Growth hormone in IVF cycles

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- Growth hormone (GH) has been used as an **adjunct to assist follicular development** and recruitment in ovulation induction for some time
- however, **its place in routine IVF and ovulation induction treatment cycles** is still being keenly debated almost 30 years later, and it does not have US Food and Drug Administration approval for this use.
- Part of the difficulty in clarifying the place (or lack of) for GH in the **treatment of female infertility** is that the drug is expensive, it is unclear what is the appropriate dose to study, when in (or before) a cycle it should be employed, or even in which subgroup of patients it should be used. The use of GH for a woman with a primary disorder of GH deficiency has a sound rationale, as many such **women will have a disorder of ovulation**
- however, GH has been used in other patient groups such as **older women**
- ***women with poor ovarian reserve***
- **women with poor embryonic development**

PHYSIOLOGICAL RATIONALE FOR THE USE OF GROWTH HORMONE IN OVARIAN STIMULATION

- The physiological effect of GH on the oocyte and folliculogenesis is presumed to be **via insulin-like growth factor 1 (IGF-1)** or by a ***direct action of GH***.
- GH **stimulates serum and follicular IGF-1** and **hepatic production of IGF-1**
- IGF-1 receptors are present within **the oocytes, granulosa, and theca cells**
- In animal models, GH improves **oocyte cytoplasmic and nuclear maturation**
- promotes **steroidogenesis in granulosa cells**
- maturation of denuded oocytes in the human in-vitro maturation

- If these physiological effects were applicable within an IVF population, it may be expected to lead to the improved **recruitment of available antral and preantral follicles during IVF stimulation** and, or, the development of more competent oocytes or embryos

- Further rationale for the use of GH in IVF treatment is that the follicular **fluid IGF-1 concentrations** of women undergoing IVF have been directly correlated to the **number of developing follicles and inversely linked to the duration of stimulation and the amount of stimulation required to reach oocyte retrieval**
- Furthermore, along with other markers in an IVF cycle, **follicular concentrations of GH have been related to the chance of a clinical pregnancy**
- and the follicular concentration of GH is greater in a follicle that leads to **successful oocyte fertilization, embryonic development, and embryo implantation**

- To reinforce the integral nature of **GH and IGF-1 in folliculogenesis,** and its presumptive beneficial role in IVF treatment, evidence derived from studies using mouse models demonstrate that **IGF-1 suppresses follicular apoptosis** is required for the progression of follicular development from the early antral stage and for acquisition of follicular stimulating hormone (**FSH) responsiveness and oocyte maturation** and at least in mice, **GH itself is essential for follicular development and inhibition of follicular apoptosis**

GROWTH HORMONE USE IN ALL IVF CYCLES GH

- is an expensive treatment; hence the benefit derived from its use as an ***adjunct to standard IVF treatment*** would have to be clinically significant to warrant its use. The rationale for the use of GH in standard IVF is that GH and IGF-1 and their receptors are present within the oocyte and granulosa cells
- They demonstrated a **significant increase in the 3 hydroxysteroid dehydrogenase and aromatase mRNA in granulosa cells of the GH-treated women and an increase in the follicular fluid testosterone concentration, suggesting an effect on the theca cells**

GROWTH HORMONE USE IN IVF CYCLES FOR WOMEN WITH POLYCYSTIC OVARY SYNDROME Women with polycystic ovary syndrome (PCOS)

- generally have higher serum concentrations of **insulin and often have elevated free androgens and an elevated serum-luteinizing hormone**
- All of these endocrine changes are known to influence IGF-binding proteins [30] and hence may be related to the perturbed follicular development often present in women with PCOS
- Consequently, the use of GH has been studied in women with PCOS, and a favorable clinical response was reported in this small subgroup studied who were undergoing IVF treatment; **these women also had a significant increase in their serum and follicular IGF-1 concentrations**
- although a study of women with PCOS resistant to ovulation induction did not derive any benefit from the addition of GH

GROWTH HORMONE USE IN IVF CYCLES FOR WOMEN WITH A POOR OVARIAN RESPONSE TO STIMULATION

- This analysis demonstrated that there is a favorable effect of the use of GH (provided in varying doses and for various duration during stimulation) with **respect to the number of oocytes collected**
- There was a favorable benefit on the time taken **to reach oocyte retrieval**
- and the ***number of fertilized oocytes obtained***
- despite this there **was no increase in the chance in having an embryo available for transfer**
- However, there was **a benefit noted with regard to the chance of a positive pregnancy test** a **clinical pregnancy**

GROWTH HORMONE IN FERTILITY AND INFERTILITY: PHYSIOLOGY, PATHOLOGY, DIAGNOSIS AND TREA

- GH is a **monomeric protein** secreted by the **pituitary** with a high molecular similarity to other lactogenic hormones like prolactin and placental lactogen.
- In the **anterior pituitary** gland, the secretion by the **somatotroph cell** is regulated by **both stimulatory** peptides [e.g., Growth Hormone Releasing Hormone (**GHRH)] and inhibitory (e.g., Somatostatin**) peptides.
- The secretion takes place in a **pulsatile way that** combines short-term variability of spikes of irregular amplitudes with a clear circadian increase, coinciding with the late non-Rapid Eye Movement (REM) periods, probably mediated by dopamine related neurotransmitters
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- This complicates the determination of optimal plasma levels. The action of GH is exerted through its **binding to the extracellular domain of a complex membrane receptor.**
- In contrast to dimeric glycoproteins like gonadotropins, two receptors are needed in order to establish a trimeric structure composed by two membrane receptors and the GH molecule.
- Thus, three recognition processes are needed for an effective downstream **activation: receptor-to-receptor and agonistic GH molecule to each of the receptors** to form the activated GH trimeric complex. This complex relationship between the hormone and the target organ makes the process of activation vulnerable to different mutations, causing different downstream effects such as the clinical diversity in the different phenotyp

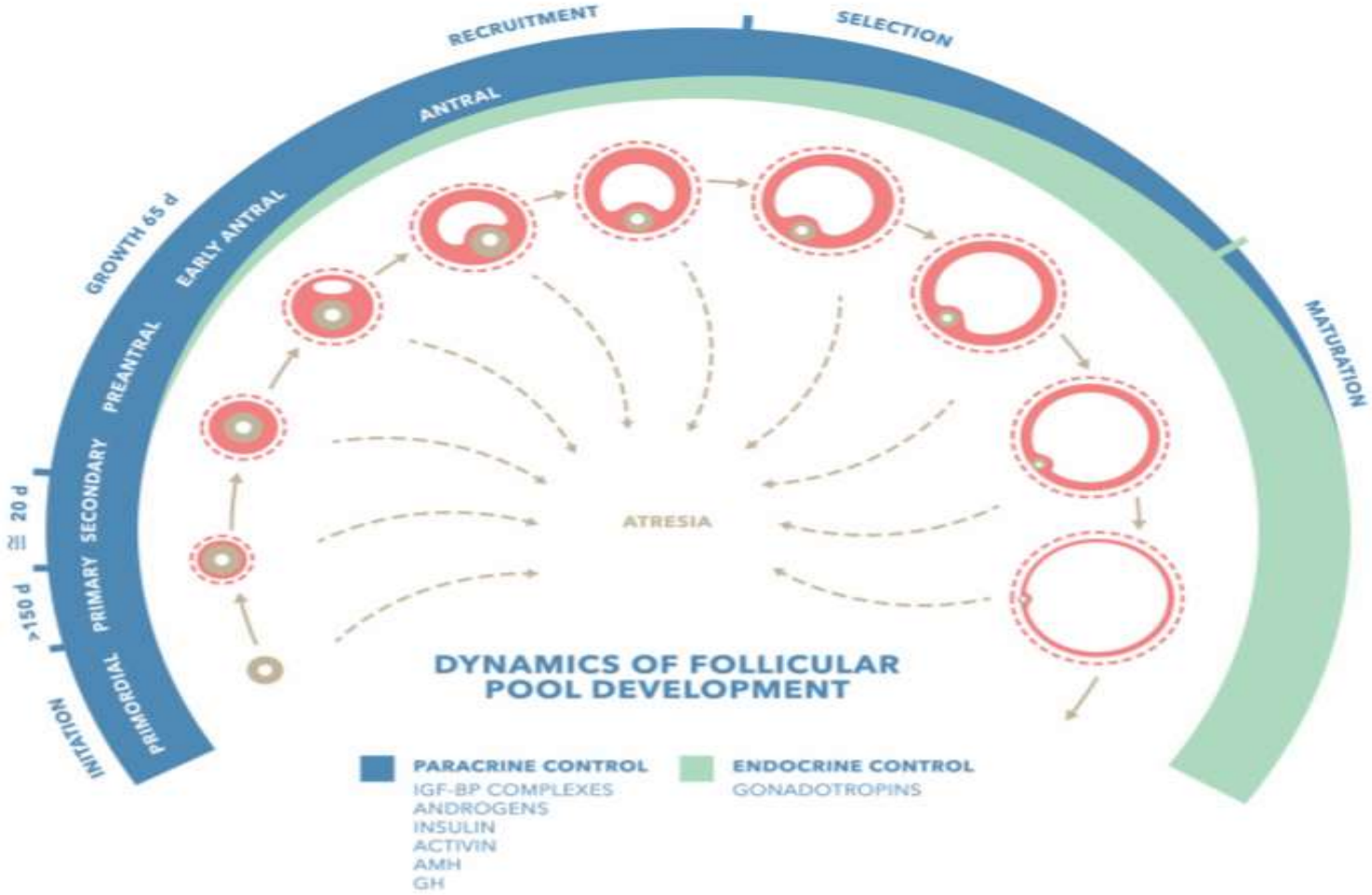


FIGURE 1 | Factors influencing dynamics of follicular pool development. Adapted from Gougeon (18) *IGF-BP complexes (Insulin Growth Factor Binding Protein Complexes); GH (Growth Hormone); AMH (Anti Müllerian Hormone)*. Blue stripe shows follicular stages predominantly influenced by paracrine factors: activators such as GH and IFG-BP complexes, insulin, androgens and activin and AMH. Green stripe shows follicular stages predominantly influenced by endocrine factors such as gonadotropins. Although there has been so far a clear differentiation between gonadotropin-independent and gonadotropin responsive/dependent stages, all these molecules have shown to take part not only at one level, but in the whole folliculo-genesis.

