FETAL AND PLACENTAL EFFECTS OF FROZEN EMBRYO TRANSFER

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ART AND FETAL/PLACENTAL EFFECTS

- Western world 6-8% of pregnancies → conceived by ART
- ART → treat moderate to severe infertility:

  IVF, ICSI, FET, oocyte donation, blastocyst culture, IUI, and PGT-A

Each of these techniques and as well as the underlying infertility pathology represents a possible confounding factor to determine a precise relationship between ART and obstetric and neonatal outcomes

Meta-analysis of 50 cohort studies: increased risk of PIH, PE, placenta praevia, abruptio, APH, oligohydramnios, PTB, caesarean section, VLBW and increased PNM *(Qin J, Liu X et al: Fertility and Sterility)*

WHY?

- Well documented → changes have been reported → placental morphology and structure, growth dynamics, imprinted and non imprinted genes, increased placental thickness, haematomas and other placental regulatory aspects. Imprinting disorders like BWS, PW and Angelman syndrome is higher.

- ART → increased risk of circa/epi-genetic alterations→ gene expression and DNA methylation in early development and in placenta

- Confounders → infertility pathology itself/ types of fertility treatments

FETAL EFFECTS AND ART

- Risk of major congenital anomalies – higher prevalence AOR of 2 (CI 1-3.8)

- ICSI → urogenital malformations – hypospadias

- Cleft lip/palate –OR of 2.4
- Oesophageal atresia –OR 4.5
- Anorectal atresia –OR 3.7
- Septal heart defects –OR 2.1
- VACTERL and oculoauriculovertebral spectrum phenotypes

PATHOPHYSIOLOGY

- 1. Abnormal hormonal milieu
- 2. Circa/Epi-genetic changes
- 3. Immune activity dysregulation
- 4. Dysmetabolism
- 5. Inflammation

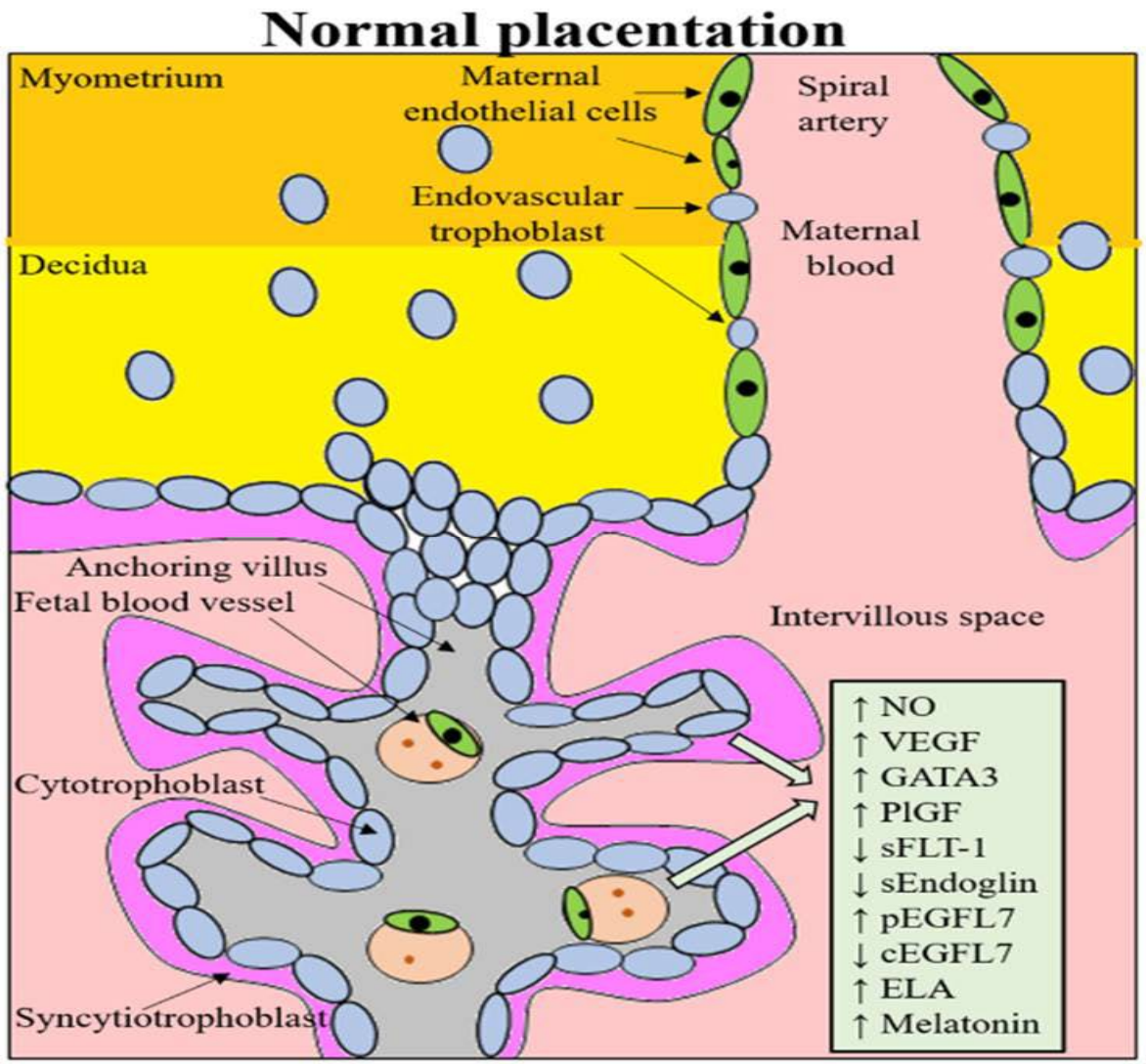
- Theoretical basis on placental changes caused by ART→ fetal, neonatal and long term diseases.

