Obesity induces ovarian inflammation and reduces oocyte quality

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Abstract

In the United States, 36.5% of women between the ages of 20 and 39 years are obese. This obesity results in not only metabolic disorders including type II diabetes and cardiovascular disease, but also impaired female fertility. Systemic and tissue-specific chronic inflammation and oxidative stress are common characteristics of obesity. This is also true in the ovary. Several studies have demonstrated that pro-inflammatory cytokines and reactive oxygen species alter estrous cyclicity, steroidogenesis and ovulation. Inflammation and oxidative stress also impair meiotic and cytoplasmic maturation of the oocyte which reduces its developmental competence for fertilization and pre-implantation embryo development. Interestingly, there is recent evidence that obesity- and/or polycystic ovary syndrome (PCOS)-dependent changes to the gut microbiome contributes to ovarian inflammation, steroidogenesis and the expression of mRNAs in the oocyte. However, several gaps remain necessitating future studies to identify inflammation, oxidative stress and gut microbiome mechanisms that reduce ovarian function and oocyte quality.

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Introduction

Obesity has progressed from a significant health risk to an epidemic in the United States. Obesity is defined as body mass index (BMI) greater than $30\,kg/m^2$ and in the US, 39.6 percent of adults (>20) were classified as obese in 2015–2016 (Hales *et al.* 2017). Reproductive age women (20–39 years) have similar obesity rates (36.5%) compared to the general population. However, there is ethnic obesity disparity such that 55% of African American and 51% of Hispanic reproductive age women are classified as obese (Hales *et al.* 2017). Obesity is characterized by increased lipid storage in adipose tissue and other metabolic organs, which leads to cellular lipid toxicity, inflammation and oxidative stress. The result is development of metabolic dysfunctions like type II diabetes, cardiovascular disease and ultimately, reduced quality and quantity of life. Importantly, the percentages of obese and overweight adults are expected to rise to 50% by 2030 (Wang *et al.* 2011, Finkelstein *et al.* 2012).

Obesity in women not only affects her metabolism but also her reproductive health. Specifically, obese women are at increased risk for ovulatory subfertility and anovulatory infertility compared to age-matched lean women (Pandey & Bhattacharya 2010, Kumbak *et al.* 2012, Penzias 2012). While anovulation can be overcome with ovarian stimulation, obese women have decreased responsiveness to gonadotropins, decreased oocyte retrieval, decreased oocyte quality, reduced rates of pre-implantation embryo development and increased risk for miscarriage compared to their lean counterparts (Kumbak *et al.* 2012, Klenov & Jungheim 2014, Broughton & Moley 2017). Current research aims to define obesity-dependent mechanisms that cause these phenotypes in order to prevent or reverse female infertility.

Obesity causes chronic inflammation, oxidative stress and changes to the gut microbiome

Acute inflammation, which is triggered by tissue damage as a result of an invading pathogen or trauma, activates the release of chemokines by resident innate immune cells. These chemokines attract additional innate immune cells from the systemic circulation (Sokol & Luster 2015). At the same time, resident and infiltrating innate immune cells produce pro-inflammatory cytokines. The cytokines initiate signaling pathways at the cellular level to stimulate expression of chemokines and cytokines as well as genes that regulate cell death, senescence and survival (Sapochnik *et al.* 2017, Varfolomeev & Vucic 2018). The end result is phagocytosis of damaged tissue and subsequent secretion of anti-inflammatory cytokines that regulate wound repair and resolution of the inflammatory response. Chronic inflammation is defined as unregulated and persistent chemokine and cytokine synthesis and secretion. This can be caused by unresolved inflammation after tissue damage (Landskron *et al.* 2014). Alternatively, environmental pressures (e.g.

allergens), abnormal metabolism (e.g. microbiome changes) or persistent necrotic cell death within a tissue (e.g. obese adipocyte) can induce *de novo* inflammatory responses (Garn *et al.* 2016, Kuroda & Sakaue 2017).

In the context of obesity, increased circulating triglycerides are stored in adipocytes resulting in adipocyte hypertrophy (Ouchi *et al.* 2011, Engin 2017). The end result is hypoxia-induced necrosis of adipocytes and infiltration of adipose tissue with circulating macrophages and T helper cells due to release of monocyte chemoattractant 1 (MCP-1/CCL2) and nicotinamide phosphoribosyltransferase (NAMPT) (Ouchi *et al.* 2011). The macrophages subsequently secrete pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α) and pro-inflammatory interleukins (ILs; e.g. IL-6). These cytokines activate the NFκB signal transduction pathway to produce more pro-inflammatory cytokines (Fig. 1). Adipocytes also produce factors (adipokines, e.g. leptin and lipocalin) which upon secretion promote additional release of TNFα and IL-6 (Ouchi *et al.* 2011). Importantly, when cytokines and adipokines are released into the circulation they induce inflammatory responses in other tissues including the ovary (Ouchi *et al.* 2011, Nteeba *et al.* 2013, Wang & Huang 2015, Xie *et al.* 2016).

