Maternal metabolic health and fertility : we should not only care about but also for the oocyte!

Review paper IETS 2023

Title: Maternal metabolic health and fertility: we should not only care about but also for the oocyte!

JLMR Leroy[1], B Meulders[1], K Moorkens[1], I Xhonneux[1], J Slootmans[1], L De Keersmaeker[1], A Smits[1], O Bogado Pascottini[3], WFA Marei[1,2]

[1] Gamete Research Centre, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium

[2] Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Egypt.

[3] Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Summary

Reduced oocyte quality, mainly due to mitochondrial dysfunction, is a key cause of subfertility in patients with metabolic diseases such as obesity. Recent fundamental understanding of the underlying mechanisms highlights the importance of developing effective preconception care strategies not only to improve metabolic health, but also oocyte quality. Minimizing mitochondrial oxidative stress either *in vivo* or *in vitro* is a promising solution, however further investigations should consider the long-term consequences on epigenetic programming and offspring health.

Abstract

Metabolic disorders due to obesity and unhealthy lifestyle directly alter the oocyte's microenvironment and impact oocyte quality. Oxidative stress and mitochondrial dysfunction play key roles in the pathogenesis. Acute effects on the fully-grown oocytes are evident, but early follicular stages are also sensitive to metabolic stress leading to a long-term impact on follicular cells and oocytes. Improving the preconception health is therefore of capital importance but research in animal models demonstrated that oocyte quality is not fully recovered. In the *in vitro* fertilization clinic, maternal metabolic disorders are linked with disappointing assisted reproductive technology results. Embryos derived from metabolically compromised oocytes exhibit persistently high intracellular stress levels due to weak cellular homeostatic mechanisms. The assisted reproductive technology procedures themselves form an extra burden for these defective embryos. Minimizing cellular stress during culture using mitochondrial-targeted therapy could rescue compromised embryos in a bovine model. However, translating such applications to human *in vitro* fertilization clinics is not simple. It is crucial to consider the sensitive epigenetic programming during early development. Research in humans and relevant animal models should result in preconception care interventions and *in vitro* strategies not only aiming at improving fertility but also safeguarding offspring health.

Keywords (8)

Maternal metabolic health, oocyte quality, assisted reproduction, epigenetic programming, oocyte mitochondria, preconception care interventions, antioxidant, mitochondria targeted therapy

Introduction

1. Maternal metabolic health in modern times and impact on fertility and on the vulnerable oocyte

Being fertile and generating healthy offspring involves a complex series of finely controlled endocrine, cellular and molecular events, which require optimal maternal health. Metabolic disorders are known to affect reproductive physiology resulting in subfertility. In humans, unbalanced diets and a sedentary lifestyle may result in obesity, type II diabetes or metabolic syndrome. The prevalence of these metabolic health disorders is dramatically increasing worldwide and have been strongly linked to this subfertility problem (WHO and UNFPA 2006; Valeggia and Ellison 2009; Practice Committee of the American Society for Reproductive Medicine 2015). The world health organization (WHO) European regional

obesity report of 2022 (WHO Regional Office for Europe 2022) stipulated that almost 60% of adults and 30% of children are obese or overweight. This prevalence seems to be further increased due to the COVID-19 pandemic and the enforced lock-down regulations. Worrying levels of overweight and obesity among men and women of childbearing age are seen across many European countries and continue to increase. In Hungary, Ireland, Portugal, Spain and the United Kingdom more than 20% of women are estimated to have obesity when they become pregnant. This percentage is similar across other European countries and is socioeconomically patterned, with the greatest burden experienced by those from lower socioeconomic backgrounds (WHO Regional Office for Europe 2022). The prevalence of infertility in obese women is up to 3 times higher compared to normal weight women, due to a higher prevalence of polycystic ovarian syndrome and oligoovulatory or anovulatory cycles, lower conception rates, and more pregnancy loss (Grodstein *et al.* 1994; Practice Committee of the American Society for Reproductive Medicine 2015). Obesity associated subfertility requests intensive and expensive fertility treatments which comes with emotional and financial costs for the patient and for the social security system (Koning *et al.* 2010).

Based on in-depth biomedical research, it is generally accepted that a deviating diet (energy or protein content and ratio, unbalanced micronutrients), an energy imbalance, but also a state of obesity or insulin resistance, seriously disrupt the finely tuned endocrine crosstalk in the hypothalamic-pituitary-ovary-uterus axis (Valeggia and Ellison 2009). Consequently, this may result in altered follicular growth patterns with oligoovulation or anovulation. Moreover, this may ultimately lead to the ovulation of a bad quality oocyte and to an increased risk for abortion, as seen in obese patients (Fedorcsak *et al.* 2000; Metwally *et al.* 2007). Epidemiological studies indicate that with each unit increase of the body mass index (BMI), the chance of spontaneous conception in ovulatory women reduces by 5% (Van der Steeg *et al.* 2008). Of course, such reduced fertility is a multifactorial problem, however, more and more research clearly indicates that reduced oocyte quality is a major factor (for review see Leroy *et al.* (2008b); Wu *et al.* (2011)). The primary importance of reduced oocyte quality in the pathogenesis of subfertility is further confirmed by the fact that embryo transfer from healthy, normal weight oocyte donors, restored pregnancy success in obese mothers (Luke *et al.* 2011).

Furthermore, the disappointing ART (Assisted Reproduction Technology) outcome as clinically reported in overweight and obese women, clearly highlights the specific importance of reduced oocyte quality in the pathogenesis of subfertility (Pandey *et al.* 2010). It remains unclear whether it is the disturbed metabolic health condition associated with

obesity and an unhealthy lifestyle (poor nutritious food, consumption of alcoholic or sweetened drinks, smoking, lack of fruit and vegetable consumption) or merely the direct changes in the oocyte environment that affect oocyte quality. Setti *et al.* (2022) confirmed very recently, in a large cohort study, that poor maternal lifestyle habits, linked to diet and smoking during the last 6 months before undergoing intracytoplasmic sperm injection (ICSI), were clearly associated with reduced oocyte morphology, fertilization rate, embryo development, clinical pregnancy and live birth rates. It is important to mention that all women included in this study were seeking clinical assistance to become pregnant for female- and/or male-associated reasons or unexplained infertility. All women were younger than 40 years, had regular menstrual cycles and had a BMI between 17.5 and 29.9. Therefore, no obese patients were included. Furthermore, all applied statistical analyses were controlled for maternal age and BMI. Such epidemiological studies clearly indicate that not only a deviating metabolic health, due to obesity or an unhealthy lifestyle, but also specific insults, through dietary or some lifestyle factors, may directly affect the oocytes microenvironment and the oocyte proper. Differentiating the indirect from a potential direct impact of a high fat diet on the follicular fluid (FF) composition and the oocyte quality remains a big challenge. Furthermore, only very little data are available about how long it takes for a specific lifestyle factor like an unhealthy diet to impact on the follicular environment and on subsequent oocyte quality.