- Activators such as GH and IFG-BP (Insulin Growth Factor Binding Protein) complexes, **Insulin, androgens and activin, might promote follicular growth, transition to antral stage or even follicular recruitment**, either by acting as anti-apoptotic factors or enhancing follicular response to gonadotropins.
- Inhibitors such as Anti Müllerian Hormone (**AMH) are** able to block initial follicle recruitment, transition to antral stage or even the gonadotropin-dependent recruitment.
- Late follicular stages are predominantly influenced by endocrine factors such as **gonadotropins: mainly FSH in the** recruitment and selection of the leading follicle and LH at later stages and last oocyte maturation and ovulation.
- Although there has been a clear differentiation between gonadotropin-independent and gonadotropin responsive/dependent stages, all the molecules mentioned have been shown to take part not only at one level, but in the entire process of folliculogenesis. Basic science studies provide biologically plausible data for **GH and IGF-1 as key factors for an optimal follicle development**.
- GH may play an activating role, either **directly or indirectly**, via for instance IGF-1 in the transition from primordial follicles to late antral stages.

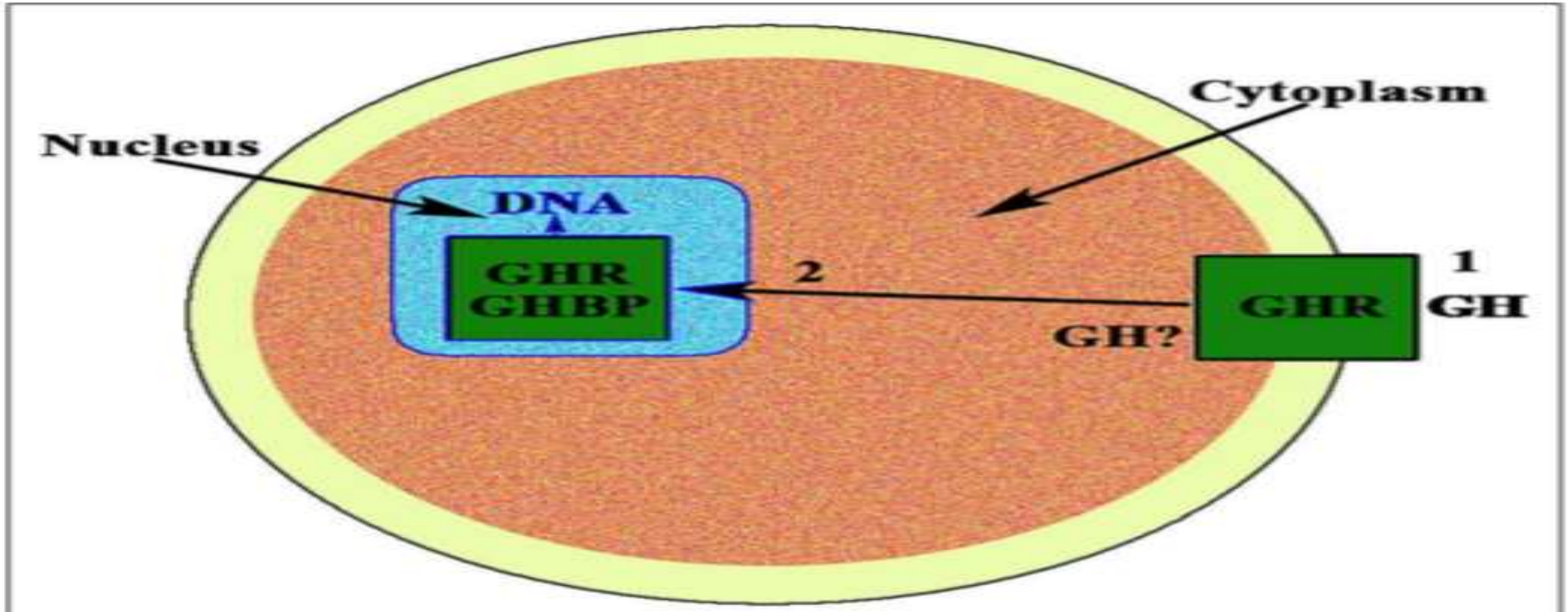


FIGURE 1 | GH receptor in the nucleus of the oocyte. In the nucleus of the oocyte, the GH receptor (GHR) and the carrier protein GHBP have been found. This means that GH, after interacting with its membrane receptor (1), has allowed the internalization of both, GH and GHR, (2) and then the receptor is translocated to the cell nucleus where it would act as a gene transcription factor. The possibility exists that the own GH is expressed in the oocyte.

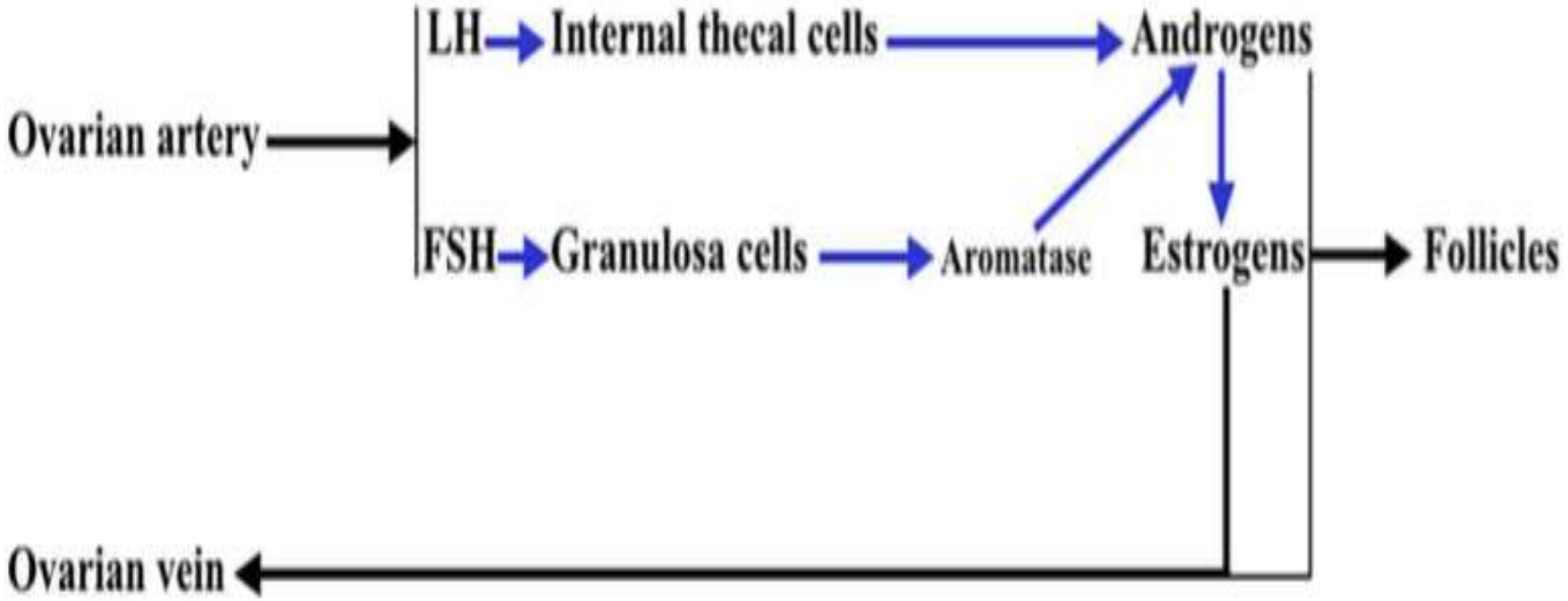


FIGURE 2 | Interactions between the theca cells of the internal layer and the granulosa cells in a growing follicle. Under the stimulus of pituitary LH, the theca cells produce androgens that reach the granulosa cells and in these, under the control of pituitary FSH, that activates aromatase, they are aromatized to estrogens (mainly estradiol). These estrogens are released into the systemic circulation and follicular fluid. Likewise, a small part of the androgens produced in the thecal cells pass into the systemic circulation.

- Later studies showed that there is a genetic expression of GHR **in cumulus cells, mature oocytes and preimplantation human embryos, in which there is a high expression of GHR from the 4-day morula onwards**
- This study led to the conclusion that, in humans, **GH plays a role in the maturation of the oocyte and embryogenesis, from its early stages. GH and GHR have been found in human ovaries from fetuses and adults** where they play a very important autocrine/paracrine role.

