Aging and the environment affect gamete and embryo potential: can we intervene?

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Optimal maturation of the oocyte depends on its environment and determines embryo competence, because the embryonic genome is not active until the cleavage stage and new mitochondria are not produced until blastulation. Adverse environmental factors include aging, andropause, oxidative stress, obesity, smoking, alcohol, and psychologic stress, whereas androgen supplementation, a prudent diet, exercise, nutritional supplements, and psychologic interventions have beneficial effects. Mitochondrial function and energy production deteriorate with age, adversely affecting ovarian reserve, chromosome segregation, and embryo competence. In aging mice, the mitochondrial cofactor coenzyme Q10 reverses most of these changes. Early human experience has been encouraging, although only a small study using a shorter duration of intervention compared with the murine model has been carried out. Mitochondrial metabolic stress can result in an abnormal compensatory increase in mitochondrial DNA, which can be assessed in biopsied blastomeres of trophectoderm as a predictive biomarker of implantation failure. Psychologic stress may reduce oocyte competence by shifting blood flow away from the ovary as part of the classic "fight or flight" physiologic response, and methods to reduce stress or the body's reaction to stress improve pregnancy success. Enhancing oocyte competence is a key intervention that promises to reduce the number of euploid embryos failing to produce viable deliveries. (Fertil Steril® 2016;105:548–59. ©2016 by American Society for Reproductive Medicine.)

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The oocyte is supported and nourished by intimate cross-talk with its surrounding granulosa cells (GCs) and by endocrine and paracrine interactions with its environment (1, 2). During controlled ovarian hyperstimulation (COH), gonadotropin stimulation results in growth of multiple follicles yielding oocytes. However, only 7% result in a term delivery (3), and of oocytes that fertilize and are found by means of comprehensive chromosome screening (CCS) to be euploid, still only approximately two-thirds result in a viable birth. During maturation, the oocyte undergoes a tremendous increase in the quantity and quality of the cytoplasm referred to as cytoplasmic maturation, which determines the capability of the resulting embryo to achieve a viable pregnancy. Oocyte mitochondria are a critical example of cytoplasmic maturation, described below in detail. Gathering evidence indicates that the maternal environment, including changes brought about by aging, have a major impact on the quality of cytoplasmic maturation and ultimate success of in vitro fertilization (IVF).

The sperm loses most of its antioxidant defenses during maturation by shedding its cytoplasm to facilitate motility, making it extremely sensitive

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to oxidative stress (OS). OS primarily affects the sperm during passage through the collecting system; exposure to OS is therefore worsened by infrequent ejaculation and minimized by retrieval of sperm directly from the testicle. Sperm membranes have a high concentration of omega-3 fatty acids (FAs), which are highly sensitive to oxidation.

AGING

Aging affects the number of oocytes that can be retrieved with the use of COH. However, the modest decline of term delivery per euploid embryo with increasing age (4) indicates that either aging of cytoplasmic quality is of minor importance or, more likely, cytoplasmic quality influences oocyte competence both by affecting chromosome redistribution as well as by other mechanisms (also, available CCS data, particularly in older women, is skewed toward a select group of more normal responders who may have inherently better cytoplasmic quality). An example illustrating these parallel effects is that treatment of the aging mouse with coenzyme Q10 (CoQ10, an essential component of the electron transport chain involved in energy generation), described in detail below, affects the quality of the oocyte spindle as well as energy available for cell divisions and other functions (5). Factors impairing cytoplasmic maturation may also reduce follicle growth. In the aging mouse, the decline in ovarian follicles and litter size with age were also prevented by CoQ10 administration, and interference with CoQ10 production caused decreased ovarian reserve (5). Follicle depletion therefore may also be influenced by decreasing cytoplasmic quality through communication with the GCs or by the general decline of CoQ10 and mitochondrial energy production with aging (5).

Another example of parallel defects of ovarian reserve and cytoplasmic quality is the impact of decreased androgen levels exposed to the follicles with advancing age (6). With increasing age, serum levels of the adrenal androgen DHEA and circulating T and free T (7) decrease. Basal T levels correlate with reduced ovarian response when controlled for age (8) (unfortunately, usual T assays are not accurate in that low range, so measuring serum T for this purpose is generally not practical). One-half of the intrafollicular T results from conversion from DHEA. T increases antral follicles, increases GC proliferation, and decreases GC apoptosis, which in turn would be expected to favorably affect oocyte competence (9). Randomized studies have shown improved stimulation with administration of oral DHEA (10), T gel (11), and T patch (12), resulting in higher circulating T levels that are modest with the use of DHEA, moderate with the use of T gel, and rising toward the low male range with the use of T patch (systemic administration of T would cause relatively small local increases of ovarian T). Consequently, it appears to require at least 2–3 months of oral DHEA, a minimum of 3 weeks with the use of T gel, and at least 5 days with the use of T patch to increase the ovarian response to stimulation. Therefore, preparation for stimulation in such patients must be planned well in advance of the start of gonadotropins. Poor responders have been the predominant group where androgen treatment has been applied (9–11), although improved oocyte competence may also occur.

Oxidative stress increases with age (13), owing in part to lowered endogenous antioxidant defenses generating tissue glutathione levels (14). OS is associated with increased GC apoptosis, which is in turn associated with reduced embryo quality and successful birth (2, 15). OS is also implicated in shortening of telomeres, most likely as a long-term process while oocytes remain in limbo during this era of delayed childbirth, and shortened telomeres contribute to aneuploidy (16).

Aging has prominent effects on sperm DNA fragmentation (17), which is correlated with OS (18), and may account for the adverse effects of male age on IVF success. The cytoplasm of younger oocytes may correct DNA fragmentation, explaining the lesser impact with the use of egg donation or a younger female partner (19). Different assays (20) or assay conditions (21) may vary in their detection of sperm DNA fragmentation; therefore, increased intake of antioxidants should be recommended for all men over the age of 40 years, regardless of testing.

OBESITY

Obesity is a state of high OS (22), including as documented in follicular fluid (23), and is associated with decreased clinical pregnancy, with the odds ratio for failure increasing from 1.26 for body mass index (BMI) 35–39.9 kg/m^2 to 1.53 for BMI $>$50 kg/m^2. The odds ratio of failing to achieve a viable birth was reduced even more significantly, varying from 1.14 with BMI 25–29.9 kg/m^2 to 2.3 with BMI $>$50 kg/m^2 (24). The effect of obesity in recipients of egg donation in some studies suggests effects on the endometrium as well as the oocyte (25). The incidence of IVF live birth is reduced even more when obese women have twins (26) or are black, Hispanic, or Asian. Strikingly, the significant adjusted odds ratios for failure to achieve live birth comparing nonwhite and white women were 1.24 for normal weight, 1.52 for overweight, and 1.86 for obese, the latter two being significant at $P<.0001$ (27). Obesity also increases miscarriage (28). Most importantly, a BMI $>$35 kg/m^2 more than doubles deliveries at $<$32 weeks, and with twins the risk of deliveries at $<$28 weeks triples (26). Authors cite multiple possible mechanisms, including systemic inflammation, as possible effectors of these adverse outcomes. These data emphasize the need to focus maximal attention on obesity in ethnic minorities to improve IVF outcomes, with particular emphasis on elective single-embryo transfer.

All obese women, particularly with a BMI $>$35 kg/m^2, should be advised of the above risks and encouraged to lose weight before starting IVF, especially women aged $\leq$38 years. Increased antioxidant intake is logical, given their systemic and ovarian OS and inflammation. The antiinflammatory effects of omega-3 FA supplements may also be beneficial (29). An exercise program may be the most promising modality. In a case-control study, exercise (including at a vigorous level) was associated with a more than threefold increase of the clinical pregnancy rate in obese women having IVF (30).

SMOKING

Smoking by the female partner reduces the IVF pregnancy rate by about one-half (31) and increases miscarriage by almost one-fourth (32). Adverse effects are also observed

with donor eggs, indicating a pelvic as well as an ovarian site of action (33). Smoking by the male partner reduces IVF and intracytoplasmic sperm injection (ICSI) success (34). Second-hand smoke has lesser, but still significant, effects (35). Smoking is a state of severe OS, so a marked increase of antioxidant intake may be helpful for those unable to quit. Given adequate time from cessation of smoking to the IVF, adverse effects appear to abate. A period of 3–6 months is likely optimal (36).

ALCOHOL

Alcohol intake by both partners reduces IVF success (37) and increases miscarriage (38). Couples should be advised to abstain before and during an IVF cycle.