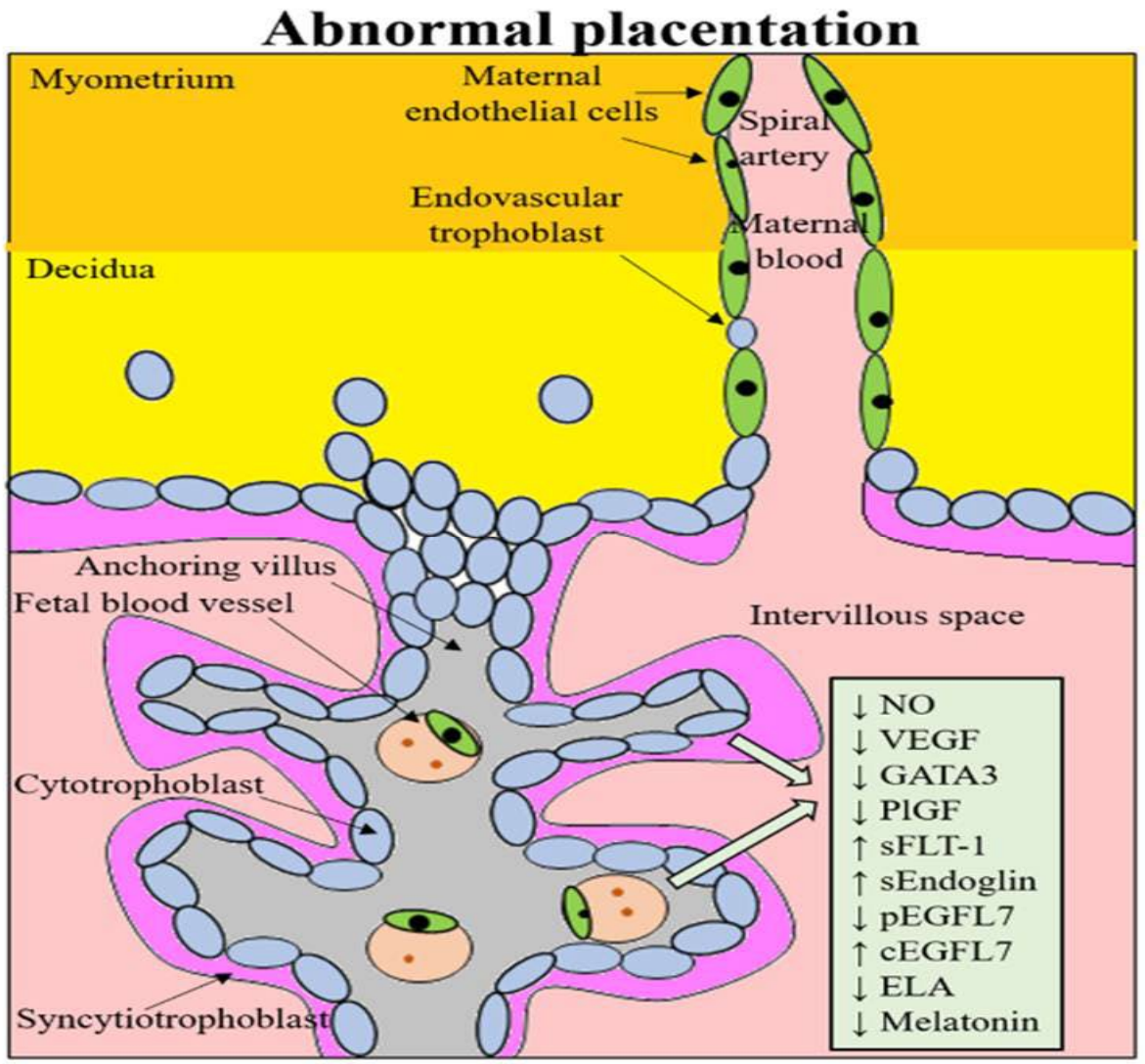
ART

- Altered expression of molecules and factors→ reduced vascular remodelling, impaired trophoblastic invasion + hypoperfusion
- Dysregulated expression of PLGF and sFlt-1→ systemic endothelial response.
- sEndoglin is markedly increased → upregulated in the abnormal placenta → antiangiogenic properties.

ABNORMAL HORMONAL MILIEU

- Supraphysiological hormonal levels caused by ovarian stimulation treatment
- High E2 levels → spiral artery remodelling, alters angiogenesis and placental insufficiency
- Controlled ovarian stimulation → in fresh cycles→ multiple CL →high amounts of progesterone→strong negative feedback on LH→ failure/ impacts on placental development
- Conversely lack of hormones may also affect placental development →as suggested for some FET protocols following hormonal endometrial preparation→ in these protocols CL is not formed → secretes protective vasoactive substances eg relaxin


Normal placentation
Myometrium
Maternal endothelial cells
Spiral artery
Endovascular trophoblast
Maternal blood
Decidua
Anchoring villus
Fetal blood vessel
Intervillous space
Cytotrophoblast
Syncytiotrophoblast
↑ NO
↑ VEGF
↑ GATA3
↑ PlGF
↓ sFLT-1
↓ sEndoglin
↑ pEGFL7
↓ cEGFL7
↑ ELA
↑ Melatonin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CIRCA/EPI -GENETIC CHANGES AFTER ART

- Circa/Epi-genome → chemical compounds→modify or mark the genome→ specific instructions
- Epigenetic regulatory mechanisms occur from several processes:
- Direct DNA methylation, non coding RNA, imprinting, post translational modification of histone proteins and chromatin remodelling
- Prolonged exposure to extracorporeal environment → anomalies or modification of these mechanisms especially DNA methylation → gene expression alterations
- Syncytiotrophoblastic stress can also affect the establishment and/or maintenance of genomic imprinting (DNA methylation silencing an allele of the one copy of the genetic sequence)
- Evidence that longer the in-vitro embryo culture lasts ie blastocyst transfer c/f cleavage stage more likely that epigenetic changes will occur

EPIGENETIC CHANGES -ART

- Placenta develops from the outer trophoblastic layer→more susceptible to circa/epigenetic regulatory changes → environmental interventions and influences during ART
- An increased incidence of autism spectrum disorders noted in IVF → epigenetic alterations