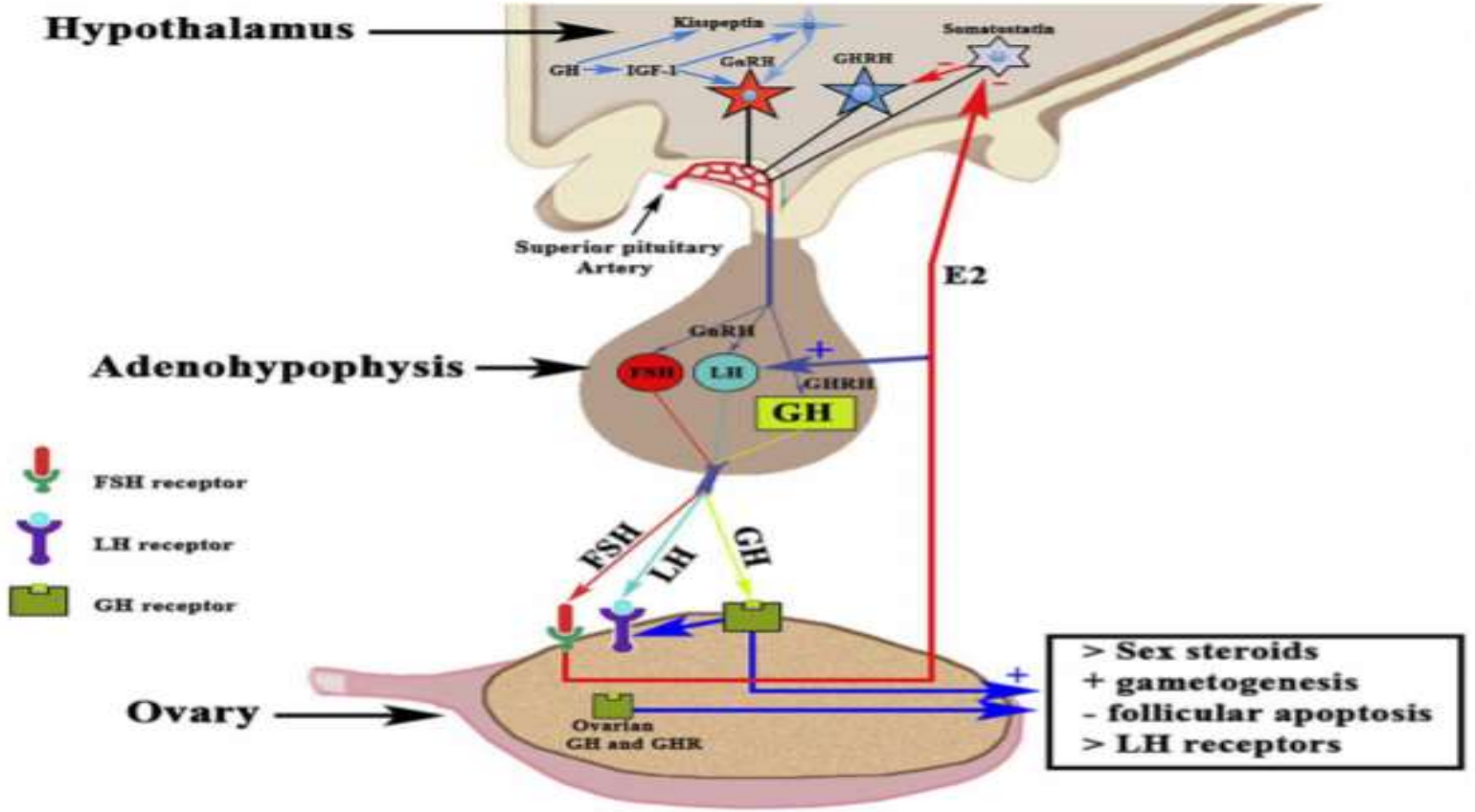
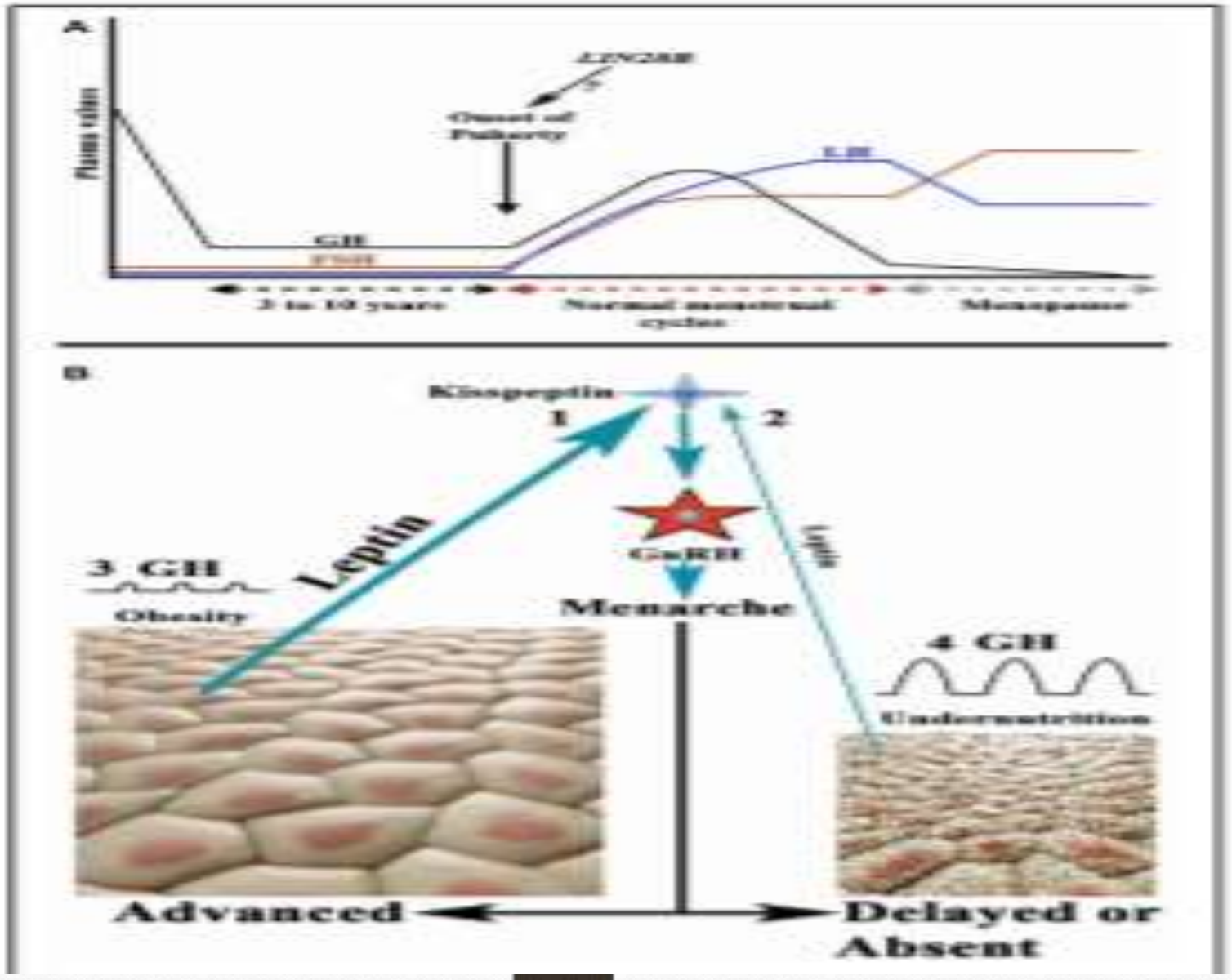