Disappointing fertility results are not only relevant in human clinical settings. In livestock, fertility results determine the farmer's income, management efficiency and environmental impact (greenhouse gasses and nitrogen emissions) (Garnsworthy 2004; von Soosten *et al.* 2020). Reproductive failure in pig and cow farming is now recognized as a main burden and has serious economic consequences. Metabolic stress due to, e.g. negative energy balance (NEB), has been strongly correlated with disappointing fertility outcome in modern dairy industry worldwide (Berry *et al.* 2016). Excessive fat mobilization in NEB cows and the resulting lipotoxic effects, higher levels of oxidative stress and a higher inflammatory state, have the potential to directly impact on the oocyte's microenvironment and, thus, may reduce oocyte quality. This review will highlight some of the above mentioned factors. It is important to mention though that some studies could not find any negative association between the cow's postpartum (pp) metabolic profile and oocyte quality and development when comparing different time points (from 21 days to 80 days pp) (Matoba *et al.* 2012) or when comparing early pp lactating cows to heifers (Rizos *et al.* 2005). Therefore, the association between metabolic health and oocyte quality appears to be dependent on other factors that may vary from one farm to another, such as nutrition, feed additives, housing, management, antioxidant (AO) status and stress.

2. The growing importance of the oocyte's culture environment in determining female fertility

ART poses an extra burden on oocyte and embryo viability. Despite the great progress in the understanding of oocyte and embryo cell physiology, birth rates per transfer remain relatively low, both in humans and animals (Hansen 2020). A whole cascade of potential stressors may impact on oocyte and embryo viability throughout the ART process causing an accumulation of cell damage. Not only the artificial *in vitro* environment, UV-light and oxygen tension, but also physical stressors due to pipetting, ICSI and biopsying, may lead to cell damage, reactive oxygen species (ROS) accumulation and oxidative stress, DNA integrity losses and altered gene expression (Truong *et al.* 2022). This may lead to developmental arrest due to apoptosis or to problems in the fetal development of the surviving embryos. Truong and Gardner (2017) clearly illustrated that even a short-term exposure to atmospheric oxygen levels can have a negative impact on embryo developmental capacity. It is remarkable that, in contrast to farm animal assisted reproduction settings, about 2/3 of the human *in vitro* fertilization centers still use 20% oxygen in only a part or throughout their entire *in vitro* embryo production (IVP) procedures (Truong *et al.* 2022). Even if culture is performed in 5% oxygen, an unfavorable exposure to ambient oxygen levels is still possible during oocyte collection procedures, ICSI and visual checks under the microscope. In addition, there is more and more investment in pre-implantation genetic testing, both in human and bovine settings. However, this technique requires embryo cryopreservation. Cryoprotectants and changes in osmolarity and temperature, are all harmful factors affecting embryo gene expression, cellular redox status, DNA repair mechanisms, and epigenetic processes, such as DNA and histone methylation and acetylation. Altered methylation patterns can lead to imprinting errors (Katari *et al.* 2009), leading to large offspring syndrome (Young *et al.* 2001), a common sequel of bovine ART, and to Beckwith-Wiedeman syndrome in humans (Maher *et al.* 2003).

The impact of *in vitro* culture (IVC) techniques on oocyte quality and further development is a very important factor to consider as we may assume that oocytes collected from metabolically compromised individuals are more sensitive to such suboptimal *in vitro* environments (Marei and Leroy 2021). On the other hand, the *in vitro* oocyte handling, and further culture may create a unique opportunity to provide a supportive and even "therapeutic" *in vitro* environment to recover the quality of oocytes collected from patients with a compromised (metabolic) health (Marei *et al.* 2019b). The rest of this overview paper will merely focus on the pathophysiology of reduced oocyte quality *in vivo* under metabolic

stress conditions and on the potential opportunities to intervene in order to rescue or to prevent low oocyte quality. The outline of the review paper is visualized in Figure 1.

3. The oocyte and the consequences for the offspring's health.

Mounting evidence points towards the importance of the periconception period for the development of non-communicable diseases later in life, which is captured in the DOHaD concept or hypothesis (Developmental Origins of Health and Disease) (Barker 2007; for a very recent review, see Peral-Sanchez *et al.* 2022). The mechanisms behind this impact have been widely studied and are based on epigenetic modifications, affecting the expression level, activity or silencing of specific genes. The main types of epigenetic modifications are DNA methylation, histone modifications and non-coding RNAs. Both the prematuration and final oocyte maturation, but also the early preimplantation embryo development and the further development in the uterine environment, all have been recognized as important windows for reprogramming of the epigenetic footprint (Fleming *et al.* 2012). The Dutch famine study was one of the first and best-known epidemiological approaches highlighting the specific vulnerability of the periconception period and how it may impact on postnatal adult health (Roseboom *et al.* 2006). Later studies confirmed this (Waterland *et al.* 2010). Ge *et al.* (2014b) illustrated the specific vulnerability of the oocyte, describing changes in oocyte epigenetic marks in maternal diabetes conditions. Paternal metabolic health and obesity has also been linked with the offspring's epigenetic marks and health, further stressing the capital importance of the gamete and its environment (Lane et al. 2015). Fleming et al. (2018) elegantly overviewed the significant impact of a disturbed metabolic preconception environment on the oocyte and indicated the importance of preconception care to safeguard the health of the next generations. This implies that adult metabolic diseases such as the metabolic syndrome, obesity and type II diabetes but most probably also reduced fertility and oocyte quality may have their origin in the maternal health before, and just after conception. This is a very important notion if we aim to design tailored made health care advice for subfertile women (or couples) aiming at pregnancy. An eye-opening opinion was published in Fertility and Sterility (Oct 2019) stating that "Preconception care should become a key component in reproductive medicine as it is the ultimate window of opportunity to improve mother's fertility and to set the stage for the child's health. It is all about winning the battle before the war has begun" (Simon 2019). Just recently, more than 95% of the fertility staff involved in a Belgian study, indicated that, while urgently needed, no structured and scientifically substantiated lifestyle modification programme is offered in their clinic (Boedt *et al.* 2021). Moholdt and Hawley (2020) also concluded that the preconception period is the window to target when focusing on lifestyle

and healthcare interventions. Whether oocyte quality benefits from such preconception lifestyle and health care interventions is not well studied and should deserve much more scientific attention.

4. Should we care about the oocyte as a target for improving fertility?

Last March (31st March, 2022) our laboratory organized a national seminar entitled: "Preconception care for the oocyte: from the well to clinical practice". At the end of the seminar, an online real-time questionnaire was proposed to the audience. Sixty-two attendees (25% clinicians, 60% scientists and 15% industry affiliated persons) were asked to anonymously respond every question within 5 minutes. The first question was: "Does the oocyte deserve centre stage in routine clinical assisted reproduction?". 51% of the respondents answered "Yes of course, no doubt about that" while 46% responded "Yes, but practically it is difficult to prove the need for that as there are a lot of practical constraints.". Only 3% of the attendants answered "No, there are other, much more important factors that need more attention first in the fertility clinic before we start to focus on the oocyte.". These figures show that more and more human fertility clinicians recognize and highlight the capital importance of optimizing or recovering maternal health before conception to maximize oocyte quality and thus fertility outcome and to safeguard the health of the next generation.

Recent insights in mechanisms linking maternal health with oocyte quality

1. The oocyte's microenvironment, a mirror of maternal health

Acquisition of oocyte developmental competence is a cumulative process that takes place in the ovarian follicle during oocyte growth and maturation (Fulka *et al.* 1998; Watson 2007). This involves a sequence of complex cytoplasmic and molecular changes that are essential to make the oocyte fertilizable, ultimately leading to viable offspring. Any perturbation in the microenvironment of the oocyte within the ovarian follicle potentially impacts on oocyte quality and developmental competence (Mermillod *et al.* 2008; Krisher 2013), which puts fertility at risk.