CAFFEINE

Although an older study had shown the best IVF outcome with ingestion of 0–2 mg/d caffeine, equivalent to no intake or one cup of decaffeinated beverage (39), two more recent studies showed no impact of caffeine on IVF outcomes (40, 41). Until further data are available, women should consider limiting caffeine intake.

ADVANCED GLYCATION END-PRODUCTS

Advanced glycation end-products (AGEs) are toxic end-products that induce OS and accumulate with age owing to increased circulating glucose and/or ingestion of foods cooked at high heat (e.g., grilling, barbecuing). Elevated levels in serum and follicular fluid have been associated with a reduced ovarian response and a reduced chance of pregnancy (42). Boiling, microwaving, and poaching are ideal ways to avoid overheating of foods.

BISPHENOL A

Urinary bisphenol A (BPA) was almost twice as high in women who were given a low-responder protocol ($P<.001$), and there was a marked trend toward lower implantation (43). Higher urinary BPA has also been correlated with miscarriage (44). BPA may also affect sperm quality (45). Sources of BPA are mainly plastic containers (number 7, polycarbonate), canned food linings, and most credit card receipts.

EXERCISE

Moderate exercise for both the male (46) and the female (47) partners is associated with improved gamete function and IVF outcomes. Moderate exercise reduces oxidative stress, in part by increasing circulating (48) and cellular (49) antioxidant defenses. Vigorous exercise in the female partner is associated with reduced IVF success (50, 51), though not in obese women (51); in the male partner, ≥ 5 hours of cycling per week reduced semen quality (52), most likely owing to heating of the testicles rather than physical activity per se.

DIET

A "prudent" diet improves fertility for both partners, i.e., less red meat and saturated fat, more seafood, and more fruits and vegetables, often referred to as the "Mediterranean diet" (53, 54). Antioxidants may explain part of this positive effect. Follicular fluid reactive oxygen species (ROS) have been correlated negatively and total antioxidant capacity (TAC) positively with clinical pregnancy rate (both $P<.001$) (55). The type of fat consumed in a "prudent" diet may explain some of the remaining impact. Omega-3 FAs are concentrated in sperm membranes and are lower in the circulating blood and spermatozoa of infertile men. Infertile men also had higher intakes of total, saturated, and trans fats and lower intake of omega-3 FAs than fertile men (56). Omega-3 FAs may also be helpful for the female partner in improving IVF outcome (57), but data are as yet limited. Because omega-3 FAs are very sensitive to oxidation, the appropriate study may be to compare omega-3 and antioxidant supplementation versus antioxidants alone.

ANTIOXIDANTS

An obvious way to counteract the adverse effects of OS is to increase intake of antioxidants, which may in part explain benefits of the "Mediterranean diet" noted above. Pomegranate, chocolate, berries, and espresso coffee are excellent sources of antioxidants. Fruits and vegetables contain varying amounts, and their content, almost without exception, is increased by cooking. Spices such as turmeric, cumin, ginger, and oregano contain up to 50 times as much as berries by weight (58, 59). Isoflavones are antioxidants that have been correlated with IVF birth rate (60). Numerous antioxidant supplements are available, of which vitamin C is the most well characterized and least expensive. A combination of vitamins C and E was shown to reduce sperm DNA fragmentation from 22 to 9% ($P<.001$) (61). Vitamin E should be avoided because it has been linked with increased all-cause mortality and with prostate cancer and it markedly sensitizes the platelets to aspirin (62). As described here, there are many stronger antioxidants that are available without such safety issues having been observed. Pycnogenol, a commercial preparation of polyphenols similar to those in berries, was shown to improve semen quality and raise T levels (63). Alpha-lipoic acid increased TAC by >50% and doubled rapid progressive motility (64). L-Carnitine, a weak antioxidant long used in male fertility supplements, has been implicated in the adverse effects of red meat on the cardiovascular system (65). It should not be recommended now that other better-studied agents are available.

NUTRITIONAL SUPPLEMENTS/DURATION OF TREATMENT

For those patients who are unwilling to increase dietary sources of antioxidants, the commercial sources discussed above are readily available online or in health food stores. For omega-3 treatment of infertile men, a period of loading may be required to substantially change sperm membrane concentrations. In a randomized study of 6 months' administration of ~1,800 mg of an omega-3 supplement compared with placebo, total sperm and sperm density increased (61.7 million/ml vs. 38.7 million/ml and 28.7 million/ml vs. 15.6

million/ml; both $P<.001$), motility increased from 18.6% to 27.4% ($P<.002$), and strict morphology increased from 7.5% to 12.8% ($P<.002$) (66). Lower doses and durations have not been studied. For men with increased DNA fragmentation, a 2-month treatment with antioxidants was used (61). A detailed discussion of lifestyle factors influencing fertility is available at www.lifechoicesandfertility.com. Because longer abstinence intervals compromise sperm quality, a detailed discussion of similar lifestyle factors influencing erectile function is also provided. Links to Pubmed abstracts provide an evidence-based approach to those materials.

MITOCHONDRIAL FUNCTION IN THE OOCYTE AND EMBRYO IS PERTURBED BY AGE AND OXIDATIVE STRESS

According to the Centers for Disease Control, which monitors clinic assisted reproductive technology (ART) success rates in the USA, one in every eight North American couples seeks medical treatment for infertility, often requiring ART. However, over the past 20 years, live birth rates following ART have not improved much beyond 30% per cycle. Embryo quality remains a strong determining factor for predicting success rates of ART (67). Mathematical modeling of death rates in human preimplantation embryos has suggested that the factors predisposing an embryo to arrest may be determined before the zygote stage (68, 69). The ability of the embryo to pass through the transition from maternal to zygotic control in vitro has been proposed to depend predominantly on cytoplasmic components either already present in the primordial oocyte or acquired during follicle growth. With the onset of the mid-cycle LH surge, gap junctions between the cumulus cells and the oocyte are disrupted and the cytoplasmic content of the oocyte is fixed with minimal impact from the newly formed zygotic genome (70). Therefore, after ovulation, survival of the mature oocyte and subsequent embryo depends almost exclusively on preexisting maternal mRNA, proteins, and other factors, especially mitochondria, that have accumulated during oocyte growth and maturation (71), and these components appear to be lacking or dysfunctional in those embryos that arrest.

Mitochondria and Energy Production

Mitochondria are thought to originate from a prokaryotic ancestor that became an eukaryotic symbiote (72). The mitochondrial genome (mtDNA) is a double-stranded circular DNA, which, unlike nuclear DNA, does not contain histones, has no introns, does not have any DNA-repair enzymes, and is, consequently, highly susceptible to mutations (73). MtDNA encodes 37 genes for 13 proteins of respiratory chain complexes involved in adenosine triphosphate (ATP) production (74), 22 transfer RNA genes, and 2 genes for ribosomal RNA. The transfer of many of the mitochondrial genes to the nuclear genome likely occurred to keep parts of the vulnerable DNA away from the nearby energy production site that generates a large amount of reactive oxygen species (ROS) (75). The inheritance of mitochondria is strictly maternal. Paternal mitochondria are eliminated by the oocyte shortly after fertilization by lysosomal degradation through allogeneic (nonself) organelle autophagy (76). Mitochondrial morphology in oocytes is also unique, with an atypical round shape, thick mitochondrial membrane, and few cristae, and the oocyte mitochondrion generally has a single mtDNA genome and spatially regulated respiratory activity (77).

An important feature of mitochondrial physiology is the amplification of mitochondrial numbers as human oocytes develop from the primordial state, when they contain about 6,000 mitochondria, to mature metaphase II (MII) eggs containing 300,000 to 400,000 or more mitochondria (78). A speculation underlying this tremendous expansion of mitochondrial numbers during oocyte maturation is that the oocyte is actively preparing for the increased energy demands of fertilization and the cleavage divisions associated with early embryonic development. This seems reasonable, because the ability for mitochondrial DNA replication is absent in the MII egg and is not reactivated in the embryo until the blastocyst stage (79). Therefore, as an embryo expands from the 1-cell zygote to a blastocyst containing 60–100 or more cells, the number of mitochondria per cell would be continually diluted. This supposition is supported by assessment of reduced mtDNA content in blastomeres compared with the oocyte during preimplantation embryogenesis (80) and reduced mitochondrial numbers per cell in blastocyst stage embryos (81).

There is evidence that older infertility patients have abnormal oocyte mitochondrial activity and reduced production of ATP, which in turn limits normal embryo development. It has been proposed that insufficient endowment of the mitochondrial pool could drive poor embryo development (82) and that supplementation of mitochondria can improve fertilization, at least in laboratory and domestic species (83, 84). Many studies have proposed a link between insufficient ATP availability in oocytes and meiotic spindle abnormalities leading to defective chromosomal segregation (85, 86). Defective spindle formation would result in the failure of zygotes produced from these ATP-compromised oocytes to form viable blastocysts (87, 88). This concept is supported by work in mice showing that disruption of mitochondrial oxidative phosphorylation in oocytes results in meiotic spindle abnormalities and decreased preimplantation embryo developmental potential (89).

