CHAPTER

23

Assisted Reproductive Technologies

TERMINOLOGY

- Assisted reproductive technology (ART): any fertility treatment in which **both egg and sperm** are handled. Accordingly, ART procedures involve the surgical removal of eggs, known as *egg retrieval*, followed by fertilization with sperm.
- ***In vitro* fertilization (IVF):** most common ART procedure. Ovarian stimulation, oocyte retrieval, and fertilization of the oocytes in the laboratory; embryos are then cultured for 3 to 7 days with subsequent transfer or cryopreservation.
- **Gamete intrafallopian transfer (GIFT):** ovarian stimulation and egg retrieval along with laparoscopically guided transfer of a mixture of unfertilized eggs and sperm into the fallopian tubes *(largely historic procedure)*
- **Zygote intrafallopian transfer (ZIFT):** ovarian stimulation and egg retrieval followed by fertilization of the eggs in the laboratory and laparoscopic transfer of the day 1 fertilized eggs (*zygotes*) into the fallopian tubes *(largely historic procedure)*
- **Donor egg IVF:** used for patients with poor egg numbers or egg quality; involves stimulation of an egg donor with typical superovulation followed by standard egg retrieval; eggs are then fertilized by sperm, and embryos are transferred in a standard IVF-like process.
- **Intracytoplasmic sperm injection (ICSI):** developed in the early 1990s to help couples with severe male factor infertility; one sperm is injected directly into each mature egg, typically resulting in a 50% to 70% fertilization rate.
- **Pregnancy rate:** can have many definitions ranging from serum or urine positive for human chorionic gonadotropin (hCG) to a live birth
- **Clinical pregnancy rate (CPR):** most commonly reported pregnancy rate from ART centers. This is the percentage of patients with at least one embryo in the uterine cavity with fetal cardiac activity.
- **Live birth rate (LBR):** percentage of patients with a live birth from an ART cycle
- **Implantation rate:** This is the chance that each embryo transferred into the uterine cavity will result in a clinical pregnancy (intrauterine pregnancy with fetal cardiac activity). Calculated by taking the number of clinical pregnancies divided by the number of embryos transferred.

EVALUATION BEFORE ASSISTED REPRODUCTIVE TECHNOLOGIES

Ovarian Reserve *(also see Chapter 22)*

- Ovarian reserve testing (AMH and antral follicle count) determines the number and possibly quality of eggs present before infertility treatment.
- Predicts ovarian response to stimulation medications/oocyte yield[1]
- Diminished ovarian reserve (DOR) is likely not associated with aneuploidy, although the literature is mixed.[2-6]

Evaluation of Uterine Cavity and Hydrosalpinx

- Evaluation techniques: sonohysterogram (SHG or SIS), hysterosalpingogram (HSG), or hysteroscopy
- Conducting the SHG in the same cycle with the embryo transfer (ET) did not adversely affect pregnancy outcomes.[7]
- Significantly lower CPRs if uterine cavity abnormalities are present (8.3% vs 37.5%)[8]
- Mixed data as to whether screening hysteroscopy prior to IVF improves LBRs[9]
  - Prevalence of unsuspected intrauterine defects found on office hysteroscopy with prior normal transvaginal ultrasound (US) ranging from 11% to 45%[10]
- Hydrosalpinx on ultrasonography[11]:
  - 50% ↓ pregnancy rate from IVF
  - 2-fold ↑ miscarriage rate

Table 23-1 Potential Mechanisms by Which a Hydrosalpinx Adversely Affects Pregnancy Outcomes

Hydrosalpinx Adverse Effects on Conception
↓ Nutrients in hydrosalpinx fluid
Toxic effect of fluid on embryos[12] and/or sperm[13]
↓ Endometrial $\alpha v\beta 3$, LIF, HOXA10[14]
↑ Endometrial peristalsis due to hydrosalpinx fluid
Embryo washout effect from fluid
↓ Endometrial and subendometrial blood flow[15]
↑ Endometrial inflammatory cells[16]

LIF, leukemia inhibitory factor.

Source: Strandell A, Lindhard A. Why does hydrosalpinx reduce fertility? The importance of hydrosalpinx fluid. *Hum Reprod*. 2002;17(5):1141-1145 by permission of Oxford University Press.

- Ligation of the hydrosalpinx or salpingectomy restores normal pregnancy rate.[17-19]
- Randomized controlled trial (RCT) for US-guided hydrosalpinx aspiration during IVF resulted in nonsignificant increase in CPR versus no aspiration, though not statistically significant (31.3% vs 17.6%, small *n*).[20]
  - Unasyn 1.5 g intraoperatively and then azithromycin 500 mg × 3 days following procedure

- RCT comparing salpingectomy versus aspiration showed nonsignificant (? underpowered) increase in CPR in the salpingectomy group (40% vs 27.5%)[21]
  - 34% of the aspiration group had reaccumulation of fluid within 2 weeks.
- Note: It remains unknown whether surgical intervention in patients with patent dilated tubes improves pregnancy outcomes before IVF.[22]

Trial Transfer (Uterine Mapping; Mock Transfer)

- Empty ET catheter is passed through the cervical canal in advance of an ET to help ensure atraumatic transfer of the embryos into the uterine cavity.
  - Uterine length, cervical and uterine angles, and any other barriers (eg, cervical crypts) encountered can be recorded.
  - Can help identify patients who may benefit from a cervical stitch (for atraumatic traction at the time of transfer) or dilation prior to ET

Evaluation of Male Partner (If Applicable)

- Semen analysis: Basic semen analysis should include volume of ejaculate, concentration, motility, and morphology using the Kruger strict criteria.
- Also want to evaluate for the presence of round cells, which could indicate infection if they are genuinely leukocytes

STIMULATION PROTOCOLS AND MEDICATIONS

Cycle Start Options

- **Oral (po) contraceptive pill (OCP) start**
  - OCPs typically started during menses and continued until ready to start stimulation.
    - Baseline US can be performed while still on OCPs, with a plan to start stimulation medications on cycle day (CD) 2 or CD3 of menses.
    - No data to support a minimum or maximum duration of OCPs prior to stimulation, although one should consider the adverse impact of prolonged (>6 months) OCP suppression on ovarian responsiveness to stimulation[23]
  - OCPs can help with scheduling of IVF cycles and may help synchronize the ovary and result in a more uniform follicular cohort.
  - May also be used to coordinate cycles between donor and recipient in reciprocal IVF or third-party reproduction using donor eggs
  - No difference in oocyte yield or ongoing pregnancy rates, but may increase the duration of stimulation and total gonadotropin dose[23]
  - Can be with combined estradiol (E_2)-progestin or progestin-only formulations[24]
  - Women with DOR may become overly suppressed with OCPs—consider low-dose pill or cold start.
  - A Cochrane review suggests decreased ongoing pregnancy and LBRs with combined OCP use prior to cycles using antagonist for suppression of ovulation.[25]
    - The European Society of Human Reproduction and Embryology (ESHRE) 2020[26] ovarian stimulation guidelines state that combined OCPs are *not* recommended prior to antagonist cycles.[26]
- **"Cold" start**
  - Start stimulation with the onset of spontaneous menses.
  - Typically, patient calls with CD1 and can come in for the baseline scan/start meds on CD2 or CD3.