It has been already well described in several studies that maternal health drastically alters the composition of the oocyte's microenvironment. Obesity, diet, lifestyle and disease all directly affect follicular growth and the composition of the FF (Leroy *et al.* 2015). Not only markers of insulin resistance, dyslipidemia, oxidative stress, systemic inflammation but also bacterial components such as lipopolysaccharides (LPS) have all been detected in FF

(Piersanti *et al.* 2019). Granulosa cells (GCs) express toll-like receptors which can be activated by LPS to produce proinflammatory cytokines that hamper oocyte quality (Bromfield and Sheldon 2011). Adipocytokines, produced by the adipose tissue, are reflected in the FF and are linked to oocyte developmental capacity. In the FF, concentrations of tumor necrosis factor α (TNFα), interleukin 6 and 10, and other inflammatory cytokines have also been related with oocyte quality and the chance of a successful pregnancy (Wyse *et al.* 2021). Like others, we generated a lot of data linking maternal metabolic health with the FF composition, both in women as in the high-producing dairy cow model. In high-producing dairy cows, it has been well described that upregulated lipolysis, due to a reduced insulin sensitivity, a low insulin status, obesity, or a NEB associated catabolic status, coincides with a significant increase in the free fatty acid concentrations in blood and FF (Leroy *et al.* 2005; Valckx *et al.* 2014b). We learned that these elevated concentrations of free fatty acids play an important role in explaining the reduced oocyte quality observed in these animals as they induce lipotoxicity at the level of the cumulus oocyte complex (COC). Mirabi *et al.* (2017) confirmed in human follicular samples that higher concentrations of saturated fatty acids (particularly palmitic acid) coincide with a lower *in vitro* oocyte developmental capacity after ICSI. The specific lipotoxicity associated pathways in the oocyte will be further discussed in detail below.

Not only maternal diet and health but also heat stress (HS) has been documented to have drastic adverse effects on oocyte quality and subsequent embryonic development in dairy cows (Sartori *et al.* 2002; Yin *et al.* 2019). This is due to the direct impact of heat at the level of the oocyte and the follicle and/or due to an indirect stress-induced reduction in dry matter intake and the concomitant (exacerbation of) NEB status (Abdelatty *et al.* 2018).

Many interesting retrospective studies compared the FF composition from oocytes that did develop until blastocyst with those that did not further develop both in humans (Jungheim *et al.* 2011; Batushansky *et al.* 2020) and in cows (Annes *et al.* 2019). In this way it was possible to propose several predictive oocyte quality markers. Moore *et al.* (2017) even found that FF metabolites (profiles of specific fatty acids and amino acids) are highly predictive for genetic merit for fertility. However, these very interesting studies only show association and thus fail to identify a causative link.

Next to the FF composition, many cumulus cell gene and proteome markers have been identified as good predictors for oocyte quality (Bunel *et al.* 2015; Alves *et al.* 2019; Si *et al.* 2021). Cumulus cell physiology is intimately linked with oocyte quality as has been elegantly overviewed in detail by Marchais *et al.* (2022).

Altering the oocyte's microenvironment can be an interesting approach to directly affect the quality of the oocyte and thus to improve fertility results. As others, we performed several studies on changing the FF composition through dietary supplementation as a first step to approach the oocyte (for overview see Valckx and Leroy 2015). In animal models (like the dairy cow) it is rather straight forward to, for example, alter the fatty acid content and profile or the concentrations of specific AOs in the follicular compartment which provides an attractive opportunity to improve oocyte quality (Leroy *et al.* 2014; De Bie *et al.* 2016). Kermack *et al.* (2021) recently reported for the first time in a clinical setting that a 6-week dietary intervention has the potential to increase omega-3 fatty acid concentrations in human FF. We furthermore showed that, for example, linolenic acid added to the final oocyte maturation environment has the potential to protect the oocyte from the lipotoxic effects of elevated saturated fatty acids (Marei *et al.* 2017). However, understanding such specific consequences for the oocyte is difficult as next to direct effects at the oocyte level, dietary interventions may induce several indirect changes, such as changes in endocrine signaling pathways, altered immune function and metabolic health, and different follicular growth patterns. A fundamental bottom-up approach in a completely controlled *in vitro* environment may be the first step to dissect a specific impact at the level of the oocyte, to discover the pathways involved and to understand the potential interactions when more than one influencing factor is altered (De Bie *et al.* 2017). However, translating these insights to and applying them in the clinic remains a significant challenge. Furthermore, individual variation and environmental factors are expected to add an extra layer of complexity as they may induce variation in the response to such interventions.

2. Extracellular vesicles as mediators of maternal health to the oocyte

The multi-directional communication between follicular cells and the oocyte is carried out via gap junctions or paracrine and autocrine secretion of molecules (Bosco *et al.* 2011). Furthermore, extracellular vesicles (EVs) are released from various cell types and play a crucial role in cell-to-cell communication, also in ovarian follicles (for a detailed overview see Raposo and Stoorvogel 2013; Simon *et al.* 2018). EVs can exert essential physiological and pathological effects on both recipient and parent cells via various functional molecules (RNAs, proteins, DNA, and lipids), either as structural or as cargo components (Valadi *et al.* 2007; Keller *et al.* 2011; Hailay *et al.* 2019). Interestingly, in both human and bovine FF, EVs have been isolated (Sohel *et al.* 2013; Santonocito *et al.* 2014) and it was found that EV microRNA (miRNA) content is associated with the developmental capacity of oocytes (Sohel *et al.* 2013). Very recently Gebremedhn *et al.* (2020) overviewed the role of EVs in modulating metabolic and environmental stress responses in the ovarian follicle. Much more

297 research is needed, however, to study the cargo composition of FF EVs from metabolically
298 stressed individuals and their potential role in reduced developmental capacity of the oocyte
299 remains to be discovered. Using a dairy cow model, Hailay *et al.* (2019) characterized the
300 EV miRNA landscape of FF from nulliparous heifers, NEB and positive energy balance
301 cows. Results showed several well-conserved known miRNAs (n=365) within EVs.
302 Furthermore, target prediction and pathway analysis revealed downregulation of 5 EV
303 miRNA (miR-2285, miR-451, miR-132, miR-486, and miR-874) in NEB compared to positive
304 energy balance cows. *In silico* analysis unraveled that these differentially expressed
305 miRNAs are implicated in various pathways, including the tumor growth factor β (TGF-β)
306 signaling pathway, known for its role in oocyte and embryo development (Yu *et al.* 2016).
307 Furthermore, 37 miRNA were differentially expressed between NEB cows and nulliparous
308 heifers EV miRNA from the FF involved in pathways linked to folliculogenesis and early
309 embryo development (Christenson 2010; Mondou *et al.* 2012). These results are
310 undoubtedly promising, but also here, direct causative links between EVs characteristics
311 and oocyte quality are still missing. Directly adding isolated EVs to the culture medium
312 seems to be a promising approach (Asaadi *et al.* 2021) to further study the rescuing and
313 protective capacity of EVs against stressors in order to improve oocyte quality
314 (Gebremedhn *et al.* 2020).

