Essay Title: “From your knowledge of reproductive biology, critically evaluate in detail, one possible cause of mass of infertility that could explain the situation in the Handmaid's Tale.”

Sophie Carroll
65/2 Spottiswoode Street, EH9 1DL, Edinburgh
07803804126

Reproductive Biology, Year 3
The University of Edinburgh

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Introduction

Margaret Atwood's 1985 novel 'The Handmaid's Tale' details the dystopian, totalitarian state of Gilead where disaster has rendered a large proportion of the female and male population infertile. The population of Gilead is divided into various institutional roles, with the fertile women being assigned the role of "Handmaids", whose duty it is to provide children to the elite and wealthy couples of society. The Handmaids are slaves to the state, and women have been reduced to accessories to male leadership. Atwood's themes, while seemingly unbelievable for our evolved and progressive civilisation, may have some scientific basis. The global birth rate has declined significantly in the past century, igniting serious concern for population turnover (1). Several reasons are cited as explanations for this decline, including women delaying pregnancy and the increase in readily available contraceptives. However, one reason that is gaining increasing attention is obesity. The obese state is associated with a complex combination of mechanisms that have negative implications for female fertility. Could this lead to mass infertility as depicted in 'The Handmaid's Tale'? This essay will discuss the impact of obesity on female reproductive function, and to what extent obesity is the reason for the declining global birth rate.

The Obese State

Obesity is described as an unnecessary accumulation of adipose tissue (AT), with a body mass index (BMI) above 30kg/m^2 considered obese (2). AT is a metabolically active, endocrine tissue that secretes signalling molecules, therefore excess adiposity leads to irregular secretion of adipokines, inducing a chronic low grade inflammatory state (3).

Leptin

Leptin is responsible for weight homeostasis by increasing satiety levels and energy expenditure (4) and is elevated in the obese individual (5). After release from adipocytes, leptin binds receptors found throughout the body, including the hypothalamus, gonadotrophic cells of the anterior pituitary, granulosa (GC) and thecal cells (TC) of the ovary and the endometrium (6, 7, 8).

A research group investigating adiposity and fertility fed mice a high fat diet (9). The DBA/2J female mice became obese and hyperleptinemic, experienced a 60% reduction in spontaneous pregnancy rates and had significantly less follicular activity. Obese, hyperleptinemic DBA/2J mice achieved normal pregnancy rates after exogenous gonadotrophin supplementation. Additionally, using RT-PCR, they established that obese DBA/2J mice had 50% less hypothalamic GnRH transcripts, indicating anovulation was of central origin (9). C57BL/6J female mice fed the same diet did not become obese, hyperleptinemic, or experience reduced follicular activity, suggesting a role for leptin in the suppression of the HPG axis, creating hypothalamic hypogonadism. This suppression depletes FSH and LH levels, abolishing estrogen activity and therefore folliculogenesis (10, 11). The researchers failed to discuss estrogen levels of the obese, sub-fertile mice, so further research is required to support dampening of the HPG axis as a reason for subfertility. However, they did identify a good model for female infertility in obesity and revealed neuroendocrine irregularities induced by extreme leptin levels.

In work using granulosa cells (GCs) extracted from ovulating women, leptin showed a dose-dependent inhibition on estradiol secretion via IGF-1 augmentation of FSH stimulation (12). Leptin had no effect on FSH-only stimulation but abolished IGF-1s action. Estradiol, an estrogen steroid hormone, is paramount to development of the dominant follicle (13) and IGF-1 is likely involved in the initiation of human follicle growth (14) therefore high circulating leptin levels may inhibit follicular development. Cells were exposed to physiological leptin levels found in obese women, mimicking ovarian cell leptin exposure in vivo, but the follicles themselves were obtained from women of BMI 26.3 ± 1.2 kg/m^2, meaning they were overweight (2). This limits the inference we can make regarding obese and morbidly obese women as follicles of overweight women might respond differently in vitro. However, this study was the first that looked at human cells rather than animal models, allowing for deeper understanding of human physiology.

Insulin and Androgens

Adiponectin is another adipocyte secreted factor. Despite high adiposity in obesity, adiponectin levels are low in obese individuals and increase with weight loss (15,16). Adiponectin is important in fuel metabolism. It inhibits gluconeogenesis in the liver and contributes to insulin sensitivity of muscle (17). Obesity and low adiponectin levels contribute to hyperinsulinemia and peripheral insulin resistance, however human GCs

remain responsive (18). Incubation with insulin of GCs obtained from "normal" and PCOS ovaries enhanced both basal and LH-stimulated production of progesterone and estradiol.

