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The role of glucagon-like peptide-1 in reproduction: from physiology to therapeutic perspective

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BACKGROUND: Glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1 RAs) have become firmly established in the treatment of type 2 diabetes and obesity, disorders frequently associated with diminished reproductive health. Understanding of the role of GLP-1 and GLP-1 RAs in reproduction is currently limited and largely unaddressed in clinical studies.

OBJECTIVE AND RATIONALE: The purpose of this narrative review is to provide a comprehensive overview of the role of GLP-1 in reproduction and to address a therapeutic perspective that can be derived from these findings.

SEARCH METHODS: We performed a series of PubMed database systemic searches, last updated on 1 February 2019, supplemented by the authors' knowledge and research experience in the field. A search algorithm was developed incorporating the terms glucagon-like peptide-1, GLP-1, glucagon-like peptide-1 receptor, GLP-1R, or incretins, and this was combined with terms related to reproductive health. The PICO

(Population, Intervention, Comparison, Outcome) framework was used to identify interventional studies including GLP-1 RAs and dipeptidyl peptidase-4 (DPP-4) inhibitors, which prevent the degradation of endogenously released GLP-1. We identified 983 potentially relevant references. At the end of the screening process, we included 6 observational (3 preclinical and 3 human) studies, 24 interventional (9 preclinical and 15 human) studies, 4 case reports, and 1 systematic and 2 narrative reviews.

OUTCOMES: The anatomical distribution of GLP-1 receptor throughout the reproductive system and observed effects of GLP-1 in preclinical models and in a few clinical studies indicate that GLP-1 might be one of the important modulating signals connecting the reproductive and metabolic system. The outcomes show that there is mostly stimulating role of GLP-1 and its mimetics in mammalian reproduction that goes beyond mere weight reduction. In addition, GLP-1 seems to have anti-inflammatory and anti-fibrotic effects in the gonads and the endometrium affected by obesity, diabetes, and polycystic ovary syndrome (PCOS). It also seems that GLP-1 RAs and DPP-4 inhibitors can reverse polycystic ovary morphology in preclinical models and decrease serum concentrations of androgens and their bioavailability in women with PCOS. Preliminary data from interventional clinical studies suggest improved menstrual regularity as well as increased fertility rates in overweight and/or obese women with PCOS treated with GLP-1 RAs in the preconception period.

WIDER IMPLICATIONS: GLP-1 RAs and DPP-4 inhibitors show promise in the treatment of diabetes and obesity-related subfertility. Larger interventional studies are needed to establish the role of preconception intervention with GLP-1 based therapies, assessing fertility outcomes in obesity, PCOS, and diabetes-related fertility problems. The potential impact of the dose- and exposure time-response of different GLP-1 RAs need further exploration. Future research should also investigate sex-specific variability of GLP-1 on reproductive outcomes, in particular on the gonads where the observations in males are most conflicting.

Key words: glucagon-like peptide 1 / glucagon-like peptide 1 receptor agonists / reproduction / infertility / hypothalamic-pituitary-gonadal axis / menstrual regularity / pregnancy rates / obesity / type 2 diabetes / polycystic ovary syndrome

Introduction

Metabolism and reproduction are fundamental and interdependent aspects of mammalian physiology (Izzi-Engbeaya et al., 2018). Adequate metabolic homeostasis is important for reproductive health. Both energy deficiency and hypercaloric conditions that result in obesity are associated with decreased fertility (Heppner et al., 2017; Silvestris et al., 2018; Comninos et al., 2014). The complexity of the mechanisms linking metabolism and reproduction is not fully understood (Izzi-Engbeaya et al., 2018). A better understanding of the relation between these two biological systems is of importance for designing strategies to address reproductive disorders accompanying metabolic abnormalities (Comninos et al., 2014), in particular due to worldwide increasing prevalence of obesity (Ng et al., 2014).

The reproductive system involves the hypothalamus, where the primary signals originate, the pituitary that subsequently releases the gonadotrophins, and the gonads, where gametogenesis and release of the gonadal sex steroids occur and—in females—a transient passage of gametes via ovarian tubes and cervix to a receptive endometrial environment. The system is regulated by a complex feedback mechanism including neuronal, endocrine, and paracrine signals. Failure at any level in this system or its tight network of regulatory signals can affect fertility (Comninos et al., 2014; Gellersen et al., 2007).

Several metabolic signals, including ghrelin, obestatin, insulin, peptide YY3–36, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), leptin, adiponectin, and resistin, interfere with the reproductive system and its regulation (Comninos et al., 2014). The extent, site, and direction of their effects are variable depending on species, sex, and pubertal stage (Comninos et al., 2014; Heppner et al., 2017). GLP-1 deserves attention because a growing body of evidence supports the modulatory activity of GLP-1 in reproduction (Lamos et al., 2017; Comninos et al., 2014). In addition, GLP-1 receptor agonists (GLP-1 RAs) have an established firm role in the treatment of type 2 diabetes and obesity because of their glucose- and weight-lowering actions (Nauck and Meier, 2018). Diabetes and obesity are associated with poorer reproductive health (Silvestris et al., 2018), at least in part due to the fact that obesity suppresses the hypothalamic–pituitary–gonadal (HPG) axis thereby interfering with ovarian function, ovulation rate, and endometrium receptivity (Silvestris et al., 2018). Obesity might cause secondary polycystic ovary syndrome (PCOS) and have negative effects on reproductive outcomes in intrinsic PCOS phenotypes that are not characterized by obesity (Balen et al., 2016; Balen et al., 1995). Furthermore, obesity and diabetes-related inflammation are involved in the derangement of major molecular mechanisms that regulate the normal biological activity of the reproductive system (Silvestris et al., 2018), negatively affecting reproductive tissues (Artunc-Ulkumen et al., 2015).

GLP-1 was identified in 1983 as part of the gene sequence encoding the preproglucagon gene (Gcg) (Bell et al., 1983), which is expressed in L cells in the gastrointestinal tract, alpha pancreatic cells, and in the brain (Nauck and Meier, 2018). It mediates its action through a G protein-coupled GLP-1 receptor (GLP-1R) (Nauck and Meier, 2018) that has been identified in many tissues, with the highest expression reported in the lung and pancreas and less expression in the stomach, intestine, kidney, heart, and brain (Pyke et al., 2014). Some recent studies identified a relevant amount of GLP-1 R also in the reproductive system (Nishiyama et al., 2018; Zhang et al., 2015a). However, an accurate mapping of GLP-1R-expressing cell types in the GLP-1 target organs is complicated by differences between rodents and humans (Körner et al., 2007) as well as by the frequent use of unvalidated histological methods (Pyke et al., 2014).

The endogenous GLP-1 (7–36) amide is rapidly degraded to GLP-1 (9–36) amide by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4) resulting in an *in vivo* half-life for the active peptide of 1–2 min (Deacon et al., 1995). Two strategies were adopted to overcome this rapid degradation in the development of viable therapies: DDP-4 inhibitors, which prevent the degradation of endogenously released GLP-1, and the development of GLP-1 RAs, which provide supra-physiological

concentrations of ligands that stimulate the peripheral and central GLP-1R (Drucker and Nauck, 2006).

The purpose of this comprehensive review is to summarize the findings regarding the role of GLP-1 in the physiology and pathophysiology of reproduction, and to address the potential therapeutic perspective of GLP-1 RAs and DPP-4 inhibitors that builds on these findings.