The oocytes, and in fact the pre-granulosa cells which give rise to the GCs and cumulus cells (CCs) are unique in the body because there is no cell division after late gestation. Therefore, in older women, the oocytes and GCs in primordial follicles will have been exposed to low levels of ROS produced by mitochondrial respiration over decades, resulting in possible cumulative damage to mitochondria and mtDNA. The functional status of the mitochondria contributes to oocyte quality (82). Higher-quality oocytes, assessed by morphology, contain significantly higher ATP levels and are more likely to progress to the blastocyst stage after fertilization (88). Inappropriate mitochondrial activity at the pronuclear stage is associated with early developmental arrest and demise (90). As a woman ages, mitochondrial activity within the oocyte steadily declines (91, 92). Furthermore, a direct correlation exists between the number of mitochondria

within the oocyte and its likelihood of fertilization and preimplantation development (93). In addition, women with a history of failed IVF attempts often have compromised mitochondrial function within the oocyte (82).

A Murine Model for Ovarian Aging

To determine if aging influences mitochondrial function in murine oocytes, in a series of mitochondrial assays, 12-month-old murine oocytes were compared with 10-week-old murine oocytes (94). From these studies, it was determined that mitochondria are not fully functional in aged oocytes, as demonstrated by the decrease in metabolic activity, increased mitochondrial membrane potential, and lower citrate/ATP ratio. In addition, many genes regulating mitochondrial function are down-regulated. The aged animals produced oocytes with increased spindle defects and frequent chromosomal scattering. Of importance, it was found that many of these mitochondrial abnormalities could be partially or completely corrected by maternal supplementation with the mitochondrial nutrient CoQ10 (94). CoQ10 delayed age-mediated oocyte loss, because the number of primordial, primary, and early secondary follicles in ovaries of CoQ10 treated dams was increased compared with age-matched control subjects, resulting in ovulation of a higher number of oocytes. Breeding trials confirmed improved breeding performance of dams exposed to CoQ10 (Fig. 1) (94).

In addition to changes in mitochondria, it was also observed that oocytes from older females were surrounded by fewer CCs, caused by an increased rate of cell death (94), suggesting that oocytes in older females could be under metabolic stress caused by insufficient "nourishment" from CC-derived factors, one of which is CoQ10. Of interest, CoQ10 treatment resulted in a higher number of CCs surrounding each oocyte, indicating that the treatment effect is not isolated to germ cells. Because CoQ10 treatment improved embryo quality and breeding performance of older mothers, the expression of CoQ synthesis genes in CCs of young and old females was studied. Results of that expression survey revealed that CCs of older females had decreased expression of several enzymes involved in CoQ10 production. *Pdss2* and *CoQ6* mRNAs were the most significantly affected in the murine CCs (94).

To determine if diminished CoQ production by oocytes could be responsible for reproductive aging, the researchers conditionally disrupted Pdss2 in oocytes of young mice by means of the Cre-lox mediated genetic approach. Phenotypically, the inactivation of *Pdss2* and subsequent inhibition of CoQ synthesis in the oocyte caused exhaustion of ovarian reserve by 3–4 months of age. The reduction of follicle number and very poor ovulation response was observed as early as 2 months of age. Those oocytes that survived and ovulated recapitulated many of the defects observed in aged oocytes, including decreased ATP production and poor mitochondrial activity (94). These outcomes confirmed that an aging phenotype could be generated in young animals by an isolated decrease in CoQ production in the oocyte and, of interest, could be reversed by supplementation of the animals with CoQ10.

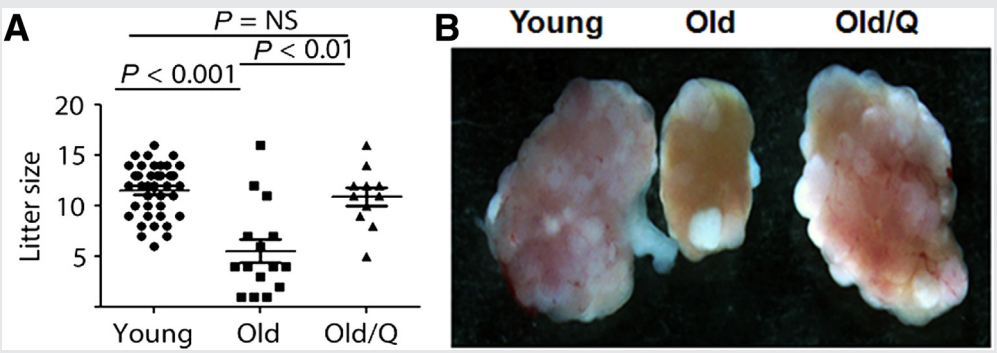
Clinical Use of CoQ10

An attempt to translate these findings was undertaken by clinically supplementing the diets of older women undergoing IVF with 600 mg/d CoQ10 for 2 months. In a randomized double-blind study performed in a small number of women, array comparative genomic hybridization (aCGH) of the oocyte polar bodies revealed no significant effect on oocyte aneuploidy, although the frequency of oocyte aneuploidy was 46.5% with CoQ10 treatment compared with 62.8% in the placebo-treated group (95). Larger, more definitive studies are required to determine if there is any positive benefit of CoQ10 on the reproductive outcome of aged women.

Oocyte Injection with Healthy Mitochondria

An alternate to mitochondrial stimulation by mitochondrial nutrients is to supplement healthy mitochondria to increase

FIGURE 1



(A) Litter size in young (n = 39), old vehicle-treated (n = 15), and old coenzyme Q10–treated mice (n = 11). The number of live pups born to dams in the 13th month of age was decreased, but it normalized after CoQ10 supplementation. Each female produced only one litter during the breeding trial. Scatter plot data are shown as mean ± SEM per female. (B) Representative images of ovaries after gonadotropin stimulation (5).

Meldrum. Environment affects gametes and embryos. Fertil Steril 2016.

ATP production in dysfunctional oocytes. Pioneering experiments with ooplasm transfer in the 1980s demonstrated that unidentified factors in the cytoplasm of healthy oocytes were capable of preventing the arrest of embryos from abnormal oocytes (96). That basic research led to controversial clinical attempts to rescue human embryos that demonstrated poor developmental potential by transferring "healthy" donor ooplasm into recipient oocytes prone to abnormal development (97). Such ooplasm transfers in humans were performed for patients with increased maternal age, repeated embryonic developmental failure, or poor ovarian reserve and resulted in the birth of several children worldwide. The success of this procedure is now thought to be the result of mitochondrial transfer from the donor ooplasm to the recipient oocyte. Unfortunately, the ooplasm transfer resulted in offspring carrying mitochondria from both the donor and recipient, thus creating mitochondrial heteroplasmy (98). Although the benefits of this mitochondrial enrichment are clearly evident during the early developmental stages, mitochondrial heteroplasmy may have unknown but potentially deleterious late physiologic consequences (99). Researchers also demonstrated the possible development of metabolic syndrome in the offspring in a mouse model of neutral mitochondrial heteroplasmy (100). Therefore, it is desirable to devise molecular strategies for treatment of poor embryo development without the creation of mitochondrial heteroplasmy.

In 2004, the Tilly lab discovered an autologous source of germ-line mitochondria (101). These mitochondria were present in egg precursor cells (PCs) found in the avascular outer lining of the ovarian cortex (101, 102). These egg PCs could be isolated from a biopsy of the ovarian cortex by tissue digestion and cell sorting using antibodies specific to human VASA, a cell surface antigen found on human egg PCs (102). Mitochondria isolated from a woman's own egg PCs appear to be similar to young oocyte mitochondria and can serve as an autologous source of mitochondria for injection into deficient oocytes, thereby avoiding the concerns raised with donor oocyte mitochondrial injection (99).

Autologous mitochondrial injection has been performed in women with previous poor reproductive performance. Egg PCs were isolated and mitochondria concentrated by differential centrifugation. These autologous mitochondria were injected at the time of ICSI into the patient's own oocytes. The autologous mitochondrial injection treatment is based on previous reports of donor oocyte cytoplasmic injections (103) as well as on multiple published animal studies that have demonstrated that the addition of mitochondria during IVF treatment is safe, increases embryo quality, and improves IVF success rates (83, 84, 104, 105). A preliminary report (106), supports the improved embryo quality and pregnancy rates seen with ooplasm injection and with animal studies of mitochondrial injection. So far, more than a dozen healthy live births or ongoing pregnancies have been achieved after autologous mitochondrial injection at the time of ICSI in a group of very-poor-prognosis women with a history of multiple failed cycles or poor embryo development. Further experience is presently being obtained, and the data regarding live births after autologous mitochondrial injection are being collected by means of a worldwide registry, which over the next few years should be able to confirm the safety of this new procedure.