FIGURE 3 | Schematic representation of the Hypothalamic-Pituitary-Ovarian axis and the effects of GH on ovarian functioning. The activation of the GnRH pulse generator leads to the release of GnRH in the portal blood from which it reaches the gonadotropic cells in which it induces the release of FSH and LH (in proportions and amounts variable throughout a menstrual cycle) in the systemic circulation. Both gonadotropins interact with their ovarian receptors, triggering the previously described effects. The release of pituitary GH is induced by hypothalamic GHRH, which is negatively regulated by somatostatin. Systemic GH interacts with its ovarian receptor GHR and induces the positive regulation of LH receptors. The activation of GHR induces, throughout its effects on thecal cells, an increase in the production of sex steroids, mainly estradiol (E2), which in addition to its actions at the ovarian level, is released into the general circulation. Estradiol increases the pituitary release of LH, and also acts by inhibiting the hypothalamic release of somatostatin, which allows GHRH to be released into portal blood and stimulate pituitary synthesis and release of GH. GH and its GHR also are produced in the ovary; therefore, the ovarian actions of GH may also depend on the GH–GHR interaction. GH acts on the oocyte and the survival of the ovarian follicles. GH is also produced in the brain, where it stimulates the synthesis of IGF-1 that activates the GnRH pulse generator. In addition, both brain GH and IGF-1 stimulate kisspeptin neurons to release kisspeptin, a key factor in the activation of the GnRH pulse generator. +, stimulation; –, inhibition.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2
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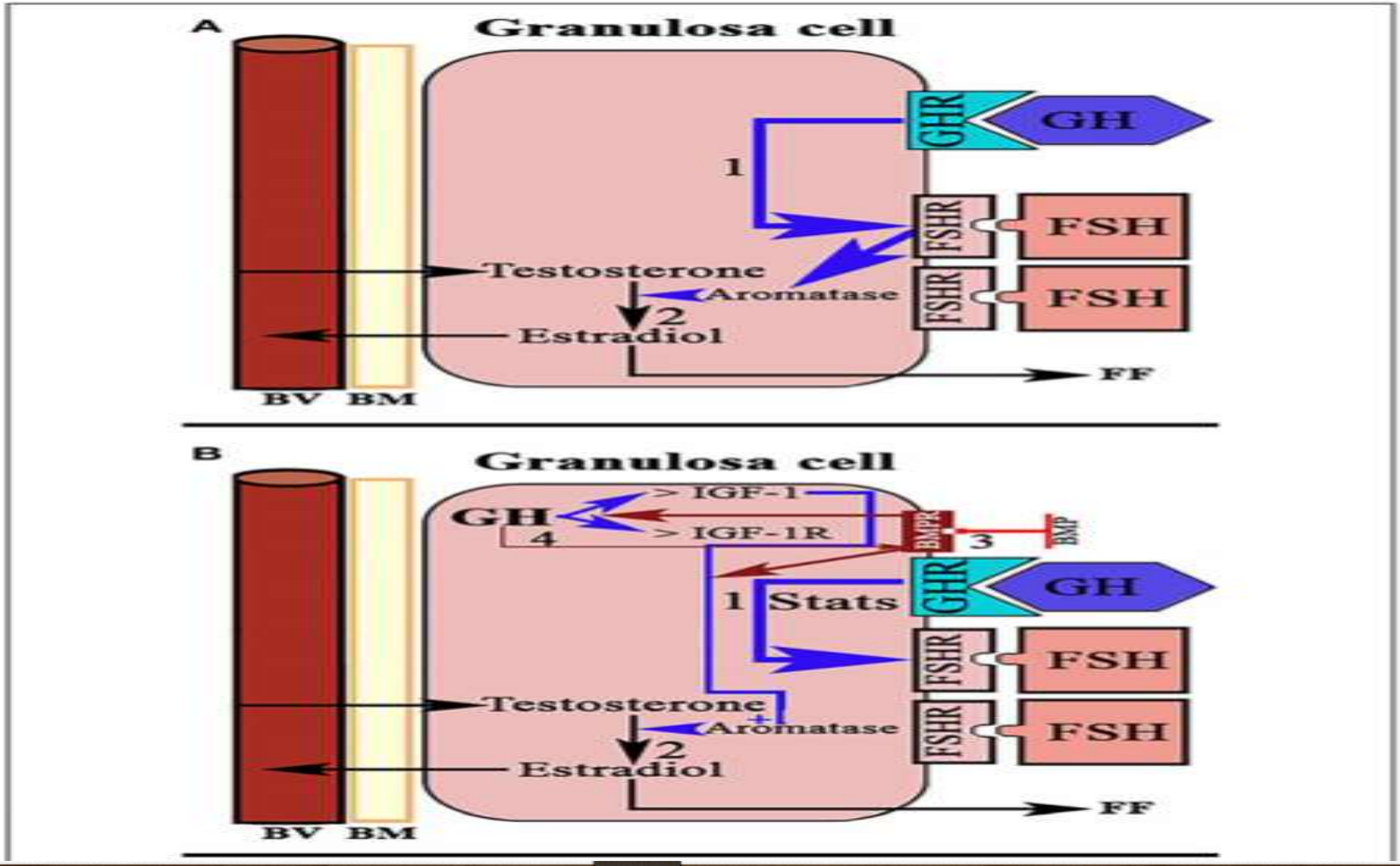
3 GH
Obesity
GnRH
Menarche
Leptin
4 GH
Undernutrition
Advanced
Delayed or Absent


A
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Granulosa cell
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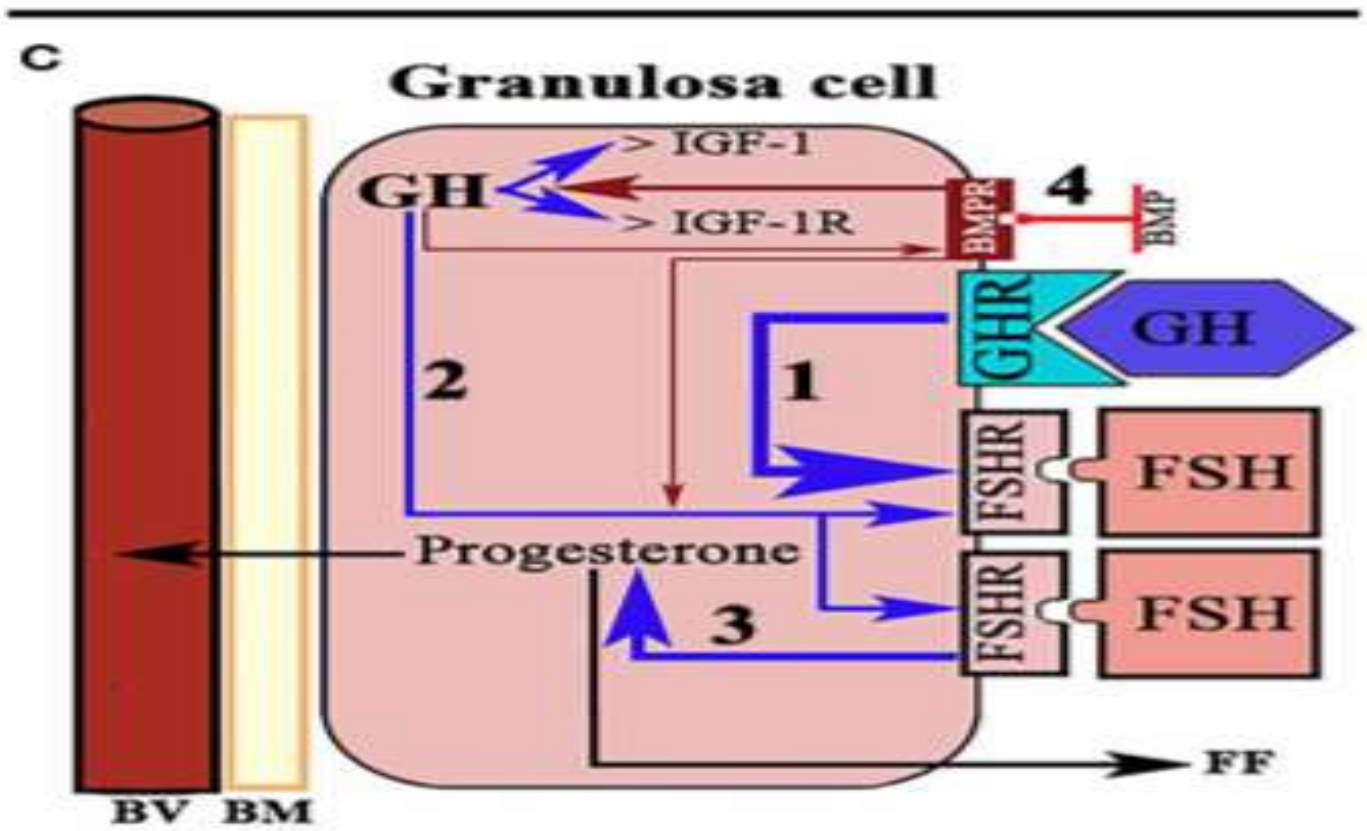

FIGURE 5 | Schematic representation of the actions of GH/IGF-1 in the ovarian granulosa cells. **(A)** In normal women, administration of GH in the early follicular phase exerts a synergistic effect with FSH on the production of estradiol. GH up-regulates the receptors for FSH (1) and increases the activation of aromatase by this hormone. The result is increased formation of

(Continued)

- FIGURE 5 | estradiol from testosterone from the theca cells (2). (B) In rats, it has been shown that the up-regulation of FSH receptors induced by GH is mediated through activation of Signal Transducers and Activators of Transcription STATS (1). Ovarian GH increases the expression of IGF-1 and its receptor, and is IGF-1 which increases FSH-dependent aromatase activity (2). Bone morphogenetic proteins (BMP) suppress the expression of the ovarian GH/IGF-1 axis (3), therefore inhibiting the aromatization of testosterone to estradiol. In turn, GH/IGF-1 down-regulate the expression of BMP receptors (4). Blue arrows: stimulation. Red arrows, inhibition. (C) Systemic GH (1) and/or ovarian GH (2) increase progesterone production in ovarian granulosa cells induced by FSH (3), while BMP blocks this effect of GH (4). Blue arrows, stimulation; Red arrows, inhibition; BV, blood vessel; BM, basement membrane; FF, Follicular

