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Review article

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Luteal phase support in assisted reproductive technology

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Abstract

Infertility affects one in six couples, with in vitro fertilization (IVF) offering many the chance of conception. Compared to the solitary oocyte produced during the natural menstrual cycle, the supraphysiological ovarian stimulation needed to produce multiple oocytes during IVF results in a dysfunctional luteal phase that can be insufficient to support implantation and maintain pregnancy. Consequently, hormonal supplementation with luteal phase support, principally exogenous progesterone, is used to optimize pregnancy rates; however, luteal phase support remains largely 'black-box' with insufficient clarity regarding the optimal timing, dosing, route and duration of treatment. Herein, we review the evidence on luteal phase support and highlight remaining uncertainties and future research directions. Specifically, we outline the physiological luteal phase, which is regulated by progesterone from the corpus luteum, and evaluate how it is altered by the supraphysiological ovarian stimulation used during IVF. Additionally, we describe the effects of the hormonal triggers used to mature oocytes on the degree of luteal phase support required. We explain the histological transformation of the endometrium during the luteal phase and evaluate markers of endometrial receptivity that attempt to identify the 'window of implantation'. We also cover progesterone receptor signalling, circulating progesterone levels associated with implantation, and the pharmacokinetics of available progesterone formulations to inform the design of luteal phase support regimens.

Sections

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Key points

- During in vitro fertilization (IVF) treatment, supraphysiological ovarian stimulation and the resultant high sex steroid levels can disrupt the luteal phase via insufficient progesterone production from the corpora lutea, shortening the luteal phase.
- Luteal phase support during IVF can support implantation and maintain pregnancy by increasing progesterone levels, which is achieved either by increasing endogenous sex steroid secretion or by directly supplementing with sex steroids.
- The presence, or not, of the corpus luteum has implications for the degree of luteal phase support required to maintain pregnancy and for the risk of pregnancy complications.
- A gonadotrophin-releasing hormone receptor agonist (GnRHa) trigger for ovarian maturation is not sufficient to support functional corpora lutea, resulting in a more disrupted luteal phase than a human chorionic gonadotrophin (hCG) trigger.
- Frozen embryo transfer (FET) can mitigate the effect of the disrupted luteal phase after ovarian stimulation, and is favoured especially if a GnRHa is used to trigger oocyte maturation.
- FET, especially via methods that do not result in the formation of a functional corpus luteum, can increase the risk of pregnancy complications such as pre-eclampsia compared with fresh embryo transfer cycles.

Introduction

Infertility, which is defined as the inability to conceive within 1 year of the start of trying, affects one in six couples[1]. Assisted reproductive technologies (ART), notably in vitro fertilization (IVF) treatment, have emerged as promising interventions to address this challenge. Although ~86% of women undergoing IVF have at least one embryo available for transfer after their mature oocytes have been collected and fertilized in vitro, only 25–30% of IVF cycles culminate in a live birth, rising to ~50% when a euploid embryo is transferred[2]. The fall in success rates even with embryos that are perceived as 'top quality' underscores that the pivotal junctures in the success of ART lie in the implantation of the embryo and subsequent sustainment of the pregnancy. Factors precipitating embryo implantation failure span a spectrum from embryonic concerns (for example, aneuploidy or developmental anomalies) to issues with the endometrium, and even problems with the intricate interplay between the embryo and endometrium. Systemic conditions, such as metabolic disorders, haemostatic imbalances or immune system irregularities, further complicate this delicate balance.

During the natural menstrual cycle, after ovulation, typically only a single corpus luteum is formed from the remnants of the dominant ovarian follicle. The corpus luteum produces several factors, most notably progesterone, that transform the endometrium to become receptive to implantation of the embryo and subsequent pregnancy. By contrast, during IVF, the ovaries undergo supraphysiological stimulation to yield multiple oocytes to increase the likelihood of obtaining at least one good-quality embryo for embryo transfer to the endometrium. After ovarian stimulation, exposure to a factor with luteinizing hormone (LH)-like activity is essential to trigger the maturation of the oocytes, facilitating their collection in a fertilizable condition. These protocols lead to a perturbed luteal phase via multiple mechanisms (discussed in detail below), which is characterized by suboptimal circulating levels of progesterone and a shortened luteal phase[3]. To counteract this effect and facilitate a healthy pregnancy, luteal phase support can be provided in the form of hormonal supplementation, which primarily comprises progestogens (agents that mimic the action of progesterone)[4].

Two primary strategies for luteal phase support can be utilized (discussed in detail below). The first is augmented LH-like exposure (for example, supplementary human chorionic gonadotrophin (hCG)) to bolster the corpora lutea to produce endogenous sex steroids. The second is direct hormone replacement with exogenous sex steroids, effectively replacing the function of the corpora lutea. The need for luteal phase support was acknowledged early in the evolution of ART, resulting in a paucity of studies comparing luteal phase support with placebo or no luteal phase support[5–9]. Notably, initial studies in women undergoing IVF that compared luteal phase support with placebo did not show an increase in either ongoing pregnancy rates or live birth rates when each outcome was analysed separately. However, an improvement was seen in a composite outcome in a Cochrane analysis including 642 women (comparing control with luteal phase support), which was labelled 'very low-quality evidence' (odds ratio (OR) 1.77, 95% confidence interval (CI) 1.09–2.86)[5,10]. The pervasive endorsement of the necessity of luteal phase support today raises ethical concerns over trials that might exclude it. The literature regarding luteal phase support exhibits considerable heterogeneity and the lack of consensus complicates the formulation of unequivocal guidance on the optimal regimen[4,11].

In this Review, we examine the physiology of the luteal phase in natural cycles and the effects of ovarian stimulation on the luteal phase. We also scrutinize methods that attempt to gauge endometrial receptivity to implantation, including omics and imaging. The role of progesterone during the luteal phase is discussed, alongside a summary of serum levels of progesterone associated with implantation, and the pharmacokinetics of available progesterone formulations to inform luteal phase support regimen design. Lastly, we identify unanswered questions and explore emerging individualized approaches to luteal phase support. Of note, the available data refer primarily to cisgender women, with limited studies assessing clinical outcomes in other subgroups of people with ovaries.

The luteal phase in natural and IVF cycles

Natural cycle

Spanning the interval from ovulation to either the onset of menses or the establishment of pregnancy, the luteal phase typically lasts for ~14 days, although it can range from 11 to 17 days[12]. The luteal phase commences with the mid-cycle surge in serum LH levels (Fig. 1a), which lasts for ~48 h and drives formation of the corpus luteum[13]. The corpus luteum stands as a cornerstone for both initiating and sustaining pregnancy during the natural cycle. While shorter durations of LH exposure may be sufficient for oocyte maturation, they might not guarantee ovulation or the formation of a functional corpus luteum[14,15]. The corpus luteum can sustain itself on endogenous LH pulses for at least 1 week, following which embryonic hCG from a developing pregnancy provides support for maintaining the corpus luteum[11,16] (Fig. 1b). However, in the absence of pregnancy, levels of prostaglandin E within the corpus luteum fall[17], leading to its demise and a decline in serum levels of progesterone that induces menstruation.

The physiological LH surge has a rapid ascending limb (~14 h) and peaks at a mean level of 56.5 IU/l (range 25–144 IU/l), followed by a plateau of similar duration (~14 h), and then a descending limb (~20 h)[13]. Progesterone reaches its zenith 6–8 days after ovulation (Fig. 1a). In women with regular cycles, the median serum concentration of progesterone rises from 1.59 nmol/l at the LH surge to a peak of 39.2 nmol/l (range 5.39–78.5 nmol/l) 7 days later[18]. However, mid-luteal levels can exhibit considerable variance even within the same patient's ovulatory cycles (range 0.03–164.1 nmol/l)[19].

During the luteal phase, the frequency of LH pulses is halved compared with the follicular phase owing to negative feedback from progesterone (8.4 versus 15.2 pulses per day)[20]. Progesterone secretion lags behind LH pulses by 25–55 min[11,20]. This pulsatile secretion pattern complicates the interpretation of a single isolated progesterone value, which can fluctuate eightfold within 90 min[11,20]. Various minimum thresholds for mid-luteal serum concentrations of progesterone have been used to indicate preceding ovulation, ranging from >9.54 nmol/l[11] to 25 nmol/l[16] (and traditionally >30 nmol/l). Variability in the duration of the luteal phase can further complicate interpretation from a single measure as mean luteal progesterone increases with longer luteal phase duration: from 34.6 nmol/l (95% CI 26.7–42.3 nmol/l) if <11 days, to 49.6 nmol/l (49.3–49.9 nmol/l) if 11–15 days, and 63.0 nmol/l (58.2–68.1 nmol/l) if >15 days[18]. Despite these limitations, in an analysis of >250 ovulatory cycles from 102 healthy women with regular menstrual cycles, a single random serum level of progesterone >15.9 nmol/l had 89.6% sensitivity and 98.4% specificity for ovulation[19].

The role of the corpus luteum as the primary progesterone reservoir during pregnancy shifts to the placenta at 8–9 weeks gestation (called the luteoplacental shift)[16]. This transition is critical, as surgical removal of the corpus luteum at ~7 weeks gestation in women with healthy pregnancies can induce spontaneous termination of pregnancy; however, pregnancy can be preserved with intramuscular progesterone supplementation[16].

Luteal phase defect in ART cycles

During IVF treatment, a supraphysiological dose of follicle-stimulating hormone is used to for ovarian stimulation, to promote the development of multiple follicles (each containing an oocyte). Two main IVF protocols are used to prevent a premature surge in LH secretion that would induce ovulation before oocyte retrieval is conducted. In the 'long protocol', persistent exposure to a gonadotrophin-releasing hormone receptor agonist (GnRHa) induces downregulation of GnRH receptors, followed by a short period of ovarian stimulation. By contrast, in the 'short protocol', ovarian stimulation is initiated first and then a GnRH antagonist commenced to competitively prevent stimulation of GnRH receptors. Both protocols prevent a premature surge in LH secretion.