We conclude that improving mitochondrial energy production in oocytes and embryos may lead to improved reproductive outcomes in cases of reproductive aging or of poor embryo development and recurrent pregnancy failure. Both supplementation of mitochondrial nutrients, such as CoQ10, and injection of autologous healthy mitochondria obtained from oocyte PCs may hold promise for improving live birth rates in the future.

MITOCHONDRIAL DNA CONTENT AS A VIABILITY MARKER IN EUPLOID EMBRYOS

Mitochondria in the Oocyte and Embryo

It is known that a high proportion of oocytes result in developmentally incompetent embryos or embryos that will fail to implant despite being chromosomally and morphologically normal. An important aspect of human embryo viability yet to be fully explored is the assessment of an adequate energy supply (107). In oocytes and early embryos, mitochondria have very few cristae, which suggests that they have a poor ability for oxidative phosphorylation (108).

Although mtDNA content and ATP synthesis are well correlated in most somatic cells and tissues, that is not the case for the germ line (109). Oocyte mitochondrial content, in terms of mtDNA copy number, has been studied on the principle that the oocyte mitochondrion contains only one or two genomes (110). Mitochondria accumulate in the mature oocyte, expanding from 6,000 per primordial germ cell to 300,000 to 400,000 at the MII stage (78), and no further replication occurs between fertilization and the early postimplantation stages (110). Therefore, the total amount of mtDNA in the oocyte must be split among cells formed during embryo division, and for that reason by day 5–6 of development each of the ~60–100 cells contains far fewer copies of mtDNA compared with the original oocyte/zygote.

The huge quantity of mtDNA accumulated in the oocyte and the fact that replication does not resume before blastulation have contributed to the concept that mtDNA content in oocytes correlates with fertilization outcome and embryo viability (82, 111, 112). Because the number of mtDNA copies is indicative of the quantity of mitochondria in the oocyte (113), an mtDNA copy number threshold that allows embryo development after fertilization has been suggested for MII oocytes (84, 110). Some authors suggest that there is a relationship between oocyte mtDNA content and fertilization (82, 84, 104), indirectly connecting lower mtDNA content in unfertilized oocytes with fertilization failure but without discerning whether it is the result or the cause. Others, however, have shown that this association is not consistent (114, 115).

Mitochondria and Embryo Energy

Although it is generally assumed that the mitochondria maintain embryonic development from early cleavage to the blastocyst stage (116), some studies have shown that this is not the

case in animal models. In a knockout mouse model for mitochondrial transcription factor A, it was demonstrated that diminishing mitochondrial number in oocytes did not affect fertilization or early embryonic development (80). Moreover, deletion of essential genes for maintenance, replication, and expression of mouse mtDNA did not show changes in embryo implantation rates but resulted in embryonic lethality (80). From a biologic point of view, it is reasonable that if the oocyte and embryo were dependent on external metabolic sources, evolution would have given a competitive advantage to embryos with increased capacity for nutrient and oxygen exchange. However, oocytes and early embryos are spheres with a geometric form with lower surface and therefore less exchange capacity. Taking all these data together suggests that the energy necessary during embryonic development is provided up to a threshold by the accumulated mitochondria present in the oocyte, and only in extreme cases of reduced metabolic fuel does the cell machinery react to increase mtDNA copy number in an attempt to produce more mitochondria that are not functional.

Mitochondria as an Energy Stress Sensor

The embryo is dependent on energy accumulation during oocyte maturation. As a result, in some cases, mtDNA copy number may be a result rather than the root cause of the oocyte's energy status. This is supported by the observation that mitochondrial dysfunction is frequently associated with significant mitochondrial hyperproliferation. Therefore, the pathogenic effect of "mitochondrial distress" is a marked increase in mitochondrial overproduction (117).

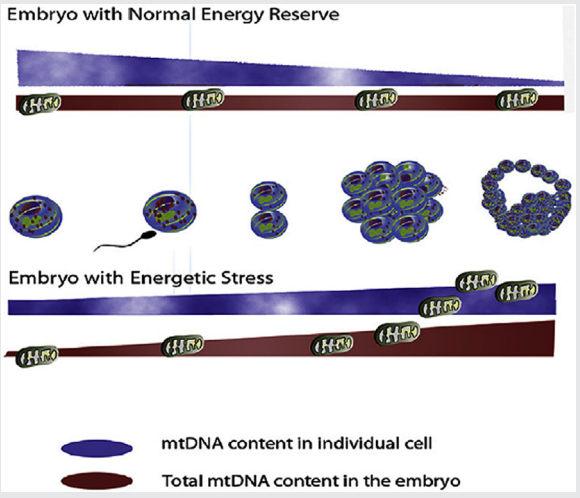
Mitochondrial biogenesis requires the regulation of numerous processes. In addition to mtDNA synthesis, biogenesis of new mitochondria requires synthesis and import of nuclear-encoded proteins and assembly of the dual genetic origin (nuclear DNA and mitochondrial DNA)–derived proteins. This regulation involves a set of nuclear transcription factors. The first recognized were nuclear respiratory factors 1 and 2, as well as estrogen-related receptor α, the cyclic adenosine monophosphate response element, and the ying yang 1 transcription factor (118).

The main organizer is the family of coactivators of the peroxisome proliferator-activated receptors (PPARs), PPAR coactivator 1α (PGC-1α) being the principal member. Multiple pathways regulating expression of nuclear-encoded mitochondrial factors connect in PGC-1α, which is considered to be the master regulator of mitochondrial biogenesis (119). Limited energy supply increases the cellular adenosine monophosphate (AMP)/ATP ratio, which is sensed by AMP-activated protein kinase (AMPK). AMPK phosphorylates and activates PGC-1α. Phosphorylation by AMPK also promotes PGC-1α–dependent induction at the PGC-1α promoter (120), inducing mitochondrial maturation and increasing mtDNA copy number.

Mitochondrial DNA Content as an Embryo Viability Marker

Consequently, an increase in mtDNA copy number in embryos would be symptomatic of metabolic stress (Fig. 2).

FIGURE 2



A proposed model based on our findings. *Top*: In healthy embryos with a normal energetic reserve, the mitochondrial biogenesis is low. The total amount of mtDNA is split between cells during division and thus the amount of mtDNA contained in individual blastocyst cells is lower than in cleavage-stage embryo cells; the overall embryonic mtDNA content remains the same. *Bottom*: In contrast, embryos with energetic stress activate mitochondrial biogenesis in an attempt to meet their energy demand; thus, the overall embryonic mtDNA content increases (122).

Meldrum. Environment affects gametes and embryos. Fertil Steril 2016.

The embryo attempts to balance the energetic deficit by increasing its mtDNA content. Energetic stress could be related to intrinsic factors during oocyte maturation or could be in response to impaired respiratory capacities due to mtDNA mutations. In agreement with this concept, a remarkable study has shown that the m.3243A>G "mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke" mutation resulted in a gradual rise in mtDNA copy number from the germinal vesicle to the blastocyst stage (121).

Two laboratories (122, 123) have independently demonstrated that high mtDNA copy number in euploid embryos is indicative of lower embryo viability in terms of implantation potential. The study by Diez-Juan et al. demonstrated the clinical usefulness of a mitochondrial score (Mitoscore [Ms]) based on the number of mtDNA copies that links embryo viability to implantation potential. The researchers analyzed the mtDNA copy number from 290 euploid embryos corresponding to 270 patients who underwent preimplantation genetic screening with single-embryo transfer.

Day 3 embryos with Ms <34 (MsA) had an implantation rate (IR) of 59% (n = 51); those with Ms 34–52 (MsB) had an IR of 44% (n = 52); those with Ms 52–97 (MsC) had an IR of 42% (n = 50); and those with Ms >97 (MsD) had an IR of 25% (n = 52). Embryos with Ms >160 (n = 22) never implanted. Day 5 embryos with Ms <18.19 (MsA) had an IR of 81%; those with Ms 18.19–24.15 (MsB) had an IR of 50% (n = 16); those with Ms 24.15–50.58 (MsC) had an IR of 62% (n = 16); and those with Ms >50.58 (MsD) had an IR of 18% (n = 17); embryos with Ms >60 (n = 7) never implanted.

