



The impact of endometriosis on embryo & oocyte and implantation

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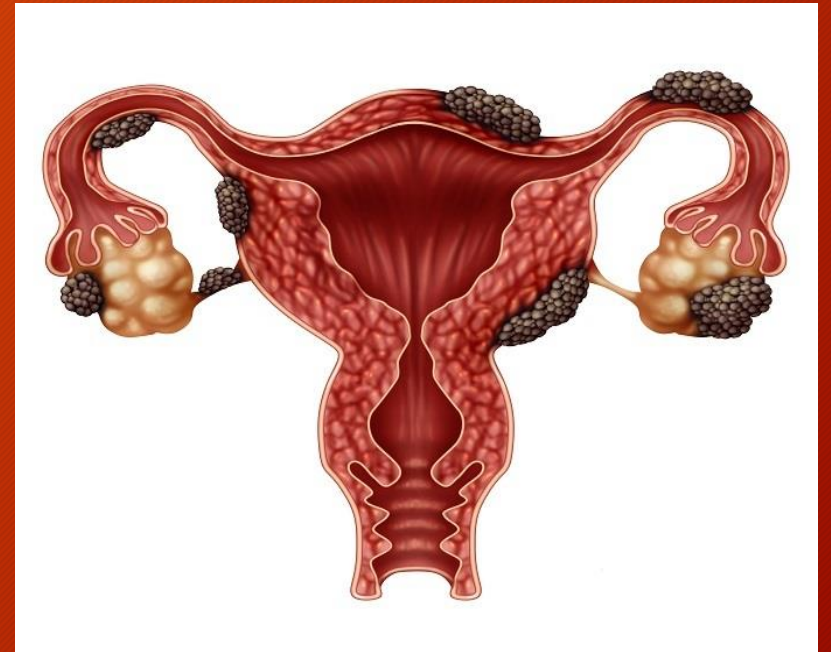
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Endometriosis

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- Endometriosis, defined by the presence of ectopic endometrial implants, affects up to 50% of women with infertility .
- In the presence of endometriosis, a couple's monthly likelihood of conception decreases from 15-20% to 2-10%.
- A number of mechanisms are thought to contribute to impaired fertility in the setting of endometriosis.





The impact of endometriosis on embryo morphokinetics: embryos from endometriosis patients exhibit delayed cell cycle milestones and decreased blastulation rates

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The impact of endometriosis on embryo



- Studies of embryo development in vitro show that embryos obtained from women with endometriosis are more likely to demonstrate aberrant and arrested growth. Similarly, studies of donor oocytes indicate that oocyte quality is impaired.
- Healthy women who receive donor oocytes from women with endometriosis have lower implantation and pregnancy rates than those who receive unaffected oocytes. Further, women with endometriosis who receive an egg from an unaffected donor have pregnancy rates equivalent to those of healthy recipients.

Time-lapse microscopy

- ❑ The advent of time-lapse microscopy (TLM) in embryology has allowed for real-time observation of embryonic development and the identification of transient morphologic features.
- ❑ The quantitative assessment of cell-cycle parameters in embryos derived from endometriosis-affected oocytes may provide further insight into the impact of the disease on embryo quality and development.

The EmbryoScope time-lapse microscopy system captured 200×images of each embryo in 5–7 different focal planes every 15 min for the duration of culture.



The timing of cell cycle events

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- ICSI: t0
- development to two-cell, t2, t3, t4, t5, t7, t8, t9
- compaction (tSC)
- morulation (tM)
- start of blastulation (tSB)
- blastulation (tB)
- expanded blastocyst (tEB)
- hatching blastocyst (tHB)

Optimal timings:

- cc2 (>5 and ≤ 11.9 h)
- s2 (≤ 1 h)
- t5 (45-57 h)
- cc3 (9.7-21 h)
- tSB (< 96.2 h)
- tEB (≤ 116 h)

The cell cycle intervals cc2 (t3-t2), s2 (t4-t3), and cc3 (t5-t3) were also assessed.

Results

The impact
of endometriosis
stage on embryo
morphokinetics

Patient
characteristics



Morphokinetic
parameters

Cycle outcomes

Known implantation
data (KID)

Morphokinetic parameters



- The development of embryos obtained from women with endometriosis is impaired.
- EE (embryos from endomethriosis patients) were slower than control embryos to complete nearly all developmental milestones evaluated, including the 2-8 cell stages($p<0.001$), compaction ($p=0.015$), morulation ($p<0.001$), start of blastulation($p<0.001$), blastulation, and expanded blastocyst($p<0.001$).
- the rate of embryo wastage, defined as the proportion of embryos discarded due to arrest or poor quality, was slightly higher in the endometriosis compared to the control group (43.9% vs 40.2%, $p=0.04$).

Morphokinetic parameters in embryos

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Parameter	Total (N= 3471)	No endometriosis (N= 2393)	Endometriosis (N= 1078)	p value
t2 (h)	27.5 ± 5.0	27.2 ± 4.7	28.1 ± 5.6	< 0.001 ^{a2}
t3 (h)	37.2 ± 6.3	36.8 ± 5.9	38.2 ± 7.1	< 0.001 ^{a2}
t4 (h)	40.0 ± 7.5	39.5 ± 7.2	41.0 ± 8.2	< 0.001 ^{a2}
t5 (h)	49.9 ± 9.7	49.4 ± 9.4	51.1 ± 10.5	< 0.001 ^{a2}
t7 (h)	58.0 ± 10.4	57.5 ± 10.2	59.2 ± 10.9	< 0.001 ^{a2}
t8 (h)	60.6 ± 11.5	60.0 ± 11.2	61.9 ± 12.0	< 0.001 ^{a2}
tM (h)	92.4 ± 11.3	92.1 ± 11.2	93.1 ± 11.5	0.036 ^{a1}
tSB (h)	101.9 ± 10.1	101.4 ± 10.0	103.2 ± 10.3	< 0.001 ^{a1}
tB (h)	107.0 ± 10.1	106.4 ± 10.0	108.4 ± 10.2	< 0.001 ^{a1}
tEB (h)	115.1 ± 10.2	114.5 ± 10.1	116.5 ± 10.3	< 0.001 ^{a1}
t9 (h)	70.7 ± 12.0	70.1 ± 11.8	72.2 ± 12.3	< 0.001 ^{a1}
tSC (h)	85.5 ± 12.1	84.9 ± 11.8	86.9 ± 12.8	< 0.001 ^{a2}
tHB (h)	121.5 ± 11.0	121.1 ± 11.2	122.3 ± 10.6	0.42 ^{a1}
cc2 (h)	11.2 [9.7, 12.3]	11.2 [9.5, 12.2]	11.3 [9.7, 12.5]	0.003 ^b
s2 (h)	0.67 [0.33, 2.3]	0.67 [0.33, 2.2]	0.67 [0.33, 2.3]	0.64 ^b
cc3 (h)	13.1 [10.9, 15.3]	13.0 [10.9, 15.1]	13.2 [11.0, 15.7]	0.046 ^b

Statistics presented as mean ± SD, median [P25, P75]. p values: a1 = t test, a2 = Satterthwaite t test, b = Wilcoxon rank sum test

The impact of endometriosis stage on embryo morphokinetics



- The only significant difference between embryos obtained from women with stages 1-2 versus stages 3-4 endometriosis, was in the timing of tSB, which was longer in the stages 3-4 cohort.
- The timing of blastulation was also delayed by approximately 2 h in the stages 3-4 group; however, this difference did not reach statistical significance.
- Additionally, there were no differences in rates of embryo wastage or progression to morula, blastocyst, or expanded blastocyst between the stages 1-2 and 3-4 endometriosis groups.