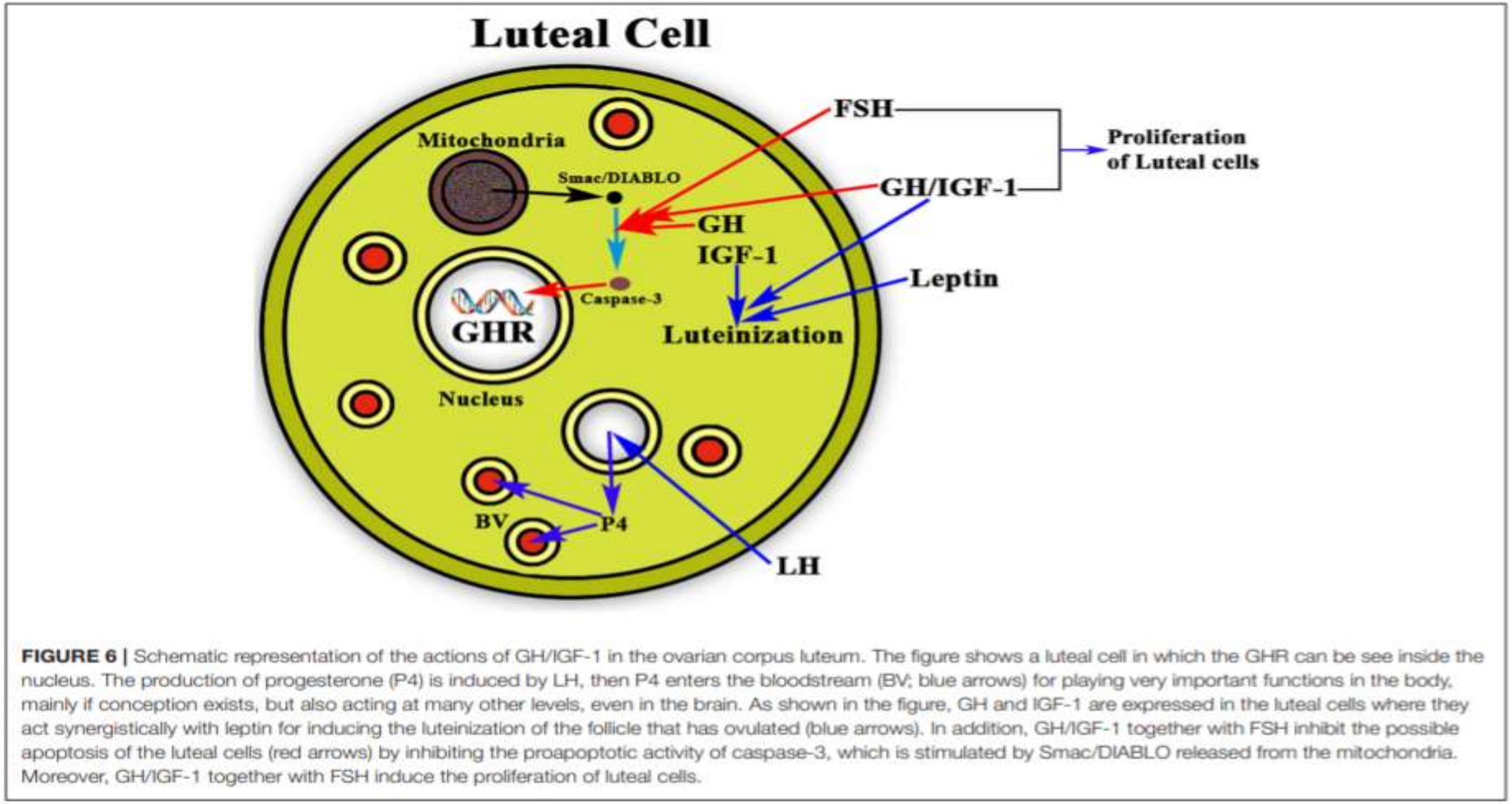

FIGURE 6 | Schematic representation of the actions of GH/IGF-1 in the ovarian corpus luteum. The figure shows a luteal cell in which the GHR can be see inside the nucleus. The production of progesterone (P4) is induced by LH, then P4 enters the bloodstream (BV; blue arrows) for playing very important functions in the body, mainly if conception exists, but also acting at many other levels, even in the brain. As shown in the figure, GH and IGF-1 are expressed in the luteal cells where they act synergistically with leptin for inducing the luteinization of the follicle that has ovulated (blue arrows). In addition, GH/IGF-1 together with FSH inhibit the possible apoptosis of the luteal cells (red arrows) by inhibiting the proapoptotic activity of caspase-3, which is stimulated by Smac/DIABLO released from the mitochondria. Moreover, GH/IGF-1 together with FSH induce the proliferation of luteal cells.

Growth Hormone and Menopause

- The pituitary secretion of GH suffers strong **changes throughout human** life: very **high release during the first year of life**, a **decrease during childhood** followed by a new **high release when puberty begins** and then a **progressive decrease in the amount of hormone released in each secretory pulse**
- **After age 20,** more or less, GH secretion is progressively declining by one-half every 7–12 years
- it is accompanied by a ***decline in plasma levels of IGF-1***, although its decrease is lesser than that occurring with GH
- Therefore, at age 50, more or less, GH secretion is residual, if it exists, and it leads to sig**nificant changes in body composition, such as reduced muscle mass, increased adiposity, reduced energy, decline in sexual activity, and increased cardiovascular risk, among other symptoms**
- Given the beneficial effects of GH, the question is: why does GH secretion declines alongside aging? Deconvolution analysis of data obtained from blood sampling every 20–30 min in humans indicate that there **are age-related alterations in the hypothalamic control of GH secretion**, its modulation by gonadal steroids, and in **GH autofeedback, leading to significantly decreased GH secretion in elder people**

Growth Hormone and in vitro Fertilization in Poor Ovarian Responders

- Based in the data presented in this review it is likely that GH administration may be useful as an adjuvant therapy in in vitro fertilization (IVF) for poor ovarian responders (POR) unable to get pregnant.
- In fact, the combined treatment with GH and gonadotropins was already used many years ago with successful results in terms of more **follicles developed, more oocytes collected, and higher urinary estrogens in patients with polycystic ovaries**
- Since those studies, several different trials have been conducted combining **GH treatment with Gns or human chorionic gonadotropin to induce in vitro fertility and embryo transfer in POR, and although some contradictory results have been reported**

- the overall conclusion is that the addition of GH significantly improves pregnancies in these infertile women and the number of positive results in terms of live birth rate. In this line, a recent study in 62 older women showed that co**treatment with GH led to the preovulatory down-regulation of FSHR, BMPR1B, and increased density of the largest follicles, and improved fertility in these older women in which there was already a significant decrease in the ovarian follicular reserve** .
- Another study analyzed the effects of 6-week pretreatment with GH in POR which were submitted to an in vitro fertilization treatment. This study, carried out in 380 POR, showed that the administration of the hormone significantly **improved the rate of utilization of oocytes and embryo quality increasing the live birth rates,** even in older patients who had previously experienced unsuccessful results from classical techniques (85). Moreover, another recent study demonstrated that co-treatment with GH in patients with **normal ovarian response significantly increased pregnancy rate**

OVARIAN ANGIOGENESIS

- As is logical, and it has been briefly described before, for a normal ovarian functioning the gland needs to receive an adequate and perfectly regulated blood supply. Follicles are the main functional structures of the ovaries; they are formed during *fetal development and are composed of a single layer of cells, granulosa cells (GCs), that surround the oocyte.*
- These are the primordial, inactive follicles; these GCs are surrounded by another type of cells, the **thecal cells (TCs) which will play a very important role, producing sex steroids, in the development of the follicles until their final stage which culminates in ovulation and luteogenesis**.
- Before the beginning of the reproductive life, a number of primordial follicles have been transformed in primary follicles. These consist in the oocyte surrounded by the pellucid zone and GCs. Around these a basal membrane separates the GCs from the TCs. In each menstrual cycle, a small number of primary follicles grows until, in general, only one of them, the dominant f follicle, suffer a proteolytic process that allows the release of the ovule, while the other follicles that had initially begun to evolve together with the dominant follicle, progressively suffer a process of atresia. The question is: why does only one follicle ends the process leading to ovulation in each menstrual cycle?