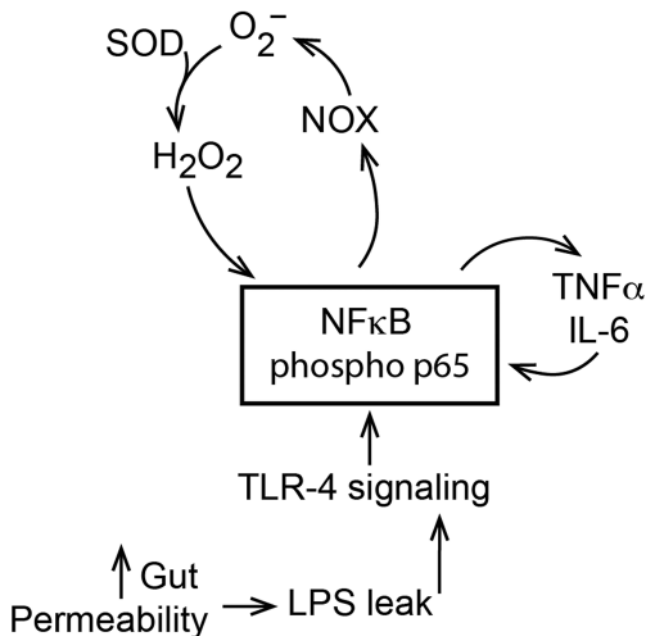Chronic inflammation also induces oxidative stress due to increased production of reactive oxygen species (ROS) that overwhelm the cell's antioxidant system (Biswas 2016, Hussain *et al.* 2016). The main ROS species are superoxide (O_2^-) and hydrogen peroxide (H_2O_2) and they are produced by multiple organelles in the cell including mitochondria, endoplasmic reticulum and peroxisomes (Brown & Borutaite 2012, Nordgren & Fransen 2014, Chong *et al.* 2017, Mailloux 2018). Inflammation-dependent increases in NFκB-p65 phosphorylation promotes expression of the redox family of NADPH oxidases (NOX) which produce O_2^- (Bedard & Krause 2007, Lu *et al.* 2010). The O_2^- is subsequently converted to H_2O_2 by superoxide dismutase (SOD) (Fig. 1). The H_2O_2 can freely move from the organelle to the cytoplasm of the cell and when in the cytoplasm activates NFκB-p65 through phosphorylation (Oliveira-Marques *et al.* 2009, Ren *et al.* 2015), thereby increasing the expression pro-inflammatory cytokines including TNFα and IL-6 (Fig. 1). Based on this interdependence of inflammation and oxidative stress, it is not surprising that obesity is also a state of chronic oxidative stress, which together contribute to the development of metabolic and neural non-communicative disease (Piya *et al.* 2013, Ertunc & Hotamisligil 2016, Rimessi *et al.* 2016).

Over the last decade, investigators have established that symbiotic relationships between different anatomical sites (e.g. skin, colon, and vagina) and colonized microbes (microbiome), which are essential for human health (Cho & Blaser 2012, Belizário & Napolitano 2015). In the gut, there is a symbiotic relationship between its microbiome, the intestinal epithelia and immune system and inflammatory responses (Fig. 2). However, environmental pressures including diet and antibiotic use cause shifts in the diversity and relative abundance of the colonizing bacteria due to microenvironmental-driven changes in bacterial growth, stasis or death (Camp *et al.* 2009,
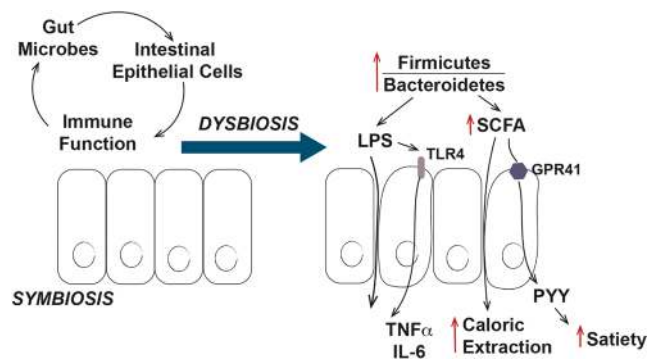

Figure 1 Schema showing relationships between increased gut permeability, inflammation and oxidative stress. H_2O_2 (hydrogen peroxide), IL-6 (interleukin-6), LPS (lipopolysaccharide), NFκB phosphor-p65 (phosphorylated p65 subunit of the nuclear factor kappa light-chain enhancer of activated B cell), NOX (NADPH oxidases), O_2^- (superoxide), SOD (superoxide dismutase), TLR-4 (toll-like receptor-4), TNFα (tumor necrosis factor alpha).

Figure 2 Schema showing symbiotic relationships between gut microbiome, immune system, and the intestinal epithelium. Obesity causes changes in the gut microbiota resulting in increased epithelial permeability, LPS leak and activation of TLR signaling in the gut, and increased caloric extraction and satiety due to increased production of SCFAs. GPR41 (G-protein receptor 41), IL-6 (interleukin 6), LPS (lipopolysaccharide), PYY (peptide tyrosine tyrosine), SCFA (short-chain fatty acid), TLR4 (toll-like receptor 4), TNFα (tumor necrosis factor alpha).