Discussion



- Embryos obtained from women with endometriosis demonstrate delayed cell cycle parameters for both early and late developmental events. Additionally, EE were significantly less likely than controls to progress to the morula, blastocyst, and expanded blastocyst stages.
- Whereas **66.2%** of control embryos became blastocysts, only **59.9%** of embryos in the endometriosis group blastulated.



Interestingly, the negative impact of endometriosis on embryonic development appears to be independent of stage and occurred regardless of whether patients had minimal-to-mild or moderate-to-severe disease.



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- Impairments in embryo development may result from the inflammatory milieu that characterizes endometriosis. It is well established that the peritoneal fluid of patients with endometriosis contains increased numbers of macrophages, prostaglandins, proteases, and cytokines, including IL-6, TNF-alpha, and VEGF.
- Exposure to this pro-inflammatory environment is thought to negatively affect oocyte and embryo quality, and may contribute to the impaired embryonic development seen in this study.

Discussion

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- Despite the notable differences in morphokinetic parameters, there were no significant differences in clinical pregnancy or live birth rates.
- This suggests that once a good quality embryo is available to transfer, pregnancy rates are equivalent between women with and without endometriosis.
- Consistent with the findings of other retrospective studies, a subgroup analysis indicates that frozen embryo transfer may result in improved pregnancy outcomes compared to fresh transfer in women with endometriosis; however, this finding requires confirmation in a larger, prospective dataset.


Discussion

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- An important finding from this study was that cycles performed in women with endometriosis were 4 times more likely to fail to produce an embryo for transfer. This finding is likely attributable in part to the lower AMH levels and smaller number of oocytes retrieved among patients with endometriosis; however,


impaired oocyte quality, resulting in impaired embryo development, may also play a role.

Novel therapeutic targets to improve IVF outcomes in endometriosis patients: a review and future prospects

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IVF clinical outcomes in patients with endometriosis

- ▶ Reduced ovarian response is also reported in endometriosis
- ▶ A higher burnout of early follicles in endometriotic ovaries was linked to premature follicle recruitment and **higher rates of atresia** (20.3% in endometriosis vs 6.3% without endometriosis), leading to a **lower antral follicle count** and **level of serum anti-Mullerian hormone**
- ▶ Ovarian endometrioma reduced the **response to ovarian stimulation** compared to the response of the contralateral normal ovary in the same patients

IVF clinical outcomes in patients with endometriosis

- ▶ Surgical treatment before ART in women with endometriosis could be a therapeutic option to improve IVF outcomes, as was reported in patients with peritoneal endometriosis (Salamun et al., 2018). However, surgery to remove ovarian endometrioma can affect oocyte reserve and quality, and subsequent IVF outcomes, suggesting that surgery before IVF cycle is not recommended (Donnez et al., 2018).
- ▶ Patients who undergo surgery have a lower antral follicle count compared to those who have no surgery, suggesting that surgery on endometrioma has a detrimental impact on ovarian reserve (Hamdan et al., 2015).
- ▶ Fertility preservation techniques before surgery may be a good solution to improve IVF outcomes in these patients.

Reduced oocyte quality

- ▶ A study evaluating oocytes that underwent IVM from women with and without endometriosis, demonstrated that a significantly lower number of immature oocytes subjected to IVM reached metaphase II (MII) in the endometriosis group compared to controls (Goud et al., 2014).