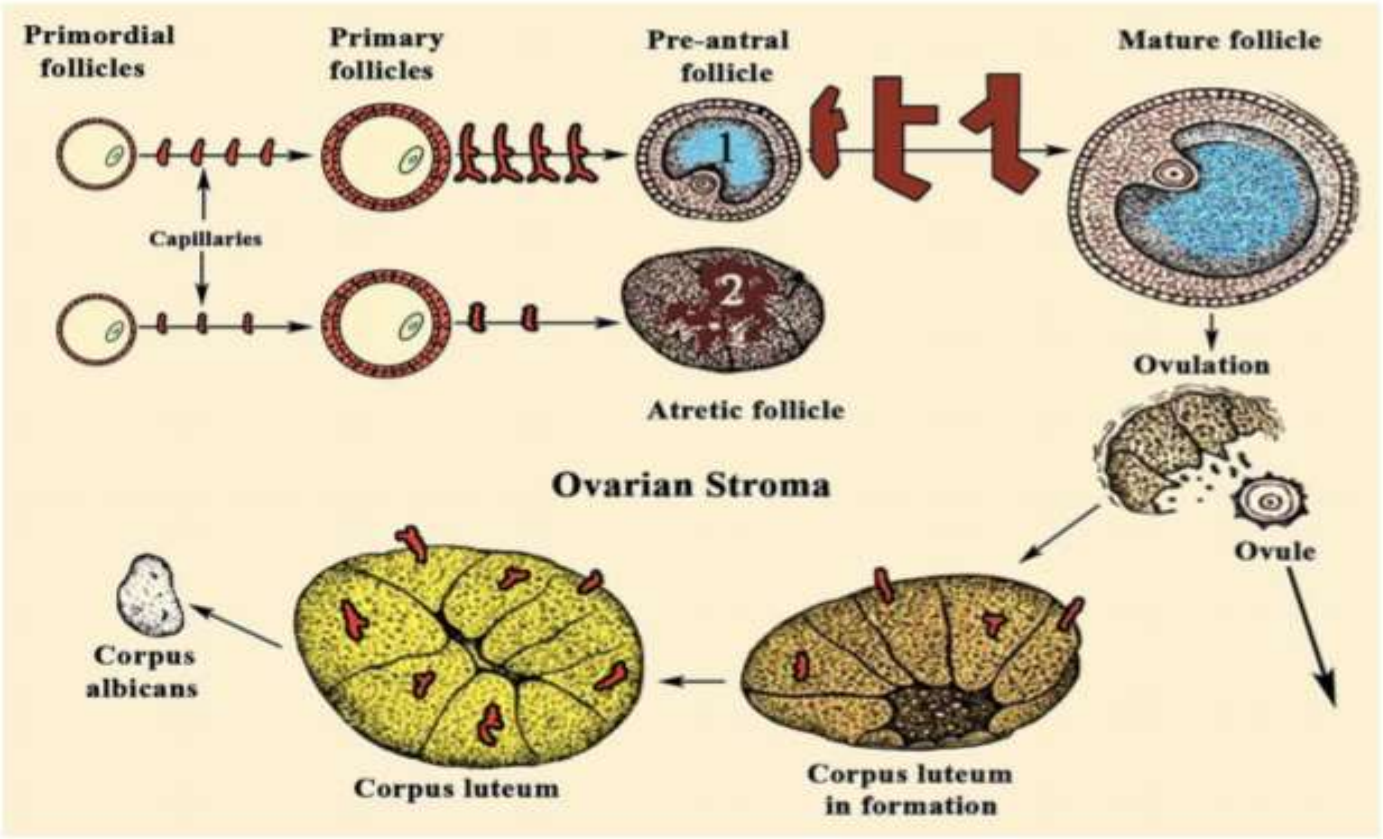

FIGURE 7 | Sequence of ovarian events during a normal menstrual cycle. During a normal menstrual cycle some primordial follicles begin to grow but, usually, only one of them reach the final maturation until it releases the ovule. It depends on the amount of VEGF-A and E2 existing in the follicular fluid. (1) Shows a dominant follicle, in which VEGF-A and E2 levels are quite higher than in the follicles that began to grow with it. These follicles degenerate and become atretic (2). Note the high number of capillaries supplying blood to follicle (1), while the atretic follicle (2) is almost privated of blood supply. VEGF-A, among other factors, is the main responsible for the generation of new small vessels supplying blood to the follicles. Similarly, after ovulation, the formation of the corpus luteum requires an increase in blood supply, as can be seen in the figure.

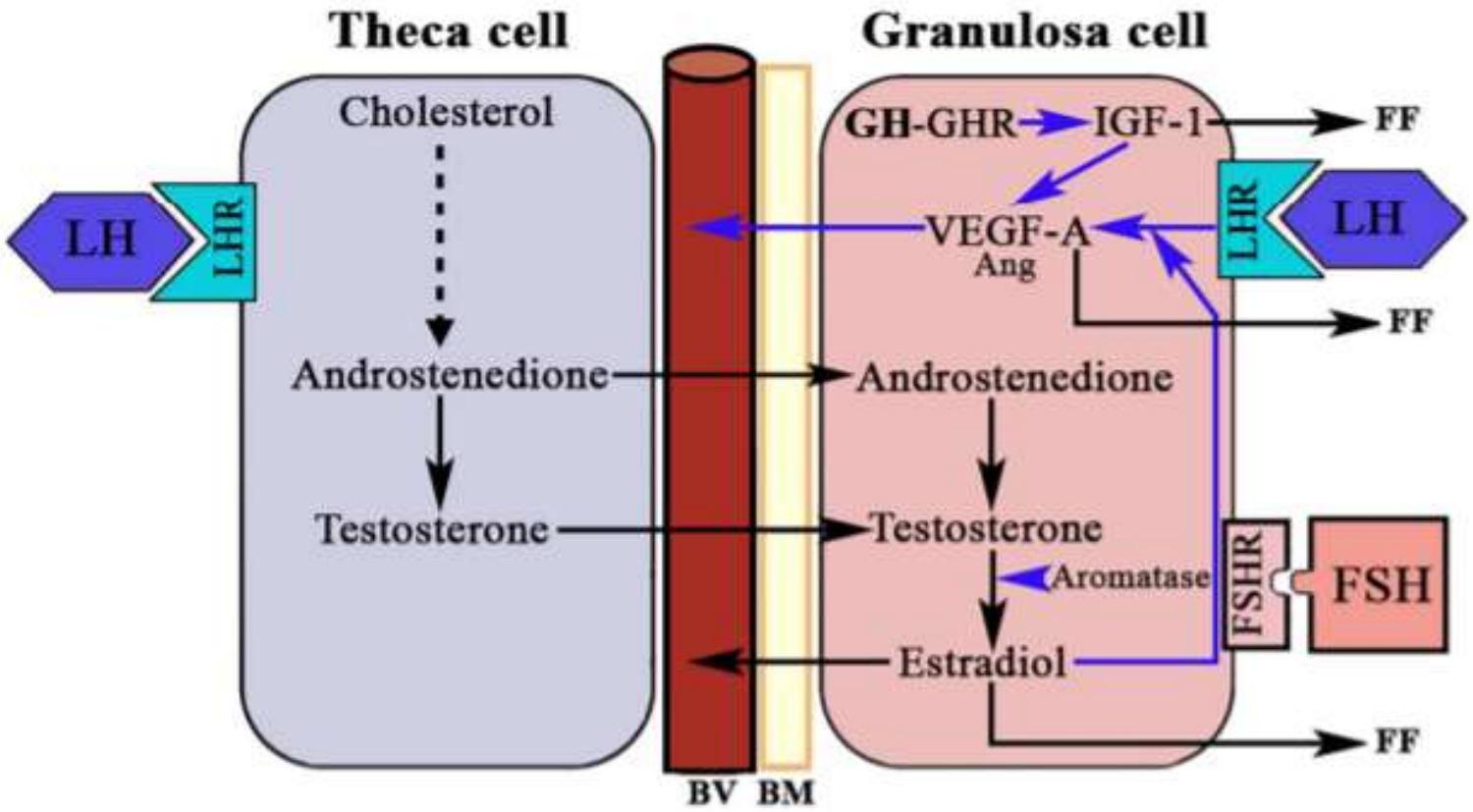

FIGURE 8 | LH, GH/IGF-1, and ovarian angiogenesis. The androgens produced in the theca cell under the stimulation of LH cross to the granulosa cell where, under the stimulation of FSH, they are aromatized to estradiol. In the growing follicle, there are receptors for LH that in combination with the produced E2 induces the expression of VEGF-A (mainly) and angiopoietin 1 (Ang). VEGF-A and Ang 1 induce the formation of new vessels (BV). There is a possibility that the GH produced in the granulosa cells induces the expression of IGF-1 who also favors the production of VEGF-A. VEGF-A and estradiol are released into small blood vessels, and both factors and IGF-1 are also released into the follicular fluid (FF). BV, blood vessels; BM, basement membrane; LHR, LH receptor; FSHR, FSH receptor; GHR, GH receptor; Blue arrows, stimulation.

Growth Hormone and Ovarian Angiogenesis

- Among the factors regulating VEGF family expression in humans GH plays a pivotal role, either **directly or by inducing the expression of other proangiogenic factors**, such as the Insulinlike growth **factors (IGF-1 and IGF-2), FGF-2,** epidermal growth factor (EGF), among others; moreover, GH is able to interact with receptors for Prolactin (PRL), which also is able to induce proangiogenic effects
- At this point, it is important to note that the pituitary secretion of GH is strongly potentiated by sex steroids, mainly E2, which in its free form (fE2) directly reaches the central nervous system (CNS), or is formed at this level from the hypothalamic aromatization of testosterone

- The possibility exists that systemic **GH could induce ovarian VEGF-A expression**, but this has not been demonstrated; perhaps because the own GH and its receptor are produced in the ovary in humans and bovines
- Moreover, the production **of GH by ovaries is higher in GCs and oocytes, avascular follicular compartments**, separated from the systemic circulation by the basal lamina
- Local expressions of GH and GHR have also been detected in the chicken ovary during sexual maturation in hens (108, 109) and fishes (110). Ovarian GH would act in an autocrine/paracrine way, so the hormone could play a role in the regulation of ovarian angiogenesis, as suggested by the fact that ovarian expression of the GH gene increases during follicular development but significantly decreases when immature follicles reinitiated meiosis