- May be preferable for patients older than 35 years or with DOR to avoid presumed suppression from OCPs

- **Random start**
  - Start stimulation medications irrespective of CD.
  - The presence of a dominant follicle or corpus luteum does not negatively impact the ovarian response to controlled ovarian stimulation.[27]
  - Multiple waves of antral follicular development occur during the menstrual cycle, challenging the traditional view that a single wave of antral follicles grows only during the follicular phase.[28,29]
  - No differences noted in random-start cycles when compared to conventional start with menses.[30,31]
  - Initially used to decrease treatment delays for medical fertility preservation but can also be used in women in whom CD is unknown (ie, those with a progestin intrauterine device [IUD] or implant) or for whom the other start options are contraindicated or inconvenient
  - Cannot be used in cycles where fresh ET is planned as endometrium will be out of sync for transfer

Gonadotropin Preparations

- Increasing the amount and duration of FSH exposure to the growing antral follicle pool allows more than one follicle to be *rescued* as a dominant follicle instead of undergoing atresia.[32]
- Steady-state serum FSH concentration with the use of exogenous FSH: 9 mIU/mL per 150 IU FSH[33]
- Debate exists as to whether individual patient characteristics should influence starting doses.
  - Multiple studies and two meta-analyses indicate that anti-Müllerian hormone (AMH) and antral follicle count (AFC) have the highest value in predicting poor response (defined by ESHRE as <3 follicles at trigger or oocytes retrieved) or hyperresponse (defined by ESHRE as >18 follicles at trigger or oocytes retrieved).[26]
    - Both age and body mass index (BMI) are insufficient to predict response alone.
    - FSH is also not strongly predictive of response and is only helpful when a high cutoff is used to predict poor response.
    - Patients with a unilateral oophorectomy seem to have a compensatory increased follicular response to ovarian stimulation in comparison with the ipsilateral ovary of age-matched controls, despite a baseline lower AMH and AFC.[34]
  - A Cochrane review suggests there may be no advantage to individualized dosing versus 150 IU FSH for all patients, regardless of ovarian reserve testing (low, average, or high).[35]
    - Note that failure to observe a difference may be due to poor quality of currently available studies.
    - There is unlikely to be a significant benefit with doses greater than 300 IU daily, even in poor responders.[26]
  - Adding luteinizing hormone (LH) to FSH in IVF cycles may be particularly beneficial for poor responders and women aged 35 to 40 years, potentially improving CPR and LBRs. However, the benefit may not be universal across all patient groups.[36,37]

Preventing Spontaneous Ovulation

Gonadotropin-releasing hormone agonists (GnRHas), gonadotropin-releasing hormone antagonists (GnRHants), or progestins are used to prevent a woman from ovulating on their own before egg retrieval (23% incidence without such medicines).[38]

Gonadotropin-Releasing Hormone Agonists (GnRHa)

- Agonists bind to and stimulate the pituitary GnRH receptor (GnRH-R) and have a long half-life.
- GnRHa binding initially causes release of stored FSH and LH ("flare effect"), but ultimately downregulates the GnRH-R by continuous binding (vs endogenous *pulsatile* GnRH), thereby decreasing FSH and LH secretion and eliminating the possibility of an LH surge with continued GnRHa administration.
- The first injection is usually administered in the luteal phase preceding the stimulation cycle to help prevent cysts from forming.
- ***Advantages***
  - Elimination of LH surge
  - ↑ Cohort synchronization
  - Improved pregnancy rates in some studies
- ***Disadvantages***
  - ↑ Cycle length
  - Possible administration during an early conception when started in the luteal phase without OCP pretreatment
  - "Flare effect" of GnRHa can lead to cyst formation.
  - Cannot use GnRHa for trigger in patients with unanticipated high response to gonadotropins; increased rates of ovarian hyperstimulation syndrome (OHSS)

Table 23-2 Gonadotropin-Releasing Hormone Agonist/Antagonist Preparations

Trade Name, Manufacturer	Formulations
Cetrotide, EMD Serono (antagonist)	250 μg/1 mL SC
Ganirelix, Merck & Co. (antagonist)	250 μg/0.5 mL SC
Leuprolide (agonist)	1 mg/0.2 mL = 20 U SC
Synarel, Pfizer (agonist)	2 mg/mL intranasal
Zoladex, AstraZeneca (agonist)	3.6 mg SC

SC, subcutaneous.

Gonadotropin-Releasing Hormone Agonist Long Protocol

- Longest standing and most well-studied IVF protocol
- Generally start with GnRHa 1 mg in the luteal phase preceding the stimulation cycle or overlapping with OCPs
- Decrease GnRHa dose with initiation of gonadotropins for stimulation or maintain dose if AFC is elevated (ie, ≥30).
- *Ultra-long* GnRHa protocols might be more beneficial in women with adenomyosis than long protocols, showing improved CPRs (odds ratio [OR] = 1.925, 95% confidence interval [CI] = 1.137-3.250) and LBRs (OR = 1.704, 95% CI = 1.012-2.859) in patients with adenomyosis.[39]

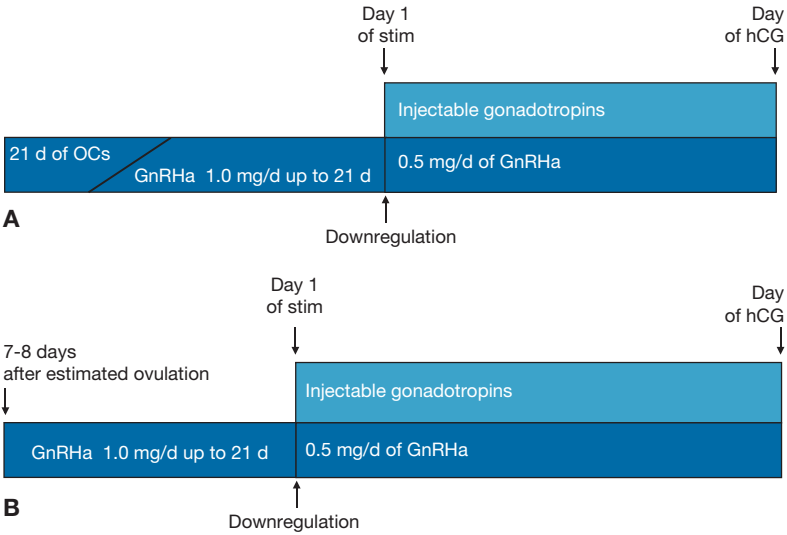

Figure 23-1 Representative illustrations of GnRHa long protocol with or without pretreatment with oral contraceptives.

GnRHa, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin; OCs, oral contraceptives.

Microdose Gonadotropin-Releasing Hormone Agonist Flare Protocol

- Capitalizes on initial flare effect from GnRHa binding to increase endogenous gonadotropin release[40]
- Generally indicated for women with lower ovarian reserve or prior poor response[41]
- GnRHa is started in the early follicular phase; this protocol takes advantage of an initial GnRHa-induced surge in endogenous LH and FSH from the pituitary, followed by downregulation.
- May see initial progesterone (P_4) elevation due to corpus luteum rescue from prior cycle or premature luteinization of granulosa cells[42]
- Start on CD3 with GnRHa 40 µg subcutaneously (SC) twice daily (bid) through the day of trigger.
  - This regimen is ideally initiated after the use of OCPs.

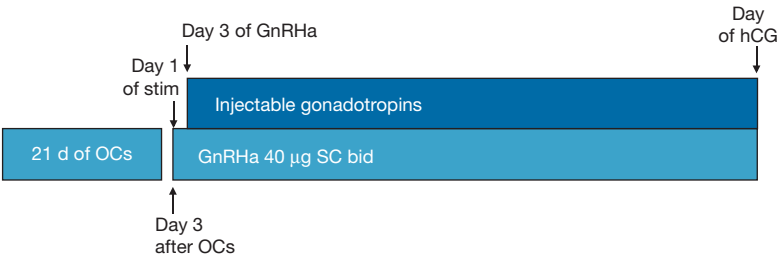

Figure 23-2 Representative illustration of microdose GnRHa flare protocol.
bid, 2 times daily; GnRHa, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin; OCs, oral contraceptives; SC, subcutaneous.