Methods

We performed a series of PubMed database systemic searches up to 21 September 2018, last updated on 1 February 2019. A search algorithm was developed incorporating the terms glucagon-like peptide-1, GLP-1, glucagon-like peptide-1 receptor, GLP-1R, or incretins, and this was combined with terms related to reproductive health (including reproduction, fertility, infertility, subfertility, polycystic ovary syndrome, PCOS, hypogonadism, gonadal axis, reproductive axis, hypothalamus–pituitary–gonadal (HPG) axis, gonadotrophic releasing hormone (GnRH) neurons, gonadotropins, luteinizing hormone (LH), follicle-stimulating hormone (FSH), androgens, sex hormone-binding globulin (SHBG), total testosterone, androstenedione, free androgen index (FAI), ovaries, folliculogenesis, ovulation, ovulatory cycles, menstrual cycle, menstrual cyclicity, menstrual regularity, endometrium, pregnancy, pregnancy rate, live births, miscarriages, embryo, embryo quality, placenta, and spermatogenesis). The PICO (Population, Intervention, Comparison, Outcome) framework was used to identify interventional studies (Table I).

The search strategy was limited to English language articles. There were no limits on year of publication. One reviewer screened the titles, abstract, and keywords of every reference found. Full articles were retrieved for further assessment if the information given suggested that the article met the selection criteria, or if it was unclear. One additional highly experienced reviewer was consulted during the screening process. Data extraction was conducted by one reviewer and revised by one co-author.

Results

Figure 1 shows the study selection process. We included 3 observational preclinical studies, 3 observational human studies, 9 interventional preclinical studies, 15 interventional human studies, 4 case reports, and 1 systematic and 2 narrative reviews. Owing to heterogeneity of the included studies, meta-analysis was not performed and the results are presented narratively.

The role of GLP-1 in reproduction

GLP-1 and the hypothalamus

GLP-1 seems to modulate the activity of hypothalamic GnRH neurons (Beak et al., 1998; Outeiriño-Iglesias et al., 2015; Farkas et al., 2016; Heppner et al., 2017). In *in-vitro* experiments with immortalized GnRH-producing GT_{1-7} neurons, GLP-1 administration resulted in a concentration-dependent increase in GnRH release. The threshold concentration of GLP-1 required to significantly stimulate GnRH release was equal to the threshold concentration required to produce a significant rise in cAMP formation in these cells. This observation

Table I The PICO framework to select relevant interventional studies.

	Include	Exclude
Population	Infertile, subfertile, PCOS	Animals without oestrous or menstrual cycles
	Animal models including animals with oestrous and menstrual cycles	
	In vitro models	
Intervention	GLP-1	
	GLP-1 RAs (liraglutide, exenatide, dulaglutide, semaglutide, lixisenatide)	
	DPP 4 inhibitors (sitagliptin, saxagliptin, vildagliptin, linagliptin)	
	Exendin-4	
Comparison	Placebo	
	Lifestyle modification	
	Metformin	
Outcomes	Reproductive*	Anthropometric measures of obesity
		Glucose and lipid metabolism–related outcomes
		Pancreatic beta cell–related outcomes
		Neurological outcomes
		Offspring-related outcomes
		Erectile dysfunction

PCOS, polycystic ovary syndrome, GLP-1 RA, glucagon-like peptide-1 receptor agonists, DPP-4, dipeptidyl peptidase-4.

*Refers to reproduction, fertility, infertility, subfertility, polycystic ovary syndrome, PCOS, hypogonadism, gonadal axis, reproductive axis, hypothalamus-pituitary-gonadal (HPG) axis, gonadotrophic releasing hormone (GnRH) neurons, gonadotropins, luteinizing hormone (LH), follicle-stimulating hormone (FSH), androgens, sex hormone-binding globulin (SHBG), total testosterone, androstenedione, free androgen index (FAI), ovaries, folliculogenesis, ovulation, ovulatory cycles, menstrual cycle, menstrual cyclicity, menstrual regularity, endometrium, pregnancy, pregnancy rate, live births, miscarriages, embryo, embryo quality, placenta and spermatogenesis.

indicates that downstream actions of GLP-1 via GLP-1R on the GnRH neurons is linked with cAMP accumulation (Beak et al., 1998). Further investigations in preclinical models demonstrated that GLP-1 might excite GnRH neurons by modulation of nitric-oxide and endocannabinoid pathways that control the presynaptic excitatory γ-aminobutyric acid (GABA)-ergic inputs to GnRH neurons (Farkas et al., 2016). Furthermore, a modulatory action of GLP-1 on GnRH neurons is likely to be mediated also via kisspeptin (Kiss)-1 neurons (Outeiriño-Iglesias et al., 2015; Heppner et al., 2017).

Kiss-1 neurons located in the hypothalamic arcuate nucleus (ARC) are essential for fertility (Gianetti and Seminara, 2008). They are key regulators of the pulsatile release of GnRH from GnRH neurons (Han et al., 2015). Similar to other ARC neurons, Kiss-1 neurons respond to different metabolic signals and transmit the information about energy availability to the GnRH neurons (Frazao et al., 2014; Nestor et al., 2014). The identification of a sensing system that regulates Kiss-1 action in response to changes in energy status remains elusive (Heppner et al., 2017). Kiss-1 neurons are potently inhibited during negative energy balance (Heppner et al., 2017), yet low leptin and insulin levels associated with negative energy balance do not appear to be responsible for this suppression of GnRH release via Kiss-1 neurons (Xu et al., 2009). On the other hand, stimulatory interactions between the GLP-1 and Kiss-1 neurons were more clearly demonstrated (Outeiriño-Iglesias et al., 2015, Heppner et al., 2017). In a preclinical study, GLP-1 administration led to an increased content of Kiss-1 and increased expression of Kiss-1 receptors in hypothalamic samples from female rats (Outeiriño-Iglesias et al., 2015). Electrophysiological experiments revealed that the long acting GLP-1 receptor agonist (RA) liraglutide increased action potential firing and caused a direct membrane depolarization of ARC Kiss-1 cells in brain slices. However, in animals that were fasting, activation of GLP-1R signaling with liraglutide was not sufficient to prevent a suppression of LH induced by negative energy balance (Heppner et al., 2017). The inability of liraglutide to increase LH release during fasting conditions might reflect the simultaneous counterbalanced activation of multiple inhibitory pathways that block GnRH release, including ghrelin (Tschöp et al., 2000), corticosterone (Dallman et al., 1999), and fibroblast growth factor 21 (Zhang et al., 2015b). It seemed likely that those inhibitory pathways were not sufficiently overridden by GLP-1 RA (Heppner et al., 2017).

Interestingly, the hypothalamic expression of GLP-1R in animal models varied in a time- and tissue-specific manner through the oestrous cycles. The highest expression level of GLP-1R mRNA in the hypothalamus was detected during the pro-oestrous phase (Outeiriño-Iglesias et al., 2015). Another group reported that plasma concentrations of GLP-1 also varied in a time-related manner and were highest during the pro-oestrous phase (Johnson et al., 2017).

Collectively, in the period close to potential conception, an increased GLP-1 level along with increased hypothalamic responsiveness to GLP-1 characterized by increased GLP-1R mRNA seems to provide a permissive signal to support fertility when metabolic homeostasis is adequate. GLP-1 stimulation of GnRH neurons is likely to be mediated by modulation of presynaptic GABA-ergic inputs and via Kiss-1 neurons. Further studies exploring multiple metabolic signals during negative and positive energy balance will be necessary to fully understand the significance of GLP-1R signaling for activation of GnRH neurons.