In the context of ART, ovarian stimulation is hypothesized to disrupt the ensuing luteal phase through multiple mechanisms. The degree of luteal phase deficiency hinges on whether functional corpora lutea form and can be sustained, which in turn is influenced by the ovarian stimulation protocol and the trigger used to induce oocyte maturation (either hCG or a GnRHa, discussed in detail in the next section) (Fig. 1c,d). Ovarian stimulation increases mid-luteal serum oestradiol levels (694 pmol/l versus 482 pmol/l) and suppresses gonadotrophins (<1 IU/l). Compared to a natural cycle, ovarian stimulation during ART induces a shortened median luteal phase from 13 (range 8–17) to 11 (9–14) days, even though mid-luteal serum levels of progesterone (50–55 nmol/l) remained similar[21].

One proposed explanation for the observed luteal phase deficiency during IVF is that serum levels of LH during the luteal phase are inadequate to maintain the function of the corpora lutea. This LH deficiency can arise as a result of supraphysiological circulating levels of sex steroids during the follicular–luteal transition that directly inhibit LH secretion[21,22]. Alternatively, endogenous LH levels can become persistently suppressed, particularly if a GnRHa is used to prevent a premature LH surge, as it can take up to 2 weeks for GnRH receptors in the pituitary to be renewed[16,23]. Potential damage to granulosa cells at the time of oocyte retrieval is another speculated mechanism. However, studies indicate that follicular flushing (the process of flushing a follicle with saline or culture medium after initial aspiration at oocyte retrieval to encourage retrieval of oocytes) does not affect the duration of the luteal phase or the levels of progesterone in treatment cycles during which only one follicle was grown[24]. Nevertheless, granulosa cells retrieved during the luteal phase of IVF cycles, particularly those triggered by GnRHa, exhibit reduced viability ex vivo and fail to maintain sex steroid production compared with granulosa cells retrieved during a natural cycle[25].

The effect of supraphysiological levels of sex steroids during IVF cycles was investigated using the aromatase inhibitor letrozole (half-life 45 h), which impedes the conversion of testosterone to oestrogen[22]. In women undergoing GnRHa-triggered oocyte donation cycles, letrozole treatment during ovarian stimulation reduced serum levels of oestradiol at day 5 after oocyte retrieval from 3,000 pmol/l to 2,000 pmol/l[22]. This reduction led to a higher level of progesterone (67.1 nmol/l versus 2.3 nmol/l) and a longer luteal phase (8 versus 5 days) in women treated with letrozole compared with a protocol without letrozole[22]. Notably, the serum concentration of oestradiol at oocyte retrieval was inversely related to the luteal phase duration[26], although not to pregnancy rates[27,28]. In 129 women undertaking IVF, letrozole treatment during ovarian stimulation reduced luteal levels of oestradiol by 69%, increased LH by 34% and progesterone by 38%, but did not enhance ongoing pregnancy rates compared with a protocol without letrozole[28]. Echoing this, in a randomized controlled trial (RCT) with 159 women published in 2023, letrozole during IVF did not significantly affect the live birth rate compared with placebo[29]. Overall, these findings suggest that supraphysiological serum levels of oestradiol during IVF can negatively feedback on LH and thus reduce mid-luteal progesterone and the duration of the luteal phase, although the use of letrozole to mitigate this effect did not improve the live birth rate.

Effect of the trigger on the luteal phase during IVF

The trigger used to induce oocyte maturation has a pivotal role in determining the available LH-like activity to support formation and survival of functional corpora lutea (Fig. 1). While hCG directly binds to the LH receptors on the corpus luteum, GnRHa stimulates the pituitary to release endogenous LH[30]. Of note, hCG has a substantially longer half-life than endogenous LH (28–30 h versus 30 min) (Fig. 1c) and exhibits a fivefold increased potency at the LH receptor in inducing a rise in intracellular levels of cAMP[31]. After administration, serum levels of hCG peak at 24 h to 120 IU/l, and revert towards baseline by 6–7 days[30]. In the absence of luteal phase support in IVF cycles, serum concentrations of progesterone peak at ~180 nmol/l on day 4 after hCG trigger; by contrast, the concentration peaks at 340 nmol/l on day 8 if additional hCG is provided as luteal phase support[23]. In this scenario, progesterone levels begin to decline once hCG levels drop below 30 IU/l[23]. Increasing the hCG dose can raise progesterone secretion during the luteal phase, but this strategy simultaneously increases the risk of ovarian hyperstimulation syndrome[32].

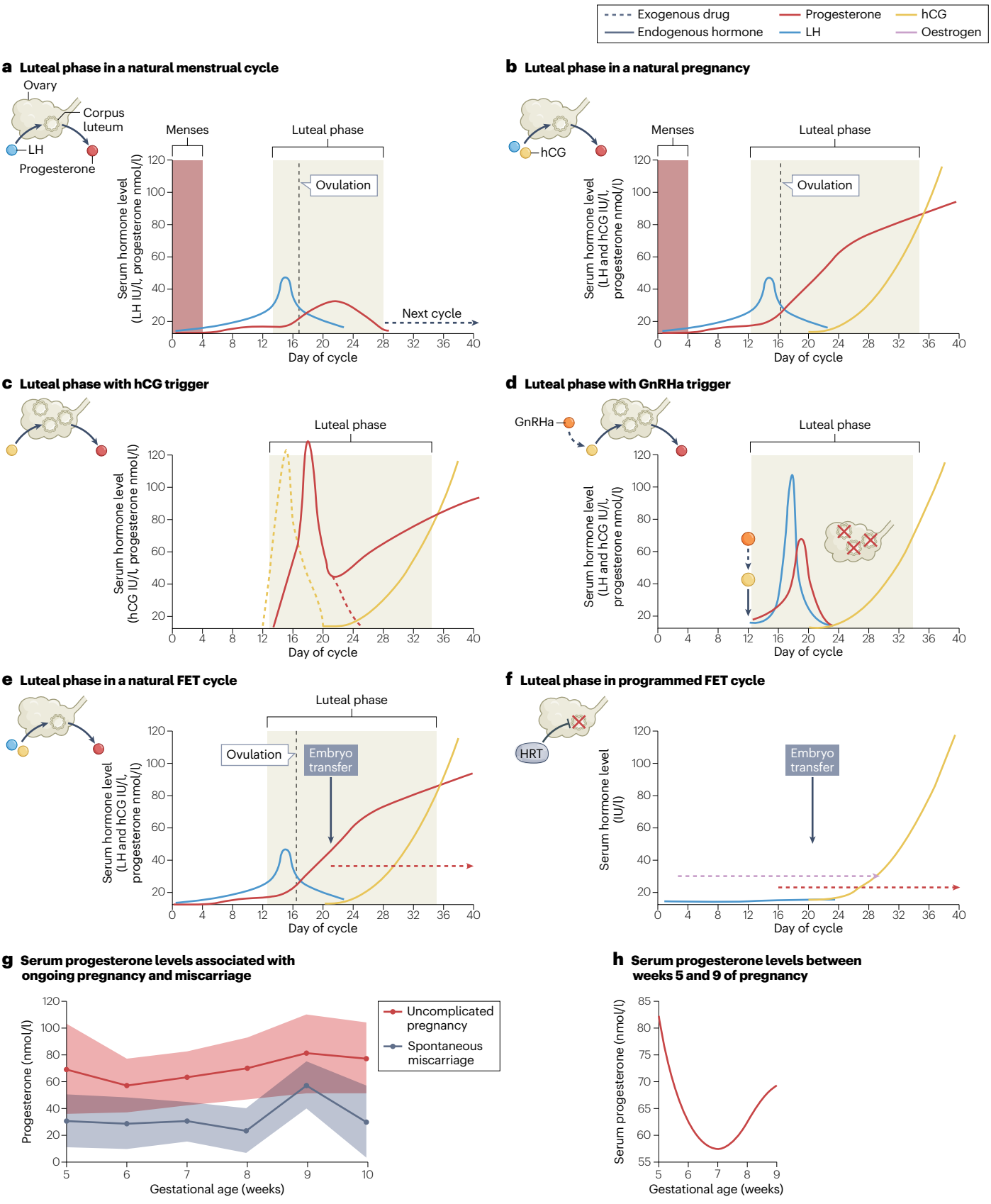

Exogenous drug
Endogenous hormone
Progesterone
LH
hCG
Oestrogen
a Luteal phase in a natural menstrual cycle
Ovary
Corpus luteum
LH
Progesterone
Menses
Luteal phase
Ovulation
Next cycle
Serum hormone level (LH IU/l, progesterone nmol/l)
Day of cycle
b Luteal phase in a natural pregnancy
hCG
Menses
Luteal phase
Ovulation
Serum hormone level (LH and hCG IU/l, progesterone nmol/l)
Day of cycle
c Luteal phase with hCG trigger
Luteal phase
Serum hormone level (hCG IU/l, progesterone nmol/l)
Day of cycle
d Luteal phase with GnRHa trigger
GnRHa
Luteal phase
Serum hormone level (LH and hCG IU/l, progesterone nmol/l)
Day of cycle
e Luteal phase in a natural FET cycle
Luteal phase
Ovulation
Embryo transfer
Serum hormone level (LH and hCG IU/l, progesterone nmol/l)
Day of cycle
f Luteal phase in programmed FET cycle
HRT
Embryo transfer
Serum hormone level (IU/l)
Day of cycle
g Serum progesterone levels associated with ongoing pregnancy and miscarriage
Uncomplicated pregnancy
Spontaneous miscarriage
Progesterone (nmol/l)
Gestational age (weeks)
h Serum progesterone levels between weeks 5 and 9 of pregnancy
Serum progesterone (nmol/l)
Gestational age (weeks)

