Abstract: Inflammation has a well-known suppressive effect on fertility. The function of gonadotropin-releasing hormone (GnRH) neurons, the central regulator of fertility is substantially altered during inflammation in females. In our review we discuss the latest results on how the function of GnRH neurons is modified by inflammation in females. We first address the various effects of inflammation on GnRH neurons and their functional consequences. Second, we survey the possible mechanisms underlying the inflammation-induced actions on GnRH neurons. The role of several factors will be discerned in transmitting inflammatory signals to the GnRH neurons: cytokines, kisspeptin, RFamide-related peptides, estradiol and the anti-inflammatory cholinergic pathway. Since aging and obesity are both characterized by reproductive decline our review also focuses on the mechanisms and pathophysiological consequences of the impact of inflammation on GnRH neurons in aging and obesity.

Keywords: GnRH neuron; estradiol; inflammation; cytokines; obesity

1. Introduction

The hypothalamic–pituitary–gonadal axis (HPG axis) regulates reproduction. Gonadotropin-releasing hormone (GnRH) neurons are the central regulators of fertility. They are small, fusiform cells scattered throughout the hypothalamus and basal forebrain (medial septum (MS) preoptic area (POA), with fibers projecting to the median eminence (ME) and the organum vasculosum of the laminae terminalis (OVLT) [1]. GnRH is a decapeptide that acts on the anterior pituitary (AP) to control the production and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which regulate gonads: Testosterone production from testes and estradiol and progesterone from ovaries.

GnRH secretion is finely governed by excitatory and inhibitory transsynaptic neuronal inputs. Kisspeptin, a KISS-1 gene product was identified as the main regulator of episodic GnRH release. Kisspeptin is a neuropeptide expressed predominantly in the rostral periventricular area of the third ventricle (RP3V) and arcuate nucleus (ARC) in rodents [2] or in the RP3V and infundibular nucleus (equivalent to the rodent ARC) in humans [3]. In addition, the role of two other neuropeptides has been described in GnRH pulse generation, neurokinin B (NKB) and dynorphin. They have been demonstrated to co-localized with kisspeptin in the arcuate nucleus creating the kisspeptin/neurokinin B/dynorphin (KNDy) neurons [4]. According to the “KNDy hypothesis” NKB initiates the pulse onset, kisspeptin is the output signal to drive GnRH secretion and finally, dynorphin serves as an inhibitory...
signal to terminate the pulse [5]. Morphological studies showed that KNDY neurons are connected with each other via axo-somatic synapses [4].

In addition to kisspeptin, gonadotropin inhibitory hormone (GnIH) is a lately discovered neuropeptide in birds that regulates the HPG axis in physiological conditions [6]. Similarly, mammalian GnIH orthologs, known as RFamide-related peptides (RFRPs) suppress the function of HPG axis. GPR147, the receptor of RFP is expressed in the hypothalamus and pituitary as well and the RFamide-related peptide-3 (RFRP3) has been shown to act on GnRH neurons in the hypothalamus and also on the pituitary to inhibit GnRH and LH release and synthesis, respectively [7]. Besides that RFRP-3 neurons regulate GnRH and pituitary neurons, they also influence LH secretion acting on kisspeptin neurons [8]. However, the effect of RFRP-3-induced actions on kisspeptin neurons is controversial and are species- and sex-dependent [9–11].

Estradiol has a critical regulatory effect upon the activity of GnRH neurons in females that is indispensable for normal reproductive functions. During the estrous cycle, GnRH is secreted in a pulsatile manner, which is mainly controlled by the negative feedback actions of estradiol secreted from the ovaries [12]. In the preovulatory stage, GnRH is secreted in a surge induced by the positive feedback effects of estradiol released from the mature ovarian follicles finally evoking LH surge and consequently ovulation [13,14]. The positive feedback effects of estradiol on GnRH neurons occur through kisspeptin neurons that project to the cell body and proximal dendrites of GnRH neurons [1]. Although the critical role of intracellular signaling molecules such as cAMP responsive element binding protein has been proposed in estradiol-induced negative feedback action on GnRH neuron the precise mechanism remains elusive [15].

Besides its well-known role in fertility, the HPG axis acts in concert with the immune system to control immune functions. The relationship between the immune system and the HPG axis is bidirectional: Gonadal hormones have an impact on the immune system, but alterations in the immune function can elicit functional modifications of the HPG axis as well.

The interaction between the immune system and the HPG axis is primarily based on their shared receptors and mediators [16]. Primary substances that mediate signals from the immune system to GnRH neurons are the cytokines such as IL-1, TNF-α, and IL-10. Cytokines are essential in maintaining homeostasis and for regulating immune responses in the brain. The unbalanced production of pro- and anti-inflammatory cytokines has been linked to the progression of various human neurological disorders. Inflammation of the central nervous system (CNS) is now associated with nearly all neurological diseases. Neuroinflammation develops via peripheral immune cells migrating into the CNS [17] or local cytokine synthesis in the brain parenchyma [18]. Some amounts of blood borne cytokines can also cross the blood–brain barrier (BBB) with a saturated transport mechanism [19].

As there have been compelling studies published recently about the functional relevance of inflammation affecting the function of GnRH neurons, our review will focus on the mechanisms and the effect of inflammation on the GnRH neurons. We will discuss the neuroinflammatory processes and the effects of inflammation on fertility. As part of the mechanism, the role of cytokines, kisspeptin, RFamide-related peptides, estradiol, and the cholinergic pathway in inflammation will be reviewed. In regard to consequences, clinical aspects will be surveyed with special attention given to the neuroinflammatory processes in aging and the effect of obesity-induced inflammation on reproductive functions.

2. Neuroinflammatory Processes: The Role of Blood–Brain Barrier (BBB), Astrocytes, and Microglia

In order to understand the effects of neuroinflammation on GnRH neurons we first discuss the role of key players in the development of neuroinflammation such as BBB, astrocytes, and microglia.

BBB restricts the passage of large molecules from the bloodstream into the brain by forming tight endothelial junctions. Under physiological conditions the access of peripheral immune cells into the CNS is prevented by the BBB. When bacterial or viral infections disrupt the BBB, circulating immune
cells, such as lymphocytes (B and T cells), monocytes and granulocytes can enter the brain parenchyma supplying a source of cytokines [20–23]. Additionally, cytokines can be secreted locally in the brain parenchyma [18], the BBB [24] and choroid plexus cells [25] during peripheral inflammation initiating inflammatory processes in the CNS.

Another possible way for cytokines to enter the brain is through the circumventricular organs. The circumventricular organs such as OVLT and ME are specialized sites lacking the BBB that allows direct communication between the blood and brain parenchyma. It has been established that most GnRH neurons send axons to the external zone of the median eminence to secrete GnRH in a pulsatile manner into the pituitary portal system to control gonadotropin release. However, the detailed structure and function of a very high density of GnRH fibers found within the OVLT has remained unexplored until recently. Herbison and his colleagues reported that the cell bodies of GnRH neurons located within 100 µm of the OVLT extend highly branched dendritic trees into the OVLT beyond the BBB [26]. This finding suggests that GnRH dendrites and cell bodies residing in the OVLT itself and approximately within 100 µm from the OVLT in the deeper parenchyma can be exposed to molecules in the peripheral circulation even without the disruption of BBB.

The formation and integrity of the BBB and the infiltration of leukocytes to the CNS parenchyma is critically regulated by astrocytes [27–29]. It has been reported that astrocytes play both harmful and protective roles in neuroinflammation [30,31]. The physical barriers composed of the BBB and astrocytic end feet (glia limitans) around small vessels provide the first line of defense of the CNS against immune attacks [32]. Astrocytes are capable of inhibiting inflammation by maintaining the expression of junction proteins thereby preserving the intact BBB [33]. However, astrocytes may also be responsible for the expansion of CNS inflammation. In the presence of immune stimuli, astrocytes lose their end feet leading to the damage of glia limitans and also secrete factors, which are contributing to disruption of BBB [34]. Astrocytes also produce chemoattractant molecules that induce leukocyte infiltration [35].

Astrocytes-derived factors can also activate microglia, while vice versa microglia secrete factors that regulate the functions of astrocytes. The microglia–astrocyte crosstalk determines neuronal functions and dysfunctions [36]. Microglia are resident macrophages supplying essential defense of the CNS. Ramified resting microglial cells keep their environment under surveillance and they are immensely sensitive to the smallest disturbances in the extracellular environment responding rapidly with morphological and consequent functional changes. In response to stressors microglia are activated by gradually retracting their processes and becoming amoeboid phagocytic cells. Activated microglia phenotypes exist in a large scale spectrum with two end-points [37]. For instance, M1 phenotype microglia are pro-inflammatory involved in pathogen elimination, tissue damage, and neuroinflammation, while M2-like microglia are anti-inflammatory taking part in the resolution of inflammation and restoration of homeostasis [38].

3. Effects of Inflammation on Fertility: LPS-Induced Functional Disturbances in GnRH Neurons

There is numerous clinical evidence that infections and inflammatory diseases often impair reproductive functions in females [39–42]. It is well known that immune-stress can lead to reproductive dysfunction, such as anovulation and amenorrhea [43]. The suppressive effect of inflammation on the HPG axis is also well-documented in animal experiments. Most studies use peripheral injection of bacterial endotoxin, lipopolysaccharide (LPS), the major molecular component of the outer membrane of Gram-negative bacteria as a model to examine the impact of inflammation on the reproductive functions as LPS initiates an inflammatory response [44].