Gonadotropin-Releasing Hormone Antagonist (GnRHant) *Protocol*

- Synthetic GnRH molecule that has antagonist properties on the GnRH-R. *Immediately* binds to and blocks GnRH binding to the receptor
- Due to likely comparable LBRs between GnRHa and GnRHant protocols[43] and the significant decrease in the risk of OHSS, the GnRHant protocol is recommended in normal responder patients.[26]
- Clinics vary on the use of fixed (antagonist started on day 5 or 6 regardless of response to stimulation) versus flexible (antagonist started based on US and/or lab criteria).
  - Two meta-analyses have suggested the highest pregnancy rate overall for fixed protocol without OCP pretreatment, but this may not be true for subpopulations, such as polycystic ovary syndrome (PCOS) or poor responders.[43,44]
  - Regardless of OCP pretreatment, some data suggest that a fixed protocol is superior to flexible for the ongoing CPR, although data are of low quality.[44]
- ***Advantages***[43]
  - Immediate onset of action
  - ↓ Cycle duration
  - ↓ OHSS risk
    - Allows for GnRHa-only trigger
    - Antagonist cycle recommended for patients with PCOS, high ovarian reserve, or prior high response/prior OHSS.
  - ↓ Number of injections (average of 5 injections vs 25 with long GnRHa protocol)
- ***Disadvantages***
  - Higher rate of spontaneous LH surge versus long GnRHa protocol (8% vs 1%)[43]
    - LH surge occurs prior to antagonist start, so the occurrence may be higher in flexible protocols.
  - Possible decrease in pregnancy rates:
    - Large systematic review and meta-analysis showed a slightly decreased ongoing pregnancy rate in the general IVF population compared to the long GnRHa protocol (23.8% vs 27.4%).[43]
      - ➔ Difference seemed to be driven by cycles with OCP pretreatment and a flexible antagonist protocol, although protocols that used a flexible antagonist protocol without OCPs also had a small decrease in CPR.
      - ➔ No difference seen in small number of studies that looked at fixed antagonist protocols versus long GnRHa
      - ➔ No differences in CPR in a subset of patients with PCOS or poor responders

- A Cochrane review showed a slight decrease in CPR (28% vs 30%), but no difference in LBR in antagonist versus long GnRHa cycles.[45]
  ➔ Superior CPR with antagonist versus minimal stimulation cycles (32% vs 25%)
  ➔ No difference in miscarriage rate

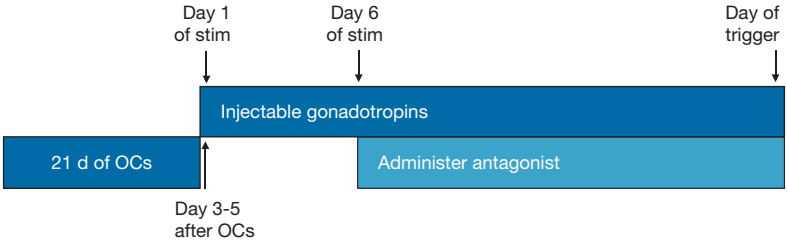

Figure 23-3 Representative illustration of GnRHant protocol.
GnRHa, gonadotropin-releasing hormone antagonist; hCG, human chorionic gonadotropin; OCs, oral contraceptives.

Progestin-Primed Ovarian Stimulation (PPOS) Protocol

- Progestins result in negative feedback on the hypothalamus and pituitary, thereby suppressing endogenous LH surge.
- Typically start progestin with gonadotropin stimulation, 10 mg/d po medroxyprogesterone acetate (MPA) sufficient to prevent LH surge.[46-49]
- ***Advantages***
  - ↓ Cost
  - Administered po
  - ↓ Cycle duration
  - ↓ OHSS risk
  - Allows for GnRHa-only trigger
- ***Disadvantages***
  - Cycles using progestin for suppression need to be "freeze all" as the early progestin exposure will advance the endometrium and affect receptivity.
- RCT comparing suppression with MPA versus GnRHant in 318 donor egg cycles with GnRHa trigger showed no difference in the number of oocytes retrieved, serum endocrine profiles in donors, or CPR or LBR in recipients.[50]

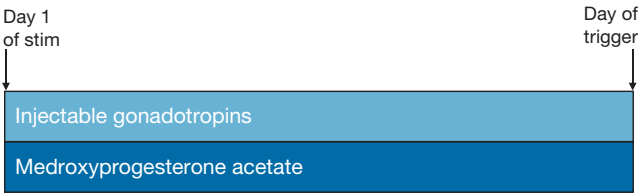

Figure 23-4 Representative illustration of progestin-primed ovarian stimulation protocol.

Alternative Protocols

DuoStim Protocol

- Allows patients to stim twice in a shortened time; maximizing oocyte yield while shortening the treatment time frame[51-53]
- Involves two consecutive ovarian stimulations and oocyte retrievals within the same menstrual cycle, targeting both the follicular and luteal phases[54]
- This protocol could benefit patients with poor ovarian response or those requiring urgent fertility preservation before gonadotoxic treatments.[55]
- Clinical outcomes from DuoStim, such as the number of mature oocytes and viable embryos, are comparable or superior to traditional single-stimulation cycles, especially in poor responders.[51,56,57]
- One open-label RCT compared DuoStim with two consecutive conventional GnRHant cycles in poor ovarian responders. The time to the second oocyte retrieval was significantly shorter in the DuoStim group ($P < .001$), but the cumulative LBR (CLBR) was not statistically different between the groups ($P = .08$). The study concluded that DuoStim does not improve the # of oocytes retrieved or the CLBR compared to two consecutive conventional stimulations, although it may shorten the time to a second retrieval.[58]
- Ovarian suppression with either GnRHant or MPA
  - If using GnRHant, continue until the day of trigger. The antagonist is to be given on the day of trigger in those at high risk of premature ovulation, that is, poor responders.
  - If using MPA, ok to use continuously from DuoStim #1 (D1) through hiatus prior to DuoStim #2.
- At the time of retrieval #1: Leave alone follicles less than 10 mm.
- The fifth day after retrieval #1: Repeat baseline and restart gonadotropins.
- Monitoring and criteria for trigger shot #2 are similar to cycle #1.

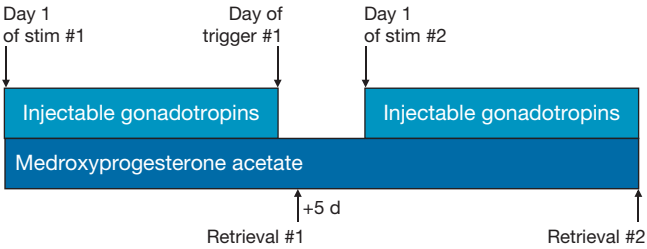

Figure 23-5 Representative illustration of DuoStim protocol.

Luteal Estradiol Priming Protocol

- May be beneficial for (1) poor responders, (2) prior premature ovulation, and (3) poor follicular synchronization in past cycles
- Estrogen priming with E_2 in the luteal phase prior to stimulation is thought to prevent an endogenous luteal FSH rise and increase in sensitivity to gonadotropins.

- Begin testing for urinary LH surge starting CD7. The day of the positive LH surge is day 0.
- 7 days after the LH surge, initiate E_2 supplementation (ie, E_2 2 mg po bid or E_2 patch 0.1 mg/d) and await menses.
  - Some protocols also include GnRHant × 3 days overlapping with luteal E_2.
- On CD2 to CD3, present for baseline testing.
- Stop E_2 on day 1 of gonadotropins.

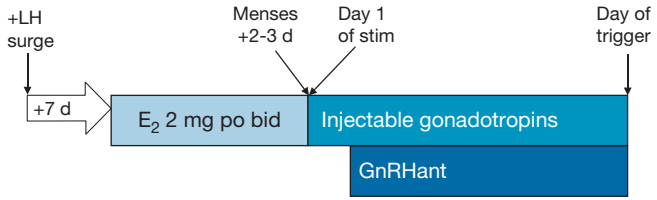

Figure 23-6 Representative illustration of luteal E_2 priming protocol.
bid, 2 times daily; E_2, estradiol; GnRHant, gonadotropin-releasing hormone antagonist; LH, luteinizing hormone; po, oral.