Fig. 1 | The luteal phase in natural and IVF cycles, and serum progesterone levels in early pregnancy. a–**f**, Hormonal changes are depicted during the luteal phase in a natural menstrual cycle (part **a**), a natural spontaneous pregnancy (part **b**), an artificial human chorionic gonadotrophin (hCG)-triggered cycle (part **c**), an artificial gonadotropin hormone-releasing hormone agonist (GnRHa)-triggered cycle (part **d**), a natural frozen embryo transfer (FET) cycle (part **e**), and a programmed FET cycle (part **f**). Day 0 depicts the onset of menses. A rise in circulating luteinizing hormone (LH) levels (blue line) occurs mid-cycle (parts **a** and **b**), which induces ovulation (parts **a** and **b**), with the formation of a single corpus luteum that produces endogenous progesterone (solid red lines)[192] during the luteal phase. In the absence of pregnancy, the corpus luteum regresses, leading to the next menstrual cycle (part **a**), whereas in pregnancy, hCG (solid yellow line) maintains progesterone production (parts **b**–**f**). In in vitro fertilization (IVF) cycles, ovarian stimulation results in multiple corpora lutea leading to disrupted endogenous progesterone production (parts **c** and **d**). In natural FET cycles, a corpus luteum is present that produces progesterone (part **e**), compared with programmed FET cycles in which hormone replacement therapy (HRT) inhibits corpus luteum formation (part **f**). The subsequent hormonal milieu of the luteal phase is considerably different across different IVF protocols (parts **c**–**d**). Exogenous hormonal support is shown with dashed lines. The horizontal dashed arrows (parts **e** and **f**) only indicate the duration of exogenous progesterone and oestrogen support, which varies in clinical practice. **g**, Mean and standard deviations of serum concentrations of progesterone in individuals with low-risk spontaneous pregnancies (red) and spontaneous miscarriage at or before 16 weeks of gestation (grey). Data were originally presented in ref. 103. **h**, Declining mean serum concentrations of progesterone in individuals with low-risk pregnancies at gestational week 5; levels reach a nadir at gestational week 7, representing the luteal–placental shift. Part **h** is reprinted from ref. 104, Springer Nature Limited.

By contrast, GnRHa induces a shorter duration of endogenous LH secretion compared with the natural LH surge (~14 h versus 48 h) or the LH-like activity provided by hCG[33] (Fig. 1d). Following administration of the GnRHa trigger, serum levels of LH rise to 140 IU/l at 4 h and then return to baseline within 36 h[30]. This strategy results in a truncated and shorter luteal phase compared with IVF using hCG as the trigger (9 versus 13 days), as well as a lower median area under the curve of serum level of progesterone (269 nmol/l versus 16 nmol/l per day)[34]. The corpus luteum can survive brief periods (up to 48 h) without LH-like activity and yet still be rejuvenated with exogenous hCG[35]. The mid-luteal level of LH is higher in natural cycles (6.0 IU/l), than in GnRHa-triggered IVF cycles (1.5 IU/l) and hCG-triggered IVF cycles (0.2 IU/l)[36]. In summary, luteal phase disruption can occur in IVF cycles, with more disruption occurring when a GnRHa trigger is used rather than hCG, necessitating greater attention to luteal phase supplementation in GnRHa-triggered IVF cycles than in hCG-triggered cycles.

Fresh versus frozen embryo transfers

An alternative strategy to mitigate the influence of ovarian stimulation on the luteal phase of the IVF cycle is to defer embryo transfer from the initial 'fresh' cycle. Instead, embryos are frozen for use in a subsequent cycle, called a frozen embryo transfer (FET). In scenarios in which the risk of ovarian hyperstimulation syndrome is not increased, exogenous hCG is used as the first-line trigger in order to perform a fresh embryo transfer. However, if the risk of ovarian hyperstimulation syndrome is high (for example, in women with polycystic ovary syndrome (PCOS) or in those aged <35 years), then the preference leans towards a GnRHa trigger followed by FET.

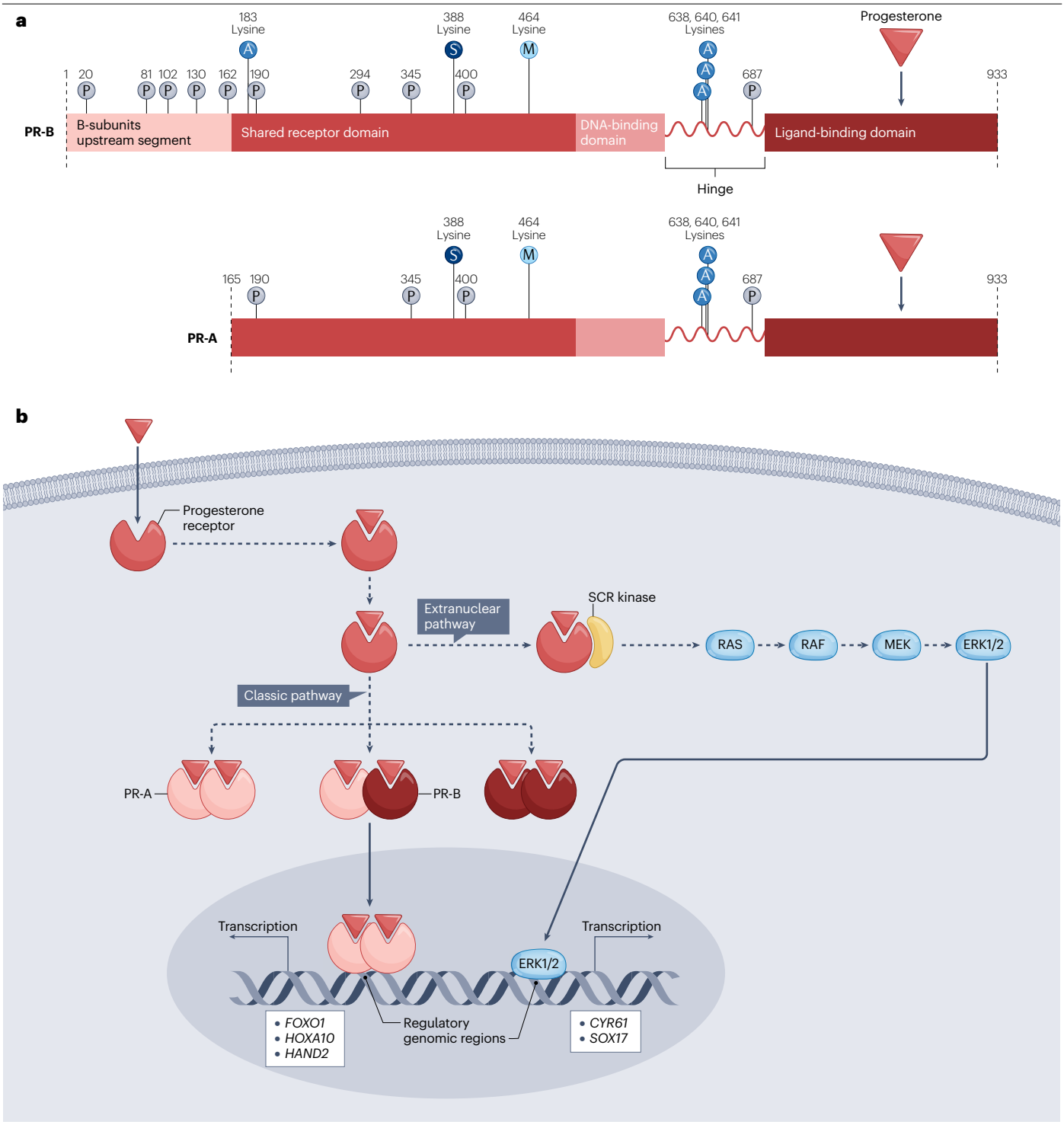
Historically, the majority of ART cycles were fresh; however, a marked shift has occurred towards FET cycles over the past decade. For example, an increase in the proportion of FET IVF has occurred in the UK, from 19% of IVF cycles in 2010 to 46% in 2021 (ref. 37), and in the USA from 24.2% in 2010 to 81.5% in 2020 (ref. 38). In terms of advantages, FET considerably reduces the risk of late ovarian hyperstimulation syndrome, allows sufficient time to conduct preimplantation genetic testing and negates the influence of the preceding supraphysiological levels of sex steroids on the luteal phase that are inherent in fresh transfers. FET can be conducted in various ways. First, a 'natural' cycle (Fig. 1e) after spontaneous ovulation and formation of a corpus luteum. Second, a 'modified natural' cycle, in which ovulation is induced using agents such as letrozole, clomiphene or gonadotrophins. Third, an 'artificial' or 'programmed' cycle (Fig. 1f), in which endogenous ovarian activity is suppressed and endometrial preparation is orchestrated using oestrogen and progesterone, leading to the absence of a corpus luteum[39].

In terms of outcomes, overall FET has comparable pregnancy rates to fresh cycles (OR 1.08, 95% CI 0.95–1.22)[40], although certain subpopulations (for example, individuals who respond more strongly to ovarian stimulation (high responders), who can include anovulatory women with PCOS[41] or women with high numbers of oocytes retrieved) could have better outcomes with FET than individuals who have a lower response to ovarian stimulation[42] or ovulatory women[43]. This disparity hints at a more pronounced disruption of the luteal phase in high responders during fresh cycles. Luteal phase support is less critical in natural cycle FET, as a corpus luteum is formed[44]. By comparison, programmed FET cycles have an increased risk of hypertensive disorders of pregnancy, fetal macrosomia and gestational diabetes mellitus[45], hypothesized to be due to the lack of a corpus luteum[45]. The corpus luteum produces various factors, including but not limited to: oestradiol, relaxin, cytokines, inhibin A, vascular endothelial growth factor and fibroblast growth factor 2 (ref. 20). However, progesterone is regarded as the most important factor and hence we describe its action and signalling in more detail.