Reduced embryo quality

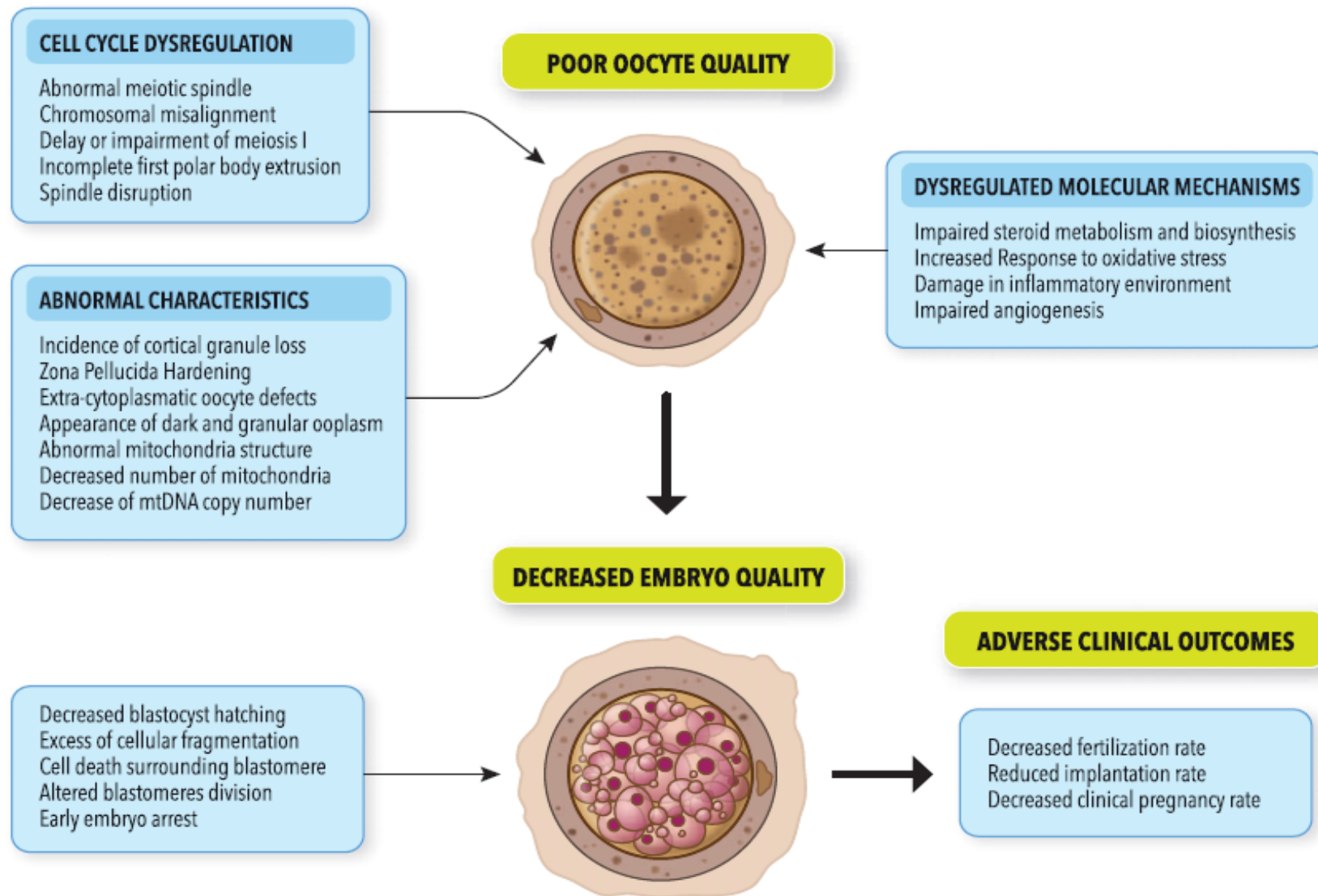


Figure 1. Poor oocyte/embryo quality and adverse IVF outcomes in patients with endometriosis. Characteristics observed in poor-quality oocytes and embryos from women with endometriosis that lead to adverse clinical outcomes in these patients. mtDNA, mitochondrial DNA.

Reactive Oxygen Species (ROS)

Ectopic endometriotic lesions



- High proliferation
- Compromized antioxidant enzyme functionality
- Lysed erythrocytes of the retrograded menstrual blood

Increased iron levels

Increased ROS and free radicals production

Proinflammatory phenomena and chronic inflammation

Impact of ROS on oocytes and embryos

- Impaired mitochondrial functionality
- Reduced ATP synthesis
- Impaired protein synthesis and enzyme functionality
- Lipid peroxidation and dysregulation of membrane architecture
- Cytoskeleton and microtubule disorganization
- Direct impairment of nuclear DNA structure

- Impaired oocyte maturation
- Impaired embryo development
- Embryo developmental arrest
- Reduced implantation potential



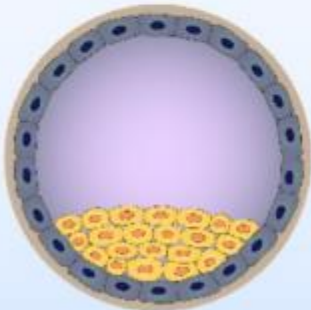
Impact of ROS and inflammation on the female reproductive system

- Dysregulation of the microenvironment of the fallopian tubes, the ovaries and the uterus
- Impact on ovarian steroidogenesis
- Dysregulation of the ovulation process
- Impaired endometrial receptivity

Oocyte



Embryo



Meiotic Spindle Disruption and Extracellular Matrix Remodeling

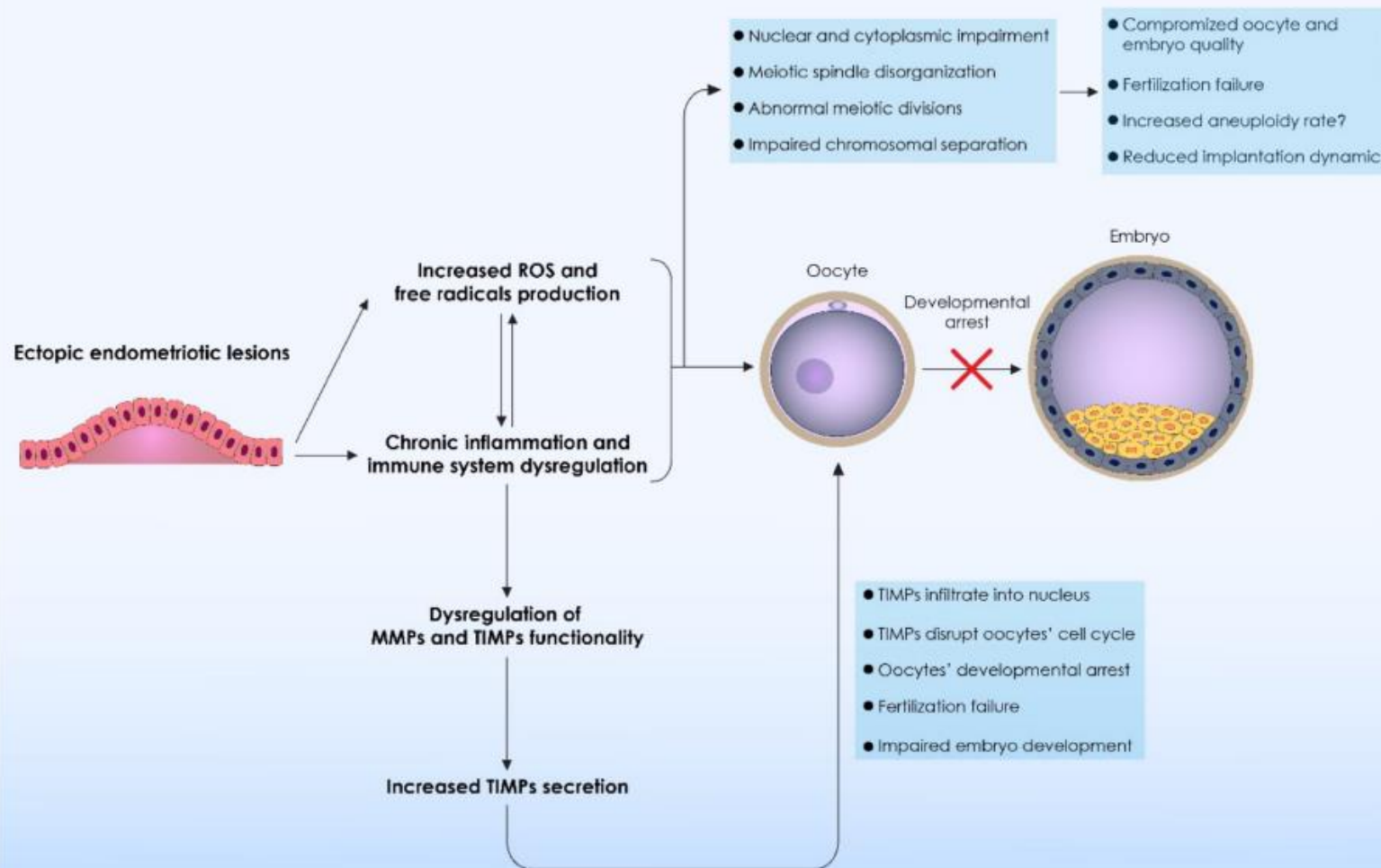


Table 3. Summary of the mechanisms involved in the meiotic spindle disruption as well as on extracellular matrix remodeling leading to impaired oocyte and embryo quality in patients presenting with endometriosis.