Minimal Stimulation Protocol

- Purported to be more cost-effective than conventional IVF protocols, yet this must be weighed against the efficacy in achieving a live birth.[59]
- Possible indications include poor responders, ultra-low AMH, AFC less than 6, and Bologna criteria for poor ovarian response.
- No standardized protocol; one example is to use clomiphene citrate (CC) or letrozole with or without lower doses of gonadotropins.
  - A study demonstrated that CC-only minimal stimulation IVF achieved CLBRs of 22.6% after the first cycle and 39.2% after the third cycle, with success rates significantly influenced by age.[60]
  - A Cochrane review found no conclusive evidence that CC or letrozole with or without gonadotropins differs from gonadotropin-only protocols in terms of LBR. However, these protocols reduce the incidence of OHSS and the amount of gonadotropins required but may increase cycle cancellation rates and reduce the # of oocytes retrieved.[61]
  - CC can be used throughout the ovarian stimulation cycle to prevent premature ovulation and reduce costs, showing noninferior outcomes compared to standard protocols with gonadotropins.[62]
- Start po agent (ie, CC 100 mg or letrozole 7.5 mg) on CD2 or CD3 for 5 days.
- Start gonadotropins (150 IU every day [qd]) on the third day of po agent.

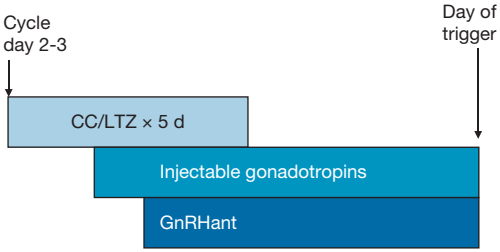

Figure 23-7 Representative illustration of Minimal Stimulation protocol.
CC, clomiphene citrate; GnRHant, gonadotropin-releasing hormone antagonist; LTZ, letrozole.

Natural Cycle In Vitro Fertilization (or "Unstimulated" In Vitro Fertilization)

- "Natural" in that no gonadotropins are administered, although hCG is used for trigger to time oocyte retrieval from the dominant follicle formed during a spontaneous cycle
  - Requires close monitoring of follicular sizes and E_2 levels, usually starting approximately CD7 to CD10; high cancellation rates without the use of GnRHant[63,64]
- Benefits to natural cycle IVF:
  - Lower costs
  - Elimination of OHSS
  - Single ET
  - Obviates ethical concerns of patients regarding excess embryos
- Cost-benefit aspect is questionable.

Oocyte Maturation Trigger Options

- Oocytes are arrested in meiotic prophase I until the LH surge, at which point meiosis resumes (Table 23-4).
  - An RCT showed that FSH supplementation (450 IU SC) along with hCG trigger may improve oocyte developmental competence and fertilization rates, although its impact on CPR and LBR remains inconclusive.[65]
- GnRHa can be used to induce LH surge (via the flare effect), or hCG can be used, given a high degree of homology with LH and a shared receptor.
  - hCG has a longer half-life than endogenous LH surge.

Table 23-3 hCG Trigger Preparations

Trade Name, Manufacturer	Source	Formulations
Novarel, Ferring	Urine of pregnant females	10,000 IU IM
Ovidrel, EMD Serono	Recombinant, Chinese hamster ovary cells	250-500 μg SC
Pregnyl, Merck & Co.	Urine of pregnant women	10,000 IU IM

hCG, human chorionic gonadotropin; IM, intramuscular; SC, subcutaneous.

Human Chorionic Gonadotropin–Only Trigger

- An Ovidrel dose of 250 µg (prefilled syringe) is sufficient to achieve ovulation trigger for most patients (equivalent mean number of retrieved oocytes and percentage of M2 oocytes).[66]
- Those with an elevated BMI ought to receive Ovidrel 500 µg.[67]
- Pregnyl and Novarel are often less costly than Ovidrel but require intramuscular (IM) injection.
  - These also have the advantage of dose adjustment since they do not come in a prefilled syringe (ie, in women at risk of OHSS).

Gonadotropin-Releasing Hormone Agonist–Only Trigger

- 4 mg (0.8 mL) SC leuprolide acetate 36 hours prior to retrieval
  - Some protocols include a second dose of leuprolide 24 hours prior to retrieval.
- Substantially decreases the risk of OHSS and all but eliminates severe OHSS, but not all patients will respond[68]
- Harbingers for suboptimal response[69]:
  - LH level less than 1 IU/L at the start of stimulation
  - LH level less than 0.5 IU/L on the day of trigger
  - High BMI or low ovarian reserve[70]
- Check labs the morning after trigger to ensure adequate response.[71]
  - Adequate if LH 15 IU/L or greater and P_4 3 ng/mL or greater
- Cannot be used with GnRHa protocols or in patients with hypothalamic amenorrhea
- Some programs will also administer a small dose of hCG (eg, 1,500 IU) with a GnRHa trigger in patients at risk of OHSS.

Dual Trigger

- 4 mg (0.8 mL) SC leuprolide acetate +2,000 IU hCG or 250 µg SC Ovidrel[72]
- The literature is mixed on the utility of a dual trigger but is sometimes used in women who have a history of poor oocyte maturity rates.[72]
- A review of RCTs found a significantly higher number of retrieved oocytes, number of mature oocytes, and LBR with dual trigger as opposed to hCG alone.[73]

Oocyte Retrieval

- Typically performed 36 hours after hCG[74]
- Performed under intravenous (IV) sedation using a 5-MHz vaginal transducer with associated needle guide. A 16- to 17-gauge, 33-cm aspiration needle is inserted transvaginally into multiple preovulatory follicles with sequential aspiration (low-grade suction) of oocytes. The aspirate is then given to the embryologist for evaluation.
- Complications are very rare (see "RISKS OF In Vitro Fertilization" section).

"ADD-ONS"

Testosterone Supplementation

- Associated with higher LBRs compared to nonsupplemented women (OR = 2.19, 95% CI = 1.11-4.32) and also improved the total number of eggs collected (weighted mean difference [WMD] = 0.88, 95% CI = 0.03-1.72)[75]

- Transdermal testosterone gel (1%, 25 mg packet or 1%, 75 g pump), start on CD3 together with OCP for 21 days, then start IVF stimulation meds 3 days off of testosterone (GnRHant) or with bleed (Min-Stim, Flare, or GnRHa protocols)
  - ½ packet = 12.5 mg applied to alternating lateral thigh, once daily, or
  - 1 pump = 12.5 mg applied to alternating lateral thigh
- Number needed to treat (NNT) approximately 12 (four RCTs)

Dehydroepiandrosterone Supplementation

Dehydroepiandrosterone (DHEA) showed a significant improvement in the number of oocytes retrieved (WMD = 0.60, 95% CI = 0.07-1.13), but not in LBR.[75]

Delayed-Start Protocol With Gonadotropin-Releasing Hormone Antagonist

- A retrospective study starting gonadotropins 3 days after GnRHant pretreatment increased the number of oocytes retrieved compared to a standard GnRHant cycle: number of mature oocytes (8 vs 5.8, $P < .001$).[76]
- Gonadotropins started 7 days after GnRHant pretreatment.[75] NNT approximately 42 (two RCTs).