315 ## 3. The oocyte suffers, but how?

316 Changes in serum metabolite concentrations are reflected in the FF surrounding the oocyte
317 as has been explained above (Leroy *et al.* 2004; Valckx *et al.* 2012). Folliculo- and
318 oogenesis are very sensitive periods to such alterations in the environment, as the oocyte
319 uses the metabolites from its microenvironment to meet its energetic and anabolic needs
320 (Valckx *et al.* 2014a; Best and Bhattacharya 2015).

321 Exposure of oocytes to lipotoxic conditions in obese mouse models but also *in vitro*
322 exposure results in an increased amount of intracellular lipid droplets (Wu *et al.* 2010; Yang
323 *et al.* 2012). The highly available intracellular fatty acids, stored in the form of triglycerides,
324 are metabolized via mitochondrial β-oxidation, which results in upregulated mitochondrial
325 activity leading to an increased ROS production and oxidative stress (Iossa *et al.* 2002;
326 Burton *et al.* 2003; Marei *et al.* 2017). By consequence, the endoplasmic reticulum (ER)
327 function will be perturbed because of structural alterations and accumulation of misfolded
328 proteins due to oxidative damage. This resulting ER stress elicits specific unfolded protein
329 responses (UPRer) (Borradaile *et al.* 2006; Diakogiannaki *et al.* 2008; Zhang and Kaufman
330 2008), which are coordinated responses that includes cell cycle arrest, transient attenuation
331 of protein synthesis and stimulation of nuclear expression of chaperons in an attempt to

maintain cellular homeostasis. Under high levels of cellular stress, this will result in the induction of apoptosis (Kaufman 1999; Rutkowski and Kaufman 2004; Runkel *et al.* 2014; Marei *et al.* 2019c).

Mitochondria are of capital importance to guarantee oocyte developmental competence as they are important for energy production, as well as regulating calcium signaling, and apoptosis (Van Blerkom 2004; Agarwal *et al.* 2008; Kirillova *et al.* 2021). There is increasing evidence that mitochondrial dysfunction plays a central role in the pathogenesis of reduced oocyte quality under metabolic stress conditions (Wu *et al.* 2010; Saben *et al.* 2016; Marei *et al.* 2019c; Marei *et al.* 2020). We have recently showed that 30% of lipotoxicity-induced proteomic alterations in oocytes are linked to mitochondrial dysfunctions (Marei *et al.* 2019c). Moreover, it has been extensively described in *in vivo* and *in vitro* mouse models that a high-fat microenvironment induces mitochondrial dysfunction in the oocyte. This has also been shown in human studies and bovine *in vitro* models. The following changes in mitochondrial functions have been reported: altered mitochondrial membrane potential (MMP) (Igosheva *et al.* 2010; Wu *et al.* 2010; Marei *et al.* 2017); altered mitochondrial DNA (mtDNA) copy numbers (Santos *et al.* 2006; Luzzo *et al.* 2012; Marei *et al.* 2020); mtDNA mutations (Larsson 2010); morphological abnormalities such as ruptured membranes, fewer cristae, disarray of cristae, swelling, decreased electron density and increased vacuolisation (Luzzo *et al.* 2012; Marei *et al.* 2020; Smits *et al.* 2020b); increased mitochondrial biogenesis (Larsson 2010; Luzzo *et al.* 2012; Boudoures *et al.* 2017) and deficient β-oxidation (Reynolds *et al.* 2015; Boudoures *et al.* 2016; Hou *et al.* 2016). Alteration in ATP dependent cytoskeletal dynamics also alters spindle formation and chromosomal segregation leading to marked increase in aneuploidy (Nakagawa and FitzHarris 2017). Similarly, HS has been shown to alter mitochondrial distribution and reduce MMP in bovine oocytes collected in summer, and to a lesser extent in fall compared to those collected in winter (Gendelman and Roth 2012). Importantly, oocytes are not capable of activating mitophagy in response to mitochondrial damage, so these mitochondria will not be cleared from the oocyte (Boudoures *et al.* 2017).

In response to oxidative stress, similar UPRs as in the ER are seen in the mitochondria (UPRmt) (Münch and Harper 2016). Extensive shotgun proteomic analysis of bovine oocytes after *in vitro* maturation (IVM) under lipotoxic conditions showed several anti-apoptotic changes such as increased abundance of mitochondrial antioxidative proteins (particularly, PRDX3, NRF2-mediated oxidative stress response, activation of p70S6K-14-3-3 signaling) (Marei *et al.* 2019c), all of which are known to be involved in the activation of pro-survival mechanisms (Chang *et al.* 2004; Lim *et al.* 2013; Amin *et al.* 2014).

4. Molecular mechanisms leading to epigenetic alteration in oocytes

The oocyte undergoes extensive epigenetic reprogramming and genomic imprinting during pre- and postnatal development, which are both key processes in establishing epigenetic patterns of the offspring (Smallwood *et al.* 2011; Pan *et al.* 2012). Due to the dynamic nature of the reprogramming, the oocyte epigenome is particularly sensitive to changes in the microenvironment. This is illustrated by different studies showing that diet-induced obesity in mice significantly altered global DNA methylation and histone modifications in fully grown oocytes (Ge *et al.* 2014a; Ge *et al.* 2014b; Hou *et al.* 2016). Studies in our lab have shown that exposure to pathophysiological NEFA concentrations during bovine IVM and IVC results in altered DNA methylation patterns in blastocysts (Desmet *et al.* 2016). Also, expression of *DNMT3b*, an essential enzyme in regulating *de novo* DNA methylation, was upregulated in blastocysts after exposure of COCs to NEFAs during IVM (Van Hoeck *et al.* 2013). Furthermore, DNA methylation patterns of several metabolism-related genes (e.g. *leptin* and *PPARα*) are changed in oocytes from obese mice and in oocytes of their offspring (Ge *et al.* 2014a). Finally, a loss of DNA methylation at the imprinted gene *PLAGL1* locus in oocytes following IVM in the presence of elevated NEFA concentrations was observed (O'Doherty *et al.* 2014). Deletion of the mitochondrial fission factor *Drp1* in murine oocytes resulted in mitochondrial dysfunction, disrupted further development and resulted in altered DNA and histone methylation patterns (Adhikari *et al.* 2022). This indicates that an affected oocyte mitochondrial function may have long lasting effects on further development and postnatal health through alterations in the epigenome. Of course, much more research is needed.

