ENZYME DISORDERS AND INFERTILITY

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Introduction

- Infertility is a global problem and according to the World Health Organization, almost one in seven couples are affected by fertility complications.

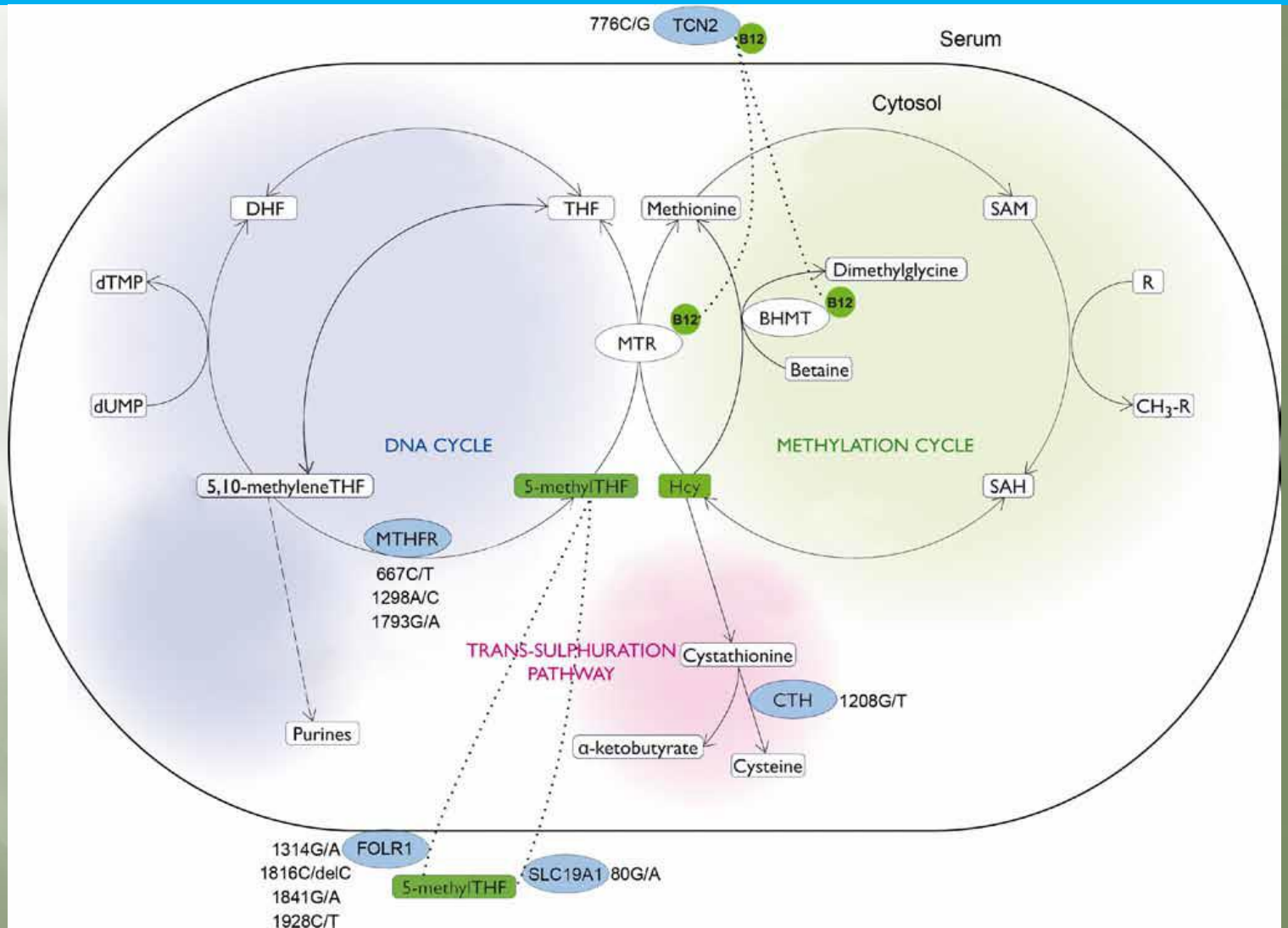
Time to pregnancy (TTP) is a measure of how long a couple takes to conceive and infertility is the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse.