Both acute and prolonged inflammation induced by single or repeated peripheral injection of LPS inhibit secretion of GnRH and LH [45–47], while endotoxin can also disturb the ovarian responsiveness to gonadotropin stimulation [48,49]. Intravenous LPS administration decreases GnRH and GnRH receptor (GnRHR) mRNAs levels in the POA and ME in anestrous ewe [50]. Endotoxin injection also downregulates GnRHR gene expression in the OVLT and mPOA during the proestrous phase in female
In the AP LPS injection reduces the sensitivity of cells to GnRH by downregulating GnRHR gene expression and decreasing the gene expression of the β subunits of LH. LPS injection suppresses tonic LH secretion and delays or prohibits the preovulatory LH surge in different species including rats, sheep, and nonhuman primates. LPS itself may affect GnRH/LH secretion directly by binding to Toll-like receptors (TLR2/4), which are found both in the hypothalamus and pituitary and indirectly by many factors like prostaglandins and opioids.

Importantly, the response to LPS is dose-dependent. A high dose of LPS induces systemic inflammation such as sepsis resulting in multi-organ failure and death. In contrast, a lower dose of LPS elicits a mild immune response without causing sepsis. However, limited number of studies examine the impact of mild inflammation on reproductive functions. Low single dose of LPS (500 ng/kg) from Salmonella Enteriditis, for instance, has been shown to dysregulate the expression of GnRH peptide in juvenile female pigs. This subclinical dose of LPS has increased the level of GnRH in the medial basal hypothalamus, the lateral hypothalamic area, the mammillary bodies, the median eminence and in the ovary without any clinical symptoms.

This result demonstrates that even an asymptomatic infection can disrupt homeostasis and cause reproductive dysfunctions. Our recently published paper also illustrates that a less severe immune-challenge may alter the integrity of HPG axis. In our experiments we selectively induced a T-cell-dependent B-cell response with fluorescein isothiocyanate/keyhole limpet hemocyanin (KLH-FITC) and presented that KLH-FITC elicits ERK1/2 phosphorylation via IL-10 in female GnRH neurons in vivo.

4. Mechanisms of LPS-Induced Anti-Gonadotropic Effect of Inflammation on the HPG Axis

The LPS-induced anti-gonadotropic effect of inflammation is primarily mediated by pro-inflammatory cytokines in the hypothalamus. Among pro-inflammatory cytokines, IL-1β is the most potent inhibitor of the GnRH-LH system, IL-1α and TNF-α are less effective, whereas the participation of IL-6 appears irrelevant. IL-1β regulates LH release primarily through modulation of GnRH neuronal activity. IL-1β might be responsible for most of the effects of LPS as intracerebroventricular (i.c.v.) injection of IL-1β has been shown to decrease GnRH mRNA level in the POA and ME. Centrally administered IL-1β also suppresses GnRH translation in the hypothalamus. Furthermore, IL-1β inhibits LH release by suppressing GnRHR gene expression in the ME and POA and by decreasing LHβ mRNA level acting directly on IL-1β receptors of the pituitary gland.

Inflammation might cause these effects via fine-tuning molecular events and the structure of GnRH neurons. A study postulates that LPS suppresses GnRH synthesis at the posttranscriptional level rather than at the transcriptional level. This theory is based on the observation that LPS robustly decreases GnRH gene expression in the ME in the follicular phase of the estrous cycle of ewe while it does not change GnRH gene expression in the hypothalamic regions containing perikarya of GnRH neurons. This finding is consistent with the characteristics of GnRH gene transcription. The amount of GnRH mRNA in the cytoplasm is higher than in the nucleus of GnRH neurons, consequently GnRH transcript continuously translocated from the nucleus to the cytoplasm. Therefore, the change in GnRH mRNA levels may arise from nuclear events such as transcription or cytoplasmic events like modification of mRNA stability. Accordingly, it is possible that the LPS-induced decrease of GnRH mRNA in the ME is a result of the degradation of cytoplasmic GnRH.

Another mechanism of action of LPS may include the inhibition of GnRH secretion via blocking GnRH mRNA transport. The transport of the GnRH transcript to the nerve terminals in the ME requires the integrity and proper functioning of cytoskeletal elements. Increasing evidence suggests that inflammatory cytokines induce cytoskeleton rearrangements in various cells such as cardiomyocytes, intestinal epithelium, or breast cancer cells. Cytoskeleton organization is also affected by cytokines in neurons. Prolongative cytokines disrupt normal actin dynamics in Alzheimer’s disease, while IL-1β impairs the dendritic spine plasticity—substantial for LTP consolidation and memory formation—in hippocampal neurons by altering actin dynamics. Although, it is
not examined yet in GnRH neurons, it is possible that inflammation inhibits GnRH transport via proinflammatory cytokines by impairing the cytoskeleton.

5. Direct Effects of Cytokines on GnRH Neurons

Based on the findings that a subpopulation of GnRH neurons and their fibers could directly sense inflammatory molecules [26] including cytokines action in circumventricular organs [76–78], cytokines might be able to modify the functions of GnRH neurons directly. Although GnRH neurons are ideally situated to integrate immune responses on reproduction, little if any attention has been given to inflammatory factors monitoring of GnRH neurons.

Microarray studies showed that receptors associated with the progression of immune responses are abundantly expressed in mouse GnRH neurons such as interleukin, prostaglandin, TNF-α and receptors [79]. More recently immunohistochemical studies have also justified that immunomodulators can have direct impact on GnRH neurons. The expression of proinflammatory cytokine receptor IL-18Rα and the anti-inflammatory cytokine receptor IL-10R have been demonstrated in a portion of GnRH neurons providing the possibility for cytokines to act directly on GnRH neurons [61,80]. IL-10, for instance, is one of the most important anti-inflammatory cytokines balancing the immune response in the brain. Clinical studies have indicated that IL-10 is substantial for normal pregnancy, fertility, and fecundity [81–83], while IL-10 deficiency is associated with pregnancy loss, preterm birth or preeclampsia [84]. Although clinical investigations have shown correlation between the levels of peripheral IL-10 and pregnancy outcome, our recently published paper suggests that IL-10 may directly alter the function of GnRH neurons. Notably, we have found that the estrous cycle is perturbed in IL-10 KO mice, indicating that the action of IL-10 on GnRH neurons might help the maintenance of the integrity of the estrous cycle in bacterial/viral infection [61].

6. Indirect Cytokine Actions on GnRH Neurons: The Role of Glial Cells

GnRH neurons receive robust glial inputs regulating GnRH neuronal activity and secretion. The perikarya of GnRH neurons are enveloped in astrocytes, while three dimensional reconstruction of confocal images has revealed that microglia are in the vicinity of GnRH neurons [85].

Although astrocytes and microglia are in an optimal position for mediating immune responses to GnRH neurons, as they directly interact with GnRH neurons, their role in translating the effects of inflammation on the function of GnRH neurons is poorly understood. Previous studies have shown that astrocytes release immune modulators such as prostaglandin E2 (PGE2) and transforming growth factor-beta (TGFβ) to increase GnRH neuron firing and GnRH secretion under physiological conditions [86,87], but it is unexplored whether astrocytes influence GnRH functions during inflammation.

Microglia also release various cytokines. M1 phenotype microglia express pro-inflammatory factors such as interleukin 1α/β (IL-1α/β), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α), while M2-like microglia produce high levels of anti-inflammatory markers like IL-10 [38]. It has also been shown that ramified, surveying microglia but not the GnRH neuron itself express COX-1, one of the rate-limiting enzymes for prostaglandin (PG) synthesis [88]. The anatomical relationship of COX-1 immunopositive microglia and GnRH neurons and the fact that PGs are among the immune mediators influencing the regulation of GnRH secretion [89], suggest that the effect of PG on GnRH release might be due to the intercellular communication between microglia and GnRH neurons and could be disturbed during inflammation. A recently published study has described an indirect cytokine effect on GnRH neurons in aging-associated hypothalamic inflammation. In early aging TNF-α produced by activated microglia has been shown to inhibit GnRH gene expression [90].

7. Kisspeptin and RFamide-Related Peptides Mediate Inflammation on GnRH Neurons

Recent data presented that the kisspeptin system is sensitive to inflammation. Systemic endotoxin injection (LPS) in female rat decreases KISS-1 mRNA expression in the hypothalamus that consequently
suppresses LH [91,92]. Moreover, intravenous (i.v.) injection of kisspeptin reverses LPS-caused LH suppression [93]. Another study using primary cultures of human fetal hypothalamic (hfHypo) cells containing 80% of GnRH neurons investigated the effect of the pro-inflammatory cytokine, TNF-α on GnRH release. They have found that TNF-α reduces GnRH secretion via downregulating kisspeptin signaling [94]. It is worth noting that GnRH and kisspeptin expressing cells do not form separate neuronal populations in hfHypo cells, but are coexpressed, suggesting that inflammation affects GnRH neurons rather directly by modifying kisspeptin signaling in hfHypo cells [94].

Other experiments also revealed that acute LPS treatment severely affects the GnRH pulse generators, KNDy neurons. In ovary-intact ewe dynorphin immunoreactive neurons are most active 6–7 h before the LH surge, while kisspeptin and NKB neurons are maximally activated during the LH surge. This activation pattern is disturbed by LPS preventing kisspeptin and dynorphin-positive cell activation leading to a failure to evoke an LH surge [95].

Inflammation may inhibit GnRH secretion via alteration of the RFRP system as LPS injection has been demonstrated to elevate hypothalamic RFRP and GPR147 mRNA levels in rodents [91,92]. Since RFRPs modulate kisspeptin signaling, inflammation might also have an effect on GnRH pulse generation via the RFRP system.