GLP-1 and the pituitary

A significant direct effect of GLP-1 on LH release in the pituitary is less likely. In comparison to the hypothalamic levels, GLP-1R mRNA in the pituitary was lower and even declined during the pro-oestrous phase (Outeiriño-Iglesias et al., 2015). GLP-1R knockout (GLP-1R−/−) mice had a completely normal number and distribution of gonadotrophic cells in the anterior pituitary in both sexes, and there were no anatomical consequences of the absence of GLP-1R at the pituitary level (MacLusky et al., 2000).

However, GLP-1 induced an increase in serum LH concentration in most functional studies (Beak et al., 1998; Outeiriño-Iglesias et al., 2015; Heppner et al., 2017), with the GLP-1 related increase in GnRH being considered as a principal mechanism to stimulate LH secretion from the pituitary. An intracerebroventricular injection of> GLP-1 using a single dose into the third ventricle in male rats resulted in a prompt increase in LH plasma concentration (Beak et al., 1998). In response to fasting, the hypothalamic GLP-1 content and plasma LH were significantly reduced. Re-feeding resulted in an increase of GLP-1, aiding the reinstatement in LH (Beak et al., 1998). Furthermore, an acute central GLP-1 administration to female rats during the pro-

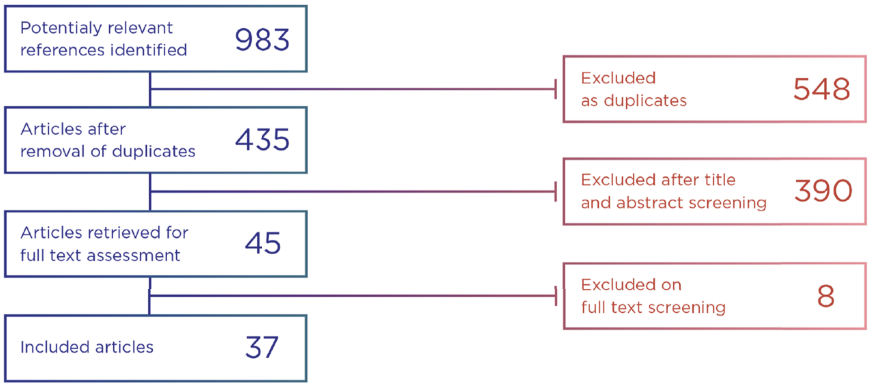

Figure 1 Flowchart of study selection.

oestrous phase doubled the amplitude of the pre-ovulatory LH surge. These changes influenced oestradiol and progesterone levels along the oestrous cycle and provoked an increase in the number of mature Graafian follicles and corpora lutea, as well as in the number of foetuses implanted and the number of offspring born (Outeiriño-Iglesias et al., 2015). The peripheral administration of GLP-1 to adult rats in the pro-oestrous phase showed even bigger LH secretory responses than those observed when centrally administered (Outeiriño-Iglesias et al., 2015).

Conversely, chronic exposure of rats to Ex4, a partial GLP-1 RA, resulted in reduced levels of LH, a significant reduction in ovarian and uterine weight, and a delay in puberty onset (Outeiriño-Iglesias et al., 2015). The inhibitory effects of Ex4 on reproductive parameters may, at least partially, reflect some of its activities distinct from GLP-1. Although Ex4 binds to the GLP-1R and most of its effects are due to GLP-1R activation, Ex4 and GLP-1 have opposite effects on some tissues. In adipose tissue of apo E/E mice, Ex4 mainly promotes lipolytic effects, whereas GLP-1 induces lipogenesis via its stimulatory effect on insulin secretion (Panjwani et al., 2013). In addition, unlike GLP-1, Ex4 dampens the elevation of ghrelin levels that is known to be an important modulator of HPG axis activity in rats under controlled starvation (Pérez-Tilve et al., 2007). Furthermore, at the doses used in the experiment by Outeiriño-Iglesias et al. (2015), Ex4 promoted a significant reduction of food intake, dropping to about 30% of control rats, whereas the effects of GLP-1 administration on LH increase were potent at low doses without changing the animals' food intake (Outeiriño-Iglesias et al., 2015). This decrease in energy input alone might hypothetically promote a blockage of the reproductive system.

A counterbalanced interplay between GLP-1 and other metabolic signals that affect the reproductive system was also indicated by the results of two small clinical studies (Selvage and Rivier, 2003; Schwartz et al., 2015). Schwartz showed that intake of a mixed glucose/protein beverage acutely decreased testosterone levels in overweight and obese peripubertal boys. The decrease in testosterone was associated with a decrease in plasma LH, and fasting testosterone was inversely correlated with fasting ghrelin level. However, they did not detect a relationship between GLP-1 and LH or testosterone change (Schwartz et al., 2015).

A 6-h continuous GLP-1 infusion in nine healthy men did not alter the pattern of LH secretion but reduced the pulsatile component of testosterone secretion with no change in mean testosterone levels (Jeibmann et al., 2005). Peripheral acute 6-h continuous GLP-1 infusion in this setting might predominantly activate catecholamine-mediated regulatory neurons resulting in some inhibitory effect on testosterone secretion independent of changes in LH (Selvage and Rivier, 2003; Ogilvie et al., 1999).

In contrast, the latest recent clinical study addressing the impact of GLP-1 RA on the HPG axis reported that treatment with liraglutide of middle-aged obese men with functional hypogonadism resulted in a significant increase in LH and total testosterone after 16 weeks of intervention and led to improved sexual function (Jensterle et al., 2019). However, it is not clear whether the increase in LH in this clinical setting was mainly mediated via weight loss or was at least partly mediated by direct interactions with the GLP-1R in the neuroendocrine reproductive axis.

In summary, the impact of GLP-1 at the level of the pituitary seems to be predominantly mediated indirectly through hypothalamic stimulation of GnRH release. In preclinical models, GLP-1 consistently induced an increase of LH release. In one clinical study, an acute GLP-1 administration had no effect on the pattern of LH release (Jeibmann et al., 2005), whereas another clinical study reported that LH was significantly increased by chronic exposure to GLP-1 RA (Jensterle et al., 2019).

GLP-1 and the gonads

GLP-1R is expressed in the ovaries (Nishiyama et al., 2018) and testes (Zhang et al., 2015a). The effects of GLP-1 on the gonads were observed in preclinical observational and interventional studies with GLP-1, GLP-1 RAs, and DPP-4 inhibitors, as described below.

GLP-1 and the ovaries

Female GLP-1R knockout (GLP-1R−/−) mice had a marginally decreased number of ovarian follicles compared to age matched GLP-1R+/+ mice. They exhibited a slight delay in the onset of puberty followed by normal cycles once the first reproductive cycle had occurred. No difference in serum estradiol, progesterone, and testosterone was detected compared to controls. The mild reproductive abnormalities were unlikely to be linked to disturbances of energy homeostasis because all animals demonstrated normal growth from birth to adulthood, normal weight, and no disturbances in control of food intake or body weight. Importantly, fertility in GLP-1R−/− knockout mice was preserved (MacLusky et al., 2000).

Rat granulosa ovarian cells maintained in culture were used to investigate the functional roles of GLP-1 in ovarian steroidogenesis (Nishiyama et al., 2018). Manipulation with both incretins GIP and GLP-1 significantly suppressed progesterone synthesis in the presence of FSH, but had no significant effect on oestrogen synthesis by granulosa cells. Accordingly, GIP and GLP-1 suppressed the expression of many progesterogenic factors and enzymes (Nishiyama et al., 2018).