These findings also support the development of strategies aimed to increase embryo energy reserves by the use of nutritional supplementation of mitochondrial nutrients during oocyte maturation (87, 95, 124). The logical clinical translation of this work is the incorporation of mtDNA copy number analysis into the routine genetic analyses performed by aCGH or now by next-generation sequencing. A larger prospective randomized trial comparing morphologic classification versus Ms in euploid embryos as an independent embryo viability marker is currently underway in our laboratories.

THE FIGHT OR FLIGHT RESPONSE IS A VASCULAR PHENOMENON AND MAY EXPLAIN EFFECTS OF STRESS ON OOCYTE COMPETENCY

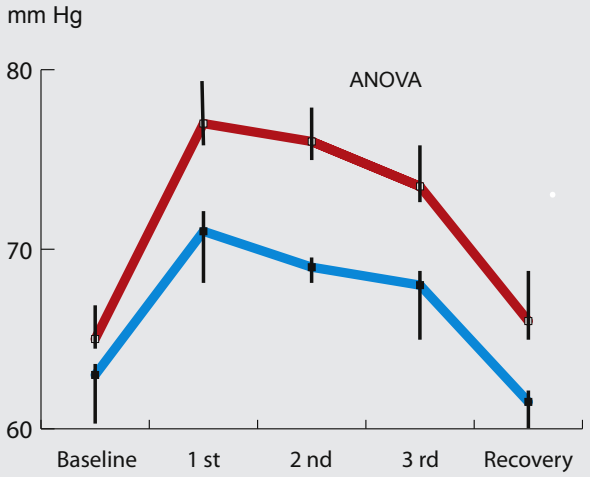
The Association of Stress with Infertility

For a woman, the stress of infertility has been compared to having a diagnosis of cancer or HIV (125). Sanders et al. found a negative impact of full-time employment, hostile mood, and higher anxiety on successful ART outcome. Multiple regression analysis also found depression to be a negative factor (126). Ebbesen et al. studied 809 women who recorded recent stressful life events within the 12 months before their IVF cycle and found that negative life events predicted IVF failure and retrieval of fewer oocytes (127). Smeenk et al. found that both anxiety and depression were correlated with IVF failure (128). Klonoff-Cohen et al. found that positive affect predicted retrieved oocytes and births with IVF/gamete intrafallopian transfer (129). The research on the impact of mood on ART outcome is inconclusive owing to a number of factors, including the fact that patient knowledge of their prognosis is likely to affect their mood. For example, a 25-year-old woman with blocked tubes not only has an excellent prognosis but also is likely aware of that prognosis and so may report a better mood. A subsequent pregnancy may or may not be related to her affect. Conversely, a 42-year-old woman with an elevated FSH level may well report high levels of anxiety and depression due to her knowledge of her low chance of success with IVF. A subsequent failed cycle may have nothing to do with her mood. The only way to truly assess the impact of mood on outcome is to recruit a large sample of women with the same prognosis, and that research has yet to be attempted.

The Likely Physiologic Mechanism Behind the Adverse Effect of Stress

The most logical physiologic mechanism to explain these effects of stress on IVF outcomes is the classic "fight or flight" response that shifts blood to the heart, brain, and muscles from "nonessential" organs, such as the ovary, to deal with a threat. Facchinetti et al. found that a greater rise in diastolic blood pressure in response to stressful stimuli predicted a lower chance of pregnancy in women about to undergo IVF (Fig. 3), supporting a vasoconstrictive mechanism, but also indicating that women who were more vulnerable to stress were less likely to conceive (130). Mendez Lozano et al. used 3-dimensional power-flow Doppler to study the relationship of blood flow surrounding the dominant follicle with natural-cycle IVF success. They found that a low flow index was associated with a dramatically ($P<.009$) lower rate of clinical pregnancy (4%) compared with a high flow index (33%). Turner et al. recently reported that anxiety and perceived stress were significantly higher and that self-efficacy in dealing with stress was lower on the day before oocyte retrieval in women failing to conceive with IVF (131). An et al. found that anxiety and depression scores and levels of circulating cortisol and norepinephrine (both $P<.001$) were all higher on the day of retrieval in nonpregnant women (132). All of these studies support a vasoconstrictive mechanism reducing blood flow to the ovary as the most logical mechanism of the effect of stress in reducing IVF outcomes.

FIGURE 3



Changes in diastolic blood pressure in infertile women submitted to the Stroop Color and Word Test. Patients showing a biochemical pregnancy are shown in *red*, whereas patients failing to conceive are shown in *blue* ($P<.0001$). First, second, and third measurements were made after reading the three sheets, respectively, of the test. Recovery measurements were made 10 minutes after the end of testing (130).

Meldrum. Environment affects gametes and embryos. Fertil Steril 2016.

Treatments Available to Counteract the Adverse Effects of Stress

A recent meta-analysis (133) included 39 studies on the impact of various psychologic interventions on pregnancy rates and negative psychologic symptoms in women undergoing infertility treatment. There was a highly significant positive impact on pregnancy rates (risk ratio 2.01) and psychologic outcome. Larger decreases in anxiety were associated with the greatest improvement in pregnancy rates. The use of cognitive-behavioral techniques (CBTs) was recommended as being particularly efficacious.

One of the CBT interventions which has been most widely studied with this patient population is the Mind/Body

Program for Infertility, which is a ten-session group program that includes relaxation training, stress-management strategies, lifestyle recommendations, and group support. In a randomized study of women attempting conception, significantly more women were successful who used the intervention (134). In a recent randomized study (135), even though only 76% of subjects had completed at least one-half of the sessions before their second IVF attempt, the pregnancy rate was 52% compared with 20% with no treatment ($P<.05$). The study also illustrated how difficult it was to persuade patients to postpone the start of treatment even with the goal of increasing their chance of being successful, and infinitely more so to participate as a subject randomized to no treatment.

An online version of the Mind/Body Program has been developed and is currently being assessed in a randomized controlled trial. Development of abbreviated and online courses could further improve outcomes and make psychologic interventions accessible to more couples. Of course, individual and group sessions, either professionally or "peer" led, available through support groups such as Resolve: The National Infertility Association, are excellent options and have also been shown to be associated with increased pregnancy rates (134).

Individuals and couples have found group, individual, and couples counseling to be very helpful in handling general sources of life stress and in preventing adverse effects of the infertility on their relationship. Providers are also likely to observe less difficulty in their dealings with couples, because irritability is a common manifestation of stress and depression. Finally, an important source of IVF failure is inability to cope with the stress of failure, resulting in inability to continue treatment. The concept of the overall burden of infertility treatment is receiving increasing attention. Methods to improve the quality of life for infertility patients include decreasing stress for patients and the health care professionals who treat them, as well as decreasing the invasiveness of treatment (136, 137). All such methods of support should include means to mitigate drop-outs, which are well recognized to be relatively independent from financial status (138). Overall, it is difficult to overestimate the importance of all IVF programs making these resources readily available to their patients.

SUMMARY

We have described a number of interventions that will increase the proportion of euploid embryos that result in viable deliveries. For the male partner, omega-3 FAs and antioxidants are most critical. For the female partner, reduction of stress and the body's response to stress, exercise and weight loss for obese women, avoidance of vigorous exercise for nonobese women, cessation of smoking for 3–6 months before IVF, and avoiding alcohol are the most important. For both partners, moderate exercise and a prudent diet would also be helpful. Because of the association of oxidative stress with aging, obesity, poor semen quality, and reduced oocyte and embryo quality, increasing antioxidant intake is a good general measure for both partners, particularly those approaching or over the age of 40 years. All of the measures described also improve vascular and erectile function and will have favorable effects on long-term health. Newer interventions, such as CoQ10, are just emerging and, according to nonhuman and limited human investigations, are very promising. For those embryos that are still stressed, biomarkers such as excessive mtDNA and others being actively investigated will allow identification of those euploid embryos most likely to result in a successful outcome. Because most of these interventions are supported by a limited number of studies, further blinded and randomized trials should be carried out.