8. The Estradiol Feedback on GnRH Neurons During Inflammation

In addition to its role as a feedback molecule on GnRH neurons, estradiol modifies the response to inflammation. As the varying level of estradiol during the estrous cycle is a key factor in regulating the secretion of GnRH neurons and estradiol is a potent immunomediator [96], it is not surprising that the effect of inflammation on GnRH neurons greatly depends on the circulating concentration of estradiol. Experiment performed in ovariectomized ewes showed that endotoxin delays the estradiol-induced LH surge [97]. Nevertheless, the LPS-induced LH surge delay is time-dependent in relation to the onset of the estradiol stimulus. LPS blocks the estradiol-induced LH surge when it is infused at the beginning of estradiol rise. In contrast, endotoxin has no effect on LH surge when it is administered at a later stage closer to the commence of the surge when an increased level of estradiol is no longer necessary [97]. Other experiments carried out in ewes have suggested that the impact of inflammation on the GnRH mRNA expression in the hypothalamus is influenced by the circulating level of estradiol. LPS might decrease GnRH content via different mechanisms depending on the circulating estradiol concentration. LPS-induced inflammation decreases the transcription of GnRH mRNA in the POA during the anestrous phase when estradiol concentration is low [50]. Contrarily, endotoxin has no effect on GnRH gene expression during the follicular phase characterized by higher estradiol level. The authors propose that the decrease in the GnRH content of the POA during the follicular phase might be due to a reduced GnRH translation [67]. Another explanation can be that endotoxin lowers plasma estradiol concentrations in the follicular phase for the time of LH surge delay thereby blocking the preovulatory estradiol rise [98].

The Role of Cholinergic Anti-Inflammatory Pathway

The cholinergic anti-inflammatory pathway is an anti-inflammatory function of the efferent vagus nerve that inhibits systemic and local inflammation [99]. As immune cells in the spleen express acetylcholine receptors, the cholinergic anti-inflammatory pathway can control cytokine secretion [67,100]. An in vitro study in human macrophage cultures indicated that ACh attenuates the endotoxin-induced release of pro-inflammatory cytokines [101]. Later, in vivo studies have reported that blocking of acetylcholine (ACh) degradation by acetylcholinesterase (AChE), the enzyme responsible for the degradation of ACh markedly attenuated IL-1β expression in mouse hippocampus [102] and LPS-induced IL-1β production in sheep hypothalamus [66]. More recent studies proved that the cholinergic anti-inflammatory pathway also has a role in hindering the effect of LPS on GnRH/LH secretion [66,67]. Peripherally administered AChEs (Neostigmine and Donepezil) eliminated the LPS-induced effects on the GnRH/LH system in the follicular phase of ewe estrous cycle. AChEs entirely abolished or reduced GnRH synthesis in the hypothalamus, while prohibited the suppression of LHβ.
gene expression and LH release and diminished the inhibition of GnRH receptor expression in the AP [67]. As parasympathetic vagus efferents are activated much faster to systemic inflammation than humoral anti-inflammatory pathways, the activation of the cholinergic anti-inflammatory pathway may serve as an important mechanism to restrict the magnitude of immune responses [101].

9. The Neuroinflammatory Processes and Function of GnRH Neurons in Aging

Aging is a gradual and general deterioration of physiological functions that affects the HPG axis. GnRH gene expression is reduced with aging leading to decreased GnRH secretion and reproductive decline [103]. The mechanism that accounts for the development of aging is unknown. Beyond its basic role in growth, development, reproduction, and metabolism, the hypothalamus has a fundamental role in systemic aging and lifespan control [104].

Aging is characterized by increased levels of circulating cytokines, pro-inflammatory markers and changes in the immune system called immunosenescence [37,105]. Similarly, mRNA levels of several cytokines and immune regulators elevated in the hypothalamus of aging mice. At the molecular level age-related inflammatory changes in the hypothalamus has been shown to be mediated by NF-κB and its upstream IkB kinase-β (IKKβ). During early aging NF-κB is activated in microglia leading to an overproduction of TNF-α. This cytokine then stimulates NF-κB signaling in hypothalamic neuronal cells. Importantly, activation of IKKβ/NF-κB inhibits GnRH release causing aging-related hypothalamic GnRH decline [90]. Interestingly, GnRH therapy or the inhibition of inflammation by blocking the activation of IKKβ or NF-κB can attenuate age-related symptoms leading to improved lifespan [90,106].

10. Pathophysiological Consequences: Effect of Obesity-Induced Inflammation on Reproductive Functions

In the last four decades, the prevalence of obesity and consequent reproductive problems have approximately tripled worldwide [13]. Optimal fat mass is indispensable for normal gonadal functions in adults, and both undernutrition and overnutrition inhibit gonadotropin production [107]. Female adult obesity is linked to menstrual cycle irregularities, ovulatory dysfunction and higher risk for miscarriages [108]. In addition to reproductive dysfunctions, chronic, low-grade systemic inflammation is also a hallmark of obesity [109]. Obesity develops when the energy homeostasis is disrupted [110]: The food intake, the energy expenditure and the energy storage become unbalanced [111]. Research over the past several decades has provided insight into control mechanisms of hypothalamus in the energy balance [112,113]. However, it is not entirely clear how obesity causes hypogonadism. Among several proposed mechanisms, one is that fat-rich diet induces hypothalamic inflammation, which in turn impairs the hormonal and neuronal circuits including the HPG axis causing reproductive disorders [114]. The mechanism by which fat-rich diet induces hypothalamic inflammation and subsequent HPG axis dysregulation is beginning to unfold. Experiments performed in dietary-induced obesity (DIO) mice suggest that hypothalamus has a prominent role in obesity-induced gonadotropin hormone level alterations. Several reports propose that hypothalamic cytokine expression pattern may have a remarkable role in the development of obesity-induced impairment of fertility [114,115]. DBA/2J mice fed high-fat diet develop dietary-induced obesity (DIO) and shows a significantly reduced pregnancy rate. It has been demonstrated that infertility manifested in DIO female DBA/2J mice is due to a suppressed GnRH expression [116]. In contrast, C57Bl/6J female mice are resistant to DIO. They require a long exposure to high-fat diet to develop obesity and even though they appear to have lengthened estrous cycles, the levels of gonadotropin hormones are not changed [115]. Ovariectomized (OVX) C57Bl/6J female mice, on the other hand, become responsive to DIO but remain resistant to gonadotropin hormone changes suggesting a protective mechanism—other than ovarian estradiol—against obesity-induced fertility problems in these female mice. It has been revealed that C57Bl/6J DIO female mice have increased levels of anti-inflammatory cytokine IL-10 in the hypothalamus, which may be able to prevent the hypothalamic inflammatory response and
a consequent HPG axis damage [115]. More recently a novel concept (GELDING—Gut Endotoxin Leading to a Decline IN Gonadal function—theory) has been proposed to provide an explanation for the development of obesity-related hypogonadism. This theory hypothesizes that high fat diet alters the gut microbiome that leads to the breakdown of the intestinal mucosal barrier and the passage of bacterial endotoxin from the gut into the blood stream [117]. As a consequence, endotoxin inhibits progesterone production in the ovary [118]. However, it is unknown whether bacterial endotoxin leaking through the gut has an effect on the GnRH neuron.

Importantly, consumption of fat-rich food triggers astrocytes and microglia to produce pro-inflammatory cytokines via the master inflammatory NF-κB signaling pathway [119,120] leading to hypothalamic inflammation. Mounting evidence suggest that long-chain saturated fatty acids (SFAs) activate glial cells to induce inflammation [121,122]. It has also been proposed that SFAs can bind to TLR4 on astrocytes, microglia and neurons as well to initiate inflammation [123–125]. However, the role of TLR4 in generating inflammation is controversial. It has been shown in human macrophages that TLR4 is not a receptor for SFAs but alters the membrane lipid composition, that is necessary for SFA-induced inflammation [126].

The role of satiety molecules such as leptin and insulin is also important in regulating the function of GnRH neurons [127–131]. These neuropeptides control reproductive functions via modulation of GnRH neurons depending on the nutritional status [132]. Leptin is a hormone mainly produced by white adipose tissue that increases energy expenditure by activating catabolic and blocking anabolic neural circuits [133]. In addition, leptin triggers the expression of GnRH and the neural activity of GnRH neurons to secrete gonadotropin hormones [134,135]. Humans and mice lacking leptin (ob/ob mice) or leptin receptor (db/db mice) become obese and infertile [136]. As inflammation induces central leptin resistance, leptin is an important link between obesity and HPG axis defects [137]. Interestingly, serum leptin levels are positively correlated with insulin resistance (IR) [138] raising the possibility that leptin is also involved in regulating IR. Indeed, leptin regulates insulin receptor substrate-1 and 2 (IRS-1, IRS2) [139], modulates glucose metabolism and the function of insulin producing pancreatic β-cells [140]. Another essential metabolic factor involved in the impairment of GnRH function by obesity-associated inflammation is insulin signaling. Obesity-induces chronic low-grade inflammation is responsible for the progression of insulin resistance and accompanying type 2 diabetes and metabolic syndrome [141]. Cytokines derived from adipocytes, inflammasomes or activated macrophages and inflammatory signaling pathways link inflammation to IR [141]. Inflammatory cytokines such as TNF-α and IL-6 increase the phosphorylation of insulin receptor substrate-1 and/or 2 (IRS-1/2) via JNK, NF-κB, TLR4, and/or JAK-STAT signaling pathways that may inhibit insulin signaling finally leading to IR. The activation of JNK and NF-κB is also engaged in the generation of pro-inflammatory cytokines, which may in turn stimulate the pathways [141]. Subsequently, IR may perturb the HPG function as it has been published in mouse: brain-specific deletion of the insulin receptor results in hypogonadotropic hypogonadism [142]. It has also been demonstrated that insulin stimulates the secretion of GnRH [143]. In summary, inflammatory signals can alter the functions of GnRH neurons via reducing insulin related mechanisms.