A total of seven randomised controlled trials (Elkind-Hirsch et al., 2008; Jensterle Sever et al., 2014; Jensterle et al., 2015a; Kahal et al., 2015; Jensterle et al., 2015b; Jensterle et al., 2015c; Nylander et al., 2017), one meta-analysis (Niafar et al., 2016), and one expert review addressed a change in androgens and SHBG in women with PCOS treated with GLP-1 RAs. Total testosterone and FAI significantly decreased, while SHBG significantly increased with exenatide (Elkind-Hirsch et al., 2008). Liraglutide resulted in significant suppression of androstenedione (Jensterle et al., 2015b; Jensterle et al., 2015c) and free testosterone (Jensterle et al., 2015c; Nylander et al., 2017) and a significant increase of SHBG when introduced to treatment-naïve patients with PCOS (Jensterle et al., 2015c; Nylander et al., 2017). In young women with PCOS, a 6-month treatment with liraglutide did not result in a significant change of total testosterone, SHBG, and FAI when compared to controls of similar age and weight (Kahal et al., 2015). In women with PCOS that were switched from metformin to liraglutide, androstenedione significantly increased after liraglutide intervention (Jensterle Sever et al., 2014). The increase of androstenedione in this study might be related to the withdrawal of metformin that is well recognized for its beneficial direct suppressive effects on androgen production (Jensterle Sever et al., 2014). Alternatively, it might be an incidental finding owing to the known methodological problems in the assessment of androgens and small sample size (Jensterle Sever et al., 2014). The results of a meta-analysis (Niafar et al., 2016) including studies published from 2008 to 2015 (Jensterle Sever et al.,

2014; Jensterle et al., 2015a; Kahal et al., 2015; Jensterle et al., 2015b) indicated that following liraglutide treatment, total testosterone decreased from 1.9 nmol/l in 97 patients to 1.6 nmol/l in 88 patients and that there was no significant increase in SHBG after treatment with liraglutide (Niafar et al., 2016). Clinical studies did not address whether the impact of GLP-1 RAs on hyperandrogenism is due to a reduction in weight or reflecting a direct action of GLP-1 RA at the ovaries.

The impact of DPP-4 inhibitors in patients with PCOS was evaluated in four clinical studies (Ferjan et al., 2017; Ferjan et al., 2018; Jensterle et al., 2017; Elkind-Hirsch et al., 2017). All studies primarily addressed metabolic outcomes, but also reported the change in androgen profiles. The first study provides evidence that DPP-4 inhibition using sitagliptin prevented weight regain in obese patients with PCOS after liraglutide cessation (Ferjan et al., 2017). All patients had been using liraglutide for weight management and not for type 2 diabetes. After liraglutide withdrawal, sitagliptin in adjunct to metformin resulted in weight maintenance, whereas a switch to metformin monotherapy resulted in a significant weight regain within a 12-week follow-up period. The ability to resist emotional eating was greater in the sitagliptin-metformin than in the metformin arm. No difference in androgen profile was observed (Ferjan et al., 2017). In another study, sitagliptin improved pancreatic beta-cell function and prevented a conversion to impaired glucose tolerance and type 2 diabetes in metformin-intolerant patients with obesity and PCOS (Ferjan et al., 2018). After metformin withdrawal, a healthy lifestyle was reinforced in all patients. Within 12 weeks, total and free testosterone increased significantly in a metformin intolerant control group that had been advised to continue only with a healthy lifestyle; by contrast, no deterioration in androgen profile was observed in a group switched from metformin to sitagliptin in addition to a healthy lifestyle (Ferjan et al., 2018). The effects of the DPP-4 inhibitor alogliptin were assessed in a small group of women with PCOS and high metabolic risk. Alogliptin alone, and in combination with pioglitazone, improved insulin resistance along with improvement of dynamic insulin sensitivity and meal-related beta cell function when added to metformin in a small group of women with PCOS that seemed to be insufficiently controlled with metformin alone (Jensterle et al., 2017). The total testosterone decreased significantly in the alogliptin-metformin and in the alogliptin-pioglitazone-metformin arm (Jensterle et al., 2017). Furthermore, treatment with a combination of the DPP4-inhibitor saxagliptin and metformin was superior to either drug alone in terms of clinical and metabolic benefits in pre-diabetic patients with PCOS. Body mass index (BMI), waist circumference, waist/height ratio, FAI, insulin sensitivity, and insulin secretion-sensitivity index improved in all groups; however, insulin secretion-sensitivity index was significantly better with the combination of saxagliptin and metformin compared to metformin alone. Triglyceride, triglyceride/high-density lipoprotein cholesterol ratio, and mean blood glucose significantly declined in the combination of saxagliptin and metformin, and saxagliptin groups only (Elkind-Hirsch et al., 2017).

Beside their role in steroidogenesis, granulosa and theca cells also secrete components that determine the biochemical characteristic of follicular fluid (FF). FF surrounds the oocyte and plays a critical role in determining oocyte quality and subsequent fertility potential as well as embryo development. GLP-1 was identified as one of the components of FF in humans (Bou Nemer et al., 2018). Nemer and colleagues demonstrated biochemical alterations in the FF of obese women undergoing in vitro fertilisation. Levels of GLP-1 as well as insulin, glucagon, C-peptide, leptin, and branched chain amino acids were elevated in the FF of obese women when compared with normal weight controls (Bou Nemer et al., 2018). No report to date has described the role of GLP-1 or GLP-1 RAs in the milieu of FF.

Three studies investigated the impact of GLP-1 RAs on morphological changes in the ovaries (Tao et al., 2015; Artunc-Ulkumen et al., 2015; Kabel et al., 2017). In a dehydroepiandrosterone-induced PCOS rat model, it was demonstrated that administration of the short acting GLP-1 RA exenatide resulted in an increased number of granulosa cell layers, a thinner theca cell layer, more corpora lutea, and decreased dilated follicles, meaning that exenatide was actually able to reverse the polycystic ovary morphology (Tao et al., 2015). Before treatment, ovarian tissue had a decreased expression of influencing silent information regulator (SIRT) 1, which might be related to ovarian insulin resistance in PCOS. SIRT-1 is a nicotinamide adenine dinucleotide–dependent histone deacetylase that promotes insulin secretion and increases insulin sensitivity by adjusting the interactions between insulin-related proteins and the insulin signal transduction pathway. Exenatide increased SIRT-1 expression and this was suggested to represent a key mechanism that led to the improvement of PCOS morphology in this experiment (Tao et al., 2015). Another study investigated the effect of exenatide on ovarian injury caused by glucose toxicity in the diabetic rat (Artunc-Ulkumen et al., 2015). In line with other animal models, diabetes caused increased ovarian stromal and follicle degeneration (Artunc-Ulkumen et al., 2015; Garris, 2005; Foreman et al., 1993). Diabetes also led to a decrease in serum anti-Müllerian hormone (AMH) levels, suggesting a decrease in ovarian reserve (Artunc-Ulkumen et al., 2015). Exenatide treatment improved the histological degeneration and stromal fibrosis in the ovaries, concomitant with the decrease in inflammatory and oxidative stress markers and with an increase in serum AMH level (Artunc-Ulkumen et al., 2015). In the third model, letrozole induced numerous PCOS-like subcapsular cysts in rats (Kabel et al., 2017). Corpora lutea were completely absent indicating anovulation. Administration of the DPP-4 inhibitor linagliptin resulted in disappearance of the cysts and appearance of healthy follicles and corpora lutea. Linagliptin also significantly decreased inflammatory cellular infiltration, tissue transforming growth factor-beta1, tumour necrosis factor alpha, and interleukin-10 expression and significantly increased expression of the antioxidant enzymes (Kabel et al., 2017).