High-Dose Gonadotropin Regimens

No difference in LBRs[75]

Screening Hysteroscopy

Currently not recommended for routine clinical use but can be considered in patients with recurrent implantation failure (RIF)[77]

Endometrial Receptivity Tests

Currently not recommended[77]

Endometrial Scratching

Currently not recommended[77-81]

Platelet-Rich Plasma

Small sample sizes studied and heterogeneous study populations with different dosages of platelet-rich plasma (PRP). Currently not recommended[77]

Steroids

Glucocorticoids are not currently recommended.[77]

Acupuncture

- Currently not recommended[77]
- Data are mixed as to the effect of acupuncture on LBRs after IVF, with some RCTs showing benefit and some showing no difference.[82]
  - May nonetheless be associated with decreased anxiety levels

Immunologic Tests and Immunomodulating Treatments

- Natural killer (NK) cells, killer cell immunoglobulin-like receptor (KIR), and human leukocyte antigen (HLA) are not currently recommended.[77]
- Intralipid, intravenous immunoglobulin (IVIG), recombinant human leukemia inhibitory factor (rh-LIF), peripheral blood mononuclear cells (PBMCs), and anti-tumor necrosis factor (TNF) are not currently recommended.[77]

Microfluidics for Sperm Sorting

Only one RCT[83]; may be considered[77]

Antibiotics

Routine antibiotic administration prior to IVF/ET is not recommended.[84,85]

Aspirin

Aspirin does not improve pregnancy rates after IVF, although it may have beneficial effects in terms of preventing preeclampsia.[86-89]

Others

Other interventions, such as letrozole, CC, growth hormone, luteal phase stimulation, dual trigger, DuoStim, LH, E_2 pretreatment, and corifollitropin alfa, did not show significant improvements in the primary or secondary outcomes.[75]

EMBRYOLOGY PRIMER

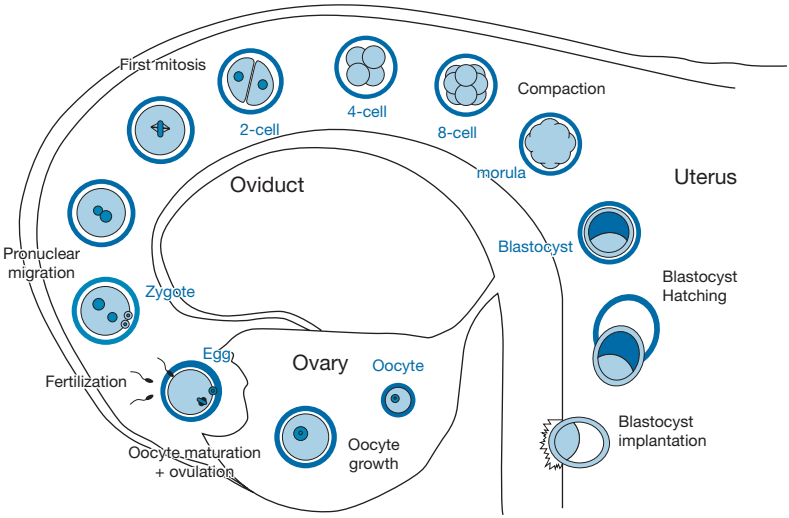

Figure 23-8 Overview of preimplantation development, from oocyte to embryo.
Source: Reprinted by permission from Clift D, Schuh M. Restarting life: fertilization and the transition from meiosis to mitosis. *Nat Rev Mol Cell Biol*. 2013;14(9):549-562.

- Retrieved eggs are identified in the follicular aspirate. Once they are identified, they are removed from the aspirate and placed in culture dishes.
- It is recommended that men provide semen specimens for oocyte retrieval with less than 24 hours of abstinence to minimize DNA fragmentation and optimize sperm quality.[90]
- Standard IVF insemination is performed by culturing the identified eggs for approximately 16 hours with 50,000 sperm/mL or greater. The next morning, the eggs are identified and evaluated for fertilization. The first sign of fertilization is two pronuclei (2PN) within the cytoplasm.
- ICSI is performed in cases of severe male factor infertility, poor or failed prior fertilization, use of surgically retrieved sperm, preimplantation genetic testing for monogenic (PGT-M), or severe antisperm antibody levels. ICSI is not routinely recommended for cases of unexplained infertility or nonsevere male factor, as studies have not demonstrated improved outcomes compared to conventional insemination.[91-93]
  - A retrospective, multicenter observational cohort study in 37,041 couples compared LBR outcomes between cycles using fresh sperm (36.4%) and those using cryopreserved sperm (32.45%). Statistically significant difference, but the use of frozen sperm is still a very viable option.[94]
  - Rescue ICSI refers to ICSI performed the day after conventional insemination to salvage cycles with total or near-total fertilization failure. Success rates vary, and it is not considered standard practice due to the risk of oocyte aging and reduced embryo developmental potential.[95-97]
  - A RCT showed that ICSI does not improve LBR compared to conventional IVF in those with total motile sperm greater than 2 million[98]; there is some evidence from a retrospective cohort study that the utilization of ICSI in non-male factor infertility may actually diminish fertilization per oocyte and euploid embryo numbers.[99]
- After identification of the eggs in the follicular aspirate, the eggs are then placed into culture dishes. For ICSI cases, the cumulus cell complex surrounding the eggs is then removed in a process called *stripping*. Once the eggs are stripped, they are evaluated for maturity. Only metaphase 2 (MII) eggs can be fertilized (Figure 23-7). All MII eggs are then inseminated by taking one motile, morphologically normal-appearing sperm and injecting it into each mature egg.

Table 23-4 Progression of a Germinal Vesicle Into a Zygote

Stage	Description	Morphology	Ploidy N, n
Germinal vesicle ***GV***	The oocyte is arrested in ***meiotic prophase I*** until LH surge contains a germinal vesicle (GV) (nucleus).		Diploid 46N 92n
LH SURGE ↓			
Metaphase I **MI**	No polar body (PB), no GV; completed prophase of meiosis I		Diploid 46N 92n
Metaphase II **MII**	First PB present, no GV; chromosomes are divided between the oocyte and PB (23 chromosomes in each); arrested until fertilization		Haploid 23N 46n
FERTILIZATION ↓			
Fertilization 	Sperm entry triggers the completion of meiosis II, resulting in the extrusion of the second PB.		
Pronuclear formation	The male and female pronuclei form.		
Zygote (2 pronuclei)	The pronuclei abut and prepare for the first mitotic division. 2 PBs.		Diploid 46N (23+23) 46n

N NUMBER: # of copies of each double stranded (ds) DNA molecule (single or double chromatids = 1, see image below)
- That is, 1N or 2N

n number: # of chromatids of each ds DNA
- That is, 46n or 96n

1N, 1n:  1N, 2n: 

PLOIDY: # of copies of each chromosome
- Haploid= 1 copy of each chromosome
- Diploid = 2 copies of each chromosome

Note: *In some stages of the cell cycle, diploid cells also have one DNA molecule per chromosome and are thus 2N.* The ploidy and N number do not always coincide.

LH, luteinizing hormone.

- Fertilization rates for ICSI typically range from 64% to 80%, depending on the source of the sperm and specific patient factors. Testicular sperm have the lowest rates.[100,101]
- Embryos are then cultured, typically for 3 to 5 days, in incubators maintained at body temperature and media specific for human embryo culture. Embryos may be evaluated on day 3 for their cell number and overall morphology, though it is likely preferable to not disturb them again until day 5.
  - It is possible for ET to be performed on day 3. However, in most centers, embryos are placed into extended embryo culture for 2 to 4 additional days, referred to as the *blastocyst stage* (D5-D6-D7).
- The utility of transferring D7 blastocyst is of great debate.[102] D7 blastocysts have a lower euploidy rate but can lead to live births (Table 23-5[103]).
- At the 32-cell stage, a fluid-filled cavity begins to form inside the embryo.[104]
- Per the American Society for Reproductive Medicine (ASRM) practice guideline, **assisted hatching** should not be offered routinely for patient undergoing fresh ET,

given evidence that it does not improve outcomes; an RCT with frozen embryo transfer (FET) failed to show any LBR improvement with assisted hatching.[105-107]

Table 23-5 D7 Blastocyst Euploidy Rate and Overall Live Birth Rate

	D7% Euploid	LBR/D7 Blastocyst (%)	Overall LBR/D7 Transfer (Untested) (%)
≤35	51.1	17.4	8.9
35-37	42.7	25	10.7
38-40	37.4	16.7	6.2
41-42	19.0	25	4.7
≥43	9.5	33.3	3.2

Note: The differences among age group rates are most likely due to disparate sample sizes.
LBR, live birth rate.
Source: Hernandez-Nieto C, Lee JA, Slifkin R, Sandler B, Copperman AB, Flisser E. What is the reproductive potential of day 7 euploid embryos? *Hum Reprod.* 2019;34(9):1697-1706 by permission of Oxford University Press.