Currently, it is not known how main metabolic peptides, including insulin and leptin influence the function of GnRH neurons as they are lacking the corresponding receptors. One hypothesis is that kisspeptin neurons are the central sensors for leptin and insulin, integrating and transmitting the metabolic signals to the GnRH neurons [135,144]. This theory is based on findings that kisspeptin neurons express leptin and insulin receptors [144–147]. Chronically obese female mice showed a decreased KISS-1 mRNA expression in the arcuate nucleus [148], whereas fasting also had a reducing effect on KISS-1 mRNA expression in the hypothalamus of female rats [149]. Diabetic female rats exhibited lowered KISS-1 mRNA levels in the hypothalamus [150]. Additionally, leptin elevates kisspeptin gene expression [151] and is able to depolarize kisspeptin neurons [152].

Interestingly studies investigating the association between obesity and estradiol levels are inconsistent in their findings [153–155]. A recently published report suggested a possible mechanism
for how estradiol affects obesity [156]. Obesity is characterized by a pro-inflammatory state and accompanied by fertility problems. Estradiol can be a potential link between these anomalies as it is an effective anti-inflammatory factor and exerts negative feedback on gonadotropin secretion. Clinical studies comparing regularly menstruating obese and normal weight women have found that mean serum LH and its amplitude was significantly lower in obese women, while its pulse frequency was not changed suggesting the importance of pituitary in the observed alterations [156]. In addition, obese women had undoubtedly higher baseline pro-inflammatory cytokine levels such as IL-6 and IL-12. Following transdermal estrogen treatment mean LH and LH pulse amplitude increased in obese but decreased in normal weight participants [156]. Besides, estradiol treatment significantly decreased the levels of IL-1β, IL-12, and IL-8 in the serum obese subjects. FSH response was different between the two experimental groups (obese versus normal) when estradiol-treated participants received a physiologic i.v. GnRH bolus. In this case mean FSH decreased in normal weight but increased in obese women. These results provide evidence that exogenous E2 priming might have a beneficial effect on HPG axis function by improving gonadotrope sensitivity and chronic, systemic inflammation in ovulatory, obese women [156].

Taken together these findings suggest that attenuating chronic inflammation may ease the burden of obesity on fertility.

11. Conclusions

As discussed in this review inflammation is one of the underlying mechanisms of many pathological conditions such as bacterial/viral infections or obesity and even physiological processes such as aging. Inflammation may cause reproductive dysfunctions like infertility, subfertility and menstrual irregularities in all these conditions. As we pointed out the function of GnRH neurons is modified during inflammation. However, it is not clear how different pathologies alter the GnRH system. Gaining more information about the mechanism of inflammation-induced changes in the function of GnRH neurons may provide a solid platform for future therapies of heterogeneous fertility problems.

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References

1. Herbison, A.E. Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. Nat. Rev. Endocrinol. 2016, 12, 452–466. [CrossRef] [PubMed]
2. Uenoyama, Y.; Inoue, N.; Kei-Maeda, I.; Tsukamura, H. The roles of kisspeptin in the mechanism underlying reproductive functions in mammals. J. Reprod. Dev. 2018, 64, 469–476. [CrossRef] [PubMed]
3. Mills, E.G.A.; Dhillo, W.S.; Comninos, A.N. Kisspeptin and the control of emotions, mood and reproductive behaviour. J. Endocrinol. 2018, 239, R1–R12. [CrossRef] [PubMed]
4. Lehman, M.N.; Coolen, L.M.; Goodman, R.L. Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin-releasing hormone secretion. Endocrinology 2010, 151, 3479–3489. [CrossRef] [PubMed]
5. Moore, A.M.; Coolen, L.M.; Porter, D.T.; Goodman, R.L.; Lehman, M.N. KNDy cells revisited. Endocrinology 2018, 159, 3219–3234. [CrossRef]
6. Tsutsui, K.; Saigoh, E.; Ukena, K.; Teranishi, H.; Fujisawa, Y.; Kikuchi, M.; Ishii, S.; Sharp, P.J. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem. Biophys. Res. Commun. 2000, 275, 661–667. [CrossRef]
7. Ubuka, T.; Tsutsui, K. Reproductive neuroendocrinology of mammalian gonadotropin-inhibitory hormone. Reprod. Med. Biol. 2019, 18, 225–233. [CrossRef]

8. Hu, K.L.; Chang, H.M.; Li, R.; Yu, Y.; Qiao, J. Regulation of LH secretion by RFRP-3 – From the hypothalamus to the pituitary. Front. Neuroendocrinol. 2019, 52, 12–21. [CrossRef]

9. Gibson, E.M.; Humber, S.A.; Jain, S.; Williams, W.P.; Zhao, S.; Bentley, G.E.; Tsutsui, K.; Kriegsfeld, L.J. Alterations in RFamide-related peptide expression are coordinated with the preovulatory luteinizing hormone surge. Endocrinology 2008, 149, 4958–4969. [CrossRef]

10. Clarke, I.J.; Smith, J.T.; Henry, B.A.; Oldfield, B.J.; Stefanidis, A.; Millar, R.P.; Sari, I.P.; Chng, K.; Fabre-Nys, C.; Caraty, A.; et al. Gonadotropin-inhibitory hormone is a hypothalamic peptide that provides a molecular switch between reproduction and feeding. Neuroendocrinology 2012, 95, 305–316. [CrossRef]

11. Henningse, J.B.; Ancel, C.; Mikkelsen, J.D.; Gauer, F.; Simonneaux, V. Roles of RFRP-3 in the daily and seasonal regulation of reproductive activity in female Syrian hamsters. Endocrinology 2017, 158, 652–663. [CrossRef] [PubMed]

12. Tsutsumi, R.; Webster, N.J.G. GnRH pulsatility, the pituitary response and reproductive dysfunction. Endocr. J. 2009, 56, 729–737. [CrossRef] [PubMed]

13. Di Cesare, M.; Bentham, J.; Stevens, G.A.; Zhou, B.; Danaei, G.; Lu, Y.; Bixby, H.; Cowan, M.J.; Riley, L.M.; Hajifathalian, K.; et al. Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. Lancet 2016, 387, 1377–1396.

14. Liu, X.; Porteous, R.; Herbsion, A.E. Dynamics of GnRH Neuron Ionotropic GABA and Glutamate Synaptic Receptors Are Unchanged during Estrogen Positive and Negative Feedback in Female Mice. eNeuro 2017, 4. [CrossRef] [PubMed]

15. Kwakowsky, A.; Herbsion, A.E.; Ábrahám, L.M. The Role of cAMP Response Element-Binding Protein in Estrogen Negative Feedback Control of Gonadotropin-Releasing Hormone Neurons. J. Neurosci. 2012, 32, 11309–11317. [CrossRef]

16. Segner, H.; Verburg-van Kemenade, B.M.L.; Chadzinska, M. The immunomodulatory role of the hypothalamus-pituitary-gonad axis: Proximate mechanism for reproduction-immune trade offs? Dev. Comp. Immunol. 2017, 66, 43–60. [CrossRef]

17. Prinz, M.; Priller, J. The role of peripheral immune cells in the CNS in steady state and disease. Nat. Neurosci. 2017, 20, 136–144. [CrossRef]

18. Vitkovic, L.; Konsman, J.P.; Bockaert, J.; Dantzer, R.; Homburger, V.; Jacque, C. Cytokine signals propagate through the brain. Mol. Psychiatry 2000, 5, 604–615. [CrossRef]

19. Maness, L.M.; Banks, W.A.; Zadina, J.E.; Kastin, A.J. Selective transport of blood-borne interleukin-1x into the posterior division of the septum of the mouse brain. Brain Res. 1995, 700, 83–88. [CrossRef]

20. Förster, C. Tight junctions and the modulation of barrier function in disease. Histochem. Cell Biol. 2008, 130, 55–70. [CrossRef]

21. Klein, R.S.; Garber, C.; Funk, K.E.; Salimi, H.; Soung, A.; Kamnoghe, M.; Manivasagam, S.; Agner, S.; Cain, M. Neuroinflammation During RNA Viral Infections. Annu. Rev. Immunol. 2019, 37, 73–95. [CrossRef] [PubMed]

22. Chen, Q.; Liu, Y.; Lu, A.; Ni, K.; Xiang, Z.; Wen, K.; Tu, W. Influenza virus infection exacerbates experimental autoimmune encephalomyelitis disease by promoting type I T cells infiltration into central nervous system. J. Autoimmun. 2017, 77, 1–10. [CrossRef] [PubMed]

23. Wilson, E.H.; Wille-Reece, U.; Dzierszinski, F.; Hunter, C.A. A critical role for IL-10 in limiting inflammation during toxoplasmic encephalitis. J. Neuroimmunol. 2005, 165, 63–74. [CrossRef] [PubMed]