Meiotic Spindle Distribution			
Mechanisms	Result	Impact on Reproductive Tissues, on Embryos and on Oocytes	Induced Endometriosis Related Infertility
Increased ROS production and chronic inflammation [74]	Data is summarized in Tables 1 and 2	<ol style="list-style-type: none">1. Nuclear and cytoplasmic impairment [69]2. Meiotic spindle disorganization [71–75]3. Abnormal meiotic divisions [71–75]4. Impaired chromosomal separation [71–75]	<ol style="list-style-type: none">1. Compromised oocyte and embryo quality and competence [78]2. Fertilization failure [78]3. Increased aneuploidy rate (remains unclear) [72,81]4. Reduced implantation dynamic [69]
Extracellular Matrix Remodeling			
Immune system dysregulation and increased cytokine secretion [74]	Dysregulation of MMPs and TIMP functionality [83,84]	<ol style="list-style-type: none">1. Impaired MMPs and TIMPs functionality leads to abnormal remodeling of ECM [83–85]2. TIMPs infiltrate into the nucleus and disrupt the overall cell cycle hindering the oocytes’ development [80,86]	<ol style="list-style-type: none">1. Compromised oocyte and embryo quality and competence [80]2. Oocyte and embryo developmental arrest [80,86]3. Impaired implantation potential [80,86]
TIMP increased secretion [83]	Direct impact on oocytes [80,86]		

ROS: Reactive Oxygen Species; MMPs: Matrix metalloproteinases; TIMPs: Tissue Inhibitors of Metalloproteinases.



Molecular mechanisms involved in endometriosis-associated infertility

Impaired steroid metabolism and biosynthesis

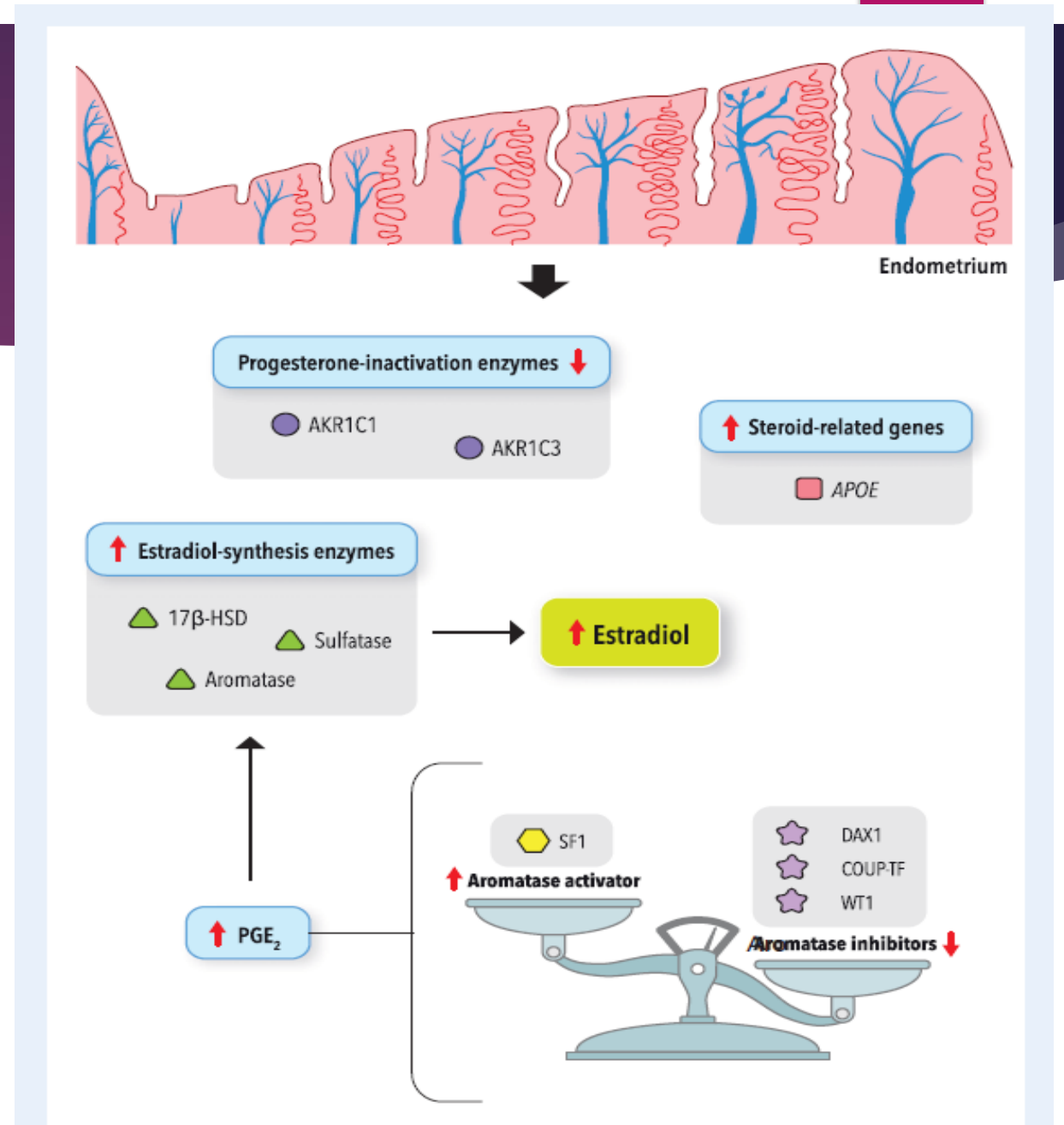
- ▶ steroid metabolism and biosynthesis functions were enriched, which leads to an **increased steroidogenic environment** in oocytes from women with ovarian endometriosis
- ▶ an upregulation of steroid-related genes, such as apolipoprotein E (APOE) (Ferrero et al., 2019), that are involved in endometrial attachment, adhesion, and invasion
- ▶ increased steroidogenic environment in human oocytes in endometriosis, which might **directly affect the oocyte microenvironment and IVF outcomes**
- ▶ Enzymes involved in estradiol formation, such as aromatase, sulfatase and 17 β -hydroxysteroid dehydrogenases (17 β -HSD), and enzymes involved in progesterone inactivation (Aldo-keto reductase family 1 member C1 and C3: AKR1C1 and AKR1C3) were significantly upregulated in ovarian endometrioma compared to controls, suggesting increased steroidogenesis in endometriosis