REFERENCES

1. Li R, Albertini DF. The road to maturation: somatic cell interaction and self-organization of the mammalian oocyte. Nat Rev Mol Cell Biol 2013;14:141–52.
2. Dumesic DA, Meldrum DR, Katz-Jaffe MG, Krisher RL, Schoolcraft WB. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. Fertil Steril 2015;103:303–16.
3. Patrizio P, Sakkas D. From oocyte to baby: a clinical evaluation of the biological efficiency of in vitro fertilization. Fertil Steril 2009;91:1061–6.
4. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014;101:656–63. e1.
5. Ben-Meir A, Burstein E, Borrego-Alvarez A, Chong J, Wong E, Yavorska T, et al. Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. Aging Cell 2015.
6. Meldrum DR, Chang RJ, Giudice LC, Balasch J, Barbieri RL. Role of decreased androgens in the ovarian response to stimulation in older women. Fertil Steril 2013;99:5–11.
7. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab 2005;90:3847–53.
8. Barbieri RL, Sluss PM, Powers RD, McShane PM, Vitonis A, Ginsburg E, et al. Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing in vitro fertilization. Fertil Steril 2005;83:302–8.
9. Vendola KA, Zhou J, Adesanya OO, Weil SJ, Bondy CA. Androgens stimulate early stages of follicular growth in the primate ovary. J Clin Invest 1998;101:2622–9.
10. Tartagni M, Cicinelli MV, Baldini D, Tartagni MV, Alrasheed H, DeSalvia MA, et al. Dehydroepiandrosterone decreases the age-related decline of the in vitro fertilization outcome in women younger than 40 years old. Reprod Biol Endocrinol 2015;13:18.
11. Kim CH, Howles CM, Lee HA. The effect of transdermal testosterone gel pretreatment on controlled ovarian stimulation and IVF outcome in low responders. Fertil Steril 2011;95:679–83.
12. Fabregues F, Penarrubia J, Creus M, Manau D, Casals G, Carmona F, et al. Transdermal testosterone may improve ovarian response to gonadotrophins in low-responder IVF patients: a randomized, clinical trial. Hum Reprod 2009;24:349–59.
13. Kregel KC, Zhang HJ. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007;292:R18–36.
14. Suh JH, Shenvi SV, Dixon BM, Liu H, Jaiswal AK, Liu RM, et al. Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid. Proc Natl Acad Sci U S A 2004;101:3381–6.
15. Tatone C, Amicarelli F. The aging ovary—the poor granulosa cells. Fertil Steril 2013;99:12–7.
16. Kalmbach KH, Fontes Antunes DM, Dracxler RC, Knier TW, Seth-Smith ML, Wang F, et al. Telomeres and human reproduction. Fertil Steril 2013;99:23–9.

17. Humm KC, Sakkas D. Role of increased male age in IVF and egg donation: is sperm DNA fragmentation responsible? Fertil Steril 2013;99:30–6.
18. Mahfouz R, Sharma R, Thiyagarajan A, Kale V, Gupta S, Sabanegh E, et al. Semen characteristics and sperm DNA fragmentation in infertile men with low and high levels of seminal reactive oxygen species. Fertil Steril 2010;94:2141–6.
19. de La Rochebrochard E, de Mouzon J, Thepot F, Thonneau P. French National IVFRA. Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. Fertil Steril 2006;85:1420–4.
20. Stahl PJ, Cogan C, Mehta A, Bolyakov A, Paduch DA, Goldstein M. Concordance among sperm deoxyribonucleic acid integrity assays and semen parameters. Fertil Steril 2015;104:56–61. e1.
21. Schmid TE, Eskenazi B, Baumgartner A, Marchetti F, Young S, Weldon R, et al. The effects of male age on sperm DNA damage in healthy nonsmokers. Hum Reprod 2007;22:180–7.
22. Matsuda M, Shimomura I. Increased oxidative stress in obesity: implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. Obes Res Clin Pract 2013;7:e330–41.
23. Bausenwein J, Serke H, Eberle K, Hirrlinger J, Jogschies P, Hmeidan FA, et al. Elevated levels of oxidized low-density lipoprotein and of catalase activity in follicular fluid of obese women. Mol Hum Reprod 2010;16:117–24.
24. Luke B, Brown MB, Missmer SA, Bukulmez O, Leach R, Stern JE, et al. The effect of increasing obesity on the response to and outcome of assisted reproductive technology: a national study. Fertil Steril 2011;96:820–5.
25. Bellver J, Pellicer A, Garcia-Velasco JA, Ballesteros A, Remohi J, Meseguer M. Obesity reduces uterine receptivity: clinical experience from 9,587 first cycles of ovum donation with normal weight donors. Fertil Steril 2013;100:1050–8.
26. Dickey RP, Xiong X, Gee RE, Pridjian G. Effect of maternal height and weight on risk of preterm birth in singleton and twin births resulting from in vitro fertilization: a retrospective cohort study using the Society for Assisted Reproductive Technology Clinic Outcome Reporting System. Fertil Steril 2012;97:349–54.
27. Luke B, Brown MB, Stern JE, Missmer SA, Fujimoto VY, Leach R. Racial and ethnic disparities in assisted reproductive technology pregnancy and live birth rates within body mass index categories. Fertil Steril 2011;95:1661–6.
28. Veleva Z, Tiitinen A, Vilska S, Hyden-Granskog C, Tomas C, Martikainen H, et al. High and low BMI increase the risk of miscarriage after IVF/ICSI and FET. Hum Reprod 2008;23:878–84.
29. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, et al. Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. J Clin Endocrinol Metab 2006;91:439–46.
30. Palomba S, Falbo A, Valli B, Morini D, Villani MT, Nicoli A, et al. Physical activity before IVF and ICSI cycles in infertile obese women: an observational cohort study. Reprod Biomed Online 2014;29:72–9.
31. Hughes EG, Yeo J, Claman P, YoungLai EV, Sagle MA, Daya S, et al. Cigarette smoking and the outcomes of in vitro fertilization: measurement of effect size and levels of action. Fertil Steril 1994;62:807–14.
32. Pineles BL, Park E, Samet JM. Systematic review and meta-analysis of miscarriage and maternal exposure to tobacco smoke during pregnancy. Am J Epidemiol 2014;179:807–23.
33. Soares SR, Simon C, Remohi J, Pellicer A. Cigarette smoking affects uterine receptiveness. Hum Reprod 2007;22:543–7.
34. Zitzmann M, Rolf C, Nordhoff V, Schrader G, Rickert-Fohring M, Gassner P, et al. Male smokers have a decreased success rate for in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 2003;79(Suppl 3):1550–4.
35. Neal MS, Hughes EG, Holloway AC, Foster WG. Sidestream smoking is equally as damaging as mainstream smoking on IVF outcomes. Hum Reprod 2005;20:2531–5.
36. de Ziegler D, Santulli P, Seroka A, Decanter C, Meldrum DR, Chapron C. In women, the reproductive harm of toxins such as tobacco smoke is reversible in 6 months: basis for the "olive tree" hypothesis. Fertil Steril 2013;100:927–8.
37. Nicolau P, Miralpeix E, Sola I, Carreras R, Checa MA. Alcohol consumption and in vitro fertilization: a review of the literature. Gynecol Endocrinol 2014;30:759–63.
38. Klonoff-Cohen H, Lam-Kruglick P, Gonzalez C. Effects of maternal and paternal alcohol consumption on the success rates of in vitro fertilization and gamete intrafallopian transfer. Fertil Steril 2003;79:330–9.
39. Klonoff-Cohen H, Bleha J, Lam-Kruglick P. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-fallopian transfer. Hum Reprod 2002;17:1746–54.
40. Choi JH, Ryan LM, Cramer DW, Hornstein MD, Missmer SA. Effects of caffeine consumption by women and men on the outcome of fertilization. J Caffeine Res 2011;1:29–34.
41. Hatch EE, Wise LA, Mikkelsen EM, Christensen T, Riis AH, Sorensen HT, et al. Caffeinated beverage and soda consumption and time to pregnancy. Epidemiology 2012;23:393–401.
42. Jinno M, Takeuchi M, Watanabe A, Teruya K, Hirohama J, Eguchi N, et al. Advanced glycation end-products accumulation compromises embryonic development and achievement of pregnancy by assisted reproductive technology. Hum Reprod 2011;26:604–10.
43. Ehrlich S, Williams PL, Missmer SA, Flaws JA, Berry KF, Calafat AM, et al. Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. Environ Health Perspect 2012;120:978–83.
44. Lathi RB, Liebert CA, Brookfield KF, Taylor JA, vom Saal FS, Fujimoto VY, et al. Conjugated bisphenol A in maternal serum in relation to miscarriage risk. Fertil Steril 2014;102:123–8.
45. Knez J, Kranvogl R, Breznik BP, Voncina E, Vlaisavljevic V. Are urinary bisphenol A levels in men related to semen quality and embryo development after medically assisted reproduction? Fertil Steril 2014;101:215–21. e5.
46. Vaamonde D, da Silva-Grigoletto ME, Garcia-Manso JM, Barrera N, Vaamonde-Lemos R. Physically active men show better semen parameters and hormone values than sedentary men. Eur J Appl Physiol 2012;112:3267–73.
47. Evenson KR, Calhoun KC, Herring AH, Pritchard D, Wen F, Steiner AZ. Association of physical activity in the past year and immediately after in vitro fertilization on pregnancy. Fertil Steril 2014;101:1047–54. e5.
48. Senti M, Tomas M, Anglada R, Elosua R, Marrugat J, Covas MI, et al. Interrelationship of smoking, paraoxonase activity, and leisure time physical activity: a population-based study. Eur J Intern Med 2003;14:178–84.
49. Farinha JB, Steckling FM, Stefanello ST, Cardoso MS, Nunes LS, Barcelos RP, et al. Response of oxidative stress and inflammatory biomarkers to a 12-week aerobic exercise training in women with metabolic syndrome. Sports Med Open 2015;1:3.
50. Gudmundsdottir SL, Flanders WD, Augestad LB. Physical activity and fertility in women: the North-Trondelag Health Study. Hum Reprod 2009;24:3196–204.
51. Wise LA, Rothman KJ, Mikkelsen EM, Sorensen HT, Riis AH, Hatch EE. A prospective cohort study of physical activity and time to pregnancy. Fertil Steril 2012;97:1136–42e1–4.
52. Wise LA, Cramer DW, Hornstein MD, Ashby RK, Missmer SA. Physical activity and semen quality among men attending an infertility clinic. Fertil Steril 2011;95:1025–30.
53. Vujkovic M, de Vries JH, Lindemans J, Macklon NS, van der Spek PJ, Steegers EA, et al. The preconception Mediterranean dietary pattern in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment increases the chance of pregnancy. Fertil Steril 2010;94:2096–101.
54. Twigt JM, Bolhuis ME, Steegers EA, Hammiche F, van Inzen WG, Laven JS, et al. The preconception diet is associated with the chance of ongoing pregnancy in women undergoing IVF/ICSI treatment. Hum Reprod 2012;27:2526–31.
55. Bedaiwy MA, Elnashar SA, Goldberg JM, Sharma R, Mascha EJ, Arrigain S, et al. Effect of follicular fluid oxidative stress parameters on intracytoplasmic sperm injection outcome. Gynecol Endocrinol 2012;28:51–5.
56. Eslamian G, Amirjannati N, Rashidkhani B, Sadeghi MR, Baghestani AR, Hekmatdoost A. Dietary fatty acid intakes and asthenozoospermia: a case-control study. Fertil Steril 2015;103:190–8.