The systemic and tissue-specific anti-inflammatory role of GLP-1 and its agonists have been postulated also in human studies (Drucker, 2016). GLP-1 may reduce inflammation indirectly through weight loss or improved glucose control as well as by targeting GLP-1R expressed on distinct populations of circulating immune cells. Alternatively, GLP-1R activation may directly reduce inflammation in organs and cell types expressing the GLP-1R (Drucker, 2016). Mechanisms linking GLP-1 to modulation of inflammation in reproductive tissues have not been explored to date.

GLP-1 and the testes

Male GLP-1R knockout (GLP-1R−/−) mice exhibited reduced gonadal weight, yet no difference in testosterone production was detected compared to controls (MacLusky et al., 2000).

The impact of GLP-1 agonism on testes was assessed in a high-fat diet (HFD)–induced obesity mice model. HFD-induced obesity led to a decreased serum level of testosterone, impairment of sperm quality, and increased inflammation of testes in mice. Abnormal physiology was partially restored by exenatide treatment, as it did in female rats. Exenatide reduced body weight without increasing serum testosterone levels but reduced the expression of pro-inflammatory cytokines and improved the quality of sperm, including sperm motility. Improved sperm quality was related to improved mitochondrial membrane potential and improved inflammatory status in the testicular tissue after exenatide treatment. Exenatide treatment restored sperm DNA damage in obese animals to the level of controls (Zhang et al., 2015a).

By contrast, a single published case report described adverse effects of the GLP-1 RA liraglutide on spermatogenesis in a 35-year-old man experiencing primary and idiopathic infertility for 1 year. His first spermiogram showed normal parameters of semen volume, sperm concentration, motility, morphology, and leucocyte count. He returned 4 months later for an intrauterine insemination of his partner. Semen analysis of the sperm collections showed no sperm motility. He reported that he had been taking 0.6-mg liraglutide per day, starting 1 month before the normal sperm analysis. He was advised to stop liraglutide. Five months after liraglutide interruption, the semen analysis showed normal values for all parameters (Fontoura et al., 2014). On 31 July 2018, the US Food and Drug Administration reported that among 25 869 people with different adverse side effects taking liraglutide, two reported having a decreased sperm count ('Who have sperm count decreased with Victoza—from FDA reports—eHealthMe,' n.d. at http: www. Ehealthme. com/ds/victoza/sperm + count + decreased).

Another case reported a deterioration of semen quality following 3 months of use of the DPP-4 inhibitor sitagliptin in a 39-year-old male with diabetes of 9 years. Semen quality recovered upon discontinuation of the drug. Subsequently, sitagliptin was re-introduced and semen quality deteriorated again, to recover again upon withdrawal of the drug (Hibi et al., 2011).

In summary, GLP-1 seems to affect steroidogenesis, gametogenesis, and morphology of gonads. The anti-inflammatory and antifibrotic effects of GLP-1 agonism observed in ovaries injured by glucose toxicity and PCOS in preclinical models as well as in testicular tissue are promising. In men, the potential negative impact of GLP-1 agonism on semen quality observed in a few anecdotal cases contrasts the observations in animal models. The reason remains unexplored. Attention on spermatogenesis might be warranted when GLP-1 RAs or DPP4 inhibitors are administrated in young male adults with a reproductive wish.

GLP-1 and the endometrium

Insulin resistance and diabetes in animal models are associated with changes in the structure and function of endometrium that might cause implantation failure, pregnancy loss, and defective placentation (Garris, 2005). Garris and colleagues observed enhancement of tissue metabolism and induced cellular toxicity in epithelial and stromal layers of endometrium in diabetic rats (Garris, 2005). Some women with PCOS present with several endometrial abnormalities that may cause implantation failure and defective implantation (Piltonen, 2016). Most markers of endometrial dysfunction in PCOS are related to steroid hormone action, insulin resistance, and inflammation (Piltonen, 2016). Exenatide decreased histological degeneration and fibrosis in the endometrium of diabetic rats, mainly by decreasing inflammation and by antagonizing oxidative stress (Artunc-Ulkumen et al., 2015). Since the decreased fibrosis and reduced inflammation likely represent a more susceptible endometrial environment for pregnancy and might increase receptivity (Artunc-Ulkumen et al., 2015), the impact of GLP-1 RA on endometrium should be further elucidated in clinical conditions such as type 2 diabetes, obesity, and PCOS.

The effects of GLP-1 on the reproductive axis are presented in Fig. 2.

The therapeutic perspective of GLP-1 RAs and DPP-4 inhibitors in human reproduction

The effect of GLP-1RAs and DPP-4 inhibitors on menstrual regularity

Alteration of the menstrual cycle can be an early symptom of dysfunctional oocyte maturation and hormone derangements that should alert to potential impaired fertility (Silvestris et al., 2018). The effect of GLP-1 on menstrual regularity is insufficiently studied. One observational study compared GLP-1 levels in women with hypothalamic amenorrhea with the levels in ovulatory women (Scheid et al., 2009). Few studies with GLP-1 RAs and DPP-4 inhibitors included menstrual regularity as an outcome (Elkind-Hirsch et al., 2008; Nylander et al., 2017; Jensterle Sever et al., 2014, Jensterle et al., 2015b; Jensterle et al., 2015c; Kahal et al., 2015; Elkind-Hirsch et al., 2017; Jensterle et al., 2017).

In an observational study with 48 women categorized into three groups, including sedentary ovulatory, exercising ovulatory, and exercising women with amenorrhea, fasting GLP-1 levels in exercising women with hypothalamic amenorrhea did not differ from GLP-1 levels in ovulatory women (Scheid et al., 2009). However, dynamic meal responses of the gut peptides were not investigated.

Elkind-Hirsch and colleagues addressed the impact of GLP-1 RA on menstrual frequency in anovulatory women with PCOS (Elkind-Hirsch et al., 2008). They randomized 42 overweight women with oligo-ovulatory PCOS to exenatide, metformin, or a combination of both for 24 weeks. They found an improved ovulation rate in all groups, with the combined treatment being superior in improving menstrual frequency and ovulation rate. The ovulation rate was 86% in the combined group compared to 50% in the exenatide only and 29% in the metformin-only group. Body weight significantly decreased in all groups. Both exenatide arms were more effective in weight loss than metformin monotherapy, with combination therapy being significantly better than metformin alone. Reduction in body weight significantly correlated with an increase in menstrual frequency ($r = 0.42$; $P < 0.006$) (Elkind-Hirsch et al., 2008).

A randomized, placebo-controlled trial investigated the effect of liraglutide on ovarian function in 72 women with PCOS after a 26-week treatment with liraglutide at 1.8 mg/d or placebo in a 2:1 ratio (Nylander et al., 2017). The group treated with liraglutide showed an improved bleeding ratio, defined as number of menstrual bleedings divided by study period in months, with 62% of women in the liraglutide group having a bleeding ratio over 0.87 compared with 28% in the placebo group. Change in bleeding ratio correlated with BMI and bleeding ratio in the last 6 months before randomization. No