- Emerging evidence also suggests a link between folate-mediated one-carbon metabolism (OCM) as a modulator of female fertility and pregnancy viability.
- There is growing evidence that folates are crucial for human reproduction.

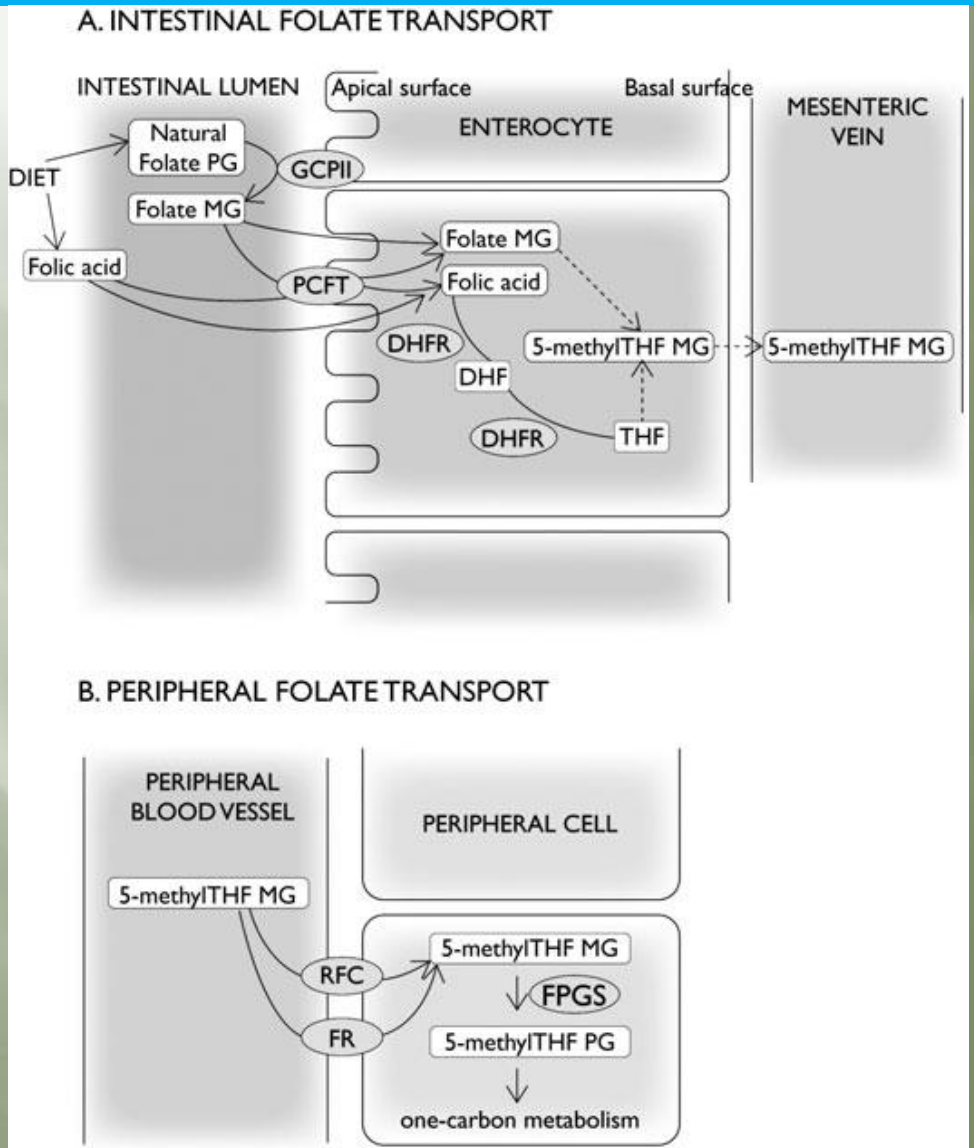
Folate functions

- Folates are folic acid compounds that occur naturally in food; they are part of the vitamin B complex and are an essential nutritional component of the human diet.
- More than 150 folate compounds are known.
- Folate is required during periods of rapid cell growth and proliferation, which occur during oocyte and follicular maturation and development.
- Folate-mediated one-carbon metabolism consists of two intertwined cycles, one producing dTMP and purine precursors for DNA biosynthesis (called DNA cycle), and the other producing and utilizing methyl group donor S-adenosylmethionine for methylation reactions (called methylation cycle).

