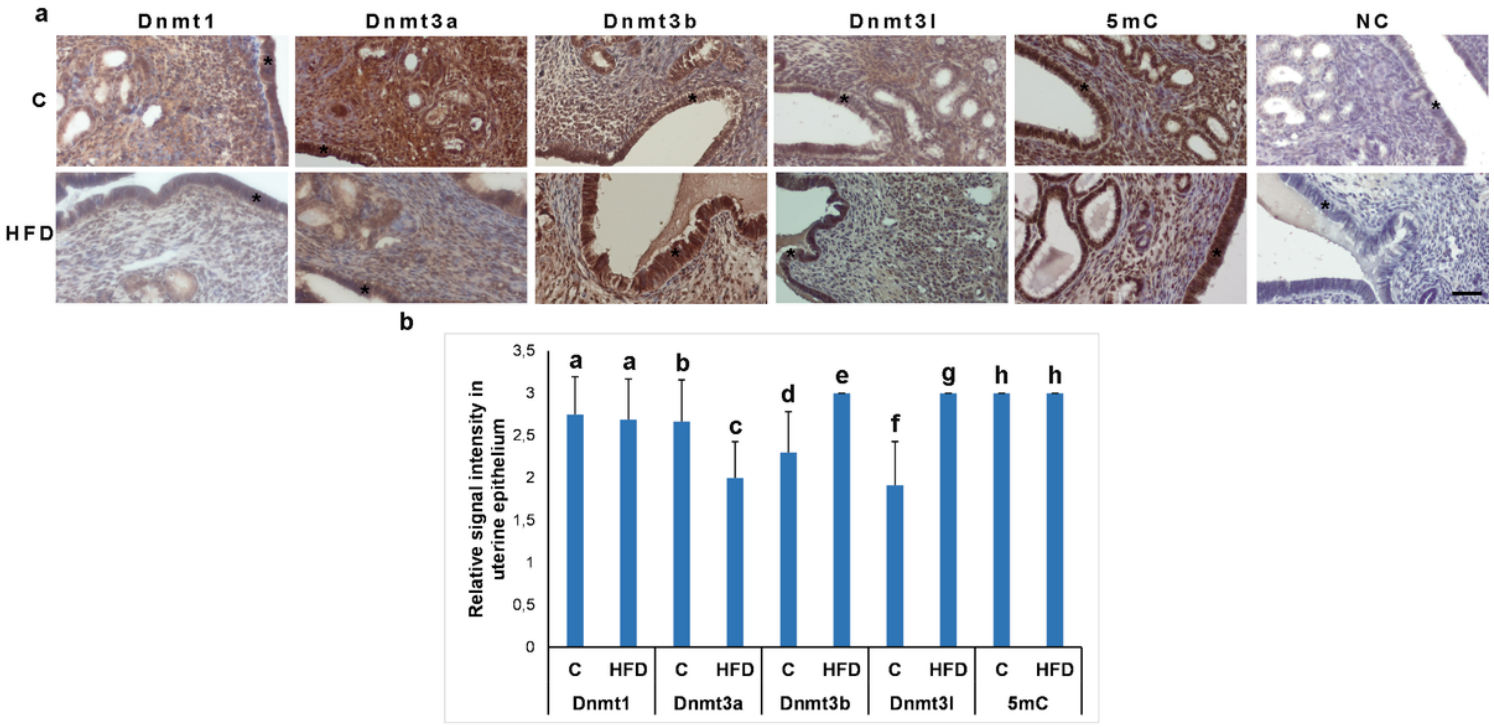


Figure 5

Relative levels of Dnmts and global DNA methylation in uterine epithelium. **(a)** Demonstrative micrographs obtained from Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and 5mC stained sections. **(b)** Dnmts and 5mC levels in immunohistochemistry (IHC) analysis. The statistical significance was determined by using one-way ANOVA followed by post hoc Tukey's test. The $p<0.05$ was considered statistically significant, shown different letters on the columns. Bars in graphs are represented as mean ± SD. Scale bar: 20 μm.