57. Kim CH, Yoon JW, Ahn JW, Kang HJ, Lee JW, Kang BM. The effect of supplementation with omega-3-polyunsaturated fatty acids in intracytoplasmic sperm injection cycles for infertile patients with a history of unexplained total fertilization failure. Fertil Steril 2010;94:S242.
58. Meldrum DR, Gambone JC, Morris MA, Meldrum DA, Esposito K, Ignarro LJ. The link between erectile and cardiovascular health: the canary in the coal mine. Am J Cardiol 2011;108:599–606.
59. Halvorsen BL, Carlsen MH, Phillips KM, Bohn SK, Holte K, Jacobs DR Jr, et al. Content of redox-active compounds (ie, antioxidants) in foods consumed in the United States. Am J Clin Nutr 2006;84:95–135.
60. Vanegas JC, Afeiche MC, Gaskins AJ, Minguez-Alarcon L, Williams PL, Wright DL, et al. Soy food intake and treatment outcomes of women undergoing assisted reproductive technology. Fertil Steril 2015;103:749–55. e2.
61. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 2005;26:349–53.
62. Meldrum DR, Burnett AL, Dorey G, Esposito K, Ignarro LJ. Erectile hydraulics: maximizing inflow while minimizing outflow. J Sex Med 2014;11:1208–20.
63. Meldrum DR, Gambone JC, Morris MA, Ignarro LJ. A multifaceted approach to maximize erectile function and vascular health. Fertil Steril 2010;94:2514–20.
64. Haghighian HK, Haidari F, Mohammadi-Asl J, Dadfar M. Randomized, triple-blind, placebo-controlled clinical trial examining the effects of alpha-lipoic acid supplement on the spermatogram and seminal oxidative stress in infertile men. Fertil Steril 2015;104:318–24.
65. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576–85.
66. Safarinejad MR. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic antioxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. Andrologia 2011;43:38–47.
67. Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. Hum Reprod 1997;12:1545–9.
68. Hardy K, Stark J. Mathematical models of the balance between apoptosis and proliferation. Apoptosis 2002;7:373–81.
69. Hardy K, Spanos S, Becker D, Iannelli P, Winston RM, Stark J. From cell death to embryo arrest: mathematical models of human preimplantation embryo development. Proc Natl Acad Sci U S A 2001;98:1655–60.
70. Goddard MJ, Pratt HP. Control of events during early cleavage of the mouse embryo: an analysis of the "2-cell block." J Embryol Exp Morphol 1983;73:111–33.
71. Dobson AT, Raja R, Abeyta MJ, Taylor T, Shen S, Haqq C, et al. The unique transcriptome through day 3 of human preimplantation development. Hum Mol Genet 2004;13:1461–70.
72. Gray MW, Burger G, Lang BF. The origin and early evolution of mitochondria. Genome Biol 2001;2. REVIEWS1018.
73. Satoh M, Kuroiwa T. Organization of multiple nucleoids and DNA molecules in mitochondria of a human cell. Exp Cell Res 1991;196:137–40.
74. von Heijne G. Why mitochondria need a genome. FEBS Lett 1986;198:1–4.
75. Gray MW. Origin and evolution of mitochondrial DNA. Annu Rev Cell Biol 1989;5:25–50.
76. Sato M, Sato K. Maternal inheritance of mitochondrial DNA: degradation of paternal mitochondria by allogeneic organelle autophagy, allophagy. Autophagy 2012;8:424–5.
77. Cummins JM. Mitochondria in reproduction. Reprod Biomed Online 2004;8:14–5.
78. Jansen RP, de Boer K. The bottleneck: mitochondrial imperatives in oogenesis and ovarian follicular fate. Mol Cell Endocrinol 1998;145:81–8.
79. Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, et al. Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nat Genet 1998;18:231–6.
80. Wai T, Ao A, Zhang X, Cyr D, Dufort D, Shoubridge EA. The role of mitochondrial DNA copy number in mammalian fertility. Biol Reprod 2010;83:52–62.
81. van Blerkom J. Mitochondria as regulatory forces in oocytes, preimplantation embryos and stem cells. Reprod Biomed Online 2008;16:553–69.
82. Santos TA, El Shourbagy S, St John JC. Mitochondrial content reflects oocyte variability and fertilization outcome. Fertil Steril 2006;85:584–91.
83. Yi YC, Chen MJ, Ho JY, Guu HF, Ho ES. Mitochondria transfer can enhance the murine embryo development. J Assist Reprod Genet 2007;24:445–9.
84. El Shourbagy SH, Spikings EC, Freitas M, St. John JC. Mitochondria directly influence fertilisation outcome in the pig. Reproduction 2006;131:233–45.
85. Eichenlaub-Ritter U, Vogt E, Yin H, Gosden R. Spindles, mitochondria and redox potential in ageing oocytes. Reprod Biomed online 2004;8:45–58.
86. Schon EA, Kim SH, Ferreira JC, Magalhaes P, Grace M, Warburton D, et al. Chromosomal nondisjunction in human oocytes: is there a mitochondrial connection? Hum Reprod 2000;15(Suppl 2):160–72.
87. Bentov Y, Esfandiari N, Burstein E, Casper RF. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. Fertil Steril 2010;93:272–5.
88. Bentov Y, Yavorska T, Esfandiari N, Jurisicova A, Casper RF. The contribution of mitochondrial function to reproductive aging. J Assist Reprod Genet 2011;28:773–83.
89. Zhang X, Wu XQ, Lu S, Guo YL, Ma X. Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. Cell Res 2006;16:841–50.
90. Van Blerkom J, Davis P, Alexander S. Differential mitochondrial distribution in human pronuclear embryos leads to disproportionate inheritance between blastomeres: relationship to microtubular organization, ATP content and competence. Hum Reprod 2000;15:2621–33.
91. Ramalho-Santos J, Varum S, Amaral S, Mota PC, Sousa AP, Amaral A. Mitochondrial functionality in reproduction: from gonads and gametes to embryos and embryonic stem cells. Hum Reprod Update 2009;15:553–72.
92. Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S. Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril 1995;64:577–83.
93. May-Panloup P, Chretien MF, Jacques C, Vasseur C, Malthiery Y, Reynier P. Low oocyte mitochondrial DNA content in ovarian insufficiency. Hum Reprod 2005;20:593–7.
94. Ben-Meir A, Burstein E, Borrego-Alvarez A, Chong J, Wong E, Yavorska T, et al. Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. Aging Cell 2015;14:887–95.
95. Bentov Y, Hannam T, Jurisicova A, Esfandiari N, Casper RF. Coenzyme Q10 supplementation and oocyte aneuploidy in women undergoing IVF-ICSI treatment. Clin Med Insights Reprod Health 2014;8:31–6.
96. Muggleton-Harris A, Whittingham DG, Wilson L. Cytoplasmic control of preimplantation development in vitro in the mouse. Nature 1982;299:460–2.
97. Cohen J, Scott R, Alikani M, Schimmel T, Munne S, Levron J, et al. Ooplasmic transfer in mature human oocytes. Mol Hum Reprod 1998;4:269–80.
98. van Blerkom J, Sinclair J, Davis P. Mitochondrial transfer between oocytes: potential applications of mitochondrial donation and the issue of heteroplasmy. Hum Reprod 1998;13:2857–68.
99. St. John JC. Ooplasm donation in humans: the need to investigate the transmission of mitochondrial DNA following cytoplasmic transfer. Hum Reprod 2002;17:1954–8.
100. Acton BM, Lai I, Shang X, Jurisicova A, Casper RF. Neutral mitochondrial heteroplasmy alters physiological function in mice. Biol Reprod 2007;77:569–76.
101. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004;428:145–50.
102. White YA, Woods DC, Takai Y, Ishihara O, Seki H, Tilly JL. Oocyte formation by mitotically active germ cells purified from ovaries of reproductive-age women. Nat Med 2012;18:413–21.
103. Barritt JA, Brenner CA, Malter HE, Cohen J. Mitochondria in human offspring derived from ooplasmic transplantation. Hum Reprod 2001;16:513–6.