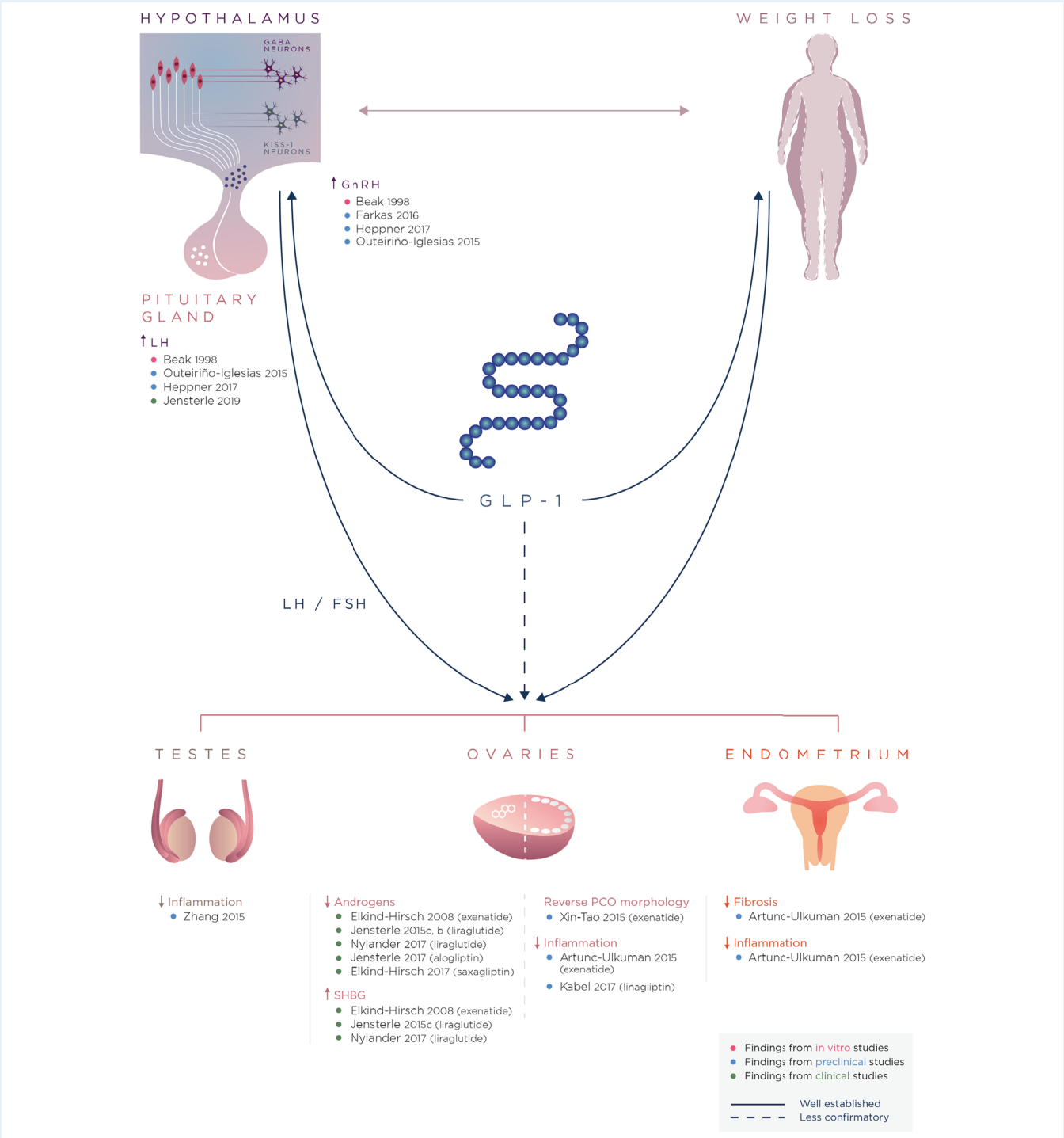

Figure 2 The effects of GLP-1 on the reproductive axis. Glucagon-like peptide-1 (GLP-1 mediates weight loss due to effects on appetite in the central nervous system. Weight loss can subsequently reverse the obesity-induced suppression of GnRH and LH. Moreover, GLP-1 seems to have direct effect on the hypothalamic–pituitary–gonadal axis, both central as well as peripheral. Most studies show stimulating effects of GLP-1 on GnRH and LH release. GLP-1 stimulation of GnRH release is likely to be mediated via GnRH neurons by modulation of presynaptic excitatory γ-aminobutyric acid (GABA)-ergic inputs and via kisspeptin (Kiss-1) neurons. The impact of GLP-1 on LH increase seems to be predominantly mediated through GnRH release. In the gonads and the endometrium, GLP-1 and its mimetics might have direct anti-inflammatory and anti-fibrotic effects. Full arrows indicate well-established relationships; the broken arrow shows a relationship that is not fully established yet and needs further evaluation. The type of research is indicated by colour of the dot before the reference. SHBG, sex hormone-binding globulin; PCO, polycystic ovary.

correlation was observed between the change in bleeding ratio after intervention and the change in waist circumference, levels of androgens, gonadotrophins, AMH, fasting glucose, or insulin resistance assessed by homeostatic model assessment of insulin resistance (HOMA-IR). In addition, there was a beneficial trend towards lower ovarian and stromal volume, which decreased by 1.6 ml with liraglutide versus placebo (Nylander et al., 2017).

Contrary to these results, several studies with liraglutide in PCOS found unaltered bleeding frequency within the observed period despite an effect on body weight (Jensterle Sever et al., 2014; Jensterle et al., 2015c; Jensterle et al., 2015b) and insulin resistance (Kahal et al., 2015). This might be due to small sample size (Kahal et al., 2015), short duration (Jensterle et al., 2015c; Jensterle et al., 2015b), and the low liraglutide dose of 1.2 mg QD (Jensterle et al., 2015b; Jensterle et al., 2015c).

The effects of DPP-4 inhibitors on menstrual regularity were reported in two studies using saxagliptin and alogliptin (Elkind-Hirsch et al., 2017; Jensterle et al., 2017). The impact of saxagliptin alone or in combination with metformin in comparison with metformin alone was assessed in patients with PCOS and impaired glucose regulation (Elkind-Hirsch et al., 2017). Menses increased in all treatment arms after 16 weeks of treatment. Return of menses was reported at a significantly higher rate with combination therapy compared with single-agent therapy with metformin. In the saxagliptin-metformin group, menses were more regular with an average (±SD) menstrual interval decreasing from 98 ± 48 days to 36 ± 11 days, compared with 121 ± 68 days to 81 ± 44 in the metformin group (Elkind-Hirsch et al., 2017). In another study that included obese PCOS women with persistent insulin resistance despite metformin in adjunct to lifestyle modification, menstrual frequency significantly improved with a triple combination of alogliptin–pioglitazone–metformin, whereas a dual combination of alogliptin and metformin did not significantly improve menstrual frequency within the 12-week observation period (Jensterle et al., 2017).

The effect of GLP-1 RAs on pregnancy rates

The role of proglucagon-derived peptides in pregnancy was first analysed in a model of homozygous glucagon-green fluorescent protein (gfp) knock in mice (GcG $^{gfp/gfp}$) that lacked all peptides derived from proglucagon, including GLP-1 (Sugiyama et al., 2012). The pregnancy rate of Gcg $^{gfp/gfp}$ females was significantly lower than that of the control females, but most of the pregnant Gcg $^{gfp/gfp}$ females could deliver at term. Approximately half of the embryos in Gcg $^{gfp/gfp}$ mice were resorbed in the second part of gestation, indicating that maternal proglucagon-derived peptides are required to fully sustain survival of embryos. Furthermore, the proportion of primiparous and multiparous mice that had lost all offspring within 36 hours after birth was compared. In primiparas, no significant difference in neonatal survival was observed between the Gcg $^{gfp/gfp}$ and control mice. However, neonatal survival was not improved by prior birthing experience in Gcg $^{gfp/gfp}$ females, whereas most of the multiparous Gcg +/+ and Gcg gfp/+ mice could raise their offspring. These results suggest that proglucagon-derived peptides are involved in breeding behaviour of the mice. Given that it has been previously shown that Glp-1R null mice (Glp 1 −/−) have defects in learning and memory (Abbas et al., 2009), the lack of Glp 1 might be responsible for the partially impaired breeding capacity of Gcg $^{gfp/gfp}$ female mice.