Korem *et al*. 2015). In the context of obesity, the ratio of Firmicutes and Bacteroidetes, two phyla of bacteria that predominate in the gut, is increased (Ley *et al*. 2005, Turnbaugh *et al*. 2006, 2008, Riva *et al*. 2017). This change has been correlated to a state of gut dysbiosis which is characterized by tight junction protein loss and increased epithelial permeability (Kim *et al*. 2012, Saad *et al*. 2016).

One consequence of microbiota-induced gut dysbiosis is increased translocation of lipopolysaccharides (LPS) into the circulation resulting in low-level endotoxemia and induction of systemic inflammation (Fig. 2) (Cani *et al*. 2007, König *et al*. 2016, Bidne *et al*. 2018). Binding of LPS to the toll-like receptor 4 (TLR-4) in the intestinal epithelial also produces cytokines including TNFα and IL-6 which also contributes to systemic inflammation and metabolic alterations (Ding *et al*. 2010, Kim *et al*. 2012, Cox & Blaser 2013, Everard *et al*. 2014). In addition to LPS, obesity-dependent gut dysbiosis results in increased dietary caloric extraction due to increased production and absorption of short-chain fatty acids (SCFAs) (Turnbaugh *et al*. 2006, Cox & Blaser 2013, Saad *et al*. 2016). Importantly, SCFAs also regulate intestinal epithelial tight junctions, epithelial permeability and gut dysbiosis. Circulating SCFAs increase lipid storage in adipose tissue and cholesterol synthesis in the liver. However, they also increase the production of the satiety hormone PYY and have anti-inflammatory function (Cox & Blaser 2013, Saad *et al*. 2016). Therefore, it is unclear how gut microbiota changes in SCFAs contributes to obesity. Together, these data have established the now well-accepted paradigm that obesity is a chronic condition characterized by low-grade inflammation, oxidative stress and increased gut permeability (Fig. 1).

Physiological cytokine and chemokine signaling in the ovary

Cytokines and chemokines play essential roles in follicular growth and ovulation during a normal estrous cycle. They arise from resident and infiltrating leukocytes that are localized in and recruited to the ovary, respectively. Specifically, Wu *et al*. localized activated T-lymphocytes, macrophages and monocytes and neutrophils in the theca layer during the follicular phase (Wu *et al*. 2006). After the ovulatory LH surge, dendritic cells are detected in the theca cells, while the numbers of neutrophils and macrophages in the theca cell layer are significantly increased indicating a role of these leukocytes during ovulation (Brännström *et al*. 1995, Cohen-Fredarow *et al*. 2014, Akison *et al*. 2018). Likewise, T-lymphocytes, granulocytes, monocytes, macrophages and dendritic cells are found in follicular fluid-derived cells of the pre-ovulatory follicle (Storeng *et al*. 2007).

The leukocytes described above as well as granulosa and theca cells secrete chemokines that recruit additional leukocytes to the ovary. Furthermore, a myriad of cytokines is produced which regulate somatic cell function during follicular growth and ovulation. For example, granulocyte-macrophage-colony stimulating factor (GM-CSF), monocyte chemoattractant protein 1 (MCP-1) and IL-8 proteins induce migration of macrophages into the ovary (Połeć *et al*. 2011, Field *et al*. 2014). Likewise, chemokine ligand 20 (CCL20) produced by the theca and granulosa cells stimulate an influx of macrophages (Al-Alem *et al*. 2015). Finally, RANTES (regulated upon activation of normal T-cell expressed and secreted) mediates influx of T-cells, eosinophils and mast cells into the ovary (Field *et al*. 2014). Cytokines regulate important functions in the ovary. For example, IL-6, interferon alpha (IFNα) and IFNβ regulate cumulus expansion after the LH surge (Fan *et al*. 2009, Dam *et al*. 2015).

In addition to acting as chemokines, GM-CSF and IL-8, acting as cytokines, also regulate the ovarian cell functions. During follicular growth, GM-CSF increases preantral follicle progression and decreases granulosa cell proliferation and synthesis of 17β-estradiol (E2) and progesterone (P4) (Gilchrist *et al*. 2000, Wang *et al*. 2005, Zhang *et al*. 2008). Interleukin-8 stimulates follicular progression from preantral to pre-ovulatory stage (Goto *et al*. 1997). However, it inhibits E2 synthesis by granulosa cells and increases P4 synthesis by theca and granulosa cells (Shimizu *et al*. 2012, 2013). These studies suggest initiation of luteinization by GM-CSF and IL-8 during the ovulatory process. Additional studies demonstrate that IL-1 induces ovulation (Brännström & Enskog 2002).