24. Johnson, R.H.; Kho, D.T.; O’Carroll, S.J.; Angel, C.E.; Graham, E.S. The functional and inflammatory response of brain endothelial cells to Toll-Like Receptor agonists. Sci. Rep. 2018, 8, 1–12. [CrossRef]

25. Mitchell, K.; Yang, H.Y.T.; Berk, J.D.; Tran, J.H.; Iadarola, M.J. Monocyte chemoattractant protein-1 in the choroid plexus: A potential link between vascular pro-inflammatory mediators and the CNS during peripheral tissue inflammation. Neuroscience 2009, 158, 885–895. [CrossRef]

26. Herde, M.K.; Geist, K.; Campbell, R.E.; Herbsion, A.E. Gonadotropin-releasing hormone neurons extend complex highly branched dendritic trees outside the blood-brain barrier. Endocrinology 2011, 152, 3832–3841. [CrossRef]

27. Engelhardt, B.; Sorokin, L. The blood-brain and the blood-cerebrospinal fluid barriers: Function and dysfunction. Semin. Immunopathol. 2009, 31, 497–511. [CrossRef]
28. Quintana, F.J. Astrocytes to the rescue! Glia limitans astrocytic endfeet control CNS inflammation. *J. Clin. Investig.* 2017, 127, 2897–2899. [CrossRef]

29. Hennessy, E.; Griffin, E.W.; Cunningham, C. Astrocytes are primed by chronic neurodegeneration to produce exaggerated chemokine and cell infiltration responses to acute stimulation with the cytokines IL-1β and TNF-α. *J. Neurosci.* 2015, 35, 8411–8422. [CrossRef]

30. Sofroniew, M. V. Astrocyte barriers to neurotoxic inflammation. *Nat. Rev. Neurosci.* 2015, 16, 249–263. [CrossRef]

31. Li, K.; Li, J.; Zheng, J.; Qin, S. Reactive Astrocytes in Neurodegenerative Diseases. *Aging Dis.* 2019, 10, 664. [CrossRef] [PubMed]

32. Cekanaviciute, E.; Buckwalter, M.S. Astrocytes: Integrative Regulators of Neuroinflammation in Stroke and Other Neurological Diseases. *Neurotherapeutics* 2016, 13, 685–701. [CrossRef] [PubMed]

33. Chapouly, C.; Argaw, A.T.; Horng, S.; Castro, K.; Zhang, J.; Asp, L.; Loo, H.; Laitman, B.M.; Mariani, J.N.; Farber, R.S.; et al. Astrocytic TYMP and VEGFA drive blood-brain barrier opening in inflammatory central nervous system lesions. *Brain* 2015, 138, 1548–1567. [CrossRef] [PubMed]

34. Varatharaj, A.; Galea, I. The blood-brain barrier in systemic inflammation. *Brain Behav. Immun.* 2017, 60, 1–12. [CrossRef]

35. Liebner, S.; Dijkhuizen, R.M.; Reiss, Y.; Plate, K.H.; Agalliu, D.; Constantin, G. Functional morphology of the blood–brain barrier in health and disease. *Acta Neuropathol.* 2018, 135, 311–336. [CrossRef]

36. Jha, M.K.; Jo, M.; Kim, J.H.; Suk, K. Microglia-Astrocyte Crosstalk: An Intimate Molecular Conversation. *Neuroscientist* 2019, 25, 227–240. [CrossRef]

37. Skaper, S.D.; Facci, L.; Zusso, M.; Giusti, P. An inflammation-centric view of neurological disease: Beyond the neuron. *Front. Cell. Neurosci.* 2018, 12, 72. [CrossRef]

38. Wang, J.; Wang, J.; Wang, J.; Yang, B.; Weng, Q.; He, Q. Targeting microglia and macrophages: A potential treatment strategy for multiple sclerosis. *Front. Pharmacol.* 2019, 10, 286. [CrossRef]

39. Li, L.X.; McSorley, S.J. A re-evaluation of the role of B cells in protective immunity to Chlamydia infection. *Immunol. Lett.* 2015, 164, 88–93. [CrossRef]

40. Qian, L.; Li, Q.; Li, H. Effect of hepatitis B virus infection on sperm quality and oxidative stress state of the semen of infertile males. *Am. J. Reprod. Immunol.* 2016, 76, 183–185. [CrossRef]

41. Malvezzi, H.; Hernandes, C.; Piccinato, C.A.; Podgaec, S. Interleukin in endometriosis-associated infertility-pelvic pain: Systematic review and meta-analysis. *Reproduction* 2019, 158, 1–12. [CrossRef] [PubMed]

42. Syriou, V.; Papanikolaou, D.; Kozyraki, A.; Goulis, D.G. Cytokines and male infertility. *Eur. Cytokine Netw.* 2018, 29, 73–82. [CrossRef] [PubMed]

43. Pauli, S.A.; Berga, S.L. Athletic amenorrhea: Energy deficit or psychogenic challenge. *Ann. N. Y. Acad. Sci.* 2010, 1205, 33–38. [CrossRef] [PubMed]

44. Bidne, K.L.; Dickson, M.J.; Ross, J.W.; Baumgard, L.H.; Keating, A.F. Disruption of female reproductive function by endotoxins. *Reproduction* 2018, 155, R169–R181. [CrossRef]

45. Cardoso, N.; Arias, P.; Szwarcarfarb, B.; Ponzo, O.; Carbone, S.; Moguilevsky, J.; Scacchi, P.; Reynoso, R.M. Reproductive axis response to repeated lipopolysaccharide administration in peripubertal female rats. *J. Physiol. Biochem.* 2010, 66, 237–244. [CrossRef]

46. Herman, A.P.; Krawczyńska, A.; Bochenek, J.; Dobek, E.; Herman, A.; Tomaszewska-Zaremba, D. LPS-induced inflammation potentiates the IL-1β-mediated reduction of LH secretion from the anterior pituitary explants. *Clin. Dev. Immunol.* 2013, 2013, 926937. [CrossRef]

47. Wojtulewicz, K.; Tomaszewska-Zaremba, D.; Herman, A.P. Endotoxin-induced inflammation suppresses the effect of melatonin on the release of LH from the ovine pars tuberalis explants—Ex vivo study. *Molecules* 2017, 22, 1933. [CrossRef]

48. Long, K.L.P.; Bailey, A.M.; Greives, T.J.; Legan, S.J.; Demas, G.E. Endotoxin rapidly desensitizes the gonads to kisspeptin-induced luteinizing hormone release in male Siberian hamsters (*Phodopus sungorus*). *J. Exp. Biol.* 2018, 221, jeb185504. [CrossRef]

49. Karsch, F.J.; Battaglia, D.F.; Breen, K.M.; Debus, N.; Harris, T.G. Mechanisms for Ovarian Cycle Disruption by Immune/inflammatory Stress. *Stress* 2002, 5, 101–112. [CrossRef]
50. Herman, A.P.; Tomaszewska-Zaremba, D. Effect of endotoxin on the expression of GnRH and GnRHR genes in the hypothalamus and anterior pituitary gland of anestrous ewes. Anim. Reprod. Sci. 2010, 120, 105–111. [CrossRef]
51. Nanni, R.E.; Rivest, S. Effect of immune and metabolic challenges on the luteinizing hormone-releasing hormone neuronal system in cycling female rats: An evaluation at the transcriptional level. Endocrinology 1997, 138, 1374–1384. [CrossRef] [PubMed]
52. Herman, A.P.; Skipor, J.; Krawczyńska, A.; Bochenek, J.; Wojtulewicz, K.; Pawlina, B.; Antushevich, H.; Herman, A.; Tomaszewska-Zaremba, D. Effect of central injection of neostigmine on the bacterial endotoxin induced suppression of GnRH/LH secretion in ewes during the follicular phase of the estrous cycle. Int. J. Mol. Sci. 2019, 20, 4598. [CrossRef] [PubMed]
53. Refojo, D.; Arias, P.; Moguilevsky, J.A.; Feleder, C. Effects of endotoxin on LH secretion in the ovariectomized monkey are prevented by naloxone but not by an interleukin-1 receptor antagonist. Neuroimmunomodulation 2000, 7, 6–15. [CrossRef]
54. Haziak, K.; Herman, A.P.; Tomaszewska-Zaremba, D. Effects of Central Injection of Anti-LPS Antibody and Blockade of TLR4 on GnRH/LH Secretion during Immunological Stress in Anestrous Ewes. Mediators Inflamm. 2014, 2014, 867170. [CrossRef]
55. Haziak, K.; Herman, A.P.; Wojtulewicz, K.; Pawlina, B.; Paczesna, K.; Bochenek, J.; Tomaszewska-Zaremba, D. Effect of CD14/TLR4 antagonist on GnRH/LH secretion in ewe during central inflammation induced by intracerebroventricular administration of LPS. J. Anim. Sci. Biotechnol. 2018, 9, 52. [CrossRef]
56. Lewis, A.J.; Seymour, C.W.; Rosengart, M.R. Current Murine Models of Sepsis. Toxins 2019, 11, 91. [CrossRef]
57. Watanobe, H.; Hayakawa, Y. Hypothalamic Interleukin-1β and Tumor Necrosis Factor-α, but Not Interleukin-6, Mediate the Endotoxin-Induced Suppression of the Reproductive Axis in Rats. Endocrinology 2003, 144, 4868–4875. [CrossRef] [PubMed]
58. Dickson, K.; Lehmann, C. Inflammatory response to different toxins in experimental sepsis models. Int. J. Mol. Sci. 2019, 20, 4341. [CrossRef]
59. Mikolajczyk, A.; Zlotkowska, D. Subclinical lipopolysaccharide from salmonella enteritidis induces dysregulation of bioactive substances from selected brain sections and glands of neuroendocrine axes. Toxins 2019, 11, 91. [CrossRef]
60. Barabásí, K.; Barad, Z.; Dénes, Á.; Bhattarai, J.P.; Han, S.-K.; Kiss, E.; Sármay, G.; Ábraháám, I.M. The Role of Interleukin-10 in Mediating the Effect of Immune Challenge on Mouse Gonadotropin-Releasing Hormone Neurons In Vivo. eNeuro 2018, 5. [CrossRef]
61. Rivier, C.; Vale, W. Cytokines Act within the Brain to Inhibit Luteinizing Hormone Secretion and Ovulation in the Rat. Endocrinology 1990, 127, 849–856. [CrossRef] [PubMed]
62. Watanobe, H.; Hayakawa, Y. Hypothalamic Interleukin-1β and Tumor Necrosis Factor-α, but Not Interleukin-6, Mediate the Endotoxin-Induced Suppression of the Reproductive Axis in Rats. Endocrinology 2003, 144, 4868–4875. [CrossRef] [PubMed]
63. Herm, A.P.; Misztal, T.; Romanowicz, K.; Tomaszewska-Zaremba, D. Central injection of exogenous IL-1β in the control activities of hypothalamic-pituitary-gonadal axis in anestrous ewes. Reprod. Domest. Anim. 2012, 47, 44–52. [CrossRef] [PubMed]
64. Kang, S.S.; Kim, S.R.; Leonhardt, S.; Jarry, H.; Wuttke, W.; Kim, K. Effect of interleukin-1β on gonadotropin-releasing hormone (GnRH) and GnRH receptor gene expression in castrated male rats. J. Neuroendocrinol. 2000, 12, 421–429. [CrossRef] [PubMed]
65. Herman, A.P.; Krawczyńska, A.; Bochenek, J.; Haziak, K.; Romanowicz, K.; Misztal, T.; Antushevich, H.; Herman, A.; Tomaszewska-Zaremba, D. The effect of rivastigmine on the LPS-induced suppression of GnRH/LH secretion during the follicular phase of the estrous cycle in ewes. Anim. Reprod. Sci. 2013, 138, 203–212. [CrossRef]
67. Herman, A.P.; Skipor, J.; Krawczyńska, A.; Bochenek, J.; Wojtulewicz, K.; Antushevich, H.; Herman, A.; Paczesna, K.; Romanowicz, K.; Tomaszewska-Zaremba, D. Peripheral Inhibitor of AChE, Neostigmine, Prevents the Inflammatory Dependent Suppression of GnRH/LH Secretion during the Follicular Phase of the Estrous Cycle. *Biomed Res. Int.* 2017, 2017, 6823209. [CrossRef]