High levels of insulin are negatively correlated with levels of sex hormone binding globulin (SHBG) as it inhibits hepatic secretion (19). Levels are even lower in women with central obesity when compared to women with peripheral obesity (20). Lower SHBG decreases transport of androgens to target tissues and is compensated for by upregulation of androgen production. Therefore, women with obesity have elevated levels of androgen production (21) and potential hyperandrogenism. Additionally, RT-PCR of subcutaneous pre-adipocytes has found a localised role of 17β-HSD type 5 in the conversion of androstenedione to testosterone in female adipose tissue (22).

Vendola et al. (23) conducted a series of experiments in which female monkeys received exogenous testosterone treatment. Histological analysis of their ovaries showed that growing pre-antral follicle number and GC/TC proliferation was increased in the treated animals, giving first evidence of a stimulatory effect of androgens on folliculogenesis in primates. This mechanism may offer explanation for the fertility issues observed in hyperandrogenic women. More follicles maturing simultaneously increases circulating estrogen levels which act via negative feedback on the HPG axis, at the anterior pituitary level. GnRH secretion and therefore LH and FSH is downregulated (10). FSH is inhibited to a greater extent than LH (24) so late follicle growth and resulting ovulation is repressed (11) creating a form of anovulation and subfertility.

Lipotoxicity, FFA levels and Reproductive Tissues

In extended periods of overnutrition, serum triglyceride and resulting free fatty acids (FFAs) levels are high. Unused FFAs are stored as lipid droplets (25), readily deposited in adipose tissue. However, in cases where triglyceride levels are elevated, tissues including reproductive tissues have limited capacity for lipid storage (25). When this capacity is exceeded, the cell enters lipotoxicity. The endoplasmic reticulum (ER) and mitochondrial membrane are injured,
leading to the release of reactive oxidative substances (ROS) that inhibit ER protein folding processes (26). In the case where cellular processes cannot be restored, Ca^{2+} release and caspase action can initiate apoptosis in the intoxicated cell (27). Altered Ca^{2+} homeostasis is used as a marker for ER stress and apoptotic cells.

Ca^{2+} concentrations of follicular fluid surrounding oocytes were measured in women of varying BMI (28). Ca^{2+} concentration, fluid FFAs, triglycerides and glucose levels were positively correlated with BMI. Secondly, the ER stress gene marker ATF4 is elevated in GCs of obese women, compared with moderate weight controls (28). This data indicates a relationship between lipotoxicity and ER stress pathways in GCs of obese women. Furthermore, increased DNA fragmentation was confirmed by DNA laddering analysis of GCs from mice fed a high fat diet compared to a control diet, suggestive of apoptosis. Both groups of mice were treated with gonadotrophins and caged with male mice. Ovulation was recorded in both groups, however in 26% of high-fat mice, zero oocytes were present in each oviduct, causing anovulation. While this effect was statistically insignificant, it does offer some indication of an effect on fertility of lipotoxicity. Furthermore, as GCs are essential to maintain oocyte development (13), impaired function is a plausible explanation for reduced fertility in obese women.

ART Outcomes in Obese Women

Assisted reproductive technology (ART) is an option for individuals who struggle to naturally conceive. While there is evidence that BMI is positively correlated with early pregnancy loss (29), other studies have shown that ART outcome is unaffected by BMI (15, 31). Despite this, much of the evidence tends towards adverse effects of obesity on conception through ART.

FSH levels must be above a threshold concentration for follicular recruitment during ovarian stimulation (32). A 2007 systematic review (33)
identified that women of BMI >25kg/m^2 required greater than 200 units of gonadotrophins more than women of BMI <25kg/m^2 in ART treatment. This requirement was further increased in obese women (BMI >30kg/m^2). Pregnancy rates were also diminished in obese and overweight groups and miscarriage rates were greater in women of BMI >30kg/m^2 than BMI <30kg/m^2.

Many studies evaluating ART outcome fail to consider confounding factors such as PCOS, smoking and age, making conclusions subject to scrutiny. Furthermore, some studies *have* considered these factors and

therefore excluded subpopulations from analysis, making systematic review and meta-analysis harder to conduct. Research with defined outcome parameters, as well as inclusion criteria are required to make reliable conclusions.

Which Mechanism?

As summarised in figure 1, there are numerous possible mechanisms tying obesity to fertility issues. The use of insulin-sensitising drugs as treatment of infertility in insulin-resistant PCOS women (34) highlights a role for insulin in infertility. However, greatest benefits were seen in those without severe obesity, and PCOS is a confounding factor that limits inferences to be made regarding obesity and insulin. Moreover, elevated androgen production resulting from decreased SHBG is counteracted by a 2-3-fold increase in androgen clearance (35), therefore insulin induced hyperandrogenism may only be relevant to centrally obese women with greater compensatory androgen production. Conversely, women with high serum insulin levels show the greatest gonadotrophin resistance (36), suggesting insulin creates challenges to ART.