Pathological cytokine and chemokine signaling in the ovary

It should be noted that the abnormalities in ovarian function have been correlated with increased infiltration of the ovary by macrophages (Skaznik-Wikiel *et al*. 2016), increased expression and signaling of pro-inflammatory cytokines (Nteeba *et al*. 2014, Xie *et al*. 2016), and increased incidence of ovarian fibrosis (de Araújo *et al*. 2018). Obesity-dependent subfertility and infertility is also associated with pathological inflammation and oxidative stress (Agarwal *et al*. 2005*a*,*b*). Recent studies show increased localization of innate immune cells, evidence of inflammatory signaling and oxidative stress in ovaries from obesity animal models as well as obese women. For example, TNFα, IL-6 and IL-8 expression and activity of their associated inflammatory signaling pathways (e.g. NFκB) are increased in ovaries from obese women and mice (Nteeba *et al*. 2013, Ruebel *et al*. 2016, Xie *et al*. 2016). Interestingly, female

TNFα-knockout mice (*Tnfa*−/−) show increased frequency of estrous cycles, increased granulosa cell proliferation and reduced oocyte apoptosis resulting in increased number of pups born over a 12-month breeding period (Cui *et al*. 2011). Finally, there is evidence of obesity-dependent oxidative stress in the ovary (Igosheva *et al*. 2010). Sources of cytokines include ovarian somatic cells and leukocytes within the ovary (Nteeba *et al*. 2014, Ruebel *et al*. 2016, 2017, Xie *et al*. 2016). There are also pro-inflammatory cytokines (IL-1, IL-6, TNFα) and oxidative stress factors (H_2O_2, oxLDL) in follicular fluid which in some cases are correlated to circulating cytokines (Bausenwein *et al*. 2010, Buyuk *et al*. 2017, Gonzalez *et al*. 2018). Interestingly, IL-6 and TNFα are positively correlated with lipid concentrations in follicular fluid of obese women. Therefore, obesity-dependent systemic inflammation and lipotoxicity may be transmitted to the ovary via follicular fluid, although additional studies are required to verify this occurrence. While physiological expression of cytokines is required for optimal ovarian functions, these data suggest that increased or aberrant expression of pro-inflammatory cytokines above a threshold level or at inappropriate time points during the estrous cycle impairs ovarian function.

Metabolic and ovarian phenotypes of PCOS

Polycystic ovary syndrome (PCOS) is an androgen excess disorder that impacts 7–10% of reproductive age women. Women with PCOS are at increased risk for development of obesity and type 2 diabetes (Rodgers *et al*. 2019) resulting in phenotypes that overlap with obese women without PCOS. For example, in a rat model of PCOS, tumor necrosis factor alpha (TNFα) and MDA, which is a metabolite of lipid peroxidation, are increased indicating chronic ovarian inflammation and oxidative stress (Furat Rencber *et al*. 2018). Interestingly, the level of cytokines and MDA are also higher in obese PCOS compared to obese non-PCOS women, although both have higher TNFα and MDA levels than lean PCOS and lean controls (Alshammari *et al*. 2017, Artimani *et al*. 2018). These data suggest that PCOS exacerbates obesity-dependent ovarian inflammation and oxidative stress (Ressler *et al*. 2015). Distinct metabolic differences between obesity and PCOS include hyperandrogenemia, that is, obese women without PCOS have circulating total testosterone concentrations similar to lean control women (Keskin Kurt *et al*. 2014, Alshammari *et al*. 2017, Usta *et al*. 2018). Other hormones increased in obese PCOS patients compared to obese women include insulin/glucose, LH and E2 (Keskin Kurt *et al*. 2014, Usta *et al*. 2018). While triglyceride concentrations are similar between PCOS and non-PCOS obese women, there are distinct differences in the types of phosphatidylcholine and lyso-phosphatidylcholine species between obese PCOS and obese non-PCOS women (Li *et al*. 2017). Based on these data, it is clear that obese women with

PCOS have similar inflammatory and oxidative stress phenotypes to obese women. It should also be noted that there are PCOS women who have a normal BMI (Alshammari *et al*. 2017, Li *et al*. 2017, Usta *et al*. 2018). Therefore, there are obese women without PCOS and lean women with PCOS.

Obesity disrupts ovarian function

Several studies using rodent models or human samples demonstrate that obesity negatively impacts ovarian function. For example, estrous cyclicity is irregular in rodents fed a high-fat diet, with longer periods of diestrus and shortened estrus periods (Nteeba *et al*. 2014, Bazzano *et al*. 2015, de Araújo *et al*. 2018). Follicle growth and development is also altered by obesity. There is depletion of the primordial follicle pool and concomitant increases in progression of follicles to the antral stage (Nteeba *et al*. 2014, Wang *et al*. 2014, Shaznik-Wikiel *et al*. 2016). There are also increased numbers of atretic follicles (Wang *et al*. 2014, Wu *et al*. 2015, de Araújo *et al*. 2018). These phenotypes have been replicated in an Ossabaw pig model of obesity. In this model, obese pigs have elongated estrous cycles and increased numbers of antral and atretic follicles compared to lean counterparts (Newell-Fugate *et al*. 2015). Together, these data suggest that obesity-dependent decreases in the number of primordial follicles and increases in the number of antral and atretic follicles may deplete the ovarian reserve and contribute to subfertility (Wang *et al*. 2014).

Granulosa cell apoptosis, which initiates follicular atresia, is increased in ovaries from obese mice and rats (Wu *et al*. 2015). In follicles that do not become atretic, it is reasonable to expect that the reduced number of granulosa cells per follicle also decreases E2 synthesis and secretion, which would directly impact both estrous cyclicity and follicle growth. Indeed, the mRNA abundance of *Cyp19a1*, which encodes the P450 aromatase protein, is decreased in ovaries from obese female mice (Nteeba *et al*. 2014, de Araújo *et al*. 2018). Likewise, the ratio of E2 to P4 and mRNA abundance of the steroidogenic acute regulatory protein (*Star*) and P450 aromatase (*Cyp19a1*) are decreased in obese mice during estrus (Nteeba *et al*. 2014, Pohlmeier *et al*. 2014).