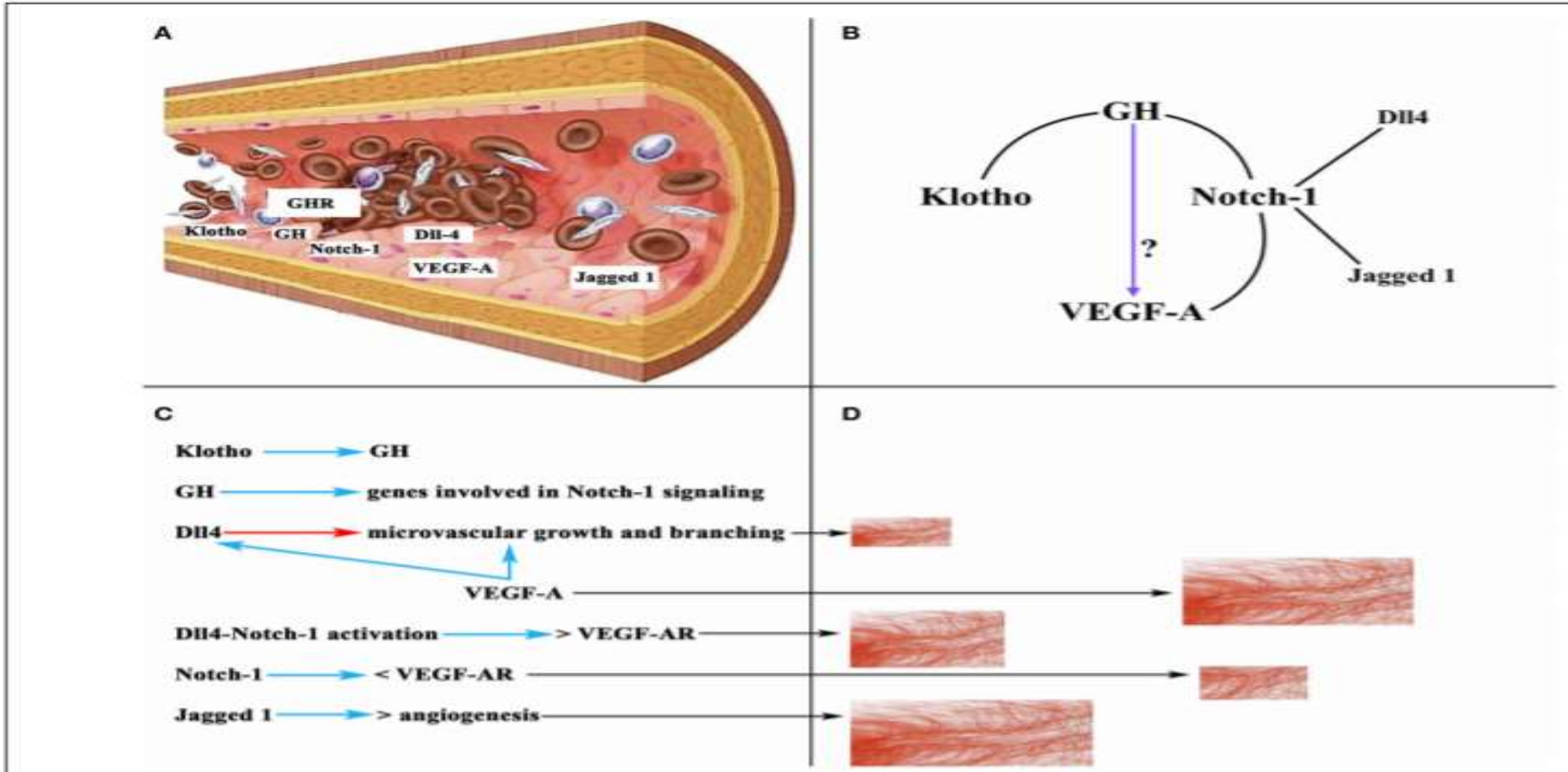

FIGURE 9 | Schematic representation of the action of VEGF-A, Notch-1 and its ligands on ovarian angiogenesis. **(A)** Section of an ovarian vascular vessel showing the expression of some factors involved in ovarian angiogenesis: Klotho, VEGF-A, Notch-1 and its ligands Dll-4 and Jagged 1, the receptor for GH (GHR), and presumably GH. **(B)** Relationships between Klotho, GH, Notch-1, and VEGF-A. These lead to the possibility that GH can act directly on the induction of the expression of VEGF-A in the endothelium of ovarian microvessels. **(C)** Summary of the actions of the factors mentioned in **(A)**. Blue arrows, stimulation; Red arrow, inhibition; <, decrease; >, increase; VEGF-AR, receptor for VEGF. **(D)** Schematic representation of the growth of ovarian microvessels produced by the action of each one of the factors showed in **(C)** (black arrows).

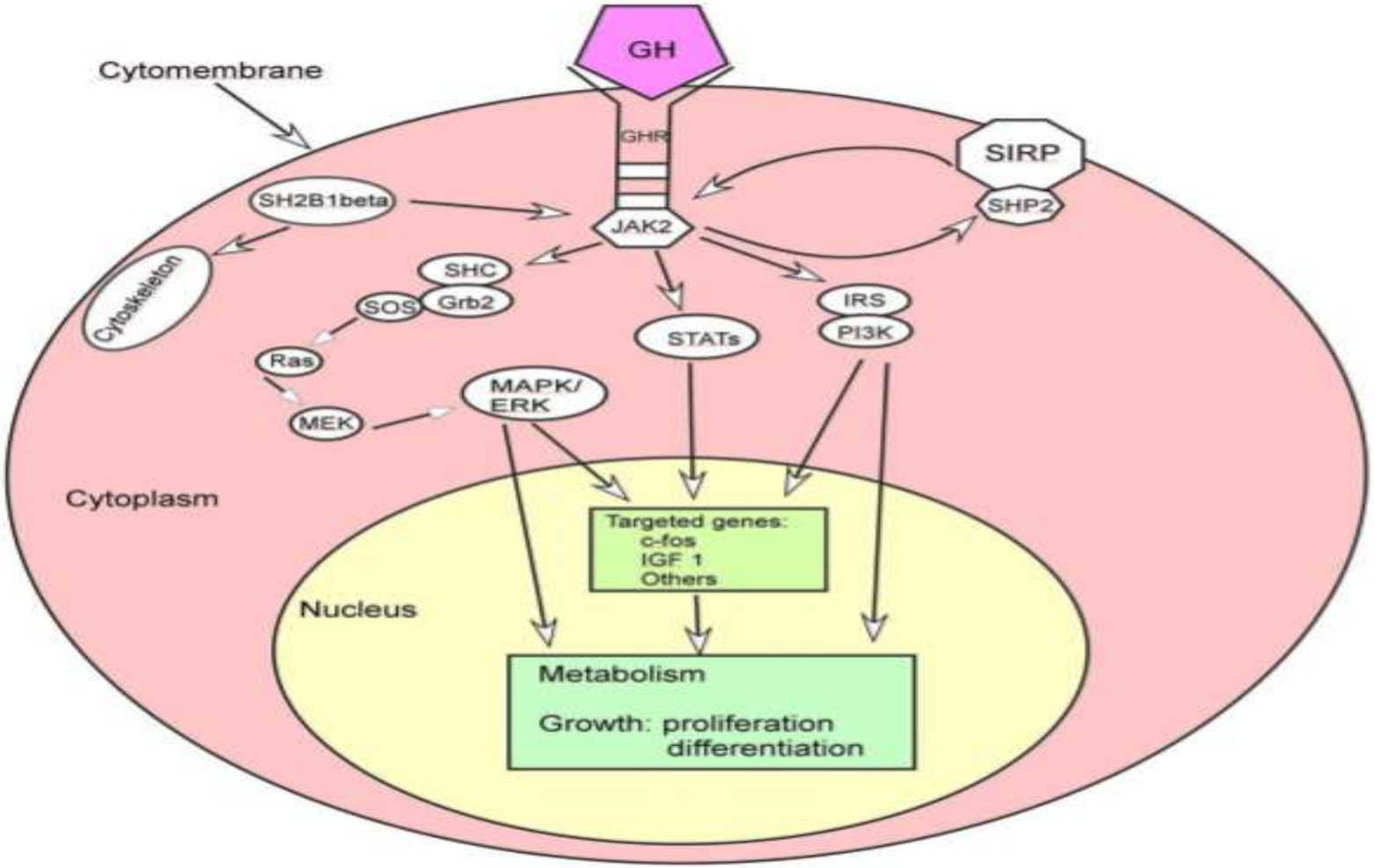

FIGURE 1 | Growth hormone (GH) acts through some signal pathways. ERK, extracellular signal-regulated kinase; GHR, growth hormone receptor; Grb, growth factor receptor-bound protein; IRS, insulin receptor substrate; IGF 1, insulin-like growth factor 1; JAK2, Janus kinase 2; MAPK, mitogen-activated protein kinase; MEK, dual specificity mitogen-activated protein kinase 2; PI3K, phosphatidyl inositol 3 kinase; SHC, SH2-domain containing transforming protein; SIRP, signal regulatory protein; SOS, son of sevenless; STAT, signal transducer and activator of transcription.