Progesterone action and signalling

Progesterone, named for its 'pro-gestational' effect, is a steroid derivative of cholesterol. This factor exerts its effects through both classic ligand-activated progesterone receptors (PR), and cell surface progesterone G protein coupled membrane receptors (PGRMCs)[46]. In female reproduction, the actions of progesterone are predominantly mediated via the classic nuclear PR[47]. Although several nuclear PR variants exist, only nuclear PR-A and nuclear PR-B (Fig. 2a) are considered physiologically relevant[48]. These two variants act through two key pathways. First, a nuclear pathway in which nuclear PR-A or nuclear PR-B bind to the progesterone response element loci in regulatory regions of the genome to affect transcription. Second, a cytoplasmic pathway in which nuclear PR-A or nuclear PR-B fine-tune the transcription profile via the MAPK kinase pathway[49] (Fig. 2b).

The dominant isoform responsible for progesterone action is nuclear PR-B, which regulates the transcription of more genes than nuclear PR-A (nuclear PR-A regulates four genes, nuclear PR-B 65 genes, and both 25 genes)[50]. Direct targets of nuclear PR signalling relevant to human implantation, decidualization and pregnancy maintenance include genes regulating angiogenesis, the immune response, the cell cycle and apoptosis[51]. Both nuclear PR-A and nuclear PR-B are co-expressed in human endometrial stromal cells, resulting in three



dimerized receptor variants. The relative abundance of nuclear PR-A to nuclear PR-B changes during the menstrual cycle. In the follicular phase, tenfold dominance is observed of nuclear PR-A over nuclear PR-B, which reduces to 5:1 as ovulation approaches, and further to 2:1 in the early luteal phase, while expression of both receptors declines in the late luteal phase[52].

Progesterone also binds to PGRMC and adiponectin family receptors[53]. However, the physiological relevance of PGRMC signalling in the context of implantation failure remains unclear[54]. Intriguingly, some single nucleotide polymorphisms in PRs are over-represented in women with recurrent pregnancy loss[55] or unexplained infertility[56].

Fig. 2 | The nuclear progesterone receptor. a, Structure of the human progesterone receptor (PR) isoforms. Nuclear PR-A and nuclear PR-B share a common sequence but differ in length, with PR-A lacking the initial 164 amino acids at the N terminus. Both nuclear PR-A and nuclear PR-B have a modular composition with three functional domains: the N-terminal domain (key for full transcriptional activity), the central DNA-binding domain and the C-terminal domain (contains ligand-binding sites). Different receptor domains are depicted with capital letters. Loci susceptible to post-translational modifications are depicted in circles: acetylation (A), methylation (M), phosphorylation (P), sumoylation (S). **b**, Genomic signalling via activation of the nuclear PR. When inactive, the PR resides in the cytoplasm bound to chaperone proteins. Progesterone binding induces a conformational change to the PR, which then acts via either the classic genomic pathway or the extranuclear genomic pathway. The specificity of the transcriptional targets via the classic pathway depends on whether receptor homodimers (PR-A + PR-A or PR-B + PR-B) or heterodimers (PR-A + PR-B) are formed. The activation of the MAPK pathway by progesterone signalling is thought to be predominantly dependent on PR-B, as this isoform more readily shuffles between the nucleus and the cytoplasm. Genes specified are examples of factors known to be involved in decidualization and implantation, whose regulatory regions contain progesterone-binding elements[193].

The action of progesterone on the various receptor subtypes affects reproductive function by acting on endometrial or myometrial tissue, by modulating uterine immune cells, or by acting systemically to induce immunomodulation[46]. During the luteal phase, the endometrium undergoes substantial cellular changes to prepare for implantation and pregnancy. Progesterone induces alterations in the apical uterine epithelial cell membrane, resulting in 5–10-µm protrusions termed pinopodes, which increase the surface area, absorb luminal uterine fluid, and promote adhesion and invasion of the blastocyst through the action of integrins and selectins[57]. Once trophoblast adhesion commences, PR expression disappears from endometrial epithelial cells, driven by progesterone-induced modulation of the transcription factor *FOXO1* and leukaemia inhibiting factor[58]. Thus, timely progesterone-driven activation and suppression of transcription factors, cytokines, growth factors and cell adhesion molecules are crucial for successful implantation[57].

In addition, progesterone signalling affects pregnancy success by acting on immune cells that infiltrate the endometrium. The endometrium must accept and tolerate a semi-allogenic fetus, which requires it to be shielded from the maternal immune system in order to enable implantation. Paradoxically, a complete absence of the immune response is also undesirable, as pro-inflammatory cells are required for implantation and account for nearly half of endometrial cells at that time[59]. During pregnancy, the exposure of T cells to progesterone reduces their proliferative capability and alters the relative abundance of secreted cytokines (more IL-4 and less IFNγ, TNF, IL-10 and IL-5)[60]. Progesterone contributes to T helper 2 (T_H2) cell dominance in pregnancy by signalling through nuclear PRs and to reduced natural killer (NK) cytotoxicity through PGRMCs[61]. Progesterone can also weakly bind to the glucocorticoid receptor, thus potentiating anti-inflammatory effects[46].

The systemic role of progesterone in early pregnancy remains a subject of ongoing debate[46,62]. Pregnant women have more circulating cytotoxic PR-expressing T cells than non-pregnant women[46,63]. Progesterone dampens pro-inflammatory cytokine production in peripheral leukocytes and activates nuclear PR in peripheral NK cells, leading to apoptosis[63]. Overall, although the predominant function of progesterone is at the endometrium, circulating progesterone could facilitate systemic immunomodulatory changes that support pregnancy.

Window of implantation and endometrial receptivity

During the luteal phase, implantation requires the blastocyst's successful apposition, attachment and invasion into the endometrium[64]. A specific window of implantation (WOI) exists during which the endometrium is optimally receptive to an embryo. This endometrial receptivity pertains to the capacity of the endometrium to nurture the incoming embryo. By contrast, endometrial selectivity describes its ability to recognize and reject less suitable embryos[65]. The WOI spans 30–36 h and occurs 6–9 days after the LH surge in natural cycles, or 4–7 days after progesterone supplementation in ART cycles[65]. Miscarriage risk increases sixfold if implantation occurs beyond this window (13% on day 9 after ovulation versus 82% beyond day 11)[66,67].

Historically, the WOI and endometrial receptivity were assessed through histological analysis of the endometrium[68]; however, over the past decades, advances have shifted focus towards omics technology[66]. In a landmark study in 1950, Noyes and colleagues meticulously analysed endometrial biopsies from women with infertility[69]. They identified markers of endometrial receptivity, such as vacuolization, oedematous stroma and pinopode formation on epithelial cells, with increased numbers of pinopodes associated with increased pregnancy rates[68] (Fig. 3). Histological grading that is at least 2 days out of synchrony from that predicted from ovulation is considered out of phase[70]. However, histological evaluations are invasive and have limited clinical utility.

Endometrial receptivity tests

The advent of transcriptomic profiling has identified genes associated with a secretory endometrium, leading to the development of next-generation sequencing-based endometrial receptivity tests (Box 1). These tests include endometrial receptivity analysis (ERA), ERMap and beREADY[71].

ERA was developed by comparing endometrial gene expression against histology across three conditions: natural cycle, ovarian stimulation and endometrium suppressed by a hormone-secreting intrauterine device[72]. From these data, ERA pinpointed 238 genes that could categorize the endometrium as 'receptive', 'pre-receptive', or 'post-receptive'. However, similar to histological analysis, the ERA requires an invasive biopsy and can only guide the timing of embryo transfer in a subsequent cycle. This limitation raises the issue of the inter-cycle and intra-cycle reproducibility of the test and how often the WOI is displaced in a fixed manner, as might occur in some women with recurrent implantation failure. Furthermore, patients with endometrial pathology such as endometriosis (often excluded from clinical trials) are more likely to have progesterone resistance, which will affect the ERA result[73]. Consequently, despite the initial promise[74], a large multicentre double-blind RCT ($n = 978$) did not demonstrate an improvement in live birth rate among women undergoing euploid FET guided by ERA compared with those without ERA testing (58.5% versus 61.9%)[65,75]. Moreover, a post hoc analysis suggested that the subgroup in which ERA led to the recommendation to change the timing of FET of >24 h had a lower (rather than higher) clinical pregnancy rate[74,76]. These findings highlight the need for robust studies demonstrating clinical benefit and generalizability before the adoption of new technologies