Oocyte quality is reduced due to obesity

Obesity-induced abnormalities in folliculogenesis and ovulation can be rescued by ovarian stimulation with exogenous gonadotropins in both mice and women (Pohlmeier *et al*. 2014, Committee and Society 2015, Xie *et al*. 2016). However, pregnancy rates remain low and miscarriage rates high indicating that oocyte quality is impaired (Kawwass *et al*. 2016). Under normal conditions, when the oocyte reaches the end of the growth phase, it acquires the ability to resume

meiosis, which is stimulated by luteinizing hormone (LH) and results in an oocyte terminated at metaphase II (Schroeder *et al.* 1990, Conti & Franciosi 2018). LH also stimulates maturation of the cytoplasmic contents of the oocyte including accumulation and re-distribution of mitochondria, accumulation of essential nutrients and selective storage and degradation of RNAs and proteins (Mao *et al.* 2014). Under optimal conditions, coordination of nuclear and cytoplasmic maturation produces a high-quality oocyte competent for fertilization, pre-implantation development and ultimately embryo/fetal viability.

Oocytes collected from diet-induced obese mice have poor quality based on reduced *in vitro* embryonic development (Colton *et al.* 2002, 2003, Minge *et al.* 2008, Pohlmeier *et al.* 2014, Hou *et al.* 2016). Furthermore, poor oocyte quality can have long-lasting effects on fetal growth and neural development (Luzzo *et al.* 2012). Interestingly, the metabolic effects of obesity, due to consumption of a high-fat diet, are reversed when mice are returned to a control diet. However, meiotic and cytoplasmic measures of oocyte quality remain poor (Reynolds *et al.* 2015). Women who are obese and use artificial reproductive technology (e.g. *in vitro* fertilization) have reduced pregnancy rates compared to their lean counterparts (Kumbak *et al.* 2012, Moragianni *et al.* 2012). However, these poor rates can be rescued by the use of donor oocytes from lean individuals (Bellver *et al.* 2007, Levens & Skarulis 2008). Based on recent data described below, reduced pregnancy rates are attributed, in part, to reduced oocyte quality and abnormalities in pre-implantation embryo development. Like the somatic cells of the ovary, obesity induces oxidative stress in the oocyte which negatively impacts both meiotic and cytoplasmic maturation of the oocyte (Table 1).

Meiotic maturation

Successful meiotic maturation requires resumption of meiosis and the faithful segregation of chromosomes. Resumption of meiosis is characterized by germinal vesicle breakdown, progression from prophase I to metaphase II and extrusion of the first polar body. Abnormalities during the resumption or progression of meiosis result in aneuploidy. Streptozotocin treatment of mice, which destroys the insulin-secreting pancreatic β-islet cells (Lenzen 2008), causes meiotic abnormalities (Colton *et al.* 2002, 2003, Wang *et al.* 2009). For example, the percentage of oocytes that undergo germinal vesicle breakdown (GVBD) is reduced when they are collected from streptozotocin-induced diabetic mice (Colton *et al.* 2002, 2003, Ratchford *et al.* 2007). There are also abnormalities in both spindle structure and chromosome alignment (Wang *et al.* 2009, Ou *et al.* 2012). It is important to note that both type 1 and type 2 diabetes results in hyperglycemia. Interestingly, when non-human primates received excess dietary sugar, meiotic resumption was impaired suggesting that hyperglycemia is an important contributor to meiotic resumption defects (Chaffin *et al.* 2014).

Disruption of meiosis, resulting in aneuploidy, has also been assessed using diet-induced obesity mouse and rat models (Hou *et al.* 2016, Wang *et al.* 2018). Like the streptozotocin-treated mice, high-fat diet-fed mice exhibit decreases in both GVBD, polar body extrusion, abnormal spindle structure and chromosome alignment (Luzzo *et al.* 2012, Zhang *et al.* 2015, Hou *et al.* 2016, Wang *et al.* 2018). Recent studies show a causative effect between accumulation of ROS in the oocyte and meiotic maturation defects. Zhang *et al.* showed that microinjection of mitochondria-localized deacetylase sirtuin 3 (*Sirt 3*) mRNA into oocytes of high-fat fed mice decreased ROS levels and rescued meiotic defects (Zhang *et al.* 2015). Likewise, knockdown of *Sirt2* results in spindle defects and chromosome misalignment (Zhang *et al.* 2014). Wang *et al.* demonstrated that loss of TP53-induced glycolysis and apoptosis regulator (*Tigar*) expression increases ROS and spindle disorganization (Wang *et al.* 2018). Alternatively, when *Tigar* is overexpressed in oocytes from high-fat-fed mice, there is a reduction in ROS and reduced structural meiotic abnormalities. These data indicate that obesity-dependent oxidative stress plays an important role in aneuploidy and ultimately embryo loss.

Interestingly, there is a significant number of first-trimester pregnancy losses in obese individuals that have a normal karyotype (Landres *et al.* 2010). This is in striking contrast to pregnancy loss in individuals with advanced maternal age which are overwhelmingly due

Table 1 Obesity effects on oocyte maturation.