Impaired steroid metabolism and biosynthesis

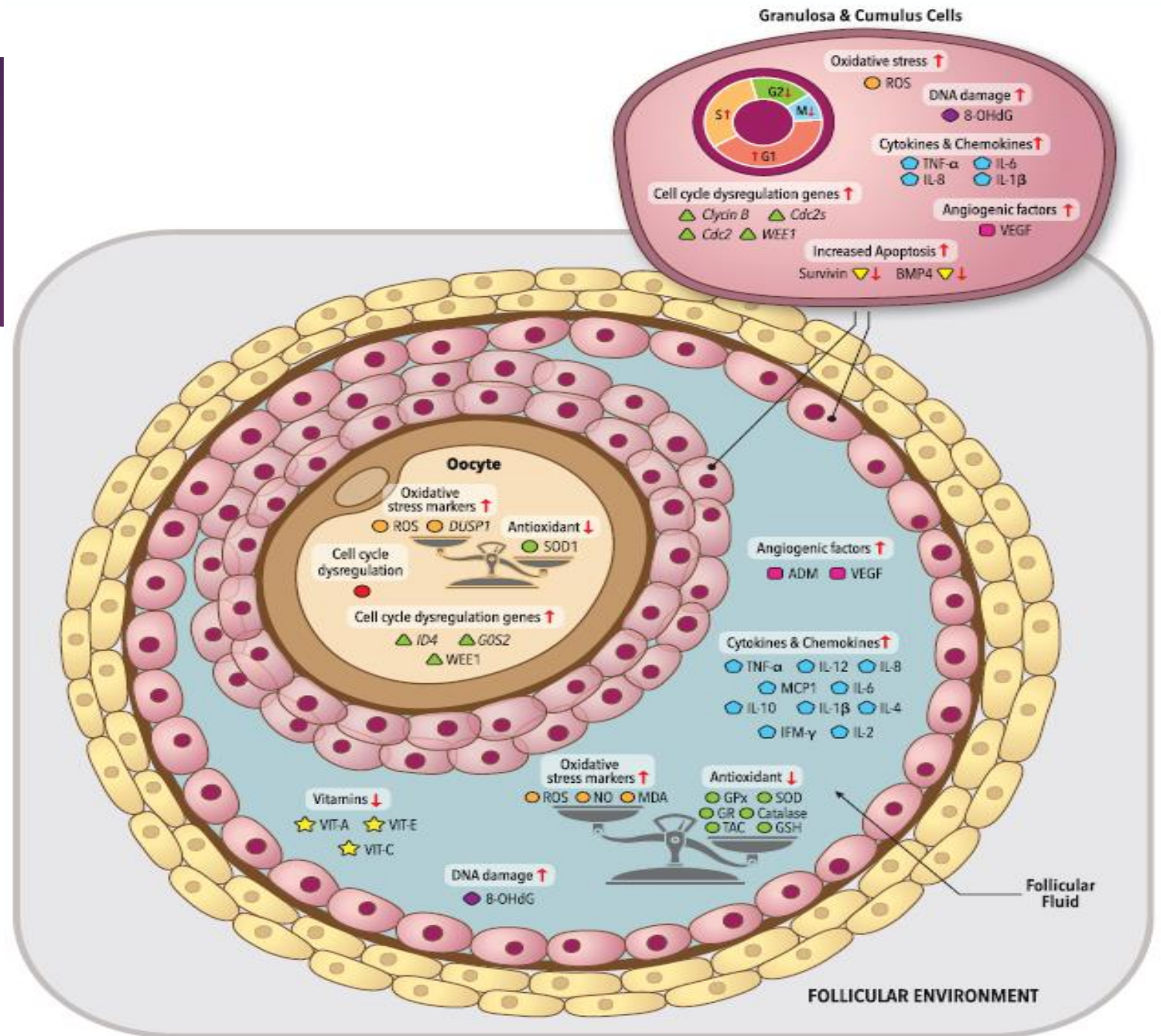
- ▶ 17b-HSDs synthesizing 17b-estradiol versus 17b-HSDs inactivating 17b-estradiol was up to 3-fold higher in ectopic compared to eutopic endometrium indicating an increased synthesis of 17b-estradiol in endometrioma and impaired estrogen metabolism.
- ▶ In addition, aromatase expression and estradiol production were higher in endometriosis implants than in normal endometrium in women with this condition, providing ectopic endometrium with an excessive proliferative stimulus such that it is able to implant and grow on peritoneal surfaces
- ▶ Furthermore, aromatase mRNA expression and enzyme activity were increased in endometriosis, and these increased levels contributed to elevated estradiol and prostaglandin E2 (PGE2) production
- ▶ PGE2 stimulates estrogen synthesis by ectopic endometrial stromal cells, and elevated PGE2 could dysregulate the production of estrogen and lead to endometriosis development.

Impaired steroid metabolism and biosynthesis

- Upregulation of estrogen impairs follicular steroidogenesis, with oocyte deficiency and lower fertilization rates along with high aromatase expression levels, which can all negatively impact IVF outcomes



1. Increased response to oxidative stress
2. Cell cycle dysregulation
3. Damage in an inflammatory environment and impaired angiogenesis



How Endometriosis Affects Oocyte Quality and Implantation

SCIENTIFIC REPORTS

► Original
► 2015

OPEN

Oocyte quality is decreased in women with minimal or mild endometriosis

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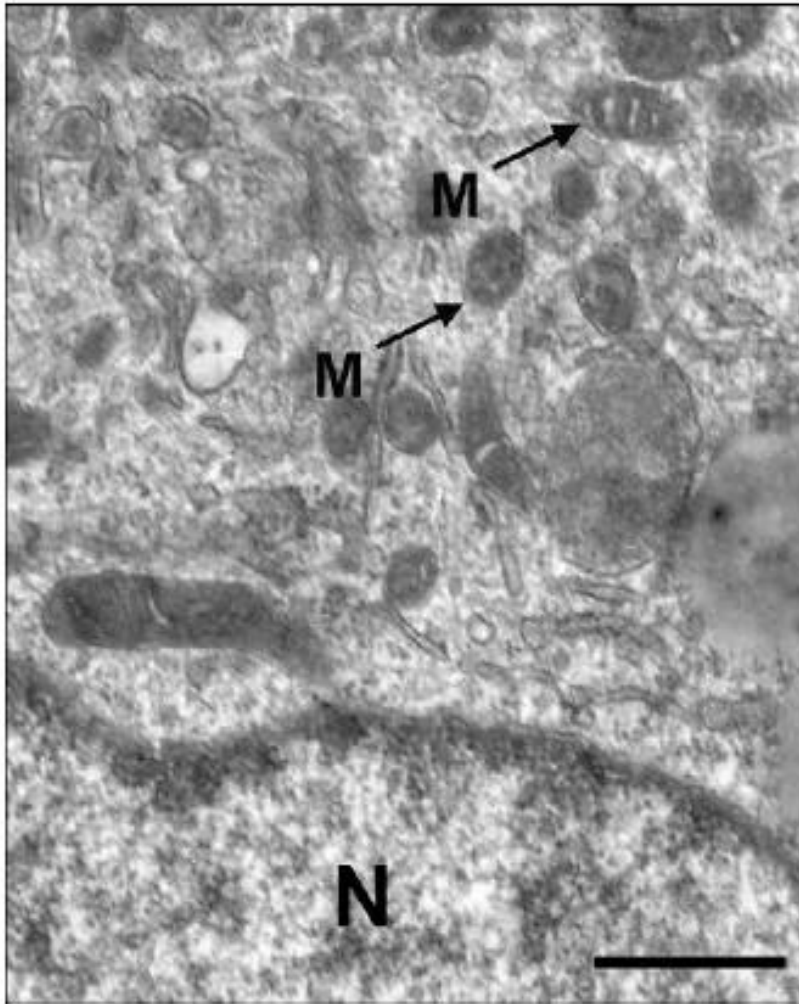
Bo Xu¹, Nan Guo¹, Xiao-min Zhang¹, Wei Shi¹, Xian-hong Tong¹, Furhan Iqbal² & Yu-sheng Liu¹