IMMUNE DYSREGULATION AT THE MATERNAL-FETAL INTERFACE

- Increased immune activity noted in IVF pregnancies at materno-fetal interface with increased incidence of villitis

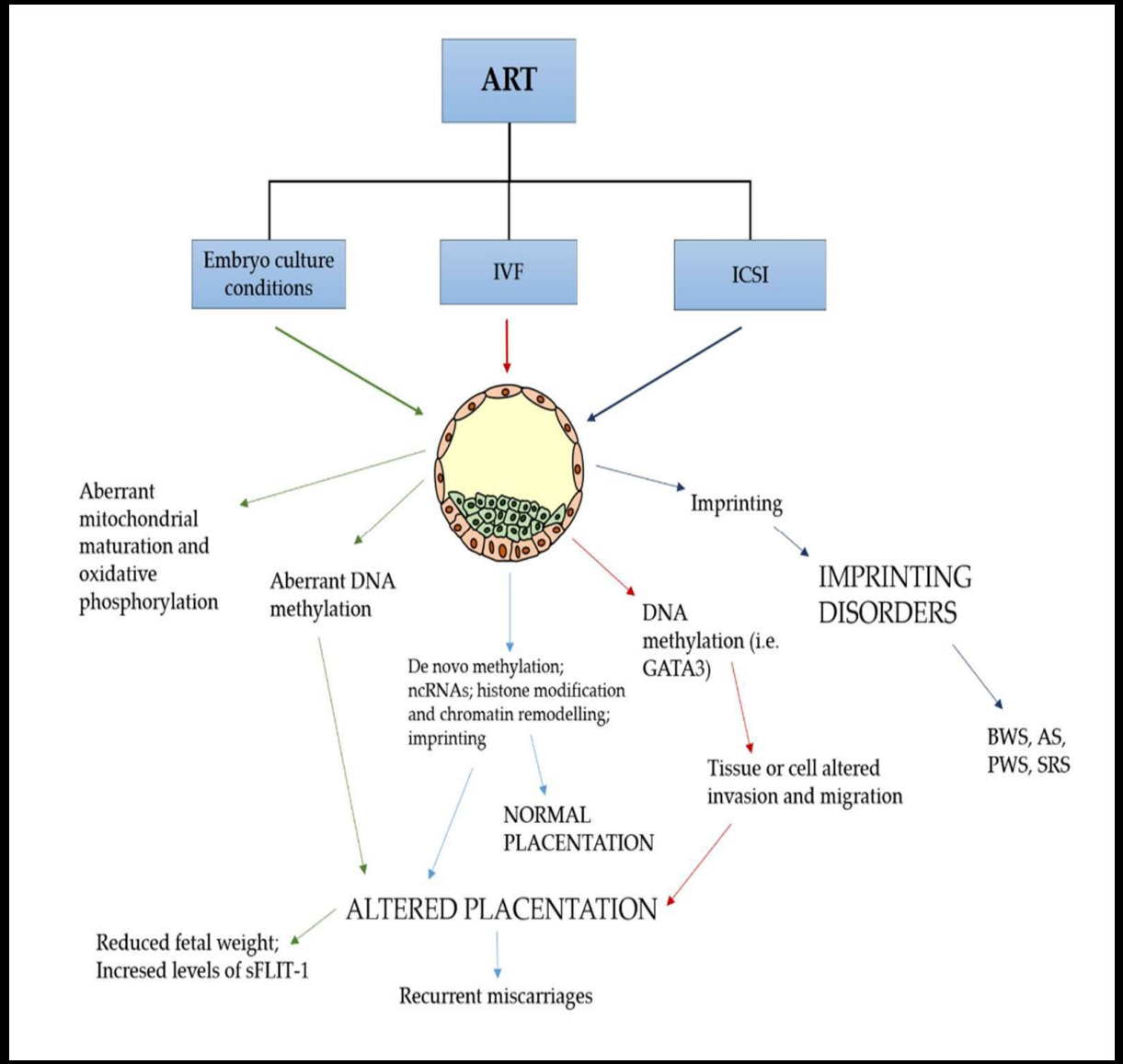
- Further impacts placental development→ fetal and neonatal outcomes