Effects	References
Mitosis	
Decreased GV breakdown and polar body extrusion	Colton *et al.* (2002, 2003), Ratchford *et al.* (2007), Chaffin *et al.* (2014), Hou *et al.* (2016), Wang *et al.* (2018)
Spindle structure and chromosome alignment abnormalities	Wang *et al.* (2009), Luzzo *et al.* (2012), Ou *et al.* (2012), Zhang *et al.* (2015), Hou *et al.* (2016)
Cytoplasm	
Mitochondria	Igosheva *et al.* (2010), Luzzo *et al.* (2012), Ou *et al.* (2012)
Endoplasmic reticulum	Wu *et al.* (2010, 2012, 2015), Yang *et al.* (2012), Sutton-McDowall *et al.* (2015), Guzel *et al.* (2017)
mRNA abundance	Wood *et al.* (2007), Pohlmeier *et al.* (2014), Hou *et al.* (2016), Xie *et al.* (2016), Ruebel *et al.* (2017)

to aneuploidy (Nagaoka *et al.* 2012). Together, these data suggest that obesity-dependent early pregnancy loss is also due to alternative oocyte abnormalities, in particular, cytoplasmic maturation of the oocyte.

Mitochondrial dysfunction

Mitochondria play an essential role in both oocyte maturation and pre-implantation embryo development. Indeed, mitochondrial dysfunction leads to abnormalities in meiotic resumption, fertilization and development of the embryo to the blastocyst stage (Van Blerkom 2011, Babayev & Seli 2015). Like somatic cells, mitochondria in the oocyte are the site of oxidative phosphorylation and production of ATP. Treatment of oocytes with an ionophore that inhibits oxidative phosphorylation, and presumably the production of ATP, results in spindle and chromosome alignment defects (Ge *et al.* 2012) indicating that the mitochondria is an important energy source for meiotic maturation. It is important to note that efficient oxidative phosphorylation requires a high mitochondrial membrane potential, which increases as the oocyte grows and matures (Van Blerkom & Davis 2007). In addition to metabolic functions, the numbers of mitochondria increase during oocyte growth to approximately 160,000 (Mahrous *et al.* 2012). However, the increase in maternally derived mitochondria are only essential for pre-implantation development but not oocyte maturation (Ge *et al.* 2012).

In the context of obesity, mitochondrial membrane potential is altered. Igosheva *et al.* and Luzzo *et al.* showed increases in membrane potential in MII oocytes and zygotes using a diet-induced model of obesity (Igosheva *et al.* 2010, Luzzo *et al.* 2012). Alternatively, Wu *et al.* showed decreased membrane potential in MII oocytes using a genetic model of obesity (Wu *et al.* 2015). Interestingly, Ou *et al.* showed increased membrane potential in germinal vesicle stage oocytes and decreased membrane potential in MII oocytes in a hyperinsulinemia model (Ou *et al.* 2012). These studies suggest that the mode of obesity development differentially dictates the metabolic potential of oocyte mitochondria. Additional studies are required to discriminate how diet, satiety suppression and hyperglycemia regulate changes in the membrane potential of mitochondria. Despite these discrepancies, all the studies showed increased levels of ROS in oocytes. Furthermore, they all showed abnormalities in spindle structure and/or chromosome alignment consistent with ionophore inhibition of mitochondrial oxidative phosphorylation.

Endoplasmic reticulum stress

As indicated earlier, obesity-dependent oxidative stress is the result of increased production of O_2^- and H_2O_2 (Fig. 1). Increased ROS also induces endoplasmic reticulum (ER) stress. The consequence is activation of the unfolding protein response (UPR) which includes increased expression of ATF4, ATF6, GRP78 and PERK (Guzel *et al.* 2017). Indeed, oocytes from obese mice or treatment of mouse oocytes with follicular fluid from obese women increases ATF6, ATF4 and GRP78 (Wu *et al.* 2010, 2015, Yang *et al.* 2012). This ER stress has been correlated with reduced ovulation, fertilization and/or pre-implantation development, which is indicative of reduced oocyte quality (Wu *et al.* 2010, 2012, Sutton-McDowall *et al.* 2015). To determine the causative effect of ER stress on the oocyte, cumulus-oocyte complexes (COCs) have been treated with thapsigargin, which induces ER stress. This treatment decreases cumulus cell expansion, which is a marker of oocyte quality, and results in poor pre-implantation development rates (Wu *et al.* 2012). Treatment of COCs with palmitic acid similarly induces ER stress, decreases cumulus expansion and reduces pre-implantation development (Wu *et al.* 2012, Sutton-McDowall *et al.* 2015). It should be noted that thapsigargin treatment decreases mitochondrial membrane potential suggesting cross-talk between ER- and mitochondrial-dependent ROS production (Wu *et al.* 2012). Therefore, to definitively demonstrate that ER stress impairs oocyte quality, COCs treated with thapsigargin or palmitic acid or collected from obese mice were cultured in the presence of the ER stress inhibitor salubrinal (Wu *et al.* 2012, Sutton-McDowall *et al.* 2015). This treatment reverses the ER stress-induced phenotypes and suggest a direct role for ER stress-dependent decreases in oocyte quality.