Endometriosis, a pathological condition in which the endometrium grows outside the uterus, is one of the most common causes of female infertility; it is diagnosed in 25–40% of infertile women. The mechanism by which endometriosis affects the fertility of females remains largely unknown. We examined the ultrastructure of oocytes from patients with minimal or mild endometriosis and control females undergoing *in vitro* fertilization (IVF) treatment by transmission electron microscopy (TEM) to investigate the physiological significance of oocyte quality for patients with minimal or mild endometriosis. The TEM results revealed that the oocytes from women with minimal or mild endometriosis exhibited abnormal mitochondrial structure and decreased mitochondria mass. Quantitative real time PCR analysis revealed that the mitochondrial DNA copy number was significantly reduced in the oocytes from women with minimal or mild endometriosis compared

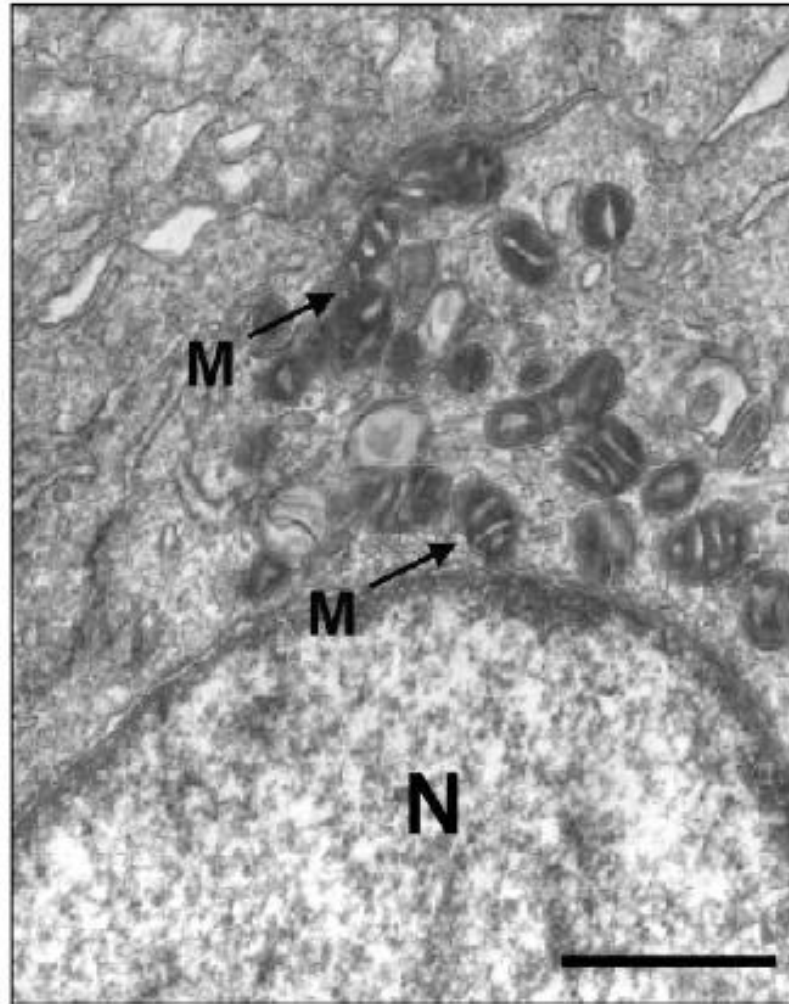
Materials and methods

- ▶ 41 women
- ▶ minimal or mild endometriosis [stage I and stage II endometriosis according to (R-AFS)]
- ▶ Evaluation of mature oocytes (MII) by Transmission Electron Microscopy (TEM)
- ▶ Mitochondrial DNA copy, number determination by quantitative real-time PCR