5. How long does it take for an oocyte to be affected?

As described above, the direct impact of maternal health or diet on oocyte quality is relatively well documented. However, how long it takes for a disease condition or for an obesogenic diet to negatively affect the oocyte remains unclear. All studies investigating the effect of high-fat diet (HFD)-induced obesity on oocyte quality performed the analysis at one timepoint after a relatively long period of exposure which varies from 4 weeks (Wu *et al.* 2010; Ruebel *et al.* 2016) to 6 weeks (Igosheva *et al.* 2010) or even 13 weeks (Marei *et al.* 2020). These studies show an increased expression of ER stress marker genes (*ATF4* and *GRP78*) in oocytes of mice after 4 weeks of feeding a HFD, together with a dramatically increased lipid content of the oocytes and reduced MMP compared to oocytes of mice fed a control diet (Wu *et al.* 2010). After 4 weeks of feeding a HFD, an increase in proinflammatory genes was shown in ovaries of Sprague Dawley rats (Ruebel *et al.* 2016). After 6 weeks of feeding an obesogenic diet, mice oocytes showed an altered mitochondrial

activity (Igosheva *et al.* 2010). Marei *et al.* (2020) reported an increase in *PRDX6* expression, a higher lipid droplet content, and an altered mitochondrial function in the oocytes of Swiss mice after 13 weeks of feeding a HFD. Different effects have been reported, however the time at which these effects start to develop in the oocyte is not known. Whether the effects might either occur as a very acute response to the diet (after hours or days), even before the development of an obese phenotype, or only after a long-term exposure to the diet (after several weeks) is not clear. It is also not known from which follicular stage onwards the oocyte is impacted by maternal disease or diet. Strategically designed animal models are needed to answer these very relevant questions. Preliminary data generated in a still ongoing study in our laboratory revealed that oocytes collected from mice fed a high-fat and high-sugar diet already showed a 60% increase in the total lipid droplet volume after 24 hours of feeding compared to the control group. This increase was persistent until 8 weeks of feeding (Moorkens *et al.*, 2021, unpubl. data). This new information clearly indicates that lipid content in oocytes is merely driven through diet and its composition and not (only) by the obese phenotype and its underlying disturbed metabolism.

6. Are preantral follicles at stake, affecting oocyte quality already many weeks before ovulation?

Cows with severe NEB lose body condition score (BCS) due to excessive fat mobilization, which leads to elevated blood NEFA concentrations mainly during the first 3 weeks pp. Direct lipotoxic effects of high NEFA concentrations on oocyte developmental competence have been described above. However, the first artificial insemination (AI) in cows usually only takes place after 50-60 days pp. By that time, energy balance is usually restored and blood NEFA concentrations are normalized (Leroy *et al.* 2004; Carvalho *et al.* 2014). Nevertheless, pregnancy rates are still affected by the severity of NEB and BCS loss (Carvalho *et al.* 2014). It has been demonstrated that cows that lose BCS during the transition period (from 21 days before to 21 days after calving, Barletta *et al.* (2017)) or during the first 3 weeks pp (Carvalho *et al.* 2014) have a significantly lower pregnancy/AI compared to those which maintained or gained BCS. In addition, cows in the highest quartile for body weight loss during the first 3 weeks pp yielded the highest percentage of degenerated embryos and the lowest percentage of transferable embryos after superovulation, AI and embryo flushing at day 60 pp, compared to cows in the other three quartiles (with less or no weight change) (Carvalho *et al.* 2014). These results strongly suggest that severe NEB and BCS loss during the early pp period have a long-term carry-over impact on oocyte quality and developmental competence later at the time of breeding.

Similar carry-over effects on oocyte quality have been described after an episode of HS (Al-Katanani *et al.* 2002; Roth 2017). As described above, HS can directly or indirectly reduce oocyte quality (Roth 2008; Torres-Júnior *et al.* 2008; Abdelatty *et al.* 2018). Importantly, like pp NEB, such negative impact of HS on oocyte developmental competence persists for at least 1-2 months after the end of the summer season before normal fertility rates are completely restored (Roth 2017).

The mechanisms of such long-term impact on oocyte quality and fertility appear to be multifactorial but not fully defined. A higher prevelance of health events in severe NEB cows together with more inflammation and endotoxemia during the pp period may have indirect effects on ovarian functions and oocyte quality (Dickson *et al.* 2020; Piersanti *et al.* 2020). On the other hand, it is now commonly accepted that the early stages of follicular development and their enclosed oocytes may be vulnerable and affected. Considering that folliculogenesis is a lengthy process that may take more than 90 days in cattle (Fair 2003), small follicles that are metabolically compromised early pp may reach ovulation at the time of breeding several weeks after the restoration of maternal health. This notion has already been postulated by the Britt hypothesis in 1992 (Britt 1992) but as it is very difficult to design a proper experimental design to study this concept, strong evidence is still lacking. The impact of HS on early follicular stages is better exemplified. Cooling of cows for 42 days prior to their slaughter in summer did not improve their oocyte developmental competence *in vitro* compared to cows that were not cooled (Al-Katanani *et al.* 2002), whereas embryo transfer bypasses the problem of reduced oocyte quality and results in a higher pregnancy rate (Roth 2017). Small ovarian follicles (0.5–1 mm in diameter) and their enclosed oocytes have indeed been shown to be highly sensitive to hyperthermia (Roth *et al.* 2000). When bovine ovarian cortex fragments were cultured *in vitro* under hyperthermic conditions for 12 hours, a lower proportion of the enclosed primordial follicles remained viable after 7 days of culture compared to controls (Paes *et al.* 2016). This was associated with an increased expression of *HSP70* and apoptosis-related genes in the affected follicles (Paes *et al.* 2016).

It is important to mention that the association between NEB, oocyte quality and fertility significantly varies among different studies and from one farm to another (Carvalho *et al.* 2014). Experimental induction of NEB by restricted feed intake in nulliparous heifers for 50 days did not influence pregnancy/AI following AI at 50 days and even increased pregnancy/AI at day 93 (several weeks after the end of the energy restriction) compared to heifers fed a maintenance diet (Parr *et al.* 2015). It is possible that the hormonal changes during pregnancy might increase the sensitivity of the cow to metabolic stress during

transition. This concept was confirmed in obese mouse models showing that pregnancy *per se* can significantly increase the severity of insulin resistance and metabolic stress in response to feeding a high-fat high-sugar diet (Pennington *et al.* 2017).

A recent study in our laboratory aimed at generating more evidence and mechanistic insights into the long-term impact of pp NEB on the follicular microenvironment and oocyte quality at the time of breeding in dairy cows (Marei *et al.* 2022). We studied the correlations between different metabolic (BCS loss, NEFAs, Glucose, and insulin growth factor 1 (IGF1)) and antioxidant parameters (βCarotene (βC); Vitamin E (Vit E); Vitamin A (Vit A); total antioxidant status (TAS); derivatives of reactive oxygen metabolites (dROM); and oxidative stress index (OSI)) in the blood at 2 weeks and 8 weeks pp, and in the FF at 8 weeks (collected by ovum pick up (OPU) after estrus synchronization, after the voluntary waiting period). We also examined the associations between these factors with changes in the GC transcriptomic (RNAseq) profile of the preovulatory follicle (before the luteinizing hormone (LH) surge) at the time of breeding (8 weeks pp) (Marei *et al.* 2022). Interestingly, such association was clearly evident with blood NEFAs, βC and Vit E at week 2. Cows in the top quartile of blood NEFA concentration at week 2 (0.86±0.16 mM) were associated with 64 differentially expressed genes (DEGs) in the GCs at week 8 compared to the lowest quartile. The upregulated DEGs were related to cellular response to stress, immune response (e.g. regulation of cytokine production), and response to lipid and ketones; while the downregulated DEGs were related to lipid catabolic processes, carnitine and Co-enzyme A metabolic process and cellular nitrogen metabolic processes. No association could be found with blood NEFA concentrations at week 8, which were decreased in all cows to basal levels.