Regulation of oocyte mRNAs

Under optimal conditions, approximately 0.5 ng of total RNA are synthesized in growing oocytes due to a high rate of transcription which correlates with diffuse chromatin distribution (Gandolfi & Gandolfi 2001). As the oocyte reaches its full-grown size, its chromatin compacts and surrounds the nucleolus (SN) at which time transcription rates dramatically decrease (Bouniol-Baly 1999). Post-transcriptional regulation of mRNA storage, translation and degradation becomes the predominate mechanism to regulate transcript abundance after the ovulatory surge of LH (Li *et al.* 2013, Svoboda *et al.* 2015). Selective degradation of mRNAs, which are initiated by the LH surge, continues after fertilization and are essential for activation of transcription from the embryonic genome. Importantly, a group of mRNAs called maternal effect genes remain relatively stable until later in development (one- to two-cell stages) and are essential for pre-implantation development (Alizadeh *et al.* 2005, Su *et al.* 2007, Svoboda *et al.* 2015).

Microarray analysis and RNA sequencing of MII oocytes from obese women with or without PCOS demonstrated differences in gene expression profiles (Wood *et al.* 2007, Ruebel *et al.* 2017). Transcripts from these analyses fell into cytokine activity, transcription

of DNA, regulation of mRNA storage and degradation, cell cycle regulators and maternal effect gene networks. Using a candidate gene approach, maternal effect genes were also increased in ovulated oocytes from diet-induced and satiety-suppressed mouse models of obesity (Pohlmeier *et al.* 2014). Similarly, the mRNA abundances of *Dppa3*, *Pou5f1* and basonuclin 1 (*Bnc1*), which are maternal effect genes, were increased in ovaries from diet-induced obese mice collected immediately after ovulation (Xie *et al.* 2016). In this same model, the STAT3 transcription factor, which is activated by inflammatory signaling, binds to the promoter of *Dppa3* suggesting that obesity-dependent inflammation regulates transcription of oocyte genes. Importantly, DPPA3 binds to di-methylated histone H3 on lysine 9 (H3K9me2), protecting 5-methycytosine to 5-hydroxymethylcytosine (Nakamura *et al.* 2007, 2012, Nakatani *et al.* 2015). Han *et al.* showed that oocytes isolated from high-fat fed mice had decreased DPPA3 protein, which altered DNA methylation (Han *et al.* 2018). Specifically, DNA methylation in the pronuclei of the zygote was altered. These data suggest that DPPA3 influences the epigenetic profile of the embryo that could have long-lasting effects on fetal development. In a preliminary experiment, Timme and Wood (unpublished data) showed that H_2O_2 induction of oxidative stress during oocyte maturation increases *Pou5f1* mRNA abundance in not only MII oocytes but also two-cell embryos (Fig. 3). Loss of maternal effect gene expression terminates pre-implantation embryonic development (Hirasawa *et al.* 2008). However, it is unclear how increases in maternal effect gene expression impacts embryo development and therefore represents an area that requires additional studies. Interestingly high-fat-fed mice have decreased H3K9-me2 in germinal vesicle stage oocytes which is correlated with oxidative stress (Hou *et al.* 2016). This suggests that gene expression during oocyte growth is altered and may have impacted the number of mRNAs in a MII-stage oocyte.

Potential role of the gut microbiome on female fertility

Interestingly, bariatric surgery of obese women normalizes ovulatory patterns, improves conception rates, reduces pregnancy complications and improves fetal health (Guelinckx *et al.* 2009, Gosman *et al.* 2010). Bariatric surgery also improves metabolic function, which is attributed in part, to changes in the composition of the gut microbiome (Murphy *et al.* 2017, Shao *et al.* 2017). These data suggest that changes to the gut microbiome may impact fertility. Toward this end, studies in *Drosophila melanogaster* were performed and showed that loss of gut microbes suppressed oogenesis (Elgart *et al.* 2016). Studies from China, Spain and
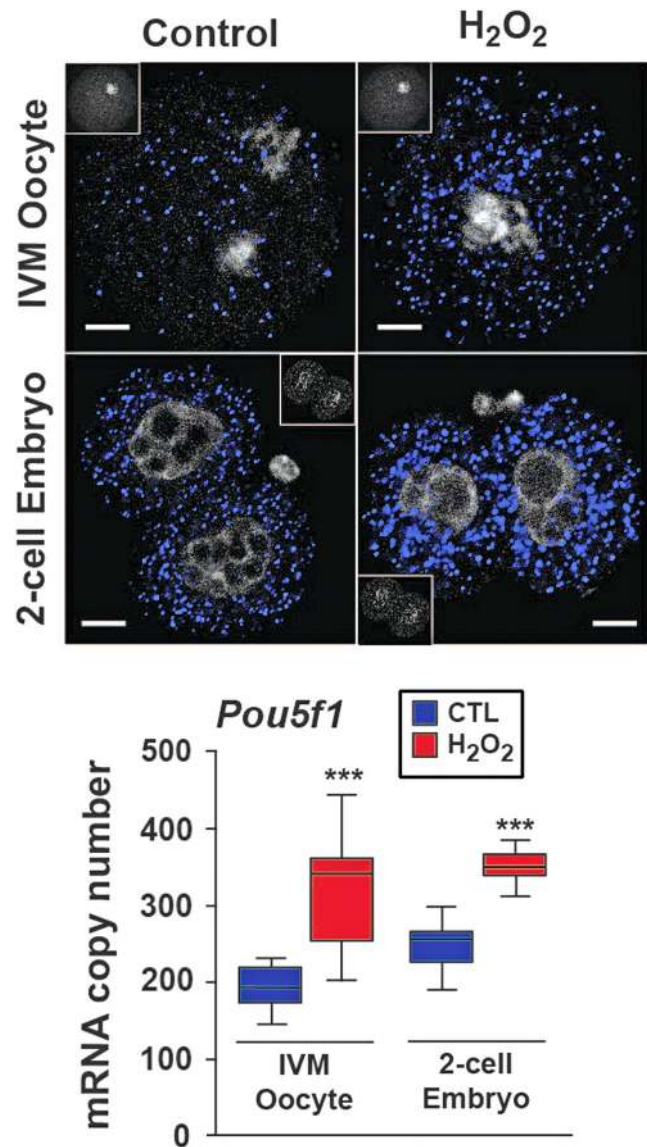
Figure 3 Oxidative stress increases the abundance of *Pou5f1* in the MII oocyte and two-cell embryo. Oxidative stress was induced by H_2O_2 during *in vitro* maturation of the oocyte. Single molecule fluorescence in situ hybridization coupled with the Spot Finding and Tracking program determined the number of mRNAs in each oocyte and embryo. The copy number of mRNAs is indicated in the graph. Significance was determined using ANOVA. $P<0.001$ are indicated by ***.