Control

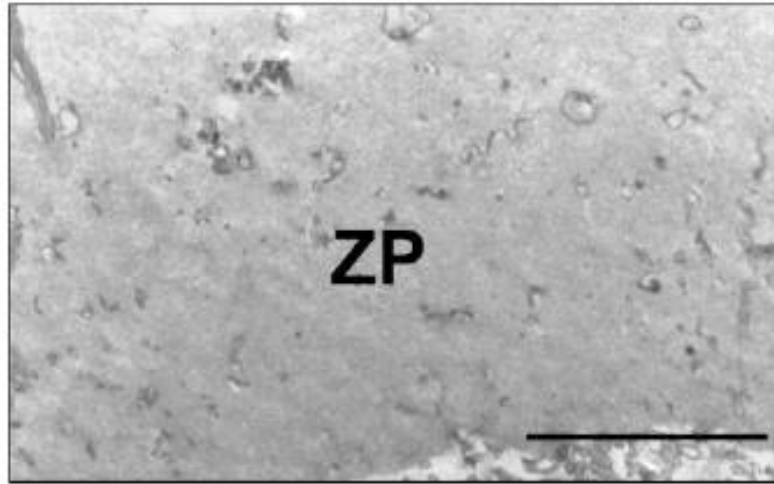


Endometriosis

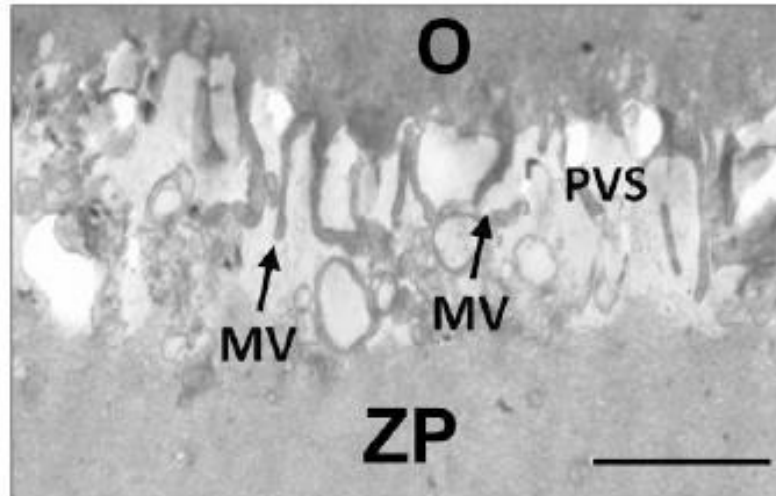
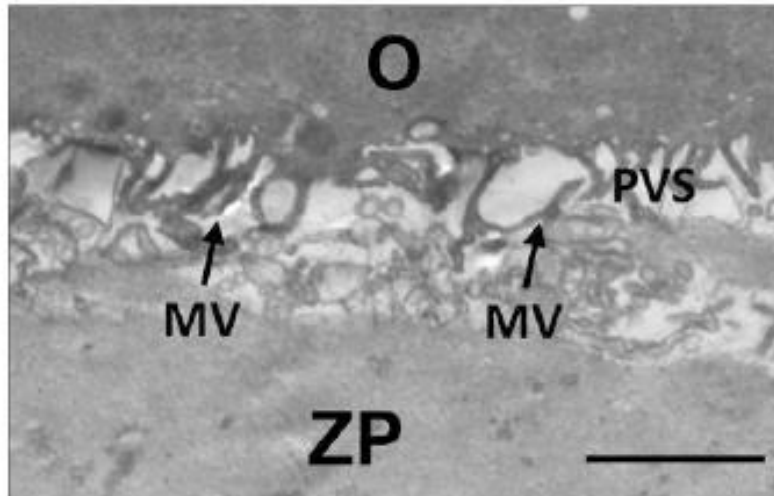
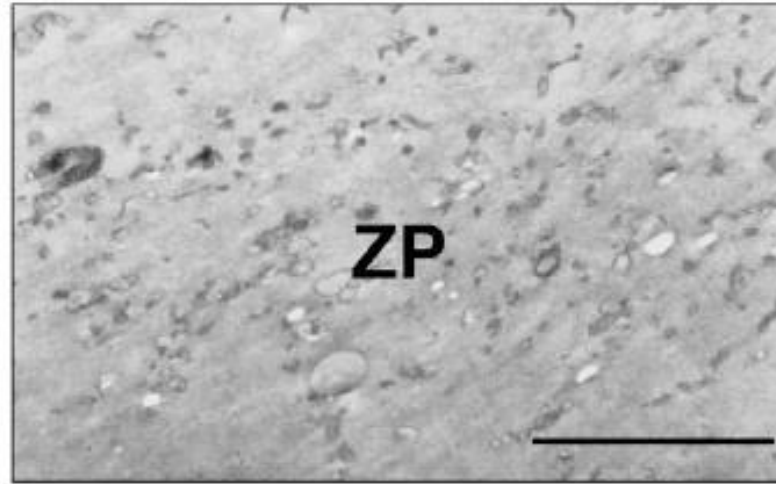


Cumulus cell of the oocytes in the endometriosis and control groups. The cumulus cells from the control group show the same ultrastructural cytoplasmic characteristics of the cumulus cells surrounding the oocytes in the endometriosis group (A,B). The tubular cristae of the mitochondria (arrows) are well developed and evenly distributed. N = nuclei; M = mitochondria; Scale bar (A,B) = 500 nm.

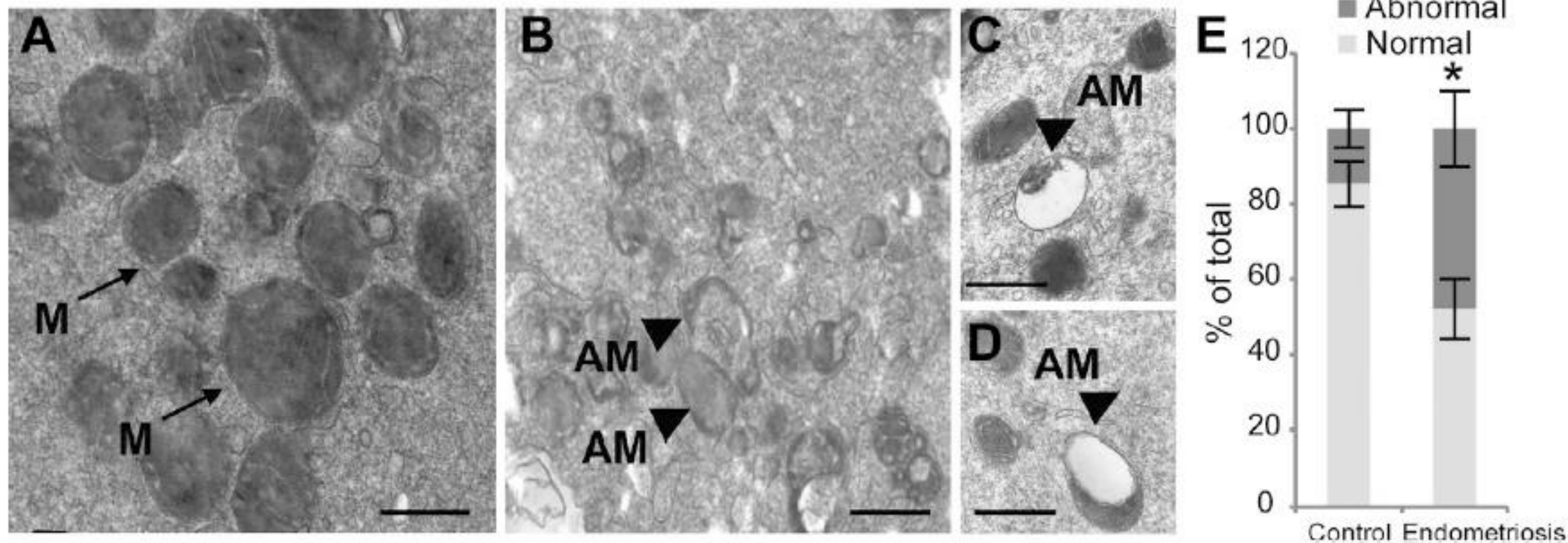
Control



Endometriosis



The electron density of the zona pellucida (A,B) and perivitelline space (C,D) in the endometriosis and control groups, respectively. No difference was observed between the endometriosis and control groups in the dense appearance of the inner aspect of the ZP, and some fibres are visible in the zona texture (A,B). The microvilli (arrows) are numerous and long on the oolemma of both groups (C,D). MV = microvilli; PVS = perivitelline space; O = oocyte. Scale bar = 500 nm.