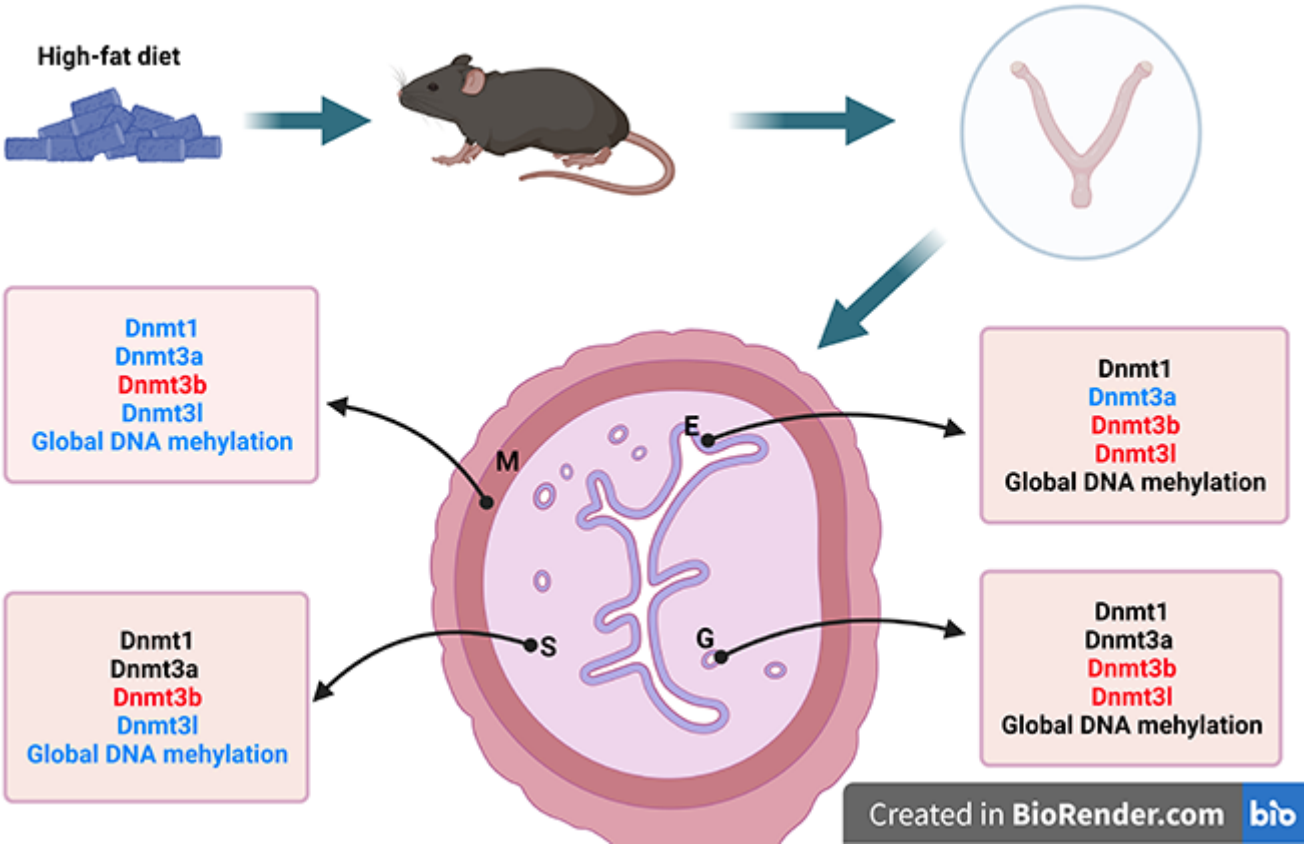


Figure 6

Representation of HFD-induced alterations in levels of Dnmts and global DNA methylation in uterus. Demonstrative figure of HFD-induced Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l and global DNA methylation levels in uterine glands (G), epithelium (E), stroma (S) and myometrium (M). Red color indicates for significantly increased level, blue for significantly decreased level in HFD group compared to control and black for no significant difference between HFD and control group. Created with BioRender.com.