104. Hua S, Zhang Y, Li XC, Ma LB, Cao JW, Dai JP, et al. Effects of granulosa cell mitochondria transfer on the early development of bovine embryos in vitro. Cloning Stem Cells 2007;9:237–46.
105. Song YZL, Chen S, Ni D, Li B, Zhou Y, Lei B, Wang T. Cytoplasm transfer in mature rabbit oocytes. Conf Proc IEEE Eng Med Bio Soc Reprod Fertil Suppl 2014:2903.
106. Fakih MHSM, Szeptycki J, dela Cruz DB, Lux C, Verjee S, Burgess CM, Cohn GM, Casper RF. The AUGMENT treatment: physician reported outcomes of the initial global patient experience. JFIV Reprod Med Genet 2015;3:154.
107. Leese HJ. Metabolism of the preimplantation embryo: 40 years on. Reproduction 2012;143:417–27.
108. Wai T, Teoli D, Shoubridge EA. The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. Nat Genet 2008; 40:1484–8.
109. May-Panloup P, Chretien MF, Malthiery Y, Reynier P. Mitochondrial DNA in the oocyte and the developing embryo. Curr Top Dev Biol 2007;77:51–83.
110. Piko L, Taylor KD. Amounts of mitochondrial DNA and abundance of some mitochondrial gene transcripts in early mouse embryos. Dev Biol 1987;123: 364–74.
111. Reynier P, May-Panloup P, Chretien MF, Morgan CJ, Jean M, Savagner F, et al. Mitochondrial DNA content affects the fertilizability of human oocytes. Mol Hum Reprod 2001;7:425–9.
112. Chappel S. The role of mitochondria from mature oocyte to viable blastocyst. Obstet Gynecol Int 2013;2013:183024.
113. van Blerkom J. Mitochondrial function in the human oocyte and embryo and their role in developmental competence. Mitochondrion 2011;11: 797–813.
114. Tamassia M, Nuttinck F, May-Panloup P, Reynier P, Heyman Y, Charpigny G, et al. In vitro embryo production efficiency in cattle and its association with oocyte adenosine triphosphate content, quantity of mitochondrial DNA, and mitochondrial DNA haplogroup. Biol Reprod 2004;71: 697–704.
115. Chiaratti MR, Bressan FF, Ferreira CR, Caetano AR, Smith LC, Vercesi AE, et al. Embryo mitochondrial DNA depletion is reversed during early embryogenesis in cattle. Biol Reprod 2010;82:76–85.
116. Leese HJ, Conaghan J, Martin KL, Hardy K. Early human embryo metabolism. Bioessays 1993;15:259–64.
117. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med 2003;348:2656–68.
118. Scarpulla RC. Nucleus-encoded regulators of mitochondrial function: integration of respiratory chain expression, nutrient sensing and metabolic stress. Biochim Biophys Acta 2012;1819:1088–97.
119. Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. Biochim Biophys Acta 2011;1813: 1269–78.
120. Canto C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. Curr Opin Lipidol 2009;20: 98–105.
121. Monnot S, Samuels DC, Hesters L, Frydman N, Gigarel N, Burlet P, et al. Mutation dependance of the mitochondrial DNA copy number in the first stages of human embryogenesis. Hum Mol Genet 2013;22: 1867–72.
122. Diez-Juan A, Rubio C, Marin C, Martinez S, Al-Asmar N, Riboldi M, et al. Mitochondrial DNA content as a viability score in human euploid embryos: less is better. Fertil Steril 2015;104:534–41.e1.
123. Fragouli E, Spath K, Alfarawati S, Kaper F, Craig A, Michel CE, et al. Altered levels of mitochondrial DNA are associated with female age, aneuploidy, and provide an independent measure of embryonic implantation potential. PLoS Genet 2015;11:e1005241.
124. Bentov Y, Burstein E, Esfandiari N, Casper RF. Use of letrozole challenge test to adjust gonadotropin dose in nondown-regulated cycles. Fertil Steril 2011;95:2492–3.
125. Domar AD, Zuttermeister PC, Friedman R. The psychological impact of infertility: a comparison with patients with other medical conditions. J Psychosom Obstet Gynaecol 1993;14(Suppl):45–52.
126. Sanders KA, Bruce NW. Psychosocial stress and treatment outcome following assisted reproductive technology. Hum Reprod 1999;14:1656–62.
127. Ebbesen SM, Zachariae R, Mehlsen MY, Thomsen D, Hojgaard A, Ottosen L, et al. Stressful life events are associated with a poor in-vitro fertilization (IVF) outcome: a prospective study. Hum Reprod 2009;24:2173–82.
128. Smeenk JM, Verhaak CM, Eugster A, van Minnen A, Zielhuis GA, Braat DD. The effect of anxiety and depression on the outcome of in-vitro fertilization. Hum Reprod 2001;16:1420–3.
129. Klonoff-Cohen H, Chu E, Natarajan L, Sieber W. A prospective study of stress among women undergoing in vitro fertilization or gamete intrafallopian transfer. Fertil Steril 2001;76:675–87.
130. Facchinetti F, Matteo ML, Artini GP, Volpe A, Genazzani AR. An increased vulnerability to stress is associated with a poor outcome of in vitro fertilization-embryo transfer treatment. Fertil Steril 1997;67:309–14.
131. Turner K, Reynolds-May MF, Zitek EM, Tisdale RL, Carlisle AB, Westphal LM. Stress and anxiety scores in first and repeat IVF cycles: a pilot study. PLoS One 2013;8:e63743.
132. An Y, Sun Z, Li L, Zhang Y, Ji H. Relationship between psychological stress and reproductive outcome in women undergoing in vitro fertilization treatment: psychological and neurohormonal assessment. J Assist Reprod Genet 2013;30:35–41.
133. Frederiksen Y, Farver-Vestergaard I, Skovgard NG, Ingerslev HJ, Zachariae R. Efficacy of psychosocial interventions for psychological and pregnancy outcomes in infertile women and men: a systematic review and meta-analysis. BMJ Open 2015;5:e006592.
134. Domar AD, Clapp D, Slawsby EA, Dusek J, Kessel B, Freizinger M. Impact of group psychological interventions on pregnancy rates in infertile women. Fertil Steril 2000;73:805–11.
135. Domar AD, Rooney KL, Wiegand B, Orav EJ, Alper MM, Berger BM, et al. Impact of a group mind/body intervention on pregnancy rates in IVF patients. Fertil Steril 2011;95:2269–73.
136. Boivin J, Domar AD, Shapiro DB, Wischmann TH, Fauser BC, Verhaak C. Tackling burden in ART: an integrated approach for medical staff. Hum Reprod 2012;27:941–50.
137. Gameiro S, Boivin J, Domar A. Optimal in vitro fertilization in 2020 should reduce treatment burden and enhance care delivery for patients and staff. Fertil Steril 2013;100:302–9.
138. Domar AD, Smith K, Conboy L, Iannone M, Alper M. A prospective investigation into the reasons why insured United States patients drop out of in vitro fertilization treatment. Fertil Steril 2010;94:1457–9.