INFERTILITY CONDITIONS; PCOS AND ENDOMETRIOSIS

- Endometriosis →Meta-analysis of 39 studies → placental mediated disease , praevia, abruption, PPH and stillbirths→ significantly associated with endometriosis→ deep disorders of placentation- → in the event of a pregnancy→ functional anomalies of a eutopic endometrium and imbalance of inflammatory and hormonal markers

- PCO:→ defective trophoblast invasion and placentation→ maternal hyperandrogenism. Associated with a chronic low grade inflammation with metabolic dysfunction→ endothelial dysfunction

- PCOS undergoing IVF itself a risk factor→higher incidences of GDM, PE, PTB, miscarriage


ART
Embryo culture conditions
IVF
ICSI
Aberrant mitochondrial maturation and oxidative phosphorylation
Aberrant DNA methylation
Imprinting
IMPRINTING DISORDERS
DNA methylation (i.e. GATA3)
De novo methylation; ncRNAs; histone modification and chromatin remodelling; imprinting
BWS, AS, PWS, SRS
Tissue or cell altered invasion and migration
NORMAL PLACENTATION
ALTERED PLACENTATION
Reduced fetal weight; Incresed levels of sFLIT-1
Recurrent miscarriages

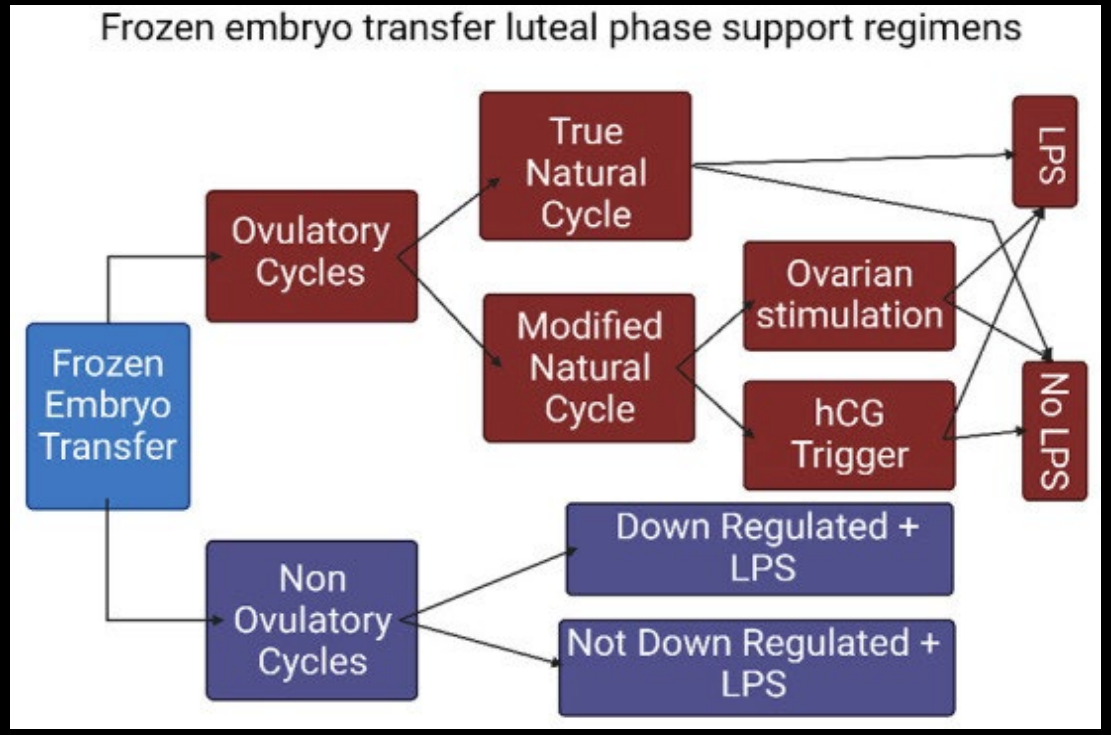
FROZEN EMBRYO TRANSFER

- Benefits:
- Permits uterine environment to return to normal after IVF medication→ natural embryo implantation conditions
- Allows genetic screening → increase chances of a successful pregnancy and healthy birth
- Preserves all viable embryos through freezing → easier to select single embryo transfer →reducing possibility of multifetal gestation
- Reduces risk of OHSS
- Is less expensive for multiple transfers than fresh IVF cycles
- Facilitates fertility preservation