Folate-mediated one-carbon metabolism consists of two cycles: DNA biosynthesis and methylation. Homocysteine is catabolized in the trans-sulfuration pathway. Some genes involved in the pathways are indicated as ellipses and some of the most studied variations are listed next to the gene. Folate, homocysteine and vitamin B12 are also indicated. Compound transport into the cell is shown in dotted line (from Laanpere et al., 2011; published with permission from Elsevier). TCN2 = transcobalamin II, DHF = dihydrofolate, THF = tetrahydrofolate, SAM = S-adenosylmethionine, MTR = 5-methyltetrahydrofolate-homocysteine methyltransferase, BHMT = betaine-homocysteine methyltransferase, MTHFR = methylenehydrofolate reductase, Hcy = homocysteine, SAH = S-adenosylhomocysteine, CTH = cystathionase, FOLR1 = folate receptor 1, SCL19A1 = solute carried family 19, member 1.



Folate absorption and transport. (A) Intestinal folate transport. Glutamate carboxypeptidase II (GCPII) hydrolyzes natural food folate polyglutamates (PGs) to folate monoglutamates (MGs) in the intestine. Folate MGs and synthetic folic acid enter enterocytes by both passive diffusion and active transport that is probably predominantly mediated by proton-coupled folate transporter (PCFT). Once inside the cell, oxidized folic acid is reduced to dihydrofolate (DHF) and tetrahydrofolate (THF) by dihydrofolate reductase (DHFR). Absorbed folic acid and natural folate MGs are converted to 5-methyltetrahydrofolate (5-methylTHF) before transport to the mesenteric vein. (B) Peripheral folate transport. Circulating 5-methylTHF MGs are internalized by peripheral cells by reduced folate carrier (RFC) or folate receptors (FRs), and polyglutamated by folylpolyglutamate synthase (FPGS).



Folate deficiency

- Folate deficiency is common in humans and it can occur as a result of poor dietary intake or malabsorption of folate.

  Low folate levels could be additionally caused by insufficient levels of micronutrients necessary for folate metabolism, such as vitamins B2, B6, B12, Fe, and Zn.

- Indeed, recent findings demonstrate that nutrients that are part of methyl group metabolism can significantly modify epigenetics.

- Further, during critical periods of development, dietary methyl-group intake can modify DNA and histone methylation, which has an impact on lifelong changes in gene expression.

- Therefore, the folate deficiency-induced processes mentioned may potentially interfere with female fertility by interrupting normal oocyte and follicular maturation, fertilization, and embryo and fetal growth and development.

Genetic basis of Folate deficiency

- One reason for inefficient folate utilization could arise from variations in folate-metabolizing genes.
- Several polymorphisms have been identified in genes involved in folate absorption and folate mediated one-carbon metabolism.
- These variations may modify the beneficial effects of folates and other micronutrients within the folate mediated one-carbon metabolism.
- The most influential polymorphism in folate-metabolizing pathway in terms of prevalence and impact seems to be 677C/T variation in the MTHFR gene.
- It results in an amino acid change at codon Ala222Val, giving rise to an unstable enzyme of 50-60% reduced activity.
- Folate-mediated one-carbon metabolism pathway with reduced MTHFR activity leads to impaired methylation reactions and accumulation of Homocysteine.

Selection of gene variants affecting folate absorption and metabolism (Modified from Laanpere et al., 2010. With permission from John Wiley and Sons).