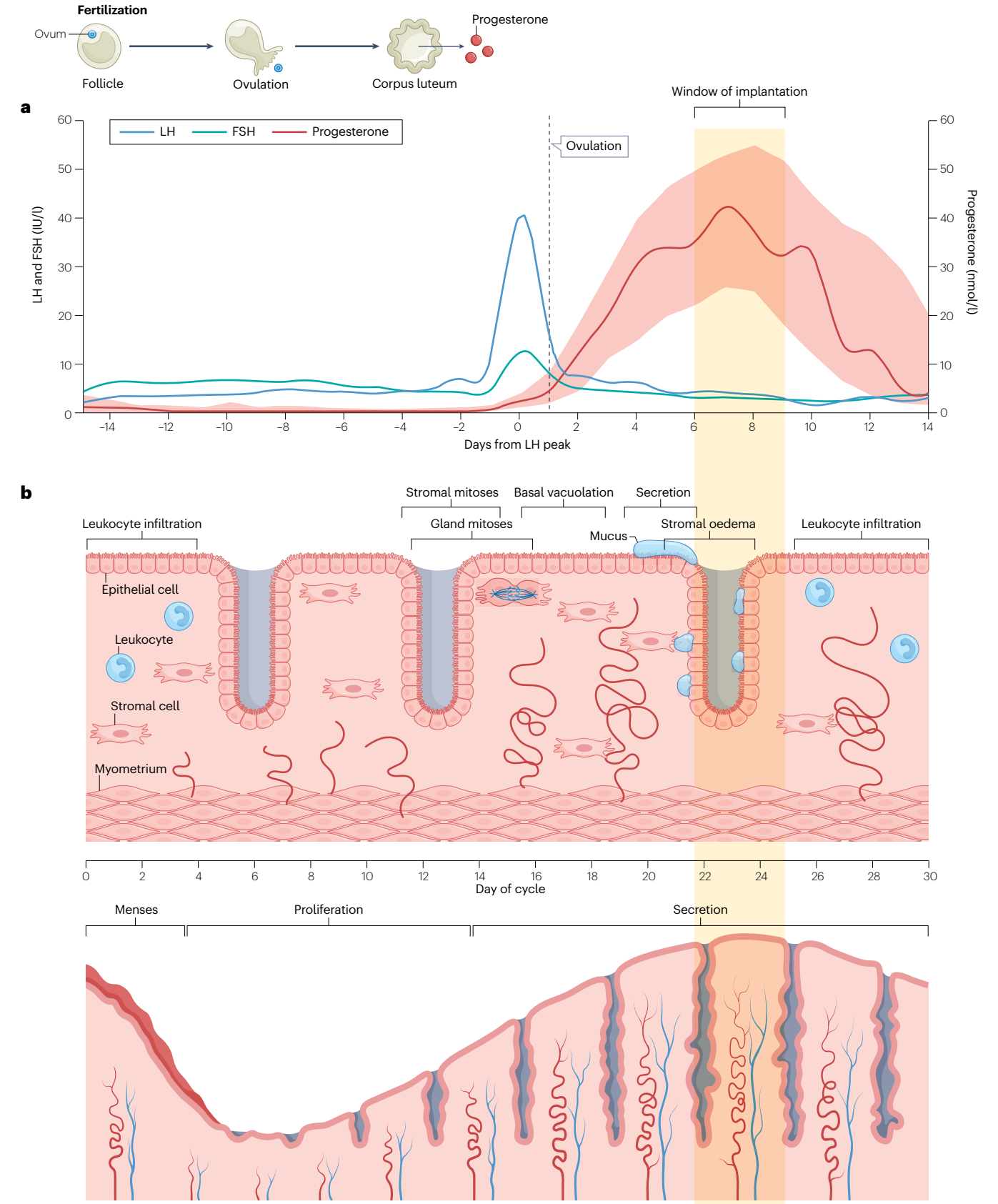

Fertilization
Ovum
Follicle
Ovulation
Corpus luteum
Progesterone
a
Window of implantation
LH
FSH
Progesterone
Ovulation
LH and FSH (IU/l)
Progesterone (nmol/l)
Days from LH peak
b
Stromal mitoses
Basal vacuolation
Secretion
Leukocyte infiltration
Gland mitoses
Mucus
Stromal oedema
Leukocyte infiltration
Epithelial cell
Leukocyte
Stromal cell
Myometrium
Day of cycle
Menses
Proliferation
Secretion

Fig. 3 | The luteal phase, endometrial receptivity and the window of implantation. a, The menstrual cycle and hormone levels. Median serum concentrations of luteinizing hormone (LH; blue line) and follicle-stimulating hormone (FSH; green line), and median serum concentration of progesterone (dark red line) including the range between the 5th and 95th centile concentrations (pink shading). Ovulation occurs after the LH peak, resulting in the formation of the corpus luteum that produces progesterone. The window of implantation is depicted from day 6 to day 9 following the LH peak (yellow shaded area). **b**, Physiological changes associated with a developing endometrium. This schematic demonstrates the histological changes and timings as described by Noyes and colleagues[69] with respect to the day of the menstrual cycle and window of implantation. The data shown in part **a** were originally presented in ref. 192.

into clinical practice. Whether non-invasive molecular tests with rapid processing can be developed in future to guide transfer in the same cycle remains an area of active research. Indeed, analysis of endometrial fluid aspirates correlates with those after endometrial biopsy[77].

Building on our understanding of endometrial receptivity, the endometrium primarily comprises two major cell types: epithelial and fibroblast-like stromal cells. Advances in single-cell RNA sequencing have unveiled a distinct transcriptomic signature for each cell type during the WOI[78]. This signature is more precise and less affected by the biopsy site than tissue-based methods. The onset of the WOI is marked by an abrupt and discontinuous transcriptomic activation in epithelial cells. This change is accompanied by transcriptomic markers of decidualization in stromal fibroblasts and pinopode formation[78]. Therefore, combining single-cell transcriptomics with tissue transcriptomics has enabled mapping of the human endometrium to identify signalling pathways and dominant cell lineages across various pathologies[78].

Transcriptomic profiling is more predictive of endometrial receptivity as determined by histology than the serum concentration of progesterone and can also identify endometrial pathologies, such as endometriosis[79,80]. ReceptivaDx is one such test that assesses overexpression of endometrial BCL6 (an inflammatory marker of endometriosis), progesterone resistance and impaired implantation. Preliminary cohort studies have shown a reduction in miscarriage rates among women with BCL6 overexpression who underwent endometriosis treatment before IVF[81]. The medical community eagerly anticipates more extensive studies that validate the efficacy of this and other tests. Intriguingly, prolactin is secreted from decidualized endometrial cells, as well as human embryonic stem cells in vitro only after 7–10 days of progesterone administration at 15 ng/ml[76], suggesting possible utility of prolactin as a marker of decidualization pending further research.

Ultrasonography assessment

Imaging by ultrasonography has been explored as a potential non-invasive tool to gauge endometrial receptivity. Various endometrial characteristics, such as thickness (>7 mm), volume (>2 ml), presence of blood flow and pattern (triple line), are possible features of a receptive endometrium using both 2D and 3D ultrasound modalities (Box 1).

During the follicular phase, oestrogen stimulates endometrial thickening, but the data on whether endometrial thickness predicts live birth in ART cycles are conflicting[65]. A large meta-analysis of 19 studies including 96,000 fresh and frozen cycles found a gradual increase in clinical pregnancy rate with increasing endometrial thickness on the day of hCG trigger[65]. Individuals with an endometrial thickness of <6 mm had half the live birth rate of those with an endometrial thickness of >8 mm on the day of trigger injection for fresh transfer cycles and at the time of progesterone initiation for frozen transfer cycles[82,83]. In fresh cycles, live birth rate increased with endometrial thickness up to 10–12 mm, whereas in FET, the live birth rate plateaued beyond 7–10 mm[84]. However, a 2019 meta-analysis that included 88,834 women concluded that endometrial thickness did not have sufficient predictive capability for pregnancy to have clinical utility[65].

Following oestradiol-induced thickening of the endometrium, progesterone induces endometrial compaction (a decrease in endometrial thickness). Endometrial compaction occurs in roughly 69% of FET cycles and although initially correlated with ongoing pregnancy rates[85], this finding was not borne out in subsequent studies evaluating live birth rate[86].

The role of progesterone in reducing uterine contractility is believed to minimize embryo displacement, thus enhancing the chances of successful implantation[87]. Measurements made using ultrasonography have shown that the frequency of uterine contractions diminishes from 4.4 per minute on the day of hCG administration to 1.5 per minute by day 7 after trigger[87]. However, the predictive power of these contractions, as well as other markers such as endometrial volume, pattern and blood flow, have not proven sufficiently reliable to serve as reliable indicators of endometrial receptivity[65,88].

Monitoring serum progesterone

Serum levels of progesterone during the natural cycle can vary due to diurnal rhythm, pulsatile secretion and poor assay reliability at low concentrations[89]. Furthermore, the same level of supplementation

Box 1

Endometrial receptivity markers

Ultrasonography

Ultrasound measurements during the late follicular phase include endometrial thickness, volume, pattern, blood flow and contractions, which might identify a receptive endometrium[65]. Characteristics that could be predictive of a receptive endometrium include:

- Endometrial thickness: >7 mm
- Endometrial volume: >2 ml
- Endometrial pattern: triple line
- Endometrial blood flow: present
- Endometrial contractions: reduced

Omics

In the future, endometrial receptivity might be assessed by transcriptomic expression of specific marker genes[72].

- Marker genes for less receptive endometrium could include *MMP7*, *THBS1*, *MMP11*, *SFRP1*, *PLAU* and *CADM1*.
- Marker genes for more receptive endometrium could include *CXCL14*, *NUPR1*, *GPX3*, *PAEP*, *MAOA* and *DPP4*.

Table 1 | Serum levels of progesterone (nanomoles per litre) at different gestational ages associated with different pregnancy outcomes

Gestational age (weeks)	Pregnancy outcome				
	Spontaneous healthy pregnancy[a] (mean (s.d.))	Spontaneous healthy pregnancy[a] (median, (10–90th centile))	Spontaneous miscarriage[a] (mean (s.d.))	Threatened miscarriage[a,b] (mean (s.d.))	Singleton programmed fresh embryo transfer with dydrogesterone luteal phase support[c] (mean (s.d.))
5	69.4 (33.7)	63.5 (29.2–126.8)	31.0 (19.7)	54.4 (36.9)	0.64 (0.32)
7	62.5 (20.1)	62.3 (34.2–89.3)	30.1 (14.6)	53.8 (24.4)	5.2 (3.4)
8	69.9 (23.4)	66.7 (42.9–102.9)	23.6 (16.6)	57.8 (21.0)	14.1 (7.6)
10	77.5 (26.6)	74.8 (50.1–107.3)	29.7 (16.0)	67.0 (22.2)	43.9 (16.3)
11–12	98.0 (24.11)	90.2 (74.0–121.9)	–	–	65.4 (20.8)

–, no data. [a]Data were originally presented in ref. 103. [b]Refers to pregnancies with vaginal bleeding. [c]Data were originally presented in ref. 106.

during luteal phase support can result in different levels of progesterone across individuals due to variations in absorption and metabolism. While these challenges exist, they also underscore the potential value of progesterone measurement in guiding luteal phase support[90]. However, data on the optimal progesterone level to target remain limited.