Ultrastructural differences in the mitochondria of normal oocytes and oocytes with endometriosis. Mitochondria with typical tubular cristae are visible in the control cytoplasm (A). A large degree of vacuolization (arrows) could be seen in the mitochondria of the endometriosis group (B-D). The rate of abnormal mitochondria was significantly lower in the control group (E). M= Mitochondria; AM= abnormal mitochondria; Scale bar (A,B,C,D)= 500 nm. The bars indicate the standard deviation (SD) of the mean. *: compared with those of the control group, the abnormal mitochondria are significantly ($P < 0.05$) increased in the oocytes from the endometriosis group. Note: Abnormal mitochondria rate= the number of abnormal mitochondria/total number of mitochondria.

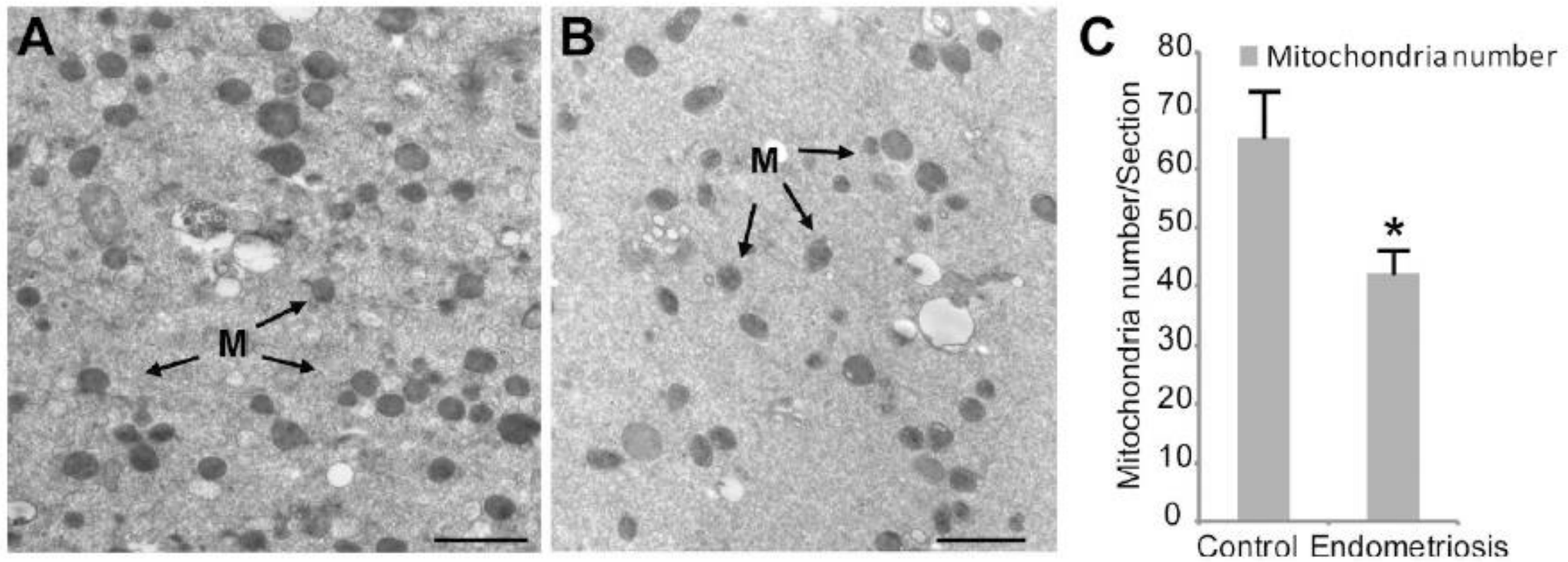


Figure 4. Comparison of the mitochondrial mass in the cytoplasm of the normal oocytes and the oocytes from the endometriosis group. The electron micrograph of the oocytes in the control group revealed abundant mitochondria in the cytoplasm (A). However, the number of mitochondria was significantly reduced in the endometriosis group (B). There were significant differences between the two groups regarding the mass of the mitochondria in cytoplasm (C). The bars indicate the standard deviation (SD) of the mean. *: compared with that of the control group, the number of mitochondria is significantly ($P < 0.05$) low in the oocytes from the endometriosis group. Note: Mitochondria mass = number of mitochondria/section. For each oocyte, the numbers of mitochondria were counted in at least 3 randomly selected TEM-oocyte sections. To eliminate errors in the mitochondria identification and counting, all of the analyses were performed in a double-blind manner by two or three individuals, and the data were pooled.

Discussion

- ▶ they suggest that minimal or mild endometriosis is specifically linked to the occurrence of impaired mitochondrial structure and reduced mtDNA copy numbers because of disorders of cytoplasmic maturation
- ▶ TEM has not previously been used for investigating the association between oocyte quality and minimal or mild endometriosis. The oocytes from the patients with minimal or mild endometriosis showed increased abnormal mitochondria and reduced mitochondria mass, which suggested that the oocyte quality was decreased in oocytes from women with minimal or mild endometriosis



Review

Endometriosis and fertility

Karolina Skorupskaite, Harish M. Bhandari

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<https://doi.org/10.1016/j.ogrm.2021.03.003>

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Abstract

Endometriosis is a chronic inflammatory condition of reproductive age which can lead to infertility and chronic pelvic pain. The pathophysiology of endometriosis-associated infertility is not well understood and it appears to be multi-factorial; mechanical, inflammatory, hormonal, genetic and environmental processes can disturb each step of the normal reproductive physiology; folliculogenesis, ovulation, sperm function, gamete transport, fertilization and implantation. Medical

- Review
- 2021
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Endometriosis-associated infertility is multifactorial:

- ▶ Mechanical
- ▶ Inflammatory
- ▶ Cell signaling
- ▶ Epigenetic
- ▶ Environmental factors
- ▶ Adverse effects on:
 - ▶ The gametes
 - ▶ Fallopian tubes
 - ▶ Endometrium
 - ▶ Implantation
 - ▶ Fecundability

Effects on the embryo

- ▶ Studies indicate that the embryos derived from oocytes retrieved from women with endometriosis have:
- ▶ fewer blastomeres
- ▶ higher rates of arrest and abnormal development compared to embryos of women with no endometriosis
- ▶ Altered immunology in peritoneal fluid and endometrial free radicals pose further negative effects on embryo viability

Take home message

- ▶ endometriosis has adversely affect each aspect of:
 - ▶ the normal reproductive physiology
 - ▶ ovarian reserve
 - ▶ oocyte quality
 - ▶ follicle development
 - ▶ oocyte release and pick up
 - ▶ sperm motility
 - ▶ Fertilization
 - ▶ dysfunctional immunological/inflammatory environment of the peritoneal cavity
 - ▶ altered endometrial receptivity
 - ▶ myometrial contractions
 - ▶ embryo
 - ▶ implantation



*Thank
You!*