68. Jakubowski, M.; Roberts, J.L. Processing of gonadotropin-releasing hormone gene transcripts in the rat brain. *J. Biol. Chem.* 1994, 269, 4078–4083.

69. Yeo, T.S.; Gore, A.C.; Jakubowski, M.; Dong, K.W.; Blum, M.; Roberts, J.L. Characterization of gonadotropin-releasing hormone gene transcripts in a mouse hypothalamic neuronal GT1 cell line. *Mol. Brain Res.* 1996, 42, 255–262. [CrossRef]

70. Gore, A.C. Gonadotropin-releasing hormone (GnRH) neurons: Gene expression and neuroanatomical studies. *Prog. Brain Res.* 2002, 141, 193–208.

71. Buoncervello, M.; Maccari, S.; Asione, B.; Gambardella, L.; Marconi, M.; Spada, M.; Macchia, D.; Stati, T.; Patrizio, M.; Malorni, W.; et al. Inflammatory cytokines associated with cancer growth induce mitochondria and cytoskeleton alterations in cardiomyocytes. *J. Cell. Physiol.* 2019. [CrossRef] [PubMed]

72. Ott, D.; Murgott, J.; Rafalzik, S.; Wuchert, F.; Schmalenbeck, B.; Roth, J.; Gerstberger, R. Neurons and glial cells of the rat organum vasculosum laminae terminalis directly respond to lipopolysaccharide and pyrogenic cytokines. *J. Comp. Neurol.* 2000, 1363, 93–106. [CrossRef]

73. Jasoni, C.L.; Todman, M.G.; Han, S.K.; Herbison, A.E. Expression of mRNAs encoding receptors that mediate stress signals in gonadotropin-releasing hormone neurons of mouse and rat forebrain. *Neurosci. Lett.* 2017, 650, 33–37. [CrossRef]

74. Walsh, K.P.; Minamide, L.S.; Kane, S.J.; Shaw, A.E.; Brown, D.R.; Pulford, B.; Zabel, M.D.; Lambeth, J.D.; Lincoln, D.; Linfield, D.; Rezaee, F.; Janigro, D.; Marchi, N.; van Boxel-Dezaire, A.H.H. IFN-γ van Boxel-Dezaire, A.H.H. IFN-γ and zonulin rapidly increase the permeability of the blood–brain and small intestinal epithelial barriers: Relevance for neuro-inflammatory diseases. *Biochem. Biophys. Res. Commun.* 2018, 507, 274–279. [CrossRef] [PubMed]

75. Tong, L.; Prieto, G.A.; Cotman, C.W. IL-1β suppresses cLTP-induced surface expression of GluA1 and actin polymerization via ceramide-mediated Src activation. *J. Neuroinflammation* 2018, 15, 127. [CrossRef]

76. Damm, J.; Luheshi, G.N.; Gerstberger, R.; Roth, J.; Rummel, C. Spatiotemporal nuclear factor interleukin-6 expression in the rat brain during lipopolysaccharide-induced fever is linked to sustained hypothalamic inflammatory target gene induction. *J. Comp. Neurol.* 2011, 519, 480–505. [CrossRef]

77. Kulkarni, A.H.; Chatterjee, A.; Kondaiah, P.; Gundiah, N. TGF-β induces changes in breast cancer cell deformability. *Phys. Biol.* 2015, 12, 65005. [CrossRef] [PubMed]

78. Ott, D.; Murgott, J.; Rafalzik, S.; Wuchert, F.; Schmalenbeck, B.; Roth, J.; Gerstberger, R. Neurons and glial cells of the rat organum vasculosum laminae terminalis directly respond to lipopolysaccharide and pyrogenic cytokines. *Brain Res.* 2010, 1363, 93–106. [CrossRef]

79. Jasoni, C.L.; Todman, M.G.; Han, S.K.; Herbison, A.E. Expression of mRNAs encoding receptors that mediate stress signals in gonadotropin-releasing hormone neurons of mouse and rat forebrain. *Neurosci. Lett.* 2017, 650, 33–37. [CrossRef]

80. Kuwahara-Otani, S.; Maeda, S.; Kobayashi, K.; Minato, Y.; Tanaka, K.; Yamanishi, K.; Hata, M.; Li, W.; Hayakawa, T.; Noguchi, K.; et al. Interleukin-18 and its receptor are expressed in gonadotropin-releasing hormone gene transcripts in a mouse hypothalamic neuronal GT1 cell line. *J. Biol. Chem.* 1994, 269, 4078–4083. [CrossRef]

81. Wegmann, T.G.; Lin, H.; Guilbert, L.; Mosmann, T.R. Bidirectional cytokine interactions in the maternal-fetal relationship: Is successful pregnancy a TH2 phenomenon? *Immunol. Today* 1993, 14, 353–356. [CrossRef]

82. Van Dunne, F.M.; de Craen, A.J.M.; Helmerhorst, F.M.; Huizinga, T.W.J.; Westendorp, R.G.J. Interleukin-10 promoter polymorphisms in male and female fertility and fecundity. *Genes Immun.* 2006, 7, 688–692. [CrossRef] [PubMed]

83. Cheng, S.B.; Sharma, S. Interleukin-10: A pleiotropic regulator in pregnancy. *Am. J. Reprod. Immunol.* 2015, 73, 487–500. [CrossRef] [PubMed]

84. Thaxton, J.E.; Sharma, S. Interleukin-10: A Multi-Faceted Agent of Pregnancy. *Am. J. Reprod. Immunol.* 2010, 63, 482–491. [CrossRef] [PubMed]

85. Fujioka, H.; Kakehashi, C.; Funabashi, T.; Akema, T. Immunohistochemical evidence for the relationship between microglia and GnRH neurons in the preoptic area of ovariecotomized rats with and without steroid replacement. *Endocr. J.* 2013, 60, 191–196. [CrossRef]
86. Clasadonte, J.; Poulain, P.; Hanchate, N.K.; Corfas, G.; Ojeda, S.R.; Prevot, V. Prostaglandin E2 release from astrocytes triggers gonadotropin-releasing hormone (GnRH) neuron firing via EP2 receptor activation. *Proc. Natl. Acad. Sci. USA* 2011, 108, 16104–16109. [CrossRef]

87. Sharif, A.; Baroncini, M.; Prevot, V. Role of glia in the regulation of gonadotropin-releasing hormone neuronal activity and secretion. *Neuroendocrinology* 2013, 98, 1–15. [CrossRef]

88. Adachi, S.; Fujioka, H.; Kakehashi, C.; Matsuwaki, T.; Nishihara, M.; Akema, T. Possible involvement of microglia containing cyclooxygenase-1 in the accumulation of gonadotrophin-releasing hormone in the preoptic area in female rats. *J. Neuroendocrinol.* 2009, 21, 1029–1037. [CrossRef]