In summary, these data suggest that proglucagon-derived peptides, including GLP-1, are not required for conception and reproduction. Nevertheless, female Gcg $^{gfp/gfp}$ mice showed a decreased pregnancy rate, smaller litter size, and defects in breeding capacity. Since multiple bioactive peptides, including glucagon and GLP-1, are produced from proglucagon, it is so far not possible to ablate one of these peptides selectively. Further studies that employ multiple genetic models, such as Gcg $^{gfp/gfp}$ and Gcg R−/−, could elucidate the distinctive role of proglucagon-derived peptides in pregnancy and reproduction (Sugiyama et al., 2012).

In the end, intact pregnancies and the occurrence of live births in human studies are the most interesting outcomes. To date, GLP-1 RAs are contraindicated in pregnancy, since human safety data in pregnancy are not available. Also, the potential role of GLP-1 RA in the preconception period in women with reproductive disorders accompanying metabolic imbalance remains to be fully explored. Two recent randomized studies addressed pregnancy rates after a course of GLP-1 RA in preconception period, both reporting higher pregnancy rates after GLP-1 RAs withdrawal in women with PCOS (Liu et al., 2017; Salamun et al., 2018).

The first randomized clinical study evaluated the effects of 12-week treatment with exenatide on the rate of natural pregnancy within 12 weeks after exenatide withdrawal (Liu et al., 2017). They randomized 176 overweight or obese women with PCOS to receive either exenatide 10 micrograms BID or metformin 1000 mg BID for the first 12 weeks. During the second 12 weeks, all patients were treated with metformin alone. Metabolic parameters were assessed at baseline and after 12 weeks. The pregnancy rate was tracked during the second 12 weeks. After the first 12 weeks of intervention, subjects treated with exenatide had significantly decreased weight and total percentage of fat, improved insulin resistance as measured with HOMA-IR, and increased menstrual frequency ratio compared to the metformin group. During the second 12 weeks, the rate of natural pregnancy of exenatide pre-treated patients was significantly higher than MET-treated patients (43.6% versus 18.70%, respectively, $P < 0.05$). This was the first study demonstrating that short-term therapy with exenatide was superior to metformin in increasing natural pregnancy rates within 3 months after exenatide withdrawal. The study was not designed to explore the underlying mechanisms, but weight loss seemed to be a key contributor that led to increased fertility (Liu et al., 2017). In their conclusion, the authors mentioned that data collection on early abortion rate, the incidence of gestational diabetes mellitus, live birth rate, and adverse maternal and neonatal outcomes continues (Liu et al., 2017), but so far these outcomes have not been reported. A successful pregnancy after 2-month preconception treatment with exenatide was reported also in a case report on a 26-year-old infertile woman with PCOS and obesity (Yang and Wang, 2016).

Another small randomized open label pilot study conducted with 28 obese women with PCOS reported that a 12-week preconception treatment with low-dose liraglutide (1.2 mg QD) in combination with metformin (1000 mg BID) was superior to metformin alone in increasing IVF pregnancy rates and the cumulative pregnancy rate including also spontaneous pregnancies after treatment in patients who had been previously resistant to lifestyle modification and first-line reproductive

Table II The effects of GLP-1 RAs and DPP-4 inhibitors on reproductive outcomes in clinical studies.

Outcome	Population	Intervention	Comparison	Duration	Effect	Reference
Ovulation rate	60 overweight and obese women with PCOS	Exenatide 10 μg BID	Metformin 1000 mg BID	24 weeks	↑Ovulation rate	Elkind-Hirsch et al., 2008
		Exenatide 10 μg BID + metformin 1000 mg BID				
Menstrual regularity	60 overweight and obese women with PCOS	Exenatide 10 μg BID	Metformin 1000 mg BID	24 weeks	↑Menstrual frequency	Elkind-Hirsch et al., 2008
		Exenatide 10 μg BID + metformin 1000 mg BID				
	40 obese women with PCOS	Liraglutide 1.2 mg QD	Metformin 1000 mg BID	12 weeks	Unaltered	Jensterle Sever et al., 2014
		Liraglutide 1.2 mg QD + metformin 1000 mg BID				
	32 obese women with PCOS	Liraglutide 1.2 mg QD	Metformin 1000 mg BID	12 weeks	Unaltered	Jensterle et al., 2015b
	41 obese women with PCOS	Liraglutide 1.2 mg QD	Metformin 1000 mg BID	12 weeks	Unaltered	Jensterle et al., 2015c
			Roflumilast 500 μg QD			
	19 obese women with PCOS versus 17 obese controls	Liraglutide 1.8 mg QD	NA	24 weeks	Unaltered	Kahal et al., 2015
	72 women with PCOS	Liraglutide 1.8 mg QD	Placebo	26 weeks	↑Menstrual frequency	Nylander et al., 2017
	34 women with PCOS with prediabetes	Saxagliptin 5 mg	Metformin 2000 mg	16 weeks	↑Menstrual frequency	Elkind-Hirsch et al., 2017
		Saxagliptin 5 mg + metformin 2000 mg				
	30 women with PCOS with insulin resistance despite treatment with metformin 2000 mg	Alogliptin 25 mg + pioglitazone 30 mg + metformin 2000 mg	NA	12 weeks	↑Menstrual frequency	Jensterle et al., 2017
		Alogliptin 25 mg + metformin 2000 mg				
Pregnancy rate	176 overweight and obese women with PCOS	Exenatide 10 μg BID	Metformin 1000 mg BID	12 weeks	↑Natural PR	Liu et al., 2017
	28 obese women with PCOS	Liraglutide 1.2 mg QD + metformin 1000 mg BID	Metformin 1000 mg BID	12 weeks	↑IVF PR	Salamun et al., 2018
	One infertile woman with PCOS and obesity	Exenatide 10 μg BID	NA	8 weeks	Pregnancy	Yang and Wang, 2016
Live birth	One woman with type 2 diabetes mellitus and PCOS	Liraglutide 1.8 mg QD	NA	1st trimester pregnancy	Healthy newborn	Greco, 2015
Spermatogenesis	One man with primary and idiopathic infertility	Liraglutide 0.6 mg QD	NA	5 months	↓Semen quality	Fontoura et al., 2014
	One man with type 2 diabetes mellitus	Sitagliptin 50 mg QD	NA	3 months	↓Semen quality	Hibi et al., 2011

Table III Questions to address in future research on the effects of GLP-1 and GLP-1 RAs on the reproductive system.

No.	Question
1	Where exactly are GLP-1 R expressed in human reproductive tissues?
2	Has expression or function of the GLP-1 R in reproductive tissues changed in populations with obesity, diabetes, and PCOS?
3	Are the effects of GLP-1 and GLP-1 RAs in reproduction mainly mediated via weight loss or by direct interactions with the GLP-1 R in reproductive tissues?
4	What is the impact of crosstalk between GLP-1 and other metabolic signals in reproductive mammalian physiology and pathophysiology?
5	Is anti-inflammatory activity of GLP-1 and GLP-1 RAs in reproductive tissues clinically relevant in fertility improvement?
6	Does GLP-1 have a role in cognitive functions and memory experiences related to breeding capacity?
7	Does preconception pharmacological stimulation of GLP-1 R improve fertility potential in populations with obesity, diabetes, and/or PCOS?
8	Is there a dose-response or an exposure time-response relationship for GLP-1 RAs and potential reproductive effects?
9	Is treatment with GLP-1 RAs in the preconception period in different obesity-related populations safe?

treatment (clomiphene citrate, letrozole, and/or ovarian drilling). The controlled ovarian stimulation was started after a 4-week medication-free period. Pregnancy rate per embryo transfer was significantly higher in the liraglutide plus metformin group (COMBI) compared to metformin alone (85.7 versus 28.6%, respectively $P = 0.03$). The cumulative pregnancy rate in a time frame of 12 months was 69% in the COMBI compared to 36% in the MET group. The observed results were remarkable, particularly since both treatment interventions resulted in a comparable reduction of body weight and visceral adipose tissue (Salamun et al., 2018), indicating that the underlying mechanisms might go beyond mere weight reduction.