On the other hand, cows in the highest quartile of week 2 blood βC and Vit E were associated with 341 DEGs in the GCs at week 8 compared to those in the lowest quartile. The pattern of expression of these genes indicated a lower ubiquitin-dependent protein catabolism, higher RNA biosynthesis and splicing, and increased expression of genes involved in response to LH and estrogen, higher steroidogenic activity and lower apoptosis, together with an increased oxidoreductase activity, mitogen activated protein kinase (MAPK) cascade, and pathways related to meiosis activation in oocyte, suggesting a higher capacity to support oocyte quality and enhance developmental competence. Pathways linked with acute inflammation, negative regulation of nuclear factor kappa light chain enhancer of activated β cells (NF-kappa β) transcription factor activity, oxidation dependent catabolic processes, sphingomyelin biosynthesis, mitochondrial fragmentation, and lipophagy were all downregulated in these cells. In other words, follicles that start to grow

in the presence of high AO concentrations (βC + Vit E) in the blood at week 2 pp seem to exhibit less inflammatory responses and less cellular stress and catabolism by the time they reach ovulation at week 8.

In addition, we examined the potential interaction between blood AOs and NEFAs on GC functions. In other words, we examined if optimal AO status may attenuate the long-term effects of NEFAs on the ovarian follicle. In cows with high concentrations of week 2 NEFAs, week 2 blood AO concentrations did not influence the GC transcriptomic profile (only 3 DEGs), whereas week 8 blood AO concentrations had a strong effect (194 DEGs). The functional annotation of these genes indicates a better cell viability, metabolic activity and oocyte supportive capacity, and lower levels of inflammation and cellular stress.

From this, we can conclude that the maternal metabolic health condition many weeks (even months) before ovulation may have a drastic long-term impact on GC functions in the preantral and early antral follicles, which may result in disappointing oocyte quality at the time of breeding. We could also conclude that such effect might be attenuated by optimal blood AOs concentration around the time of breeding.

Opportunities to target the oocyte for treatment or prevention

1. The importance of antioxidants

As described above based on our GC transcriptome study in dairy cows, AOs have the capacity to alter follicular physiology. AOs are molecules that can neutralize free radicals coming from ROS. The AO defense system contains AO enzymes, endogenous non-enzymatic compounds, metal sequestration proteins and dietary AO such as Vit E, carotenoids, α-lipoic acid and acetyl L-carnitine. Vit E is an important dietary AO and is present in plasma membranes, protecting cells against ROS. βC is the precursor of the non-AO retinol or Vit A and has also main functions in cellular growth, differentiation and regulation of development (Marshall *et al.* 1996; Gómez *et al.* 2006). α-lipoic acid has positive effects on oocyte maturation, fertilization and embryo development and acetyl L-carnitine ameliorates energy supply to the cells (Agarwal *et al.* 2003; Agarwal *et al.* 2012). These examples are just the tip of the iceberg and it is clear that an optimal AO defense system at the oocyte level requires sufficient AO intake to sustain the balance between ROS and AO.

An increase in oxidative stress is linked with subfertility (Leroy *et al.* 2008a; Leroy *et al.* 2008b; LeBlanc 2010a; Leblanc 2010b; Van Hoeck *et al.* 2014), stating the importance of a proficient cellular AO defense system. This has been also confirmed at the level of the

oocyte's microenvironment (Nishihara *et al.* 2018). Oocytes are very sensitive to such imbalances due to their long maturation process in contact with their environment. More specifically, oxidative stress insults in the oocyte can induce perturbations in the one carbon cycle hampering DNA methylation processes and also affects chromosome stability and segregation and thus may lead to aneuploidy (for review, see Dattilo *et al.* (2016)). Women with abdominal obesity suffer from hyperlipidemia with significant higher amounts of lipid peroxide markers in serum and in FF compared to women without abdominal obesity (Nasiri *et al.* 2015). This leads to an increased ROS accumulation and lower fertility rates due to the lipotoxic oocyte environment. Similarly, the metabolic stress seen in transition dairy cows lead to higher oxidative stress levels in the oocyte microenvironment. Furthermore, early pp dairy cows have a higher need for AOs in order to cover for the high systemic oxidative stress levels. In a Flemish case study (De Bie *et al.* 2014; De Bie *et al.* 2019), De Bie *et al.* reported in 2019 that one third of the Flemish dairy cows had deficient circulating plasma levels of βC and Vit E concentrations (Baldi 2005; Calsamiglia and Rodríguez 2012). Similar findings were reported in a larger European study (Mary *et al.* 2021). The main factors influencing plasma βC and Vit E levels are lactation status of the cow, the type of farm, the season, the dietary supplemented vitamins and the cow's parity. It is now generally accepted that optimal Vit E and βC concentrations significantly support reproductive outcome in dairy cows (Meyer *et al.* 1975; Lotthammer 1979; Miller *et al.* 1993; Baldi *et al.* 2000; Pontes *et al.* 2015). More specifically, we could show that daily βC supplementation substantially improved βC and retinol availability in the oocyte's microenvironment both in negative and positive energy balance cows. This creates an opportunity to directly target the oocyte through strategically designed dietary interventions (De Bie *et al.* 2016). Fundamental insights from the well confirmed that oocyte maturation in presence of high AO concentrations may have a protective impact resulting in embryos that are more resilient to a metabolic stress insult (De Bie *et al.* 2021)

While several *in vitro* AO supplementation studies seem to yield promising results, clinical prospective data clearly showing positive effects of oral AO intake are weak (Showell *et al.* 2020). There is a large heterogeneity in study design and clinical and social background of the patients may vary considerably. Too strong AO supplementation strategies may even lead to a disruption of essential regulatory processes during oocyte maturation, ovulation and fertilization. Also the composition of the diet can be an important disturbing factor as it may alter bioavailability of the AO in the gastro-intestinal tract. Most probably, patient tailored approaches are the sole way forward.

As it has been explained earlier, the importance of ART is still increasing every year. The *in vitro* environment and handling procedures are a significant source of oxidative stress. Supplementation of AO to compensate for the negative effects of this artificial environment has been tested extensively (for review see Zarbakhsh (2021)). For example, Vit E has positive effects on oocyte maturation and developmental competence of oocytes and embryos (Dalvit *et al.* 2005; Marques *et al.* 2008; Natarajan *et al.* 2010; De Bie *et al.* 2021), as well as retinoids, which increase cellular growth and cell differentiation (Ikeda *et al.* 2005). Also, Truong *et al.* (2022) showed that the AO combination of α-lopoic acid, acetyl L-carnitine and N-acetyl-cysteine improved murine blastocyst rate and quality to a level similar to the *in vivo* controls. The same AO combination increased the murine *in vitro* embryo development as well, together with a reduction of the apoptotic cell index of cryo-preserved embryos (Truong *et al.* 2022). One major bottleneck of in vitro AO applications is that only water-soluble AO can be used without the need to include solvents. Taken together, AO supplementation to the patient or in the *in vitro* well forms an important gateway to improved oocyte quality and may be able to compensate for insults through diet or a disturbed maternal health. However, much more, especially *in vivo* research is necessary to carefully modulate and personalize these supplementation strategies.