In natural cycles, progesterone levels vary between cycles[89]. Furthermore, the serum concentration of progesterone can fluctuate between 16 nmol/l and 127 nmol/l within 90 min due to pulsatile secretion, complicating the interpretation of single measures[20]. Despite this variation, a mid-luteal progesterone threshold of >29.9 nmol/l has been proposed as optimal for natural conception[91]. Endometrial dating refers to the assessment of histology in endometrial samples to verify the stage of the menstrual cycle. Based on histological dating of the endometrium, in natural cycles with a mid-luteal progesterone level of >6.4 nmol/l, 75% had normal histology, rising to 90% when progesterone exceeded 15 nmol/l[92]. In programmed FET cycles (with no corpus luteum or endogenous progesterone), a higher live birth rate (RR 1.47) and a reduced miscarriage rate (RR 0.62) were observed when progesterone was >31.8 nmol/l on the day of embryo transfer[93].

Luteal phase deficiency is defined as a short luteal phase (<10 days) or a mid-luteal serum level of progesterone below various thresholds between 15.9 nmol/l and 31.8 nmol/l[11,94]. While up to 8.4% of healthy ovulatory women can have progesterone levels of <31.8 nmol/l, this deficiency is only recurrent in 2% of healthy ovulatory women[95], but it is more often recurrent in infertile women (~6% of infertile women)[96]. Women with recurrent miscarriage consistently exhibit lower mid-luteal serum levels of progesterone (by half) and endometrial progesterone (by 200-fold) than unaffected women[97]. However, whether the luteal phase deficiency causally contributes to reduced implantation remains unclear, as luteal phase support with progesterone[98], oestrogen[99] or hCG[99] have not demonstrated notable benefits in RCTs in women with recurrent miscarriage.

A systematic review of ovulation induction cycles found that lower mid-luteal to late luteal progesterone (less than thresholds between 25.4 nmol/l and 47.7 nmol/l) was associated with lower live birth rate (RR 0.6–0.73)[39]. In a study involving 335 women undergoing ovulation induction with gonadotrophins who ovulated (279 women), mid-luteal serum concentration of progesterone was 72.5 ± 3.5 nmol/l (range 25.1–617 nmol/l), and was higher in those with live birth compared with those who failed to achieve pregnancy (71.2 nmol/l versus 59.1 nmol/l)[100]. The live birth rate increased with mid-luteal serum concentrations of progesterone: from 8% for concentrations of 25.1–31.8 nmol/l, reaching a plateau of 29–32% for concentrations >79.5 nmol/l[100]. In 340 patients undergoing fresh IVF cycles, a fall in the serum concentration of progesterone from 3 to 5 days after oocyte retrieval was associated with a halving of ongoing pregnancy rates compared with individuals with an increase in progesterone (33.6% versus 49.1%)[101] consistent with the presence of functional corpora lutea.

Progesterone levels during early pregnancy

The practice of continuing luteal phase support throughout the first trimester is adopted by many practitioners[5]. However, when hCG is used as the trigger for oocyte maturation, extending luteal phase support beyond 2 weeks has not shown any added benefit[5].

In individuals with healthy pregnancies, serum levels of progesterone increase from 35–50 nmol/l (2.5–97.5th centile) during the luteal phase to 25.4–152.6 nmol/l during the first trimester and further to 175–811 nmol/l in the third trimester[102] (Table 1). Individuals with a twin pregnancy exhibit progesterone levels ~1.7-fold higher than those with a singleton pregnancy. By contrast, individuals with a pregnancy that ends in miscarriage have lower serum levels of progesterone that rise from 19.0 nmol/l to only 30.3 nmol/l during the first trimester[103]. Moreover, women with a threatened miscarriage have progesterone levels that are on average 9.98 nmol/l lower than gestation-matched controls[103] (Fig. 1g). However, serum levels of progesterone in healthy pregnancies can overlap with those in pregnancies complicated by miscarriage, with levels as low as 9.8 nmol/l at 6 weeks still being consistent with viable pregnancy[103].

Between 5 and 7 weeks gestation, there is a sharp drop in the serum concentration of progesterone, probably signifying the luteoplacental shift (Fig. 1h). This drop is followed by a more gradual increase at a lower trajectory during the remainder of the first trimester[104]. Notably, the takeover in progesterone synthesis from the corpus luteum by the placenta is independent of hCG or human placental lactogen[105]. In a study in 88 women undergoing programmed FET cycles (with no corpus luteum) in which dydrogesterone (which does not cross-react with assays for endogenous progesterone) was used for luteal phase support, serum levels of progesterone indicative of endogenous placental production following the luteoplacental shift began to increase from 7 weeks gestation, reaching 65.4 nmol/l by the end of the first trimester[106]. In this context, those with a progesterone level >13.7 nmol/l at ~6 weeks after embryo transfer had a 99% chance of ongoing pregnancy[106].

Whether low serum levels of progesterone are a consequence or cause of pregnancy failure is debatable. The efficacy of progesterone

supplementation during the first trimester, especially in threatened miscarriage, remains a topic of contention. Progesterone supplementation (400 mg vaginal twice daily) during the first trimester seemed to predominantly benefit only women who have had three or more previous miscarriages[107]. By contrast, the STOP trial, which used 400 mg oral progesterone in women with threatened miscarriage, was stopped prematurely owing to lack of efficacy[108]. The PROMISE trial in women with recurrent miscarriage also did not corroborate these findings[109]. In fresh IVF cycles with progesterone luteal phase support, serum levels of progesterone as low as 2 nmol/l at 4 weeks gestation have been compatible with live birth[110].

Premature progesterone rise during ovarian stimulation

In IVF cycles, the flip-side to insufficient progesterone during the luteal phase is a premature rise in progesterone secretion at the end of ovarian stimulation. Here, progesterone can prematurely mature the endometrium such that it is out of sync with the embryo by the time of embryo transfer. High serum levels of progesterone on the day of trigger can lead to advanced endometrial receptivity features on histology of biopsy samples and also affects gene expression profiles, with overexpression of 13 from 25 endometrial receptivity genes[111]. The threshold for the serum level of progesterone on the day of trigger that is considered detrimental typically ranges between 2.5 nmol/l and 7.2 nmol/l[112] (Table 2; Supplementary Table 1). In an analysis of >55,000 fresh IVF cycles, raised progesterone of 4.8–5.6 nmol/l was associated with a ~10% reduction in pregnancy rates[113]. High responders could be more tolerant of premature progesterone rises than low responders[114], with increased serum progesterone thresholds of 5.72 nmol/l[115] to 7.16 nmol/l[116] endured before implantation rates are affected. Others have proposed that the duration of elevated progesterone is more important than a single raised level[117].

Of note, the serum progesterone threshold sufficient to induce premature maturation of the endometrium (2.54–7.16 nmol/l) is markedly lower than that reportedly required during the mid-luteal phase (31.8 nmol/l) for optimal pregnancy rates (Table 2). Furthermore, in women with low mid-luteal serum levels of progesterone, the live birth rate could be improved with a single daily subcutaneous bolus of progesterone on the morning of embryo transfer[118,119]. Given the limited time for this intervention to influence endometrial maturation or receptivity, this finding suggests that systemic effects of progesterone, such as immune tolerance[46] might be more important at the time of embryo transfer than direct actions on the endometrium.

Progesterone pharmacokinetics and adverse effects

A variety of progestogens and administration routes, including vaginal, rectal, intramuscular and subcutaneous, have been utilized in luteal phase support regimens (Supplementary Table 2). Initial concerns about potential teratogenic effects of synthetic progestins (such as dydrogesterone[120]), which were later proven unfounded[121–123], led to widespread use of vaginal natural micronized progesterone as the preferred option. Micronized refers to the process of reducing the particles to sizes under 50 µm to increase bioavailability.

Owing to the uterine first-pass effect, vaginal progesterone achieves higher endometrial levels but lower steady-state serum levels from 6 h after administration[124]. While all vaginally administered formulations can cause vaginal irritation[125], gels are less favoured than pessaries owing to their susceptibility to leakage[124]. Vaginal inserts achieve higher steady-state serum levels than vaginal gels, while pessaries can also be administered rectally rather than vaginally, resulting in similar serum levels of progesterone (77.0 nmol/l for vaginal versus 95.6 nmol/l for rectal administration of pessaries), but with less perineal irritation (21.3% for vaginal versus 2.2% for rectal administration)[126].

Intramuscular progesterone (50 mg dissolved in oil) achieves higher serum levels than vaginal formulations (intramuscular 51.2 nmol/l, vaginal 21.0 nmol/l)[127], but lower endometrial levels (intramuscular 4.5 nmol/l, vaginal 36.6 nmol/l, unsupplemented natural luteal phase 1.0 nmol/l)[127,128]. However, intramuscular progesterone might require assistance to administer[129], can be painful and might occasionally lead to abscesses[128]. As such, fewer patients consider intramuscular formulations convenient than consider vaginal preparations convenient: 82.6% of patients using a vaginal progesterone insert found the administration route convenient versus 44.9% of those receiving progesterone oil injections[129].

Healthy young female volunteers receiving 10 mg or 40 mg of intramuscular progesterone achieved serum levels of progesterone of 10.5–57.6 nmol/l and had normal histological endometrial dating, as found in healthy cycling women; by contrast, women receiving 2.5 mg had both grossly delayed histological development and aberrant endometrial gene expression[130]. Notably, more endometrial genes were upregulated in women after receiving 40 mg than after receiving 2.5 mg progesterone[130].

Subcutaneous progesterone is better tolerated than intramuscular progesterone[131], but induces lower serum levels of progesterone[132]. Daily subcutaneous administration of progesterone (25 mg in 1 ml of water) resulted in a nadir serum progesterone concentration of 19 nmol/l[132],

Table 2 | Serum levels of progesterone associated with pregnancy during IVF

Timing of progesterone measurement	Progesterone range (nmol/l)	Effect on outcomes with different progesterone levels (nmol/l)
Day of trigger (assess premature progesterone elevation)	2.48–6.36	LBR reduced if >2.48–4.77 (refs. 168–170); CPR reduced if >2.54–6.36 (refs. 171,172); implantation rate reduced if >4.77 (ref. 169); duration of elevation (0, 1 or >1 day prior to day of trigger) might further reduce LBR[170]
Day of embryo transfer (for FET)	15.9–47.7	LBR reduced if progesterone falls below threshold 15.9–37.62 (refs. 173,174)
Between day of OPU and OPU plus 7 days	Day of OPU, not associated with pregnancy outcomes; early luteal phase (OPU plus 2–3 days), 60–252; mid-luteal phase (OPU plus 5 to 7 days), 60–250	Varied evidence throughout, probably dependent on type of luteal phase support and timing of determination; LBR improved if: early luteal progesterone is 60–252 (OPU plus 2 or 3 days)[175]; mid-luteal progesterone is 150–250 (OPU plus 5 days)[136], mid-luteal progesterone is 130.4–190.8 (OPU plus 7 days)[176]

CPR, clinical pregnancy rate; FET, frozen embryo transfer; IVF, in vitro fertilization; LBR, live birth rate; OPR, overall pregnancy rate; OPU, oocyte pickup.