Austria demonstrated association of PCOS with reduced diversity of the gut microbiome (Guo *et al.* 2016, Lindheim *et al.* 2017, Liu *et al.* 2017, Insenser *et al.* 2018). Furthermore, reduced diversity was most severe in obese women with PCOS. Using a mouse model of obesity, Xie *et al.* showed positive correlations between increases in the bacterial family Lachnospiracae and increased ovarian *Tnfa* expression (Xie *et al.* 2016). Importantly, Lachnospiracae was increased in high-fat-fed C57BL/6J mice.
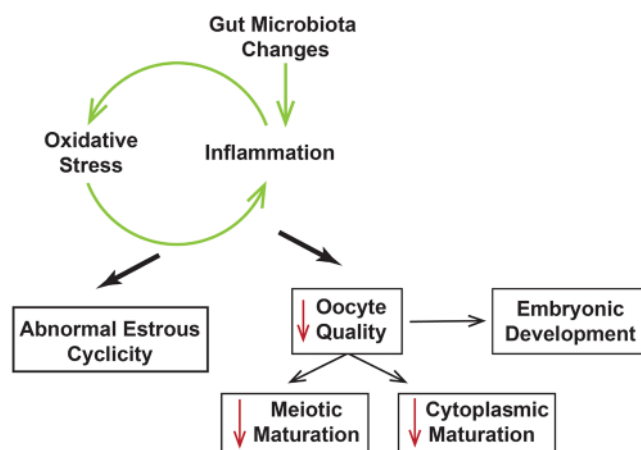
Figure 4 Model showing how changes in the gut microbiota, inflammation and oxidative stress affect ovarian estrous cyclicity and oocyte quality.

As described earlier, one consequence of gut microbiome changes is increased gut permeability due to loss of tight junctions resulting in leak of LPS and endotoxemia (Cani *et al.* 2007, 2012, König *et al.* 2016, Bidne *et al.* 2018). Interestingly, LPS is increased in the follicular fluid of cystic follicles (Shimizu *et al.* 2018). When bovine granulosa cells are treated *in vitro* with LPS, there is increased expression of IL-6, IL-8 and IL-1β, which is a result of increased activation of TLR4 signal transduction (Sheldon & Bromfield 2011, Price *et al.* 2013). These cytokines decrease expression of P450 aromatase (CYP19A1), which regulates synthesis and secretion of E2 by the bovine granulosa cells (Price *et al.* 2013, Magata *et al.* 2014, Shimizu *et al.* 2018). Furthermore, LPS disrupts meiotic resumption and spindle structure, mitochondrial membrane potential and decreases blastocysts rates and the ratio of trophectoderm and inner cell mass cell numbers (Sheldon & Bromfield 2011, Magata & Shimizu 2017).

Together, these studies suggest that changes in the gut microbiome, which increase LPS concentration, impact both ovarian steroidogenesis and oocyte quality. However, studies showing a causative effect of changes in the gut microbiome on obesity-dependent changes in the ovary are required.

Conclusions

In the last decade, the negative impact of obesity on ovarian function and oocyte quality has become evident. It is specifically tied to increases in inflammation and oxidative stress and potentially obesity-dependent changes in the gut microbiome (Fig. 4). However, future studies are required to delineate the mechanisms by which inflammation, oxidative stress and gut microbiome changes affect the ovary and oocyte. The impetus of these studies is to identify targets that can be used to therapeutically improve fertility in women who are obese or have metabolic dysfunction.

Declaration of interest

Funding

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