89. Tomaszewska-Zaremba, D.; Herman, A. The role of immunological system in the regulation of gonadoliberin and gonadotropin secretion. *Reprod. Biol.* 2009, 9, 11–23. [CrossRef]

90. Zhang, G.; Li, J.; Purkayastha, S.; Tang, Y.; Zhang, H.; Yin, Y.; Li, B.; Liu, G.; Cai, D. Hypothalamic Fergani, C.; Routly, J.E.; Jones, D.N.; Pickavance, L.C.; Smith, R.F.; Dobson, H. KNDy neurone activation by lipopolysaccharide in female rats. *Horm. Behav.* 2014, 66, 309–316. [CrossRef] [PubMed]

91. Lee, C.Y.; Li, S.Y.; Li, X.F.; Stalker, D.A.E.; Cooke, C.; Shao, B.; Kelestimur, H.; Henry, B.A.; Conductier, G.; O’Byrne, K.T.; et al. Lipopolysaccharide reduces gonadotrophin-releasing hormone (GnRH) gene expression: Role of RFamide-related peptide-3 and kisspeptin. *Reprod. Fertil. Dev.* 2019, 31, 1134–1143. [CrossRef] [PubMed]

92. Iwasa, T.; Matsuzaki, T.; Murakami, M.; Shimizu, F.; Kuwahara, A.; Yasui, T.; Irahara, M. Decreased expression of kisspeptin mediates acute immune/inflammatory stress-induced suppression of gonadotropin secretion in female rat. *J. Endocrinol. Investig.* 2008, 31, 656–659. [CrossRef] [PubMed]

93. Sarchielli, E.; Comeglio, P.; Squecco, R.; Ballerini, L.; Mello, T.; Guarnieri, G.; Idrizaj, E.; Mazzanti, B.; Vignozzi, L.; Gallina, P.; et al. Tumor necrosis factor-αVignozzi, L.; Gallina, P. et al. Tumor necrosis factor-α impairs kisspeptin signaling in human gonadotropin-releasing hormone primary neurons. *J. Clin. Endocrinol. Metab.* 2017, 102, 46–56. [CrossRef]

94. Fergani, C.; Routly, J.E.; Jones, D.N.; Pickavance, L.C.; Smith, R.F.; Dobson, H. KNDy neurone activation prior to the LH surge of the ewe is disrupted by LPS. *Reproduction* 2017, 154, 281–292. [CrossRef]

95. Bupp, M.R.G.; Potluri, T.; Fink, A.L.; Klein, S.L. The confluence of sex hormones and aging on immunity. *Front. Immunol.* 2018, 9, 1269. [CrossRef]

96. Battaglia, D.F.; Beaver, A.B.; Harris, T.G.; Tanhehco, E.; Viguié, C.; Karsch, F.J. Endotoxin disrupts the estradiol-induced luteinizing hormone surge: Interference with estradiol signal reading, not surge release. *Endocrinology* 1999, 140, 2471–2479. [CrossRef]

97. Fergani, C.; Safullizam, A.K.; Routly, J.E.; Smith, R.F.; Dobson, H. Estrous behavior, luteinizing hormone and estradiol profiles of intact ewes treated with insulin or endotoxin. *Physiol. Behav.* 2012, 105, 757–765. [CrossRef]

98. Pavlov, V.A.; Wang, H.; Czura, C.J.; Friedman, S.G.; Tracey, K.J. The Cholinergic Anti-inflammatory Pathway: A Missing Link in Neuroimmunomodulation. *Mol. Med.* 2003, 9, 125–134. [CrossRef]

99. Rosas-Ballina, M.; Ochani, M.; Parrish, W.R.; Ochani, K.; Harris, Y.T.; Huston, J.M.; Chavan, S.; Tracey, K.J. Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proc. Natl. Acad. Sci. USA* 2008, 105, 11008–11013. [CrossRef]

100. Borovikova, L.V.; Ivanova, S.; Zhang, M.; Yang, H.; Botchkina, G.I.; Watkins, L.R.; Wang, H.; Abumrad, N.; Eaton, J.W.; Tracey, K.J. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 2004, 405, 458–462. [CrossRef]

101. Pollak, Y.; Gilboa, A.; Ben-Menachem, O.; Ben-Hur, T.; Soreq, H.; Yirmiya, R. Acetylcholinesterase inhibitors reduce brain and blood interleukin-1β production. *Ann. Neurol.* 2005, 57, 741–745. [CrossRef]

102. Gruenewald, D.A.; Naai, M.A.; Marck, B.T.; Matsumoto, A.M. Age-Related Decrease in Hypothalmic Prostaglandin—Norway Rat. *Blood* 2000, 110, 72–84.

103. Kim, K.; Choe, H.K. Role of hypothalamus in aging and its underlying cellular mechanisms. *Mech. Ageing Dev.* 2019, 177, 74–79. [CrossRef]

104. Michaud, M.; Balardy, L.; Moulis, G.; Gaudin, C.; Peyrot, C.; Vellas, B.; Cesari, M.; Nourhashemi, F. Proinflammatory cytokines, aging, and age-related diseases. *J. Am. Med. Dir. Assoc.* 2013, 14, 877–882. [CrossRef] [PubMed]
106. Purkayastha, S.; Cai, D. Disruption of neurogenesis by hypothalamic inflammation in obesity or aging. *Rev. Endocr. Metab. Disord.* 2013, 14, 351–356. [CrossRef]

107. Rosenfield, R.L.; Bordini, B. Evidence that obesity and androgens have independent and opposing effects on gonadotropin production from puberty to maturity. *Brain Res.* 2010, 1364, 186–197. [CrossRef] [PubMed]

108. Nelson, S.M.; Fleming, R. Obesity and reproduction: Impact and interventions. *Curr. Opin. Obstet. Gynecol.* 2007, 19, 384–389. [CrossRef] [PubMed]

109. Xu, H. Obesity and metabolic inflammation. *Drug Discov. Today. Dis. Mech.* 2013, 10, 21–25. [CrossRef]

110. Hill, J.O.; Wyatt, H.R.; Peters, J.C. Energy balance and obesity. *Circulation* 2012, 126, 126–132. [CrossRef]

111. Lainez, N.M.; Jonak, C.R.; Nair, M.G.; Ethell, I.M.; Wilson, E.H.; Carson, M.J.; Coss, D. Diet-induced obesity and inflammation and energy balance disruptions: Spotlight on chemokines. *Front. Endocrinol.* 2017, 8, 197. [CrossRef] [PubMed]

112. Obri, A.; Claret, M. The role of epigenetics in hypothalamic energy balance control: Implications for obesity. *Cell Stress* 2019, 3, 208–220. [CrossRef] [PubMed]

113. Morelli, A.; Sarchielli, E.; Comeglio, P.; Filippi, S.; Vignozzi, L.; Marini, M.; Rastrelli, G.; Maneschi, E.; Cellai, I.; Persani, L.; et al. Metabolic syndrome induces inflammation and impairs gonadotropin-releasing hormone neurons in the preoptic area of the hypothalamus in rabbits. *Mol. Cell. Endocrinol.* 2014, 382, 107–119. [CrossRef] [PubMed]

114. Dalvi, P.S.; Chalmers, J.A.; Luo, V.; Han, D.Y.; Wellhauser, L.; Liu, Y.; Tran, D.Q.; Castel, J.; Luquet, S.; Wheeler, M.B.; et al. High fat induces acute and chronic inflammation in the hypothalamus: Effects of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons. *Int. J. Obes.* 2017, 41, 149–158. [CrossRef] [PubMed]

115. Fritsche, K.L. The Science of Fatty Acids and Inflammation 1–3. *Adv. Nutr.* 2015, 6, 293–301. [CrossRef] [PubMed]

116. Dalvi, P.S.; Chalmers, J.A.; Luo, V.; Han, D.Y.; Wellhauser, L.; Liu, Y.; Tran, D.Q.; Castel, J.; Luquet, S.; Wheeler, M.B.; et al. High fat induces acute and chronic inflammation in the hypothalamus: Effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons. *Int. J. Obes.* 2017, 41, 149–158. [CrossRef] [PubMed]

117. Zhao, Y.; Li, G.; Li, Y.; Wang, Y.; Liu, Z. Knockdown of Tlr4 in the Arcuate Nucleus Improves Obesity Related Metabolic Disorders. *Sci. Rep.* 2017, 7, 7441. [CrossRef] [PubMed]

118. Huang, S.; Rutkowski, J.M.; Snodgrass, R.G.; Ono-Moore, K.D.; Schneider, D.A.; Newman, J.W.; Adams, S.H.; Hwang, D.H. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. *J. Lipid Res.* 2012, 53, 2002–2013. [CrossRef] [PubMed]

119. Gupta, S.; Knight, A.G.; Gupta, S.; Keller, J.N.; Bruce-Keller, A.J. Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. *J. Neurochem.* 2012, 120, 1060–1071. [CrossRef] [PubMed]

120. Lancaster, G.I.; Langley, K.G.; Berglund, N.A.; Kammoun, H.L.; Reibe, S.; Estevez, E.; Weir, J.; Mellett, N.A.; Pernes, G.; Conway, J.R.W.; et al. Evidence that TLR4 Is Not a Receptor for Saturated Fatty Acids but Mediates Lipid-Induced Inflammation by Reprogramming Macrophage Metabolism. *Cell Metab.* 2018, 27, 1096–1110. [CrossRef] [PubMed]