Blastocyst Scoring Guide

- Grading of frozen embryos is detailed in Figure 23-9. Blastocysts are frozen on day 5 to 7 of growth. Extended embryo culture to the blastocyst stage helps to identify embryos with a better prognosis for pregnancy.
  - They are given a numerical score of 1 to 6 based on how expanded they are (1 being least expanded and 6 being fully hatched), followed by two alphabetical letters:
  - The first letter grades the inner cell mass (ICM) (A-D), which becomes the fetus.
  - The second letter grades the trophectoderm (A-D), which becomes the placenta.
  - Freeze criteria vary by laboratory. Some programs elect not to cryopreserve or transfer blastocysts with ICM or trophectoderm grades of D, due to historically low implantation and LBRs associated with these embryos.

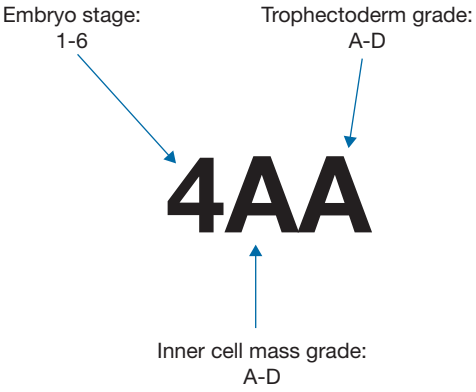

Figure 23-9 Blastocyst scoring.
Source: Adapted from Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril.* 2000;73(6):1155-1158, with permission from Elsevier.

Parameter Measured	Score	Description of Embryo Grade
Expansion status (embryo stage)	1	**Early blastocyst:** cavity less than ½ the volume of the embryo
	2	**Blastocyst**, cavity ½ or higher the volume of the embryo
	3	**Full blastocyst**, cavity completely fills the embryo
	4	**Expanded blastocyst**, cavity larger than that of the early embryo, with a clearly thinning zona
	5	**Hatching blastocyst**, trophectoderm starting to herniate through the zona
	6	**Hatched blastocyst**, blastocyst has completely escaped from the zona
ICM	A	ICM prominent, easily discernable, and consisting of many cells that are compacted and tightly adhered together
	B	Easily discernible, with several cells that are loosely grouped together
	C	Very few cells visible
	D	No visible cells, or presence of degenerating cells
Trophectoderm	A	Many cells forming a cohesive epithelium
	B	Moderate number of cells forming a loose epithelium
	C	Few and larger cells with poor epithelial formation
	D	Sparse of degenerating cells surrounding the ICM

PERCENT FERTILIZATION BASED ON FOLLICLE SIZE AT TIME OF RETRIEVAL

Table 23-6 Oocyte Diameter at Recovery and Associated Chance of Retrieving an M2 Oocyte or Chance of Ending Up with a 2PN (Zygote) per Punctured Follicle

M2 oocyte/Punctured Follicle (size, mm)					
	<10	***10-12.5***	***13-15.5***	***16-18.5***	***≥19***
IVF-ICSI	14%	25%	36%	45%	68%
2PN (zygote)/Punctured Follicle (size, mm)					
IVF-ICSI	9%	19%	28%	34%	34%

2PN, two pronuclei; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

Source: Reprinted from Shapiro BS, Rasouli MA, Verma K, et al. The effect of ovarian follicle size on oocyte and embryology outcomes. *Fertil Steril.* 2022;117(6):1170-1176, with permission from Elsevier.

POSTRETRIEVAL HORMONAL MANAGEMENT

- Luteal support is required for patients planning a fresh ET following oocyte retrieval (between the evening of the day of retrieval and day 3 postoocyte retrieval)[108,109]; typically continued until 9 to 12 weeks of gestational age
- Insufficient luteal P_4 levels were initially thought to be due to the use of GnRHa or GnRHant and the removal of granulosa lutein cells at the time of retrieval; however, subsequent studies debunked those theories, and it is now thought to be due to negative feedback on pituitary LH secretion from stimulation-induced elevated E_2 levels.[110]

- The vast majority of programs replace P_4 directly (po, vaginally [pv], by rectum [pr], IM, or SC) but can also directly stimulate lutein cells to produce P_4 with hCG administration or GnRHs.[110]
  - 50 mg qd IM P_4
  - 90 mg qd vaginal P_4 gel
  - 200 mg 3 times daily (tid) micronized vaginal progesterone-in-oil (PIO) capsules
  - 100 mg bid or tid micronized vaginal P_4 in starch suppositories
- There seems to be similar efficacy between IM and vaginal formulation for luteal phase support. A Cochrane review compared options[110]:
  - No difference in pregnancy, LBR or miscarriage rate between supplementation with P_4 versus hCG versus hCG plus P_4; groups with hCG had a higher risk of OHSS
  - No difference in live birth or miscarriage between routes of P_4 administration or types of vaginal preparations, as well as micronized versus synthetic P_4
  - LBRs were superior for luteal support with P_4 + GnRHa (single or multidose) versus P_4 alone
- Some centers also replace E_2, which is normally concomitantly secreted by the corpus luteum.
  - Studies are limited, but 2015 Cochrane review suggested no benefit to adding po E_2, but possible benefit for transdermal E_2 or transdermal plus po E_2; findings with vaginal E_2 were mixed.[110]
- If a fresh ET is not planned, no postretrieval hormones are necessary, but can give a 10- to 14-day course of P_4 to produce a more predictable menses and less spotting (particularly if planning to proceed to an FET in the next cycle).

ENDOMETRIAL LINING

- Current evidence does not support modifying clinical management solely based on endometrial thickness measurements below an arbitrary threshold.[111]
- A retrospective study after euploid FETs adjusted for factors such as age, embryo quality, day of trophectoderm biopsy, and BMI concluded that there was no identified threshold of ET (lining range of study: 4-12 mm) that precluded live birth or below which LBR decreased significantly.[112]
- Note: A decrease in endometrial thickness (endometrial compaction) is found after the initiation of P_4.[113]
- Adjuvants for thin endometrium[114]:
  - Aspirin: no significant difference in thickness or LBR[115]
  - Luteal E_2: no significant difference in thickness or LBR[116]
  - Sildenafil: 25 mg 4 times per day, intravaginally; it may improve endometrial blood flow and receptivity rather than an increase in endometrial thickness. Its effectiveness is uncertain.[117,118]
  - Granulocyte colony–stimulating factor (G-CSF): Although data indicate that intrauterine infusion of G-CSF may improve endometrial thickness, there is no controlled study demonstrating improved LBR; moreover, the potential adverse effects necessitate careful consideration.
  - Pentoxifylline, hCG, or platelet-rich plasma in FETs: no controlled studies
  - Vitamins C/E and L-arginine: small, poorly controlled studies
  - Tamoxifen (20-40 mg CD3-CD7) may be effective in improving endometrial thickness and increasing the LBR in patients with thin endometrium undergoing FET, making it a viable alternative to hormone replacement therapy.[119] However, there are no controlled studies.