Table 3 | RCTs in fresh cycles that reported live birth rate as an outcome

Type of comparison	Comparison made	Study	Study population (*n*)	Significant difference in live birth rate?
Route	Oral synthetic progesterone vs vaginal progesterone	LOTUS trial (Griesinger et al. (2018)[122]; Tournaye et al. (2017)[121])	1,034	No
	Oral natural progesterone vs vaginal progesterone	Pouly et al. (1996)[177]	283	No
	Oral synthetic progesterone vs intramuscular progesterone	Iwase et al. (2008)[178]	40	No
	Intramuscular vs vaginal progesterone	Abate et al. (1999)[147]	104	Yes (intramuscular > vaginal: 22.1% vs 8%)
		Propst et al. (2001)[149]	201	Yes (intramuscular > vaginal: 39.4% vs 24.5%)
		Dal Prato et al. (2008)[144]	441	No
		Zegers-Hochschild et al. (2000)[146]	505	No
	Subcutaneous vs vaginal progesterone	Baker et al. (2014)[131]	800	No
		Lockwood et al. (2014)[179]	683	No
	Rectal vs vaginal progesterone	Tay and Lenton (2005)[180]	126	No
Formulation	Gel versus tablets (vaginal)	Bergh et al. (2012)[181]	1,983	No
	Gel versus insert (vaginal)	Doody et al. (2009)[182]	1,211	No
Timing	Start: day of ovum pickup versus 1 day after ovum pickup	Gao et al. (2018)[183]	233	No
	Start: trigger vs ovum pickup vs embryo transfer	Mochtar et al. (2006)[152]	385	No
	Stop: positive pregnancy test or 3 weeks thereafter	Nyboe Andersen et al. (2002)[153]	303	No
	Duration: 11 days or 6 weeks	Goudge et al. (2010)[151]	101	No

RCT, randomized controlled trial.

sufficient for endometrial transformation. The serum progesterone concentration peaked within 1 h of subcutaneous administration to 184 nmol/l, and levels were dose-dependently increased (328 nmol/l after 50 mg, 748 nmol/l after 100 mg), and even higher when progesterone in water was given intramuscularly (1,400 nmol/l)[132]. This profile compares favourably to that following intramuscular administration of 100 mg progesterone in oil, which induces peak serum levels of 359 nmol/l at 6.7 h[132]. In a small study in 40 women with infertility, daily subcutaneous administration of 50 mg progesterone was associated with pregnancy rates similar to those after vaginal administration of 800 mg, with the odds of clinical pregnancy increasing by 20% for every 31.8 nmol/l increase in serum concentration of progesterone on the day of transfer[133].

Historically, oral progesterone was less favoured due to extensive first-pass metabolism, variable bioavailability[134] and the need to administer large doses associated with adverse effects (for example, somnolence, dizziness and headaches). However, the use of synthetic progestins such as oral dydrogesterone is gaining momentum. Dydrogesterone[121] has a higher specificity and selectivity for nuclear PRs[135], causing endometrial transformation at 10–20-fold lower doses than oral micronized natural progesterone, thus minimizing adverse effects[135]. In large trials[121,122], dydrogesterone achieved comparable outcomes to vaginal progesterone and was better tolerated.

Transdermal synthetic progesterone preparations have been used in contraception and menopausal hormone therapy but are not yet used in luteal phase support. One proposed limitation is the breakdown of progesterone by 5-α-reductase in the skin[128], but this effect was shown to not significantly affect progesterone levels in healthy women. However, serum levels of progesterone did not exceed 4.5 nmol/l after 80 mg/g body weight of progesterone cream in healthy women[134], indicating that this preparation is insufficient for luteal phase support.

Can we give too much progesterone?

With the high progesterone levels achieved in some luteal phase support regimens, the question arises: can one give too much progesterone and can this excess be detrimental? An observational study in 602 women undergoing IVF treatment found that high early luteal to mid-luteal serum progesterone (>400 nmol/l) was associated with a lower live birth rate (38%) than intermediate levels (150–250 nmol/l) (54%)[136]. Similarly, in FET cycles, evidence indicates that high serum progesterone levels might be linked to reduced pregnancy rates, especially when subcutaneous progesterone is used exclusively[88].

Furthermore, findings suggest that early treatment with exogenous progesterone for prevention of preterm birth, especially before 20 weeks gestation, is associated with an increased risk of gestational diabetes mellitus compared with no progesterone use (68.8% versus 39.4%)[137]. Other adverse effects could potentially arise that are not routinely monitored and could go unnoticed[138]. These data imply that a balanced approach to luteal phase support, tailored to individual needs, might be more beneficial than simply assuming that 'more is better'.

Impact of BMI on progesterone requirements

Obesity might influence progesterone requirements during pregnancy. Obesity in ovulatory women with subfertility is associated with an increase in the time to spontaneous conception[139] by 4% per 1 kg/m^2 (ref. 140). Serum levels of progesterone in early pregnancy tend to be lower in individuals with obesity, with levels below 47.7 nmol/l

observed in 25% of those with obesity, as opposed to just 3% in control individuals[141]. Similarly, serum levels of progesterone on the day of embryo transfer are typically lower in women with obesity (average 34.2 nmol/l) than in women with normal BMI (41.1 nmol/l)[142]. These observations suggest that women with obesity might benefit from higher dose luteal phase support regimens[104].

Luteal phase support in fresh ART cycles

Despite numerous studies, no consensus exists on the optimal luteal phase support regimen for improving live birth rates. A 2015 Cochrane meta-analysis[5] and several subsequent RCTs[121,122] have not demonstrated a clear advantage for any specific luteal phase support regimen (Table 3; Supplementary Table 3). While the majority of these studies have found similar efficacy between intramuscular and vaginal formulations of luteal phase support[129,143–146], a few studies favoured the intramuscular route[147–149]. Notably, large RCTs have shown that oral dydrogesterone is at least non-inferior to vaginal progesterone luteal phase support[121,122]. Given the lack of definitive evidence favouring one regimen over another, the choice of luteal phase support route or formulation is guided by patient and clinician preference. A global survey revealed that a notable majority (80%) of IVF practitioners currently favour the exclusive use of vaginal luteal phase support[4].

Timing of luteal phase support initiation and duration

The timing of luteal phase support initiation can influence outcomes. A systematic review highlighted that initiating luteal phase support before ovum pickup resulted in a 5–12% lower clinical pregnancy rate[150]. Comparable live birth rates have been reported when luteal phase support is started between ovum pickup and embryo transfer[151,152]. While support can be continued until the luteal–placental shift at 8–9 weeks[104] or even longer, studies have shown no benefit in the live birth rate if luteal phase support is continued beyond the time of the biochemical pregnancy test[151,153–155]. Nonetheless, 35% of clinicians opt to continue luteal phase support until 8–10 weeks and 52% until 12 weeks[156]. Of note, most studies on this topic utilized a hCG trigger (71% hCG; 0% GnRHa; 29% not specified)[5], which is less dependent on exogenous luteal phase support than GnRHa-triggered cycles. Adopting individualized luteal phase support approaches[119] might facilitate shorter courses than used currently; for instance, no benefit was observed of continuing luteal phase support if serum levels of progesterone exceeded 110 nmol/l on the day of the biochemical pregnancy test[157]. Overall, European Society of Human Reproduction and Embryology guidelines recommend that any of several luteal phase support preparations can be initiated at any time within 3 days of oocyte retrieval and should be continued at least until a positive pregnancy test is obtained[4].

Luteal phase support in FET cycles

The global rise in the number of FET cycles has prompted an increasing focus on their luteal phase and the relative merits of each of the FET strategies (Table 4; Supplementary Table 4). Those undergoing natural FET have a corpus luteum and are thus less dependent on exogenous luteal phase support. Yet studies have shown that luteal

Table 4 | RCTs in FET cycles that reported live birth rate as an outcome

Type of FET	Comparison	Study	Study population (*n*)	Luteal phase support starting point	Luteal phase support stop point	Significant difference in live birth rate?
Natural FET	No luteal phase support vs progesterone	Bjuresten et al. (2011)[184]	435	Embryo transfer	NA	Yes; vaginal progesterone > no luteal phase support (30% vs 20%)
		Wånggren et al. (2022)[161]	488	Embryo transfer	6 weeks	Yes; progesterone > no luteal phase support (34% vs 24%)
	Progesterone vs progesterone + GnRH	Seikkula et al. (2016)[185]	98	Embryo transfer	Biochemical pregnancy test	No
	Placebo luteal phase support vs hCG	Lee et al. (2017)[186]	459	Two doses: dose 1 on day of FET, dose 2 6 days after FET	No	No
Modified natural cycle FET	No luteal phase support vs progesterone	Horowitz et al. (2021)[187]	59	Ovulation day	Biochemical pregnancy test	No
Programmed cycle FET	Oral synthetic progesterone vs vaginal natural progesterone vs intramuscular natural progesterone	Pabuccu et al. (2022)[188]	163	Once endometrial thickness ≥7 mm	12 days after embryo transfer	No
	Duration of oestrogen luteal phase support (progesterone + oestrogen)	Ghaffari et al. (2022)[189]	64	Oestrogen, days 2–3; progesterone, once endometrial thickness >7 mm	Oestrogen, 12 weeks or 6 weeks; progesterone, both groups at 12 weeks	No
	Route (intramuscular progesterone vs vaginal progesterone vs oral synthetic progestin)	Rashidi et al. (2016)[190]	180	Once endometrial thickness >8 mm	Week 12 of pregnancy	No
	Route (intramuscular versus vaginal progesterone)	Devine et al. (2021)[162]; Devine et al. (2018)[191]	1,125	Once endometrial thickness >7 mm	Week 10 of pregnancy	Yes; vaginal progesterone inferior to intramuscular or the combination or both (27% vs 44% vs 46%)

FET, frozen embryo transfer; GnRH, gonadotropin hormone-releasing hormone; hCG, human chorionic gonadotrophin; NA, not available.