VETRIFICATION/ EMBRYO CRYOPRESERVATION

- Improvements in perinatal outcome
  Highest quality of embryos for freezing
  More physiological hormonal milieu → FET process

- Less risk for SGA/ LBW
- Paradoxically Increased risk for Pre-eclampsia
- Does vitrifying alters the IPD risk?
- Protocol –FET done TNC or mNC (CL present)/Programmed (CL not present)
- Hormonal milieu vs cryopreservation


Frozen embryo transfer luteal phase support regimens
Frozen Embryo Transfer
Ovulatory Cycles
Non Ovulatory Cycles
True Natural Cycle
Modified Natural Cycle
Ovarian stimulation
hCG Trigger
LPS
No LPS
Down Regulated + LPS
Not Down Regulated + LPS

FET

- *John and Hacker et al: Fertility and Sterility*
- Lower risk of IUGR and IUFD/placental insufficiency in FET (n=271) c/f fresh embryo cycles (n=1861) → even when this was looked at singletons alone
- PE commoner in FET → not statistically significant
- Depends whether FET → NC/mNC or programmed FET

3D ULTRASOUND, VIRTUAL REALITY TECHNIQUES (VOCAL) AND DOPPLER (*DUIJN ET AL:HUMAN REPRODUCTION*)

- Longitudinal placental development – different modes on conception
- Is there a difference in FET (n=32), fresh (n=56) or naturally conceived cycles (n=126)
- 214 patients: 32 (FET), 56 (fresh) and 126 naturally conceived cycles
- 3D volume studies at 7,9, 11 and 13 weeks: PV and UPVV studies
- Uterine artery PI at 7, 9, 11, 13, 22 and 32 weeks

FINDINGS

- FT parameters of placental development ie PV, UPVV, Uterine artery PI and RI were comparable across all modes conception techniques.
- Ut A PI and RI was significantly lower in the frozen ET than fresh ET in 2nd and 3rd trimesters
- Beneficial impact on frozen-thawed ET

FET VS FRESH EMBRYO TRANSFER

- Emerging evidence that " endocrine uterine environment" crucial role
- → maternal vascular adaptation of pregnancy → placental development
- FET -Hormonally induced cycles → HPA suppressed→prepare endometrium for transfer → corpus luteum is absent
- Presence of corpus luteum → essential for optimal maternal hormonal environment for implantation and haemodynamic adaptation to pregnancy
- Therefore there was no difference in natural conceived cycles to the frozen-thawed cycles using natural cycle as the CL is present in the majority of frozen thawed natural ET cycle in terms of placental developmental parameters

FET: NATURAL CYCLE VS HORMONALLY INDUCED

- An RCT of 1508 women with PCO that risk of PE higher in the hormonally induced frozen thawed cleavage ET (lacked the CL) than after natural cycle FET and not observed in 2157 ovulatory women (Chen et al:2016)

- Supports hypothesis that placental development after frozen thawed natural cycle is superior to frozen thawed hormonal cycle ET.

PLACENTAL MEDIATED DISEASE IN RELATION SPECIFIC ENDOMETRIAL PREP REGIMENS

- *Wang F, Wang Qi et al: Frontiers in Endocrinology*
- 2186 pregnancy women with singleton live births → divided into 3 groups:
- Naturally conceived group (1334 patients)
- Modified natural cycle group (mNC) –FET (frozen thawed embryo cycle) [217]
- Programmed FET group (635)

WANG F, WANG QI ET AL: FRONTIERS OF ENDOCRINOLOGY

- mNC cycles: a dose of HCG trigger was given based on the ultrasonic measurements of the dominant follicle + luteal support-CL present

- Programmed FET group: endometrial prep- oral oestradiol valerate followed by progesterone gel + oral dydrogesterone and combinations thereof – no CL

RESULTS: *WANG F, WANG QI ET AL: FRONTIERS OF ENDOCRINOLOGY*

- After adjusting for age and parity no difference in PMD between naturally conceived cycles and mNC (modified natural conceived cycles) vs programmed FET cycles → higher incidence of placental disorders.