Leptin inhibition of IGF-1 action in GCs (12) and its association with hypothalamic hypogonadism (9) may also contribute to the gonadotrophin resistance seen in ART, as more FSH is required to elicit the same level of estradiol secretion.

Lipotoxicity leads to metabolic dysfunction in numerous tissues throughout the body, including the pathogenesis of peripheral insulin resistance and resulting hyperinsulinemia (37). As this may cause hyperandrogenism and gonadotrophin resistance, and lipotoxicity effects oocyte viability, lipotoxicity is a probable mediator of fertility in obesity.

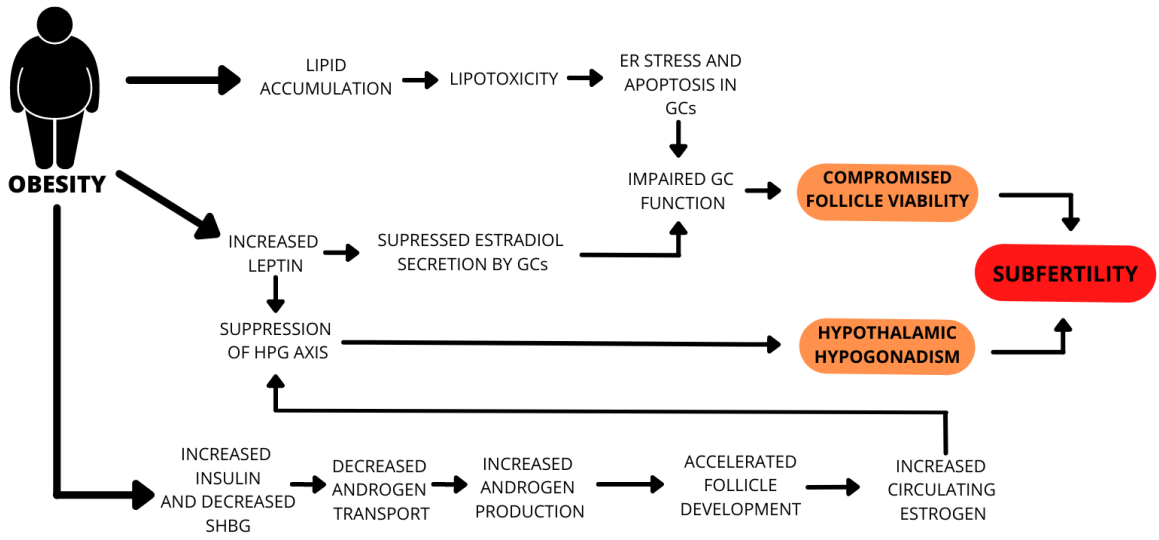

Figure 1.
Summary of discussed mechanisms. GCs; granulosa cells, SHBG; sex hormone binding globulin, ER; endoplasmic reticulum.

A Serious Threat?

The UK birth rate has reduced from 1.98 in 2009, to 1.65 in 2019 (1); nearly 17% in 20 years. The global total fertility rate is steadily dropping to below the critical replacement level of 2.1 and is forecasted to reach 1.6 in 2100, halving the population of 23 nations, causing substantial economic and environmental costs (38). As of 2009, the mean use of contraceptives was 62.7% globally; highest in the most developed countries (39). The average age of first-time mothers is also increasing, rising to 29.1 in 2020 from 23.7 in 1970, in England and Wales (40). Both may contribute to decreased birth rates, yet women are still having children later in life.

The situation depicted in 'The Handmaid's Tale' is severe and caused by a combination of factors, therefore obesity alone is unlikely to produce something so destructive. However, due to the clear tie between obesity and subfertility, and the upward projection of obesity rates, it is undoubtably an important factor and presents a serious threat. Obesity pervasiveness is increasing, with 86.3% of the US population projected to be

overweight by 2030 (41). ART does remain an option for obese women who struggle to conceive, but it is more difficult, therefore weight loss should be the first intervention.

Conclusion

In closing, the detrimental fertility rates observed in the Handmaid's Tale are much lower than those seen currently, however birth rates are trending downward globally. Obesogenic diets can induce lipotoxicity in ovarian cells, reducing oocyte viability, and high levels of insulin and leptin can contribute to a state of hyperandrogenism or hypothalamic hypogonadism. ART offers some hope in assisting fertility in obese females. Despite it being more difficult, there are still babies being born to overweight and obese mothers. The mechanisms by which obesity causes subfertility in women is not wholly understood, but we do know it is a complex interaction of numerous factors including hyperinsulinemia, hyperleptinemia and lipotoxicity. While there are other issues to consider when it comes to fertility, obesity is an epidemic that will continue to decrease birth rates without sufficient intervention. We must act now to mitigate its deleterious effects.

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