2. Mitochondria as a key target to improve oocyte quality

We explained earlier that stress conditions elicit pro-survival mitochondrial and ER UPRs in the oocyte, which are expected to increase embryo survival after fertilization (Marei *et al.* 2019c). However, embryos derived from metabolically-compromised oocytes have higher rates of fragmentation and developmental arrest during early development, and higher rates of blastomere apoptosis (Marei *et al.* 2019b). This illustrates that the endogenous UPR mechanisms are not sufficient to combat the damage or prevent its further aggravation after fertilization, leading to failure of embryo development, usually before blastocyst formation (Diskin *et al.* 2011; Marei *et al.* 2019b; Marei and Leroy 2021). The increased intracellular levels of ROS and MMP may persist after fertilization, resulting in carry-over effects during early embryo development (Marei *et al.* 2019b). The surviving embryos exhibit persistent mitochondrial dysfunction (lower MMP due to mitochondrial uncoupling) and oxidative stress (Marei *et al.* 2019b), which is associated with altered cellular metabolism, and altered cell lineage and differentiation at the blastocyst stage (Van Hoeck *et al.* 2011; Leary *et al.* 2015; Van Hoeck *et al.* 2015). Only recently, we reported in Human Reproduction that even after transfer to a healthy uterus, these bovine embryos exhibit growth retardation, altered embryo-maternal communication and long-lasting cellular dysfunctions (Desmet *et al.* 2020).

609 Controlling mitochondrial ROS production and improving the capacity of mitochondria to
610 resist cellular stress can be an effective approach to improve oocyte and early embryos
611 quality, or at least to reduce stress to tolerable levels until mitochondrial biogenesis is
612 enabled at later stages after blastocyst formation (Lima *et al.* 2018), i.e. for mitochondrial
613 damage to be self-repaired (Marei and Leroy 2021). Conventional antioxidants are usually
614 effective in prevention of ROS accumulation, but have limited capacity to alleviate oxidative
615 stress or restore mitochondrial functions in oocytes when metabolic stress is ongoing (Smits
616 *et al.* 2020a; De Bie *et al.* 2021). In contrast, mitochondria-targeted AOs such as
617 Mitoquinone (MitoQ) have been developed and approved to ameliorate metabolic
618 syndrome-related disorders in many tissues and cell types (Feillet-Coudray *et al.* 2014).
619 MitoQ is composed of Co-enzyme Q10 (CoQ10, a potent ROS scavenger naturally
620 occurring the mitochondrial electron transport chain) bound to a strong cationic carrier.
621 MitoQ can thus accumulate within the mitochondria and prevent (progression of)
622 mitochondrial oxidative damage (Milagros Rocha and Victor 2007). Very promising recent
623 studies in our laboratory have shown that *in vitro* supplementation with MitoQ during IVM
624 under lipotoxic conditions could rescue mitochondrial functions in bovine oocytes, and
625 completely alleviate the impact of lipotoxicity on subsequent embryo development (Marei *et*
626 *al.* 2019a). More importantly, supplementation with MitoQ during IVC of embryos derived
627 from metabolically compromised oocytes could significantly reduce embryo fragmentation
628 and apoptosis and restore normal blastocyst rates and quality (Marei *et al.* 2019b). Similarly,
629 CoQ10 supplementation during IVM restored mitochondrial distribution patterns and
630 developmental competence of oocytes collected during fall (which exhibit moderate level of
631 HS) (Gendelman and Roth 2012; Roth 2018). However, CoQ10 turned out to have no effect
632 on bovine oocytes collected during summer, probably due to a too high level of stress
633 (Gendelman and Roth 2012; Roth 2018).

634 Besides reduced oocyte quality linked to metabolic disorders and HS, aging has also been
635 strongly linked with reduced oocyte quality and infertility in humans and animal models
636 (Moghadam *et al.* 2022). The reduction in oocyte quality is mainly manifested as age-related
637 defects in microtubule dynamics and compromised spindle formation, leading to marked
638 increase in aneuploidy (Eichenlaub-Ritter *et al.* 2004; Nakagawa and FitzHarris 2017; Ma
639 *et al.* 2020). These defects appear to be mainly driven by accumulation of mtDNA mutations
640 and mitochondrial dysfunction (Ma *et al.* 2020). MitoQ supplementation during IVM of
641 oocytes collected from aged mice (18 months old) could significantly reduce the occurrence
642 of chromosomal misalignments from 78% to rates similar to those observed in young mice
643 (1 months old) (22%) (Al-Zubaidi *et al.* 2021).

While *in vitro* results are indeed promising, specific delivery of mitochondrial targeted AOs to the ovary to manipulate oocyte quality *in vivo* can be challenging. Several biological barriers may prevent these molecules from reaching the oocyte such as the blood follicle barrier, the compact cumulus cell layers, and the zona pellucida. Various pharmaceutical preparations such as liposomes, and polymeric nanoparticles have been developed to modify the mitochondrial protein import machinery which allows specific targeting of mitochondria (Wang *et al.* 2017). We have recently demonstrated that polymeric poly(lactic-co-glycolic acid) (PLGA) nanoparticles are taken up by the cumulus cells in COCs, and accumulate at the transzonal projection endings in the sub-zonal region in the oocyte without any negative impact on the oocyte developmental capacity (Goncalves *et al.* 2021). Modification of these particles to specifically target the ovarian follicles may become a very efficient tool to deliver mitochondrial targeted molecules to the oocyte *in vivo*.

3. Preconception care interventions and the impact on oocyte quality

We already highlighted the preconception period as a crucial window for women aiming for pregnancy. Preconception care interventions (PCCI) should improve the maternal metabolic health in the weeks and months before conception as important processes like folliculogenesis take place (3-4 months in human, 3 weeks in mice) (Clarke 2017). We do not know yet whether such improvement of the maternal metabolic health before conception has the potential to improve or even restore the quality of oocytes that has eventually already been hampered during the early phases of follicular growth under unhealthy metabolic conditions. However, if these early follicular phases are not affected by a bad maternal metabolic health, then the implementation of such PCCI may be ideal to prevent oocyte damage and thus to rescue the oocyte during the late follicular growth phase. Nowadays, overweight and obese women who are having issues with getting pregnant are advised by their fertility specialist to lose weight before conception to increase their chance of a healthy, successful pregnancy (Pasquali 2006; Jungheim and Moley 2010; Lassi *et al.* 2014). However, up until now, there are no clear evidence-based guidelines regarding preconception care in these overweight and obese infertile women as many of these clinical studies are underpowered due to high drop-out rates and are confounded by the unknown social background of the patients included (Sim *et al.* 2014; Mutsaerts *et al.* 2016; Einarsson *et al.* 2017). Designing sound preconception care strategies for obese future mothers is almost impossible in a pure clinical setting, albeit very needed and important. There is a clear need for more fundamental research, investigating the impact of preconception interventions on fertility in general and on oocyte quality more specifically in order to obtain

crucial insights towards clear preconception guidelines. Can oocyte quality be rescued or even restored in metabolically compromised women?