121. Muccioli, G.; Lorenzi, T.; Lorenzi, M.; Gh, C.; Arnoletti, E.; Raso, G.M.; Castellucci, M.; Gualillo, O.; Meli, R. Beyond the metabolic role of ghrelin: A new player in the regulation of reproductive function. *Peptides* 2011, 32, 2514–2521. [CrossRef]
128. Fernández-Fernández, R.; Tena-Sempere, M.; Navarro, V.M.; Barreiro, M.L.; Castellano, J.M.; Aguilar, E.; Pinilla, L. Effects of Ghrelin upon Gonadotropin-Releasing Hormone and Gonadotropin Secretion in Adult Female Rats: In vivo and in vitro Studies. *Neuroendocrinology* 2005, 82, 245–255. [CrossRef]

129. Reynoso, R.; Ponzo, O.J.; Szwarcfarb, B.; Rondina, D.; Carbone, S.; Rimoldi, G.; Scacchi, P.; Moguilevsky, J.A. Effect of leptin on hypothalamic release of gnrh and neurotransmitter amino acids during sexual maturation in female rats. *Exp. Clin. Endocrinol. Diabetes* 2003, 111, 274–277. [CrossRef]

130. DiVall, S.A.; Williams, T.R.; Carver, S.E.; Koch, L.; Brüning, J.C.; Kahn, C.R.; Wondisford, F.; Radovick, S.; Wolfe, A. Divergent roles of growth factors in the GnRH regulation of puberty in mice. *J. Clin. Investig.* 2010, 120, 2900–2909. [CrossRef]

131. Celik, O.; Aydin, S.; Celik, N.; Yilmaz, M. Peptides: Basic determinants of reproductive functions. *Peptides* 2015, 72, 34–43. [CrossRef] [PubMed]

132. Evans, M.C.; Anderson, G.M. Neuroendocrine integration of nutritional signals on reproduction. *J. Mol. Endocrinol.* 2017, 58, R107–R128. [CrossRef] [PubMed]

133. Stofkova, A. Leptin and Adiponectin: From energy and metabolic dysbalance to inflammation and autoimmunity. *Endocr. Regul.* 2009, 43, 157–168. [PubMed]

134. Quennell, J.H.; Mulligan, A.C.; Tups, A.; Liu, X.; Phipps, S.J.; Kemp, C.J.; Herbison, A.E.; Grattan, D.R.; Anderson, G.M. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology* 2009, 150, 2805–2812. [CrossRef]

135. Tsatsanis, C.; Dermitzaki, E.; Avgoustinaki, P.; Malliaraki, N.; Mytaras, V.; Margioris, A.N. The impact of adipose tissue-derived factors on the hypothalamic-pituitary-gonadal (HPG) axis. *Hormones* 2015, 14, 549–562. [CrossRef]

136. Sadaf Farooqi, I.; O’Rahilly, S. Leptin: A pivotal regulator of human energy homeostasis. *Am. J. Clin. Nutr.* 2009, 89, 980–984. [CrossRef]

137. De Git, K.C.G.; Adan, R.A.H. Leptin resistance in diet-induced obesity: The role of hypothalamic inflammation. *Obes. Rev.* 2015, 16, 207–224. [CrossRef]

138. Osegbe, I.; Okpara, H.; Azinge, E. Relationship between serum leptin and insulin resistance among obese Nigerian women. *Ann. Afr. Med.* 2016, 15, 14–19. [CrossRef]

139. Niswender, K.D.; Baskin, D.G.; Schwartz, M.W. Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends Endocrinol. Metab.* 2004, 15, 362–369. [CrossRef]

140. Marroquín, L.; Gonzalez, A.; Nencio, P.; Caballero-Garrido, E.; Vieira, E.; Ripoll, C.; Nadal, A.; Quesada, I. Role of leptin in the pancreatic β-cell: Effects and signaling pathways. *J. Mol. Endocrinol.* 2012, 49, R9–R17. [CrossRef]

141. Chen, L.; Chen, R.; Wang, H.; Liang, F. Mechanisms Linking Inflammation to Insulin Resistance. *Int. J. Mol. Sci.* 2015, 2015, 508409. [CrossRef] [PubMed]

142. Bruning, J.C.; Gautam, D.; Burks, D.J.; Gillette, J.; Schubert, M.; Orban, P.C.; Klein, R.; Krone, W.; Muller-Wieland, D.; Kahn, C.R. Role of brain insulin receptor in control of body weight and reproduction. *Science* 2000, 289, 2122–2125. [CrossRef] [PubMed]

143. DiVall, S.A.; Herrera, D.; Sklar, B.; Wu, S.; Wondisford, F.; Radovick, S.; Wolfe, A. Insulin receptor signaling in the GnRH neuron plays a role in the abnormal GnRH pulsatility of obese female mice. *PLoS ONE* 2015, 10, e0119995. [CrossRef] [PubMed]

144. Qiu, X.; Dao, H.; Wang, M.; Heston, A.; Garcia, K.M.; Sangal, A.; Dowling, A.R.; Faulkner, L.D.; Molitor, S.C.; Elias, C.F.; et al. Insulin and leptin signaling interact in the mouse Kiss1 neuron during the peripubertal period. *PLoS ONE* 2015, 10, e0121974. [CrossRef]

145. Smith, J.T.; Acohido, B.V.; Clifton, D.K.; Steiner, R.A. KiSS-1 neurones are direct targets for leptin in the ob/ob mouse. *J. Neuroendocrinol.* 2006, 18, 298–303. [CrossRef]

146. Cravo, R.M.; Margatho, L.O.; Osborne-Lawrence, S.; Donato, J.; Atkin, S.; Bookout, A.L.; Rovinsky, S.; Frazão, R.; Lee, C.E.; Gautron, L.; et al. Characterization of Kiss1 neurons using transgenic mouse models. *Neuroscience* 2011, 173, 37–56. [CrossRef]

147. Qiu, X.; Dowling, A.R.; Marino, J.S.; Faulkner, L.D.; Bryant, B.; Brüning, J.C.; Elias, C.F.; Hill, J.W. Delayed puberty but normal fertility in mice with selective deletion of insulin receptors from kiss1 cells. *Endocrinology* 2013, 154, 1337–1348. [CrossRef]
148. Quennell, J.H.; Howell, C.S.; Roa, J.; Augustine, R.A.; Grattan, D.R.; Anderson, G.M. Leptin deficiency and diet-induced obesity reduce hypothalamic kisspeptin expression in mice. *Endocrinology* **2011**, *152*, 1541–1550. [CrossRef]

149. Castellano, J.M.; Navarro, V.M.; Fernández-Fernández, R.; Nogueiras, R.; Tovar, S.; Roa, J.; Vazquez, M.J.; Vigo, E.; Casanueva, F.F.; Aguilar, E.; et al. Changes in hypothalamic KiSS-1 system and restoration of pubertal activation of the reproductive axis by kisspeptin in undernutrition. *Endocrinology* **2005**, *146*, 3917–3925. [CrossRef]

150. Castellano, J.M.; Navarro, V.M.; Roa, J.; Pineda, R.; Sánchez-Garrido, M.A.; García-Galiano, D.; Vigo, E.; Dieguez, C.; Aguilar, E.; Pinilla, L.; et al. Alterations in Hypothalamic KiSS-1 System in Experimental Diabetes: Early Changes and Functional Consequences. *Endocrinology* **2009**, *150*, 784–794. [CrossRef]

151. Guzmán, A.; Hernández-Coronado, C.G.; Rosales-Torres, A.M.; Hernández-Medrano, J.H. Leptin regulates neuropeptides associated with food intake and GnRH secretion. *Ann. Endocrinol.* **2019**, *80*, 38–46. [CrossRef] [PubMed]

152. Qiu, J.; Fang, Y.; Bosch, M.A.; Rønnekleiv, O.K.; Kelly, M.J. Guinea pig kisspeptin neurons are depolarized by leptin via activation of TRPC channels. *Endocrinology* **2011**, *152*, 1503–1514. [CrossRef] [PubMed]

153. Stanikova, D.; Luck, T.; Pabst, A.; Bae, Y.J.; Hinz, A.; Glaesmer, H.; Stanik, J.; Sacher, J.; Engel, C.; Enzenbach, C.; et al. Associations between anxiety, body mass index, and sex hormones in women. *Front. Psychiatry* **2019**, *10*, 479. [CrossRef] [PubMed]

154. Colleluori, G.; Chen, R.; Napoli, N.; Aguirre, L.E.; Qualls, C.; Villareal, D.T.; Armamento-Villareal, R. Fat mass follows a U-shaped distribution based on estradiol levels in postmenopausal women. *Front. Endocrinol.* **2018**, *9*, 315. [CrossRef] [PubMed]

155. Lukanova, A.; Lundin, E.; Zeleniuch-Jacquotte, A.; Muti, P.; Mure, A.; Rinaldi, S.; Dossus, L.; Micheli, A.; Arslan, A.; Lenner, P.; et al. Body mass index, circulating levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: A cross-sectional study in healthy women. *Eur. J. Endocrinol.* **2004**, *150*, 161–171. [CrossRef] [PubMed]

156. Al-Safi, Z.A.; Liu, H.; Carlson, N.E.; Chosich, J.; Lesh, J.; Robledo, C.; Bradford, A.P.; Gee, N.A.; Phang, T.; Santoro, N.; et al. Estradiol priming improves gonadotrope sensitivity and pro-inflammatory cytokines in obese women. *J. Clin. Endocrinol. Metab.* **2015**, *100*, 4372–4381. [CrossRef]

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