Effect of GH on Ovarian Reactivity

- As one of the targets of GH action, the ovary can be **directly regulated by GH for its reactivity to gonadotropins.**
- At the same time, **GH can indirectly influence the ovarian function through IGF-I**.
- Ovarian *granulosa cells produce IGFs* and express IGF receptors, and the IGFs and the receptors form a paracrine/autocrine system together with IGF binding proteins
- Binding of **IGF-I with its receptor can activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling** pathway to stimulate and regulate normal follicular growth and development in synergy with gonadotropins to increase the luteinizing hormone receptor level, consequently raising the ovary sensitivity to the follicule-stimulating hormone (FSH)

Effect of GH on Follicle Development

- GH is a potent activator **of proliferation and differentiation of the ovarian follicles**, and its administration generally increases ***ovarian weight and follicular size and number but inhibits follicular atresia***
- GH is necessary for optimal follicular maturation and survival because **GH addition to in vitro maturation medium of primordial and immature follicles can promote activation,** survival and development of preantral follicles originating from sheep, goats and mice
- Besides enhancing proliferation of the thecal and granulosa cells in the immature preantral follicles of mice
- GH can improve **the oocyte retrieval and fertilization rate in human oocytes** subjected to in vitro maturation (44) and promote cumulus expansion and subsequent embryo development in rhesus macaque

- Expression of GH in transgenic mice and adult sheep can promote follicular development besides **increasing the ovary weight, ovulation rate, and the size and health of ovarian follicles**
- In goat antral follicles, **GH can indirectly adjust the early development stage but control the late stage formation of follicles through the GHR**
- This is because **GH can induce granulosa and thecal cells to produce IGF-I**, which can regulate the ovarian function to resume meiosis of the oocytes through autocrine/paracrine function. **GH can also improve the mitochondria activity to directly ameliorate the oocyte quality**
- With aging, the number of functional mitochondria will be decreased, leading to impaired separation of chromosome associated with failed fertilization. Administration **of GH in older women can upregulate expression and activity of GHRs**, beneficial to improving the mitochondrial function, quality of oocytes and fertilization rate

Effect of GH on Endometrial Receptivity

- Good endometrial receptivity is the precondition for embryo implantation.
- During the treatment process of ovulation promotion with IVF, **supra-physiological levels of estrogen regulate effects of endogenous hormones on endometrial thickness and pattern** and expression of receptors and related factors to subsequently affect the endometrial receptivity
- The uterus produces GH, which in turn adjusts **the uterus , increases endometrial blood flow and expression of related cytokines and subsequently improves the endometrial receptivity**
- GH can also promote expression of endometrial vascular endothelial growth factor (**VEGF)-1,** leukemia necrosis factor, and matrix metalloproteinase 9, resulting in proliferation of endometrial glands, expansion of glandular cavity, blood vessel ***formation, and differentiation, thickening of endometria and endometrial mesenchyme***
- The endometrial receptivity is consequently improved. In addition, GH can increase synthesis of IGF-I in the ovary, enable the pituitary gland to secrete more FSH and promote secreting function of the granulosa cells so as to increase the estrogen level for improving the endometrial thickness and pattern

APPLICATION OF GH IN IVF

- In 1988, Homberg et al. (58) found that GH increases the **ovary sensitivity to the ovulation-inducing effect of gonadotropins,** with significant reduction in the amount, duration of treatment and daily effective dose of human menopausal gonadotropin caused by GH addition.
- Some studies suggested that pretreatment of GH could increase ovarian response to gonadotropins, improve oocyte quality and consequently be applied in the pituitary downregulation cycle or in poor ovarian response to gonadotropins in the IVF
- Other authors did not support GH as an effective adjuvant for infertility treatment because the live birth rate was not increased even though some benefits might have been achieved through the use of GH

GH Application for Improving Ovarian Response

- GH application combined with gonadotropins for ovulation promotion can improve the pregnancy outcome in most patients with poor ovarian responses. Lattes et al.
- It was found that daily administration of low-dose (0.5 IU) GH from the first day of the GnRH agonist until the day of hCG application could significantly increase the clinical pregnancy rate (34.4 vs. 0%)

- In a prospective controlled trial investigating the efficacy of within an antagonist protocol in IVF/ICSI (intracytoplasmic sperm injection) cycles in poor responders, use of low-dose (4IU/d) GH on the start of ovarian stimulation could significantly decrease **the effective dose of gonadotropins** (median 750, low quantile (LQ) 533.3 and upper quantile (UQ) 1312.5 for GH group vs. 1375, 862.5, and 2962.5 for non-GH group, respectively) **and duration of stimulation** (median 8d, LQ 7d and UQ 10 d for GH group vs. 9d, 8d, and 10d for non-GH group, respectively), but increase the **total number of** oocytes (median 4, LQ 3 and UQ 7 for GH group vs. 3, 2 and 4 for non-GH group, respectively), **metaphase II stage oocytes** (median 2, LQ 1 and UQ 6 for GH group vs. 1, 0 and 2 for nonGH group, respectively***), two pronucleus zygotes*** (median 2, LQ 0 and UQ 3 for GH group vs. 1, 1 and 2 for non-GH group) and good-quality transferred embryos (median 1.5, LQ 1 and UQ 2 for GH group vs. 0, 0 and 1 for non-GH group, respectively), with ultimate increase in the clinical pregnancy rate (21.74 vs. 0%)

- either high- (12IU/d or 24 IU/qod) or low-dose (2IU/qod) GH could significantly improve the clinical pregnancy (RR 1.76, 1.25–2.48) and the live birth rate (RR 1.91, 1.29–2.83) in poor ovarian responders even though GH supplementation in the middle of luteal phase did not increase the pregnancy and live birth rates
- the clinical pregnancy rate was not significantly different among the four different protocols (36.7 vs. 23.2, 25.9, and 30.4%, $P > 0.05$) even though there was a difference in favor of the long GH agonist.
- Since the long GH agonist protocol required significantly greater gonadotropin dose and longer duration of stimulation, low-dose GH was suggested for GH supplementation protocol because low-dose GH could improve the reactivity of the ovary

GH Application for Improving Oocyte Quality

- In a sequential crossover study of IVF to evaluate GH supplementation in poor-prognosis patients based on the past failure to conceive due to low response to high-dose stimulation (< 0.05) per fresh transfer and per frozen-thawed embryo derived from GH cycles leading to a highly significant productivity rate (30 vs. 14%, $P < 0.001$).
- GH effects were significant across all age groups, especially in **younger patients** (24 vs. 10% for patients 40 years), and independent of stimulation modality or number of transfers.
- GH (10 IU) was injected in the previous cycle on days 7, 14 and 21 with a final injection on day 2 of the treatment cycle for the first 4 years of the study, and for the last 2 years of the study, patients received six injections with the first beginning on day 21 of the preceding cycle and the subsequent injections being on days 2, 6, 8, 10 and 12 at the dose of 10 IU.

- Nonetheless, it was suggested that **longer pretreatment (4–6 weeks before gonadotropin start) with low physiological dose of 2 IU/d GH might be more beneficial to follicular growth and development.**
- GH is beneficial to the **repair of oocytes and quality improvement of ova in older patients**
- because it can upregulate expression of IGF-I in the ovary and stimulate production of oocyte-derived growth and differentiation factor and bone morphogenetic protein-15

- After studying the outcomes of poor responders following GH pretreatment (4.5 IU GH administered once every 2 days since day 16 of the previous cycle for six times and once every 2 days since stimulation day 1 for three times) with IVF/ICSI mild stimulation protocol (100 mg Clomiphene citrate administered daily from day two or three of menstrual cycle) in a retrospective analysis of 132 patients whose data were prospectively enrolled and maintained, Chu et al found that GH supplementation could significantly increase the goodquality embryo rate in either IVF (68.1 vs. 51.5%, P = 0.008) or ICSI (53.9 vs. 36.7%, P = 0.045) group (61). In an observational study investigating GH adjuvant therapy in patients with three or more IVF failures (15), GH **supplementation in the dose of 8 IU administered from day 1 of stimulation until the trigger day could significantly increase the pregnancy rate (25.7 vs. 18.2%, P < 0.01**) per retrieval in these patients, with the pregnancy rate being elevated to a level similar to that observed in the study center for the whole population. An improvement of cytoplasmic competence is proposed as an explanation for this.

Adequate thickness of the endometrium

- is the key to successful implantation, and a thin endometrium is critical in implantation failure
- A thin endometrium may be caused by impaired endometrial growth which is closely related to angiogenesis and uterine blood flow.
- Angiogenesis is necessary for endometrial growth following menstruation and can provide a vascularized **receptive endometrium for implantation**
- Uterine blood flow is also an important factor for endometrial growth and is closely related to endometrial vascular development (86, 90, 91). Low uterine **blood flow may cause a decreased pregnancy rate in patients with IVF-ET (embryo transfer), suggesting a close relationship of uterine blood flow with uterine receptivity**
- High blood flow impedance of the uterine and radial **arteries, poor growth of glandular epithelium**, decreased VEGF and poor vascular development have all been confirmed to be characteristic of a thin endometrium
- High blood flow impedance in the uterine and radial arteries may impair glandular epithelium growth and decrease endometrial V

- **VEGF level, and low VEGF level may result in poor vascular development, further decreasing the endometrial blood flow.**
- This is a vicious circle which may lead to a **thin endometrium** associated with poor endometrial receptivity. In a randomized controlled trial investigating effects of GH on uterine receptivity in women with repeated implantation failure in an oocyte donation program, it was demonstrated that administration of GH (dose and timing were not mentioned in the study) could significantly ($P < 0.05$) increase the endometrial thickness (9.3 ± 1.5 vs. 8.6 ± 1.0 mm), implantation rate), pregnancy (54.3 vs. 17.1% with the OR of 6.9 and 95% CI 2.2–22.5) and live birth rates (51.4 vs. 17.1%, with the OR 6.4 and 95% CI of 2.0– 20.9), with no abnormality detected in any of the babies born (1). C

- ADMINISTRATION PROTOCOL OF GH Currently, the addition protocols of GH for poor ovarian responders in IVF include addition at 4–6 weeks before hCG administration, in the luteal phase of the preceding menstrual cycle (in the pituitary down-regulation phase within a gonadotropin-releasing hormone agonist long protocol), at the time of hCG administration, and at the middle and late follicular phases. The dose for GH is from 0.5IU/d to 12 IU/d, but a small dose was preferred in a recent study (3-6 IU/week)