Box 2

Directions for future research

- Non-invasive rapid methods of assessing endometrial receptivity that do not require an endometrial biopsy to assess endometrial receptivity.
- Evaluation of endometrial receptivity in a non-binary manner, as a gradation in receptivity might exist as opposed to only non-receptive and receptive.
- High resolution imaging to assess the endometrium during the early luteal to mid-luteal phase and identify features that predict implantation.
- Demonstration of the effect of such tests on clinical outcomes in randomized controlled trials prior to adoption into clinical practice.
- More sophisticated preclinical models of implantation; for example, using synthetic embryo models, organoids or microphysiological systems to study implantation.
- Detailed characterization of the role of systemic levels of progesterone on the immune system as compared with local uterine effects.
- Given the widespread use of higher doses of progesterone luteal phase support, dedicated studies are needed to assess for risk of harm at high serum progesterone levels.
- Further characterization of the action of different progesterone receptors and their importance for implantation.
- More specific data on the actions of different progestogen formulations at different progesterone receptors.
- Determination of predictors of the variation in pharmacokinetic properties including absorption and distribution after the use of different progestogen preparations (for example, ethnicity and BMI).
- Endometrial progesterone resistance has been described in polycystic ovary syndrome and endometriosis. It would be relevant to investigate whether such a phenomenon can be characterized in women undergoing in vitro fertilization and if so, whether progesterone resistance affects the success of implantation.

supplementation with progesterone in women undergoing natural FET can reduce the risk of miscarriage[158] and improve live birth rate when compared with control pregnancies achieved by natural FET cycles with no luteal phase support[158]. A 2023 meta-analysis[159] of four RCTs including 1,116 women undergoing natural or modified natural FET cycles found that vaginal progesterone luteal phase support improved the live birth rate when compared with no luteal phase support (RR 1.42)[159], with more pronounced benefit in natural FET than modified natural FET cycles.

Despite these positive findings, a considerable proportion of clinicians (44%) do not prescribe luteal phase support for natural FET cycles. Of those who do, the majority (~68%) lean towards vaginal preparations[160]. In women receiving vaginal luteal phase support in natural or modified natural FET cycles, higher luteal levels of serum progesterone (>31.8 nmol/l) were associated with higher live birth rates (RR 1.47)[93]. However, this threshold is not a consistent finding, with one RCT indicating that although luteal serum levels of progesterone are not predictive of pregnancy rates in those undergoing natural FET, luteal phase support is still associated with increased live birth rates[161].

In individuals undergoing programmed FET cycles, who lack a corpus luteum, progesterone supplementation is routinely continued until 10–12 weeks gestation. Some studies have suggested that intramuscular progesterone might be more effective than vaginal preparations in programmed FET. An RCT in 1,125 women randomized to either vaginal progesterone (400 mg daily), intramuscular progesterone (50 mg daily) or vaginal progesterone daily plus intramuscular progesterone every 3rd day, found that vaginal administration alone resulted in lower live birth rate (vaginal 27% versus intramuscular 44% versus both 46%)[162]. Notably, serum levels of progesterone on the day of pregnancy test were considerably lower with vaginal delivery alone (median 21.4 nmol/l, range 11.2–35.0 nmol/l) than with vaginal plus intramuscular administration (32.4 nmol/l, 18.6–57.3 nmol/l) or daily intramuscular administration (52.8 nmol/l, 27.0–89.6 nmol/l).

However, serum levels of progesterone do not necessarily mirror endometrial levels of progesterone or the outcomes of the ERA test[79]. In 79 women undergoing FET cycles with vaginal progesterone, more women had a receptive endometrium as assessed by ERA if endometrial progesterone levels were >40.07 µg/ml (78% with receptive endometrium if endometrial progesterone >40.07 µg/ml, 35% if progesterone was below this threshold)[79]. A serum concentration of progesterone of >31.8 nmol/l on the day of embryo transfer in programmed FET cycles is most commonly reported to be associated with optimal outcomes (Table 2). In an RCT of 400 programmed FET cycles, clinical pregnancy rate was lower after 10 mg oral dydrogesterone twice daily (9%), than 400 mg vaginal progesterone twice daily (20%), 10 mg oral dydrogesterone twice daily combined with intramuscular GnRHa on the embryo transfer day and 3 and 6 days after the embryo transfer (25%), or dydrogesterone combined with intramuscular hCG on the embryo transfer day and 3 and 6 days after the embryo transfer (17%)[163]. As an explanation, having serum levels of dydrogesterone below the 25th centile on the day of embryo transfer in programmed FET was associated with one-fifth lower ongoing pregnancy rate than in those with higher levels[164].

Individualized luteal phase support

The concept of individualized luteal phase support has gained traction over the past few years, with researchers exploring the potential benefits of tailoring regimens based on measured serum levels of progesterone. Labarta and colleagues assessed serum levels of progesterone on the day of embryo transfer in 2,275 programmed FET cycles[119]. Women with progesterone levels >29.3 nmol/l (n = 1,299) received standard luteal phase support (400 mg vaginal twice daily), while those with levels <29.3 nmol/l received either standard luteal phase support (n = 426) or standard support plus an additional 25 mg of subcutaneous progesterone once daily (n = 976). In women with

progesterone <29.3 nmol/l, subcutaneous progesterone rescued the live birth rate to the same level as in those with higher progesterone[119].

Further insights have emerged suggesting that adequate progesterone can be endogenously produced from the corpora lutea with hCG stimulation, eliminating the need for supplementary luteal phase support[165]. In individuals undergoing GnRHa cycles, microdosing of hCG during the luteal phase guided by mathematical modelling has been shown to achieve satisfactory endogenous luteal serum levels of progesterone[166]. This approach resulted in higher mid-luteal serum progesterone than standard luteal phase support, with no difference in live birth rate[167]. Additionally, GnRHa has been used as luteal phase support in addition to vaginal progesterone, which improved the live birth rate in women undergoing IVF treatment (OR 1.39, 95% CI 1.08–1.78)[10].

While the potential drawbacks of excessive progesterone usage remain somewhat ambiguous, it is imperative to continue exploring individualized luteal phase support strategies. The aim of such research endeavours is to strike a balance, ensuring that patients receive adequate, yet not excessive, doses and durations of luteal phase support in clinical settings.

Conclusions

Luteal phase support is a critical element of successful ART to support implantation, maintain pregnancy and optimize live birth rates. Overall, the reasons for luteal phase insufficiency remain poorly defined. IVF cycles with transfer of fresh embryos have a disrupted luteal phase due to supraphysiological levels of sex steroid from the preceding ovarian stimulation. The trigger used to induce oocyte maturation affects the likelihood of sufficient LH-like exposure being available to form and maintain functional corpora lutea, and in turn the degree of luteal phase support required to support and maintain pregnancy. Indeed, more intensive luteal phase support is required if a GnRHa trigger is used than if a hCG trigger is used. One approach to avoiding the adverse effects of the disrupted luteal phase in the fresh cycle is to freeze embryos and transfer them in a subsequent cycle. However, FET cycles (especially via hormonal cycles that do not result in the formation of a functional corpus luteum) can increase the risk of pregnancy complications such as pre-eclampsia.

Luteal phase support is typically administered as a progestogen, which can lead to excess circulating progesterone, but whether this surplus is harmful is unclear. The aim should be to provide sufficient luteal phase support, rather than excess, when considering the perspective of cost and practicality. One approach to individualizing luteal phase support is to measure serum levels of progesterone and adjust luteal phase support accordingly. However, progesterone is a pulsatile hormone with diurnal variation, such that the value of a single serum measurement can vary during the natural cycle. In addition, progesterone assays can be unreliable, especially at low levels, and serum levels might not proportionately reflect endometrial levels. During FET cycles, optimal serum levels of progesterone should be ≥30 nmol/l; however, optimal progesterone levels in fresh embryo cycles remain less well defined. In the future, omics technology could enable more precise evaluation of endometrial receptivity using non-invasive methods and could be used to assess the sufficiency of luteal phase support and guide embryo transfer. However, current invasive methods have yet to demonstrate the benefit of such endometrial testing in RCTs to enable clinical utility.

Despite the importance of luteal phase support, many unanswered questions remain (Box 2). As the scientific community delves deeper into the nuances of the luteal phase, we anticipate a future in which monitoring becomes more precise and luteal phase support methodologies are honed to perfection, paving the way for even greater success rates in reproductive treatments.

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Author contributions

A.G., A.P.Z., A.C.Y., R.A., R.C., A.H. and A.I. researched data for the article. A.G., A.P.Z., A.C.Y., S.M.N., A.V.B. and A.A. contributed substantially to discussion of the content. A.G., A.P.Z., A.C.Y. and A.A. wrote the article. S.M.N., A.V.B., W.S.D. and A.A. reviewed and/or edited the manuscript before submission.

Competing interests

A.A. and W.S.D. have consulted for Myovant Sciences Ltd. S.M.N. has participated in Advisory Boards and received consultancy or speakers' fees from Access Fertility, Beckman Coulter, Ferring, Finox, Merck, Modern Fertility, MSD, Roche Diagnostics, and The Fertility Partnership. The other authors report no competing interests.

Additional information

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