Gene symbol	Gene name	Polymorphism	Phenotypic effect	Reference
CTH	cystathionase	1208 G/T (Ser403Ile, rs1021737)	higher Hcy levels	Altmäe *et al.*, 2010
FOLH1	folate hydrolase 1	1423 C/T (His475Tyr, rs61886492)	reduced enzyme activity	Devlin *et al.*, 2006
FOLR1	folate receptor 1	1816 delC (rs3833748) 1841 G/A (rs1540087)	delC/A haplotype Hcy-raising effect, lower folate	Nilsson *et al.*, 2012
MTHFR	methylenetetrahydrofolate reductase	677 C/T (Ala222Val, rs1801133) 1298 A/C (Glu429Ala, rs1801131)	reduced enzyme activity, low folate levels and high Hcy[4]. 677 C/T also DNA hypomethylation, reduced uracil misincorporation	Laanpere *et al.*, 2010
MTR	5-methyltetrahydrofolate-homocysteine methyltransferase	2756 A/G (Asp919Gly, rs1805087)	reduced Hcy	Fredriksen *et al.*, 2007
MTRR	5-methylenetetrahydrofolate-homocysteine methyltransferase reductase	66 A/G (Ile22Met, rs1801394)	higher Hcy levels	Gaughan *et al.*, 2001
SLC19A1	Solute carrier family 19, member 1	80 G/A (Arg27His, rs1051266)	higher Hcy, impaired folate translocation into cells	Chango *et al.*, 2000; Dufficy *et al.*, 2006

MTHFR C677T Mutation

- MethyleneTetraHydroFolate Reductase (MTHFR) SNPs, especially the C677T, is a significant inducer of perturbations in methylation that affect fertility.
- This is now well established for women as the preimplantation development is, during the 3–4 days totally dependent of the oocyte quality in term of maternally derived mRNAs and metabolites and nutrients supplied by the oviduct.
- The MTHFR 677C>T polymorphism elevates Hcy levels in the blood, especially during periods of folate insufficiency.
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- When MTHFR activity is reduced, SAM production and cellular methylation reactions are expected to be impaired.
- Patients >35 years old who were homozygous for the MTHFR 677C major allele required significantly less follicle-stimulating hormone (FSH) in ovarian stimulation, produced significantly more oocytes, and had higher serum estradiol concentrations than heterozygous or homozygous carriers of the 677T allele.

MTHFR 1298A>C (rs1801131) mutation

- The second common polymorphism in the coding region of the MTHFR gene that has been studied extensively is 1298A>C (rs1801131).
- It causes a p.Glu429Ala change in the regulatory C-terminal domain of the MTHFR protein.
- Although the original investigations suggested that the polymorphism resulted in mildly decreased MTHFR activity, a later study found the recombinant protein to have exactly the same biochemical properties as the wild-type enzyme.

Cont…

- Earlier studies did not find significant differences in serum Hcy levels between MTHFR 1298A>C genotypes.
- However, both our recent study and a large-scale epidemiological study showed that when evaluated within the stratum of 677CC, the 1298 C allele raises Hcy levels.
- Another study of IVF patients found that the MTHFR 1298C allele was associated with both higher basal FSH levels, indicative of lower ovarian follicular reserve, and diminished responses to ovarian stimulation.
- It was consequently hypothesized that proliferation of granulosa cells could be inhibited in individuals carrying the MTHFR 1298C allele.

MATERNAL FOLATE STATUS, FETAL FOLATE-METABOLIZING GENE VARIANTS, AND FETAL VIABILITY

- **Several findings indicate that the fetal MTHFR 677C>T polymorphism may influence fetal viability and that its effect is modulated by maternal folate status.**
- **In the presence of a diet containing adequate levels of folate, the MTHFR 677T allele has been suggested to increase embryo viability.**