- Programmed FET → main manifestation was abnormal placental attachment including placental adhesion and accreta. Additionally increased incidence of PE, PPH and caesarean section. LBW and HBW was also increased in this group

RESULTS: *WANG F, WANG QI ET AL: FRONTIERS OF ENDOCRINOLOGY*

- Programmed FET cycles that was linked to PMD/ morphology and structural issues independent of fertility type, total dose of Femostone and endometrial thickness
- This study also shows that a lack of CL in programmed cycles → produce vasoactive molecules like prorenin, relaxin which contribute to global placental changes earlier on and prevent PMD.
- Premature elevated levels of oestradiol in programmed cycles → suppress trophoblastic transformation of the spiral arterioles-IPD
- Abnormal placentation and attachment → combination of these factors → lead to accreta, PPH and increased rates of caesarean section.
- Important in counselling
- Explore mechanisms and potential interventions to reduce risk of abnormal placentation in programmed cycles.

PGT-A/ PGT-M : MECHANICAL STRESS ON EMBRYO AND PLACENTAL DEVELOPMENT

- Increasingly performed → USA constitutes 40% of all IVF cycles
- Requires in-vitro development of embryos up to blastocyst stage→ collect 5-10 trophoblast cells
- Concerns: trophectoderm biopsy--? disturb embryo development and negatively influence later phases of placentation → concern for abnormal placentation – morphological and functional

PGT-A

- In a comparative study *(Jing et al)* → PGT-A FET 9% increase in HDP c/f 2.3% with fresh embryo transfer cleavage-stage biopsy ? Placental injury on the trophectoderm
- In another comparative study *(Feldman et al)* →
- 6.9% HDP and 12.4% SGA in PGT-A c/f
- 4.7% HDP and 4.5% SGA in IVF without PGT-A and
- 2.3% HDP and 3.9% SGA in spontaneous conception
- Is hormonal prep a confounder? In a binary logistic regression model Makhijani et al showed risk associated with PGT A remained higher

TROPHECTODERM BIOPSY

- Is it worthwhile to look for embryonic aneuploidies?
- Amongst embryos with aneuploid cells → 31% were meiotic and 74% were mitotic → maximum aneuploidy at day 4-5 and falling to 5-6% on day 7
- Important to consider the environment influence on the mitotic origin of aneuploidies in developing embryos
- PGT A does not improve IVF outcomes and neither miscarriage rates and increases PMD risks
- Healthy babies have been born after transfer of mosaic aneuploid embryos
- Is the "self correction" ability of aneuploid embryos
- Humans this process is promoted by bone morphogenetic protein 4 (BMP 4)

TROPHECTODERM

- These mechanisms together with results obtained from a trophectoderm biopsy may not be representative of the embryo begs the question " should all aneuploidy embryos be eliminated" –depends what the aneuploidy is – Is it of a mitotic nature/mosaic as opposed to an established aneuploidy
- That is just for discussion purposes
- The Scientific and Clinical Advances Advisory Committee recently changed the rating to red meaning there is no evidence that the treatment is effective and safe
- Controversial

CONCLUSIONS

- Huge number of ART cycles in the world ***-6-8%*** in the West
- Epigenetic alterations → primary mechanism→ placental/fetal effects-ART
- Abnormal hormonal milieu→ placental transformation
- FET has overcome fresh embryo transfer in US and Australia
- FET group: 3 groups: True natural cycle/ mNC or Programmed cycles
  Natural cycles/mNC → better outcomes and similar outcomes to natural conception
  Programmed FET → PMD + placental disorders
- PGT –A/PGT-M → trophectoderm biopsy → have higher PMD risk: (40% of IVF cycles) and may not be representative: balance against knowing euploid embryo transferred.