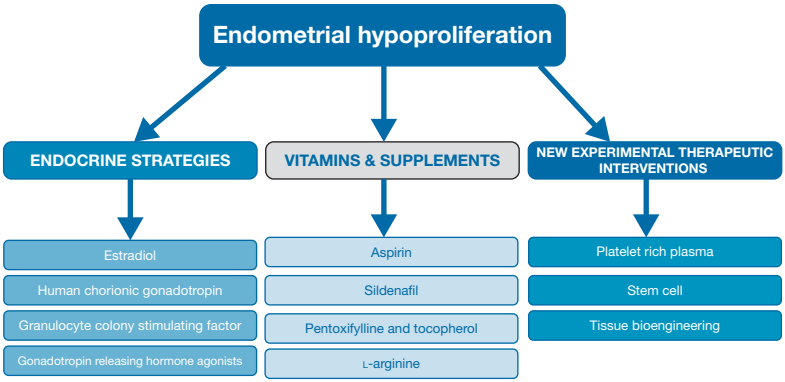

Table 1. Endocrine Strategies, Vitamins, & Supplements: Dosages Commonly Used

Endocrine strategies	
Estradiol	FET: E valerate + E transdermal patch (If ES <7 mm on D14) E (17-β estradiol) 100 μg transdermal patch starting on D2, continued every other day and each patch was removed after four days E valerate 4 × 2 mg po starting on D2 + Estradiol vaginal tablet 10 μg bid starting on D3 E valerate 3 mg bid on po D3-6 followed by 4 mg bid po on D7-10 po. If ES <6 mm, and micronized estradiol 2 mg intravaginal daily for 5-7 d (continuing the oral medications). If ES does not reach 7 mm, increase to micronized estradiol 4 mg intravaginal daily for 14-20 d
hCG	FET: hCG 150 IU SC on D7-14 hCG 150 IU IM on D8-12
G-CSF	FRESH: G-CSF 30 mU (300 μg/1 mL) intrauterine 6-12 h before hCG. Repeat on the day of OPU if ES <7 mm G-CSF 100,000 IU/kg SC daily for 15 days + 30 mU (300 μg/1 mL) intrauterine 6-12 h before hCG FET: G-CSF (300 μg/1 m) intrauterine infusion on D14 of the FET cycle G-CSF 1.5 mg/kg/d SC from the ET day to the day of β-hCG test
GnRHa	FRESH: Triptorelin 0.1 mg sc injections 3 times (OPU day/ ET day/ ET+3)
Vitamins & supplements	
Aspirin	FET: Aspirin 81 mg/d starting 1 w before initiation of E treatment. Continue until 9 w after ET in case of a positive pregnancy
Sildenafil	FRESH: Sildenafil 25 mg × 4/d intravaginal from Day 1 of stimulation until the day of hCG administration FET: Sildenafil (25×4/dav intravaginal) from Day 1 of E until the day of P start
Pentoxifylline Tocopherol	FRESH or FET: PTX (800 mg/d po) + Vit E (1000 IU/d) start 3-4 mo prior to cycle and continue until serum hCG confirms pregnancy Vit E (3*600 mg/d/po) D1-throughout the menstrual cycle.
L-Arginine	FRESH: L-arginine (4x6 g/d po); Day 1 of stimulation until the day of hCG injection

Bid, 2 times daily; E, estradiol; FET, frozen embryo transfer; G-CSF, granulocyte colony-stimulating factor; GnRHa, gonadotropin -releasing hormone agonist; hCG, human chorionic gonadotropin; OPU, oocyte pick-up; po, orally; SC, subcutaneous.

Figure 23-10 Alternate options for treating thin endometrial linings.
Source: From Cakiroglu Y, Tiras B, Franasiak J, Seli E. Treatment options for endometrial hypoproliferation. *Curr Opin Obstet Gynecol.* 2023;35(3):254-262.

EMBRYO TRANSFER

- ET is typically performed under US guidance. A full bladder helps provide an acoustic window and decreases the anterior bend of the cervix in patients with an anteverted uterus.
  - US guidance improves CPR and LBR.[82]
- Better outcomes with soft transfer catheters than rigid[82]

- There are data to suggest that the catheter tip should be placed in the middle to upper uterine cavity, greater than 1 cm from the fundus.[82]
- Higher pregnancy rates were seen when the position of the air bubble from the fundal endometrial surface was less than 10 mm; the inner catheter tip should be placed 1.5 to 2 cm from the fundal endometrium.[120]
- CPR is not sacrificed by the afterload technique (Table 23-8).

Table 23-7 Clinical Pregnancy Rate Based on Technique and Blastocyst Embryo Transfer (ET) Difficulty

	OR (95% CI)	*P* Value
Direct easy ET	1	
Afterload easy ET	0.97 (0.82-1.14)	.69
Afterload difficult ET	0.85 (0.67-1.08)	.20
Direct difficult ET	0.62 (0.49-0.77)	<.001

Note: Reference value: direct easy ET (OR = 1).
CI, confidence interval; OR, odds ratio.
Source: Reprinted by permission from Cirillo F, Immediata V, Ronchetti C, et al. Steps forward in embryo transfer technique: a retrospective study comparing direct versus afterload catheters at different time frames. *J Assist Reprod Genet.* 2023;40(12):2895-2902.

Table 23-8 ASRM Practice Guideline Recommendations for Limits on Number of Embryos to Transfer

Prognosis	*Age <35*	*Age 35-37*	*Age 38-40*	*Age >40*
Cleavage-Stage Embryos				
Euploid[a]	1	1	1	1
Other favorable[b]	1	1	≤3	≤4
Embryos not euploid[a] or favorable[b]	≤2	≤3	≤4	≤5
Blastocysts				
Euploid[a]	1	1	1	1
Other favorable[b]	1	1	≤2	≤3
Embryos not euploid[a] or favorable[b]	≤2	≤2	≤3	≤3

ASRM, American Society for Reproductive Medicine; FET, frozen embryo transfer; IVF, in vitro fertilization; LB, live birth.
[a]Demonstrated euploid embryos, best prognosis.
[b]Other favorable = any ONE of these criteria: *Fresh cycle*: expectation of ≥1 high-quality embryo(s) available for cryopreservation or previous LB after a prior transfer with sibling embryo(s); *FET cycle*: availability of vitrified D5 or D6 blastocysts, euploid embryos, first FET cycle, or previous LB after an IVF cycle.
Source: Reprinted from Practice Committee of the American Society for Reproductive Medicine and the Practice Committee for the Society for Assisted Reproductive Technologies. Guidance on the limits to the number of embryos to transfer: a committee opinion. *Fertil Steril.* 2021;116(3):651-654, with permission from Elsevier.

- Justification for transferring more embryos than recommended by ASRM guidelines should be clearly documented in the medical record.
- Following the injection of the embryo(s), one should (a) keep pressure on the syringe plunger until withdrawal of the catheter and (b) slowly withdraw the catheter (while rotating) in order to avoid negative pressure.[121,122]

- Following ET, the catheter should be carefully examined under the microscope by the embryologist to confirm that the embryo has been expelled. Retained embryos occur in approximately 0.5% of cases and should prompt immediate retransfer.[123]
  - Embryo retention: outcomes not affected by retained embryo with immediate retransfer[82,124]
- Bedrest (even for 10 minutes) after ET is not recommended, with some data suggesting superior pregnancy rates with immediate ambulation.[82]

TWINNING

- A retrospective cohort analysis found that while the raw incidence of monozygotic twinning (MZT) was higher in the blastocyst transfer group (2.4%) compared to the cleavage-stage transfer group (1.9%), this difference was not significant when controlling for factors such as patient age, time period, and embryo cohort quality.[125-128]
- Most twins are a result of dizygotic twinning, occurring when two different oocytes are fertilized independently. MZT occurs in only one other vertebrate—armadillo. MZT develops from a single fertilized oocyte.
- 70% of twins = DIZYGOTIC; 30% of twins = MONOZYGOTIC
- MONOZYGOTIC distribution:
  - 75%: monochorionic diamniotic (cleavage D3-D8)
  - 25%: dichorionic diamniotic (cleavage D1-D2)
  - Less than 1%: monochorionic monoamniotic (cleavage D9-D12)

Figure 23-11 Schematic of twin placentations.

FROZEN EMBRYO TRANSFER

- The success rates of fresh ET versus FET have been extensively studied, with varying results depending on patient characteristics and study design.
- In ovulatory women, a multicenter RCT found no significant difference in LBR between fresh ETs and FETs (48.7% vs 50.2%).[129]
- A meta-analysis indicated that high responders (ie, patients with OHSS) had a significantly higher LBR with FETs compared to fresh transfers (relative risk [RR] = 1.18).[130]
- A retrospective cohort study (Society for Assisted Reproductive Technology Clinic Outcome Reporting System [SART CORS] database) showed that FETs had higher LBRs (48.3% vs 39.8%) and CLBRs (74.0% vs 60.0%) compared to

fresh transfers. This study also found that the advantage of FET increased with advancing maternal age.[131]

- A retrospective study with euploid embryos and IM P_4 found no correlation with P_4 value on the day of transfer and LBR.[132]

FROZEN EMBRYO TRANSFER PROTOCOLS

Programmed FET (no Lupron)
Lupron FET
Stimulated FET
Natural FET
Modified natural FET

- An RCT investigated the efficacy of different P_4 administration routes for luteal phase support in *programmed* FET cycles (ie, no LH surge or endogenous P_4 production). The study concluded that vaginal-only P_4 replacement resulted in a significantly reduced LBR. **The combination of daily vaginal and q3 day IM P_4 (PIO) was found to be noninferior to daily PIO,** providing an effective alternative with fewer injections (Table 23-9[133]). Nevertheless, the simplest, most effective protocol is daily PIO.