The effect of GLP-1 RAs on live births

A single published case report reported an uneventful pregnancy and birth of a healthy child in a 37-year-old women with type 2 diabetes mellitus and PCOS who was exposed to GLP-1 RAs during the first trimester of pregnancy (Greco, 2015).

Various clinical effects of GLP-1 agonism on reproductive outcomes are summarized in Table II.

Conclusion

In the present review, we discussed the multifaceted effect of GLP-1 and its agonists on the reproductive axis. The anatomical distribution of GLP-1R throughout the reproductive system and observed effects of GLP-1 in preclinical models and in a few clinical studies indicate that GLP-1 might be an important modulating signal connecting the reproductive and the metabolic systems.

The preclinical results suggest that GLP-1 has mostly a stimulatory effect on the HPG axis and that pharmacological stimulation of GLP-1R might be able to reverse functional suppression of gonadotrophins in hypo- as well as in hypercaloric states of metabolic imbalance. In biological systems, the final effects of GLP-1 on the HPG axis seem to depend on synergistic and counterbalanced interplays with other metabolic signals, including kisspeptin. In addition to the neuroendocrine impact, GLP-1 seems to have anti-inflammatory and antifibrotic effects in different peripheral reproductive tissues, such as ovaries, endometrium, and testes, which may be injured in obesity, diabetes, and PCOS.

The second decade of GLP-1–based therapies has established their firm role in the treatment of type 2 diabetes and obesity. Although type 2 diabetes and obesity are frequently associated with diminished reproductive health, the potential therapeutic perspective of GLP-1 agonism in reproduction remains largely unaddressed. The preliminary data on the effects of GLP-1RAs on clinically relevant reproductive outcomes in overweight and obese women with PCOS are promising. The ovulation rate and menstrual frequency were consistently improved in studies with the GLP-1 RAs exenatide and liraglutide that were designed with menstrual regularity as a primary outcome. Furthermore, short-term preconception intervention with exenatide resulted in an increase of natural pregnancy rates in overweight and obese women with PCOS when compared to the metformin-treated group. A pilot study comparing short-term preconception treatment with metformin to the treatment with low-dose liraglutide in combination with metformin resulted in a significantly increased IVF pregnancy rate in obese infertile women with PCOS who were treated with liraglutide and had been poor responders to lifestyle modification and first-line reproductive treatments previously.

Taken together, the current data suggest that preconception treatment with GLP-1 RAs might provide some novel strategies in obesity, diabetes, and PCOS-associated subfertility, yet this unexplored field raises many clinically relevant questions.

Unanswered questions and future perspective

In future research, some unanswered questions, summarized in Table III, need to be addressed.

Although GLP-1R has been identified throughout the reproductive system in preclinical models, as discussed in this review, the identification of GLP-1R in human reproductive tissues, in particular in the gonads and the endometrium, needs to be specifically explored because of several examples of species differences between rodents and humans (Körner et al., 2007). Since identification of GLP-1R merely by using antibodies might result in false positive results (Pyke et al., 2014), the studies exploring the presence of GLP-1R in different human reproductive tissues should be based on both mRNA and protein expression. Using validated quantitative histological

methods (Pyke et al., 2014), GLP-1R expression in the reproductive organs in subjects with obesity, diabetes, and PCOS should be compared with that in the reproductive organs of healthy normal weight controls.

Future studies should address the differential impact of GLP-1 RAs on reproductive health mediated via weight loss and improvement of metabolic status as opposed to the direct tissue-specific effects of GLP-1 RAs that go beyond mere weight-lowering potential. Comparing different approaches for weight reduction, including diet, exercise, GLP-1 RAs, and bariatric surgery, might additionally enlighten the complex cross-talk among gut, brain, and the reproductive axis. When exploring the impact of GLP-1 agonists on the HPG axis, the relation between GLP-1 and other metabolic signals, such as ghrelin, insulin, GIP, leptin, adiponectin, and resistin, needs to be taken into account. The magnitude and the rate of weight loss independent of the weight loss strategy might well play an important role in the determination of the final effect of weight loss on the HPG axis and represent another issue needed to be addressed in future research.

The clinical relevance of anti-inflammatory potential of GLP-1 RAs in the gonads and endometrium of patients with obesity, diabetes, and PCOS should be further evaluated. We encourage the assessment of the impact of GLP-1 RAs on endometrium quality and receptivity by timed biopsy obtained during the window of implantation, in particular in women in reproductive age that are already using GLP-1 RAs for type 2 diabetes and obesity.

To investigate whether preconception treatment with GLP-1 RAs improves fertility potential in women with obesity, diabetes, and/or PCOS, we need larger multicentre randomized placebo controlled studies with uniform protocols targeting reproductive outcomes. A low- versus high-dose regimen of GLP-1 RAs, short-term versus long-term exposure to GLP-1 RAs as well as the use of different GLP-1RAs should be addressed in future studies. We also need to evaluate the impact of GLP-1 RAs in younger men with diabetes and/or obesity on their fertility potential including gonadotrophin profile and semen sample analysis.

Regarding safety, GLP-1 RAs have a well-established favourable risk-benefit balance in patients with type 2 diabetes and in obese individuals without diabetes (Bethel et al., 2018). No important safety issues have been reported in a recent larger clinical study evaluating the effects of preconception intervention with exenatide in overweight/obese patients with PCOS (Liu et al., 2017) and in a pilot study using a short-term intervention with low-dose liraglutide in combination with metformin before proceeding to IVF in obese women with PCOS (Salamun et al., 2018). Using careful monitoring, preconception treatment with GLP-1 RAs seems to be acceptable to continue with the research in this field.

Finally, another very interesting observation was made in a preclinical model, reporting that proglucagon-derived peptides, including GLP-1, might be involved in breeding behaviour of the rodents (Sugiyama et al., 2012). In line with the recognized role of GLP-1 in learning and memory (Abbas et al., 2009) and with the latest potential new indications for GLP-1 RAs for Parkinson' and Alzheimer's diseases, the impact of GLP-1 on cognitive functions and memory experiences related to breeding capacity might open another new perspective in understanding the complex interactions between reproductive and metabolic systems in mammalian reproduction.

Thus, there exist many unsolved questions and they implicate an exciting new research field with potential wider clinical implications for reproductive scientists and clinicians.

Authors' roles

M.J. performed the literature search, extracted data, wrote the manuscript, and gave final approval of the manuscript. A.J., E.F., J.H.D.V. and E.B.V. provide substantial contribution to interpretation of data, critically reviewed the manuscript and contribute an important intellectual content, and gave final approval of the manuscript. S.E.S. created outline, supervised the writing, critically reviewed the manuscript, and gave final approval of the manuscript.

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Conflict of interest

The authors declare that the current research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. M.J., E.F., E.B.V., and S.E.S. have no conflicts of interest. A.J. has served as a consultant and is on Speakers Bureaus for Astra Zeneca, Boehringer Ingeheim, Eli Lilly, MSD, Sanofi, and Novo Nordisk. J.H.D.V. sat on advisory panels of Novo Nordisk on GLP-1.

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