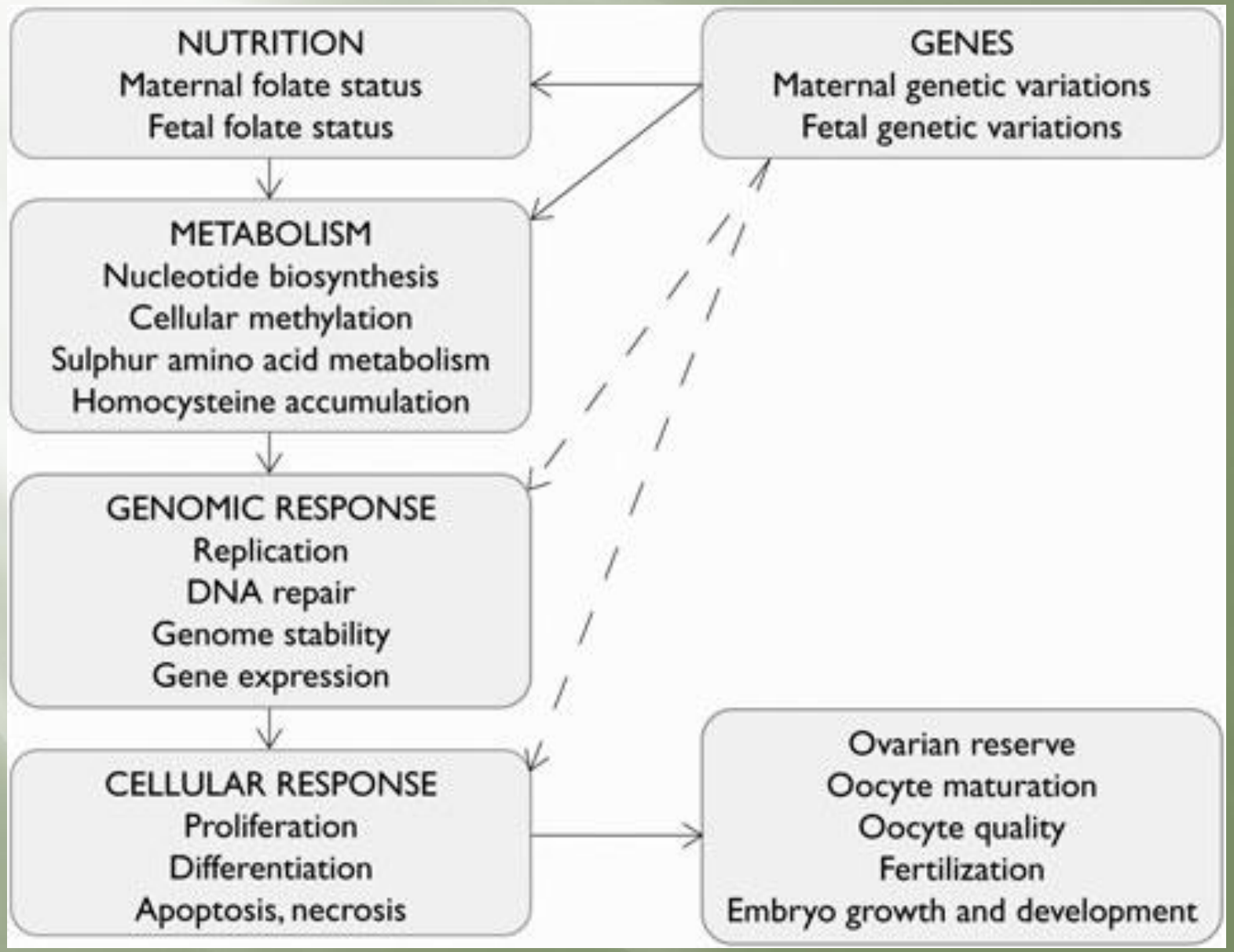
FAVORABLE EFFECTS OF MATERNAL AND FETAL MTHFR 677CT HETEROZYGOUS GENOTYPES ON FEMALE FERTILITY AND FETAL VIABILITY

- In women receiving IVF the heterozygous MTHFR 677CT genotype is the most advantageous for achieving a pregnancy and having a live birth.
- In line with these findings, we have demonstrated a lower occurrence of MTHFR 677CT heterozygosity in the female partners of infertile couples who lack any obvious reason for their infertility and are thus classified as women with unexplained infertility.
- Some of the studies conducted in spontaneously aborted embryos show that embryos with the MTHFR 677CT genotype are more viable than embryos with homozygous genotypes.

CONCLUSION

- Adequately functioning folate-mediated one-carbon metabolism supports DNA synthesis, repair, and integrity, and provides methyl groups for DNA and histone methylation, thus ensuring chromosome stability and proper gene expression.
- All of these processes are fundamental for female reproductive physiology, including gonadal function, fertilization, and embryo and fetal growth and development.
- Therefore, sufficient folate intake and uninterrupted folate metabolism may be essential for female fecundity.

CONCLUSchematic overviewof factors affecting one-carbon metabolism, the genomic and cellular responses evoked by altered folate status, and subsequent phenotypic outcomes that may affect female fertility and fetal viability. Modified with permission from Beaudin and Stover (2007)SION



Thank you