Earlier research showed a beneficial impact of dietary interventions on metabolic health by improving body composition, plasma lipids, insulin sensitivity etc. (Andersen and Fernandez 2013; Cui *et al.* 2013; Aksungar *et al.* 2017; Vangoitsenhoven *et al.* 2018). However, up until now, very limited information is available on the impact of such a preconception diet on oocyte quality (Tsagareli *et al.* 2006; Reynolds *et al.* 2015). Severe weight loss, as a result of a caloric restriction diet, resulted in significantly increased lipid mobilization with a possible significant negative impact on fertility (Jensen *et al.* 2014; Legro 2017). Therefore, severe weight loss right before conception has been discouraged in clinical settings (Legro 2016), suggesting that diet normalization might be a more suited approach. However, direct comparisons were never made before. In addition, the most suited time period for this intervention is not known. Folliculogenesis in mice lasts for 3 weeks (Clarke 2017) which is a very important notion when aiming to investigate if PCCI can rescue and/or restore oocyte quality and how long such intervention should last. The past years, our research laboratory investigated the impact of dietary PCCI for different time periods (2, 4 or 6 weeks) on both metabolic health and oocyte quality using an obese outbred mouse model. To investigate this, obese outbred mice were switched from a high-fat diet to two different diets: 1) an ad libitum control diet or 2) a severe calorie restricted control diet where both dietary composition was changed and calorie intake was significantly reduced (by 30%) compared to the control group.

Based on the results obtained during this research, undergoing diet normalization for a period of at least four weeks in mice seemed to be the most promising approach to improve both metabolic health and oocyte quality (Smits 2022). A caloric restriction diet as applied in our model showed to be a too extreme intervention, especially with regards to metabolic health (Smits *et al.* 2021). Diet normalization resulted in a more gradual weight loss (13%) and restoration of almost all metabolic health parameters assessed (serum lipid profile and glucose tolerance) after four weeks on the diet. Although some improvements were present with regards to oocyte quality, it is clear that dietary interventions do not result in complete restoration of the oocyte quality. Especially mitochondrial abnormalities in the oocytes from the intervention groups were not completely restored. Boudoures *et al.* (2016) described very similar results in inbred obese mice which were subjected to a voluntary exercise intervention for 6 weeks, but which remained on the high-fat diet. Altogether, these results indicate that the primordial follicle pool might be damaged and that a complete recovery based on diet normalization or exercise is not possible. Targeting the oocyte mitochondria

may be an important step to move forward. Also, when these oocytes are processed *in vitro* during assisted reproduction services, a tailored IVM (or even prematuration) environment should be considered to alleviate or at least avoid further cellular damage and support mitochondrial functions. Finally, awareness programs should communicate these fundamental scientific findings in order to stress the importance of prevention. Caring for the oocyte should start long before we consider using the oocyte!

Conclusions

In conclusion, there is strong evidence showing that reduced oocyte quality plays a key role in subfertility in humans, especially in conditions of reduced maternal health or unhealthy lifestyle. Obesity, diet, stress, inflammation and infection can directly hamper the oocyte's microenvironment, lowering oocyte quality. Similar effects are documented in farm animals due to NEB, HS and pp diseases. Such deterioration in oocyte quality appears to involve a long-term impact on the growing oocyte during folliculogenesis. Fully-grown oocytes exhibit mitochondrial structural and bioenergetic dysfunctions and oxidative stress with several molecular consequences during subsequent embryo development. This also affects epigenetic programming and puts the offspring health at risk. The ideal solution to prevent such deterioration in oocyte quality is to alleviate the primary cause before oocyte quality is affected, i.e. to improve preconception health. However, while some of these PCCI appear to improve metabolic health, oocyte quality is not completely recovered. Interventions aiming at improving the follicular microenvironment by e.g. increasing its AO capacity are promising techniques to influence the oocyte, however assessing the specific impact on oocyte quality, and its further development is much more complicated. The *in vitro* environment during ART procedures forms an ideal window during which the oocyte or at least the early embryo can be rescued, however some ART steps can themselves form an extra burden for incompetent embryos which may already carry defective mitochondria and increased cellular stress levels from the oocyte. This may impact embryo developmental capacity, but more importantly may influence epigenetic reprogramming and postnatal health. Supplementing mitochondrial targeted AO during embryo culture has been shown to minimize cellular stress and restore mitochondrial functions in embryos derived from metabolically-compromised bovine oocytes. Application of such research in human settings is very difficult to perform due to ethical and practical limitation, again stressing the importance of well-designed *in vitro* and animal experiments. Translating these fundamental findings into clinical application should be done in a multidisciplinary context. Importantly, it is crucial to consider the sensitive epigenetic programming during early development.

747 Research in further development of PCCI and *in vitro* treatments should not only aim at
748 improving embryo yields and fertility, but also safeguarding offspring health.

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752 Conflicts of interest

753 The authors declare no conflicts of interest.

754 Declaration of funding

755 This research did not receive any specific funding.

756 Data availability statement

757 Data sharing is not applicable as no new data were generated or analysed for this
758 review paper.

Figures

Title figure 1: Illustrative summary of the review content. Oocyte quality is affected by a deviating maternal metabolic health. Several opportunities exist to alleviate or even restore oocyte quality in order to improve fertility and safeguard offspring's health.

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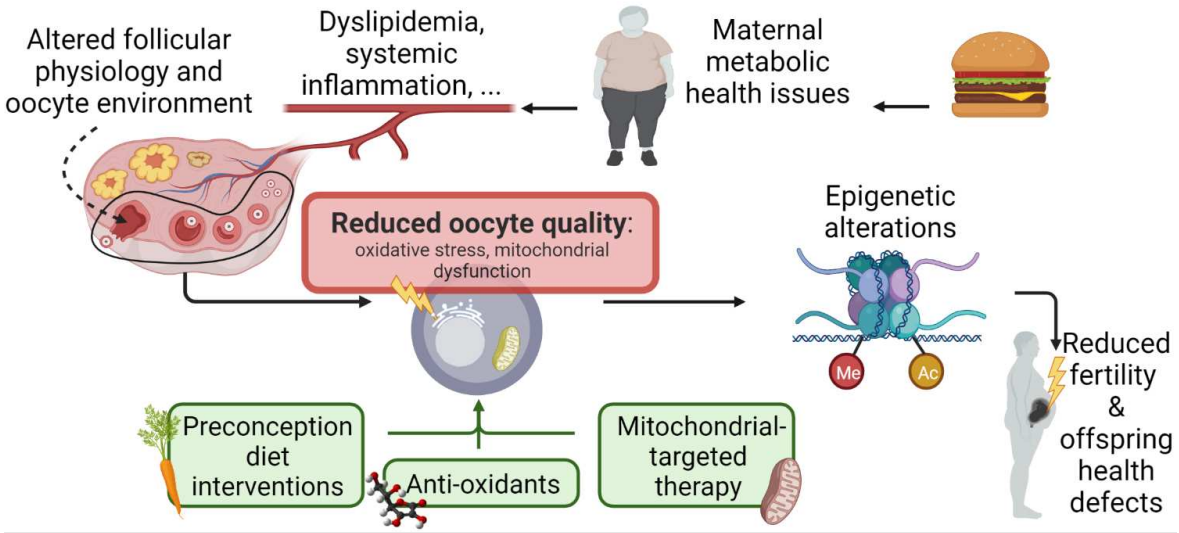
Altered follicular physiology and oocyte environment
Dyslipidemia, systemic inflammation, ...
Maternal metabolic health issues
Reduced oocyte quality:
oxidative stress, mitochondrial dysfunction
Epigenetic alterations
Me
Ac
Reduced fertility & offspring health defects
Preconception diet interventions
Anti-oxidants
Mitochondrial-targeted therapy

Figuer 1