Table 23-9 Alternate Regimen Using Alternate Dosing of Vaginal and PIO

D1	D2	D3	D4	D5	D6
PIO			PIO		
PVS	PVS	PVS	PVS	PVS	PVS

Note: On day 1, the patient gets both PIO and progestin vaginal suppositories (PVSs); on day 2 and 3, the patient gets only PVS. On day 4, the patient gets both PIO and PVS; on day 5 and 6, the patient gets only PVS and onward.
PIO, progesterone in oil.

- If pregnant, hormone supplementation continues until 10 to 12 weeks of the estimated gestational age.
- Data are conflicting about whether a true natural cycle has superior outcomes to a modified natural cycle (in terms of CPR and miscarriage rate).
- Largest RCT to date suggests no difference in LBR between true natural cycle, modified natural cycle, and programmed cycle, but higher cycle cancellation rates in both natural cycle groups.[134]
- A 2020 Cochrane review concluded that there is insufficient evidence to recommend one endometrial preparation method over another, but low-quality data suggest a stimulated FET may have superior pregnancy rates over programmed FET.[135]

Programmed Frozen Embryo Transfer (No Lupron)

- Regular ovulating patients
  - May use OCPs (prefer monophasic pill with 30 to 35 µg ethinyl estradiol) starting on CD1 to CD4 for 14 to 28 days to initiate withdrawal bleed and schedule cycle
- Patients with greater than 40 day per irregular cycles
  - Period may be induced after obtaining a negative urine pregnancy test.
- On CD1, begin Estrace 2 mg tid or 4 Vivelle dot 0.1 mg patches changed qd to every 3 days for 12 to 20 days.

- 0 to 2 days prior to the tentative start of P_4, schedule US for endometrial thickness and follicular activity. Draw a serum P_4.
- If P_4 greater than 2 ng/mL, cancel cycle.
  - If endometrial thickness 7 mm or greater (or decision has been made to proceed with ET if <7 mm), schedule start for PIO 50 mg at noon (may use progestin vaginal suppositories [PVSs] if requested for noon dose only). Repeat 50 mg PIO at 7 PM and thereafter. Continue estrogen replacement. (P_4 start should occur according to Figure 23-12).

DAY	1	2	3	4	5	6
Ultrasound ~D14—good lining						
Progesterone NOON[a]	50					
7 PM	50	50	50	50	50	50
Transfer Day (D6 of P_4)						✓

Figure 23-12 Sample PIO administration calendar for programmed FET. FET, frozen embryo transfer; PIO, progesterone in oil.
[a]NOON start and ~11 AM FET = 5 days − 1 hour = ~119 hours.
P_4 NOON: PIO 50 mg IM or PVS, thereafter, evening PIO 50 mg IM.

Lupron Frozen Embryo Transfer

- Regular ovulating patients
  - May use OCPs (prefer monophasic pill with 30-35 µg ethinyl estradiol) starting on CD1 to CD4
- Patients with greater than 40 day or irregular cycles
  - Period may be induced after obtaining a negative urine pregnancy test.
- Start leuprolide SC at approximately the same time daily. The starting dose will be 20 units. The leuprolide may be started as early as after 15 days of OCPs or as late as after 35 days of OCPs. Overlap OCPs and leuprolide by 4 days.
  - If OCPs are contraindicated, in patients with regular cycles, leuprolide may be started 7 to 8 days after a +LH surge (or estimated menses −7 days).
  - If OCPs are contraindicated, in patients with irregular cycles, induce bleed and begin leuprolide on the day of the PIO or up to day #6 of MPA use.
- 11th **to** 18th day of leuprolide—check E_2 level and US.
  - If E_2 less than 70 pg/mL and endometrial thickness less than 8 mm, eligible to start estrogen replacement
  - If E_2 greater than or equal to 70 pg/mL or greater, repeat 2 to 3 days later as clinically indicated.
  - ↓ leuprolide to 0.5 mg (0.1 mL or 10 U) SC daily
    - Day 1 to 5: Estrace or equivalent—2 mg/d
    - Day 6 to 9: Estrace or equivalent—4 mg/d
    - Day 10 to 14: Estrace or equivalent—6 mg/d
    - **Thereafter: Estrace or equivalent—4 mg/d**
- On day 12 to 14, schedule US for endometrial stripe (ES).
  - If ES 7 mm or greater (or decision has been made to proceed with ET if between <7 mm), schedule start for PIO 50 mg at noon (may use PVSs if requested for

noon dose only). Repeat 50 mg PIO at 7 PM and thereafter. Continue estrogen replacement. (P_4 start should occur according to Figure 23-12.)

- **Leuprolide stops** the day before PIO starts or the day PIO starts if it is taken in the morning.

Stimulated Frozen Embryo Transfer

- FET following controlled ovarian hyperstimulation (COH) to induce endogenous E_2 production to thicken the lining
- May use letrozole, clomid, gonadotropins, or hybrid
- Managed identically to a COH cycle with baseline US and monitoring
  - Some programs will use GnRHant to prevent spontaneous ovulation with protocols involving gonadotropins.
- Day of "trigger:"
  - Criteria for trigger
    - Trilaminar lining 7 mm or greater
    - Dominant follicle 18 mm or greater, and
    - P_4 less than 1.5 ng/mL

    NOTE: There are data suggesting the trigger can be administered flexibly when the mean follicle size is between 13 and 22 mm, provided an endometrial lining is 7 mm or greater and serum P_4 is less than 1.5 ng/mL.[136,137]

    ➔ If ovulation predictor kit (OPK) negative: give trigger in the evening. Start PIO 36 hours after trigger (Figure 23-12). (Endometrin tid is an acceptable alternative.)

    – If E_2 less than 200 pg/mL, supplement with Estrace 1 mg po bid with trigger.

    ➔ If OPK positive: give trigger in the evening. Start PIO the following day (Figure 23-12). (Endometrin tid is acceptable alternative.)

    – If E_2 less than 200 pg/mL, supplement with Estrace 1 mg PO bid with trigger.

Natural Frozen Embryo Transfer

- CD2 to CD3, baseline US and bloodwork (hCG, E_2, P_4, LH). Ok to start if:
  - US: endometrium 8 mm or less or still bleeding actively; no concerning cysts on ovaries
  - Labs: E_2 less than 70 pg/mL, P_4 less than 1.5 ng/mL, hCG negative (LH is for later comparison only.)
- CD10, start LH testing with OPKs (or 4 days prior to estimated LH peak).
- CD12, schedule return US and labs (E_2, P_4, LH) every 1 to 2 days depending on the results/size of dominant follicle, labs, and lining appearance.
- Await endogenous LH surge (increase in serum LH at least 3X baseline LH)
- Note that ET should occur 1 day *earlier* after endogenous LH surge than it would with an hCG trigger.
- Supplement with Prometrium 200 mg pv or equivalent starting 48 to 72 hours after trigger.

Modified Natural Frozen Embryo Transfer

- Identical to natural cycle protocol, but hCG is used to trigger ovulation and time transfer
- Criteria for trigger
  - Trilaminar lining 7 mm or greater
  - Dominant follicle 13 mm or greater, and
  - P_4 less than 1.5 ng/mL

  NOTE: There are data suggesting the trigger can be administered flexibly when the mean follicle size is between 13 and 22 mm, provided an endometrial lining is 7 mm or greater and serum P_4 is less than 1.5 ng/mL.[136,137]

RISKS OF IN VITRO FERTILIZATION

- Complications are very rare and include intra-abdominal bleeding (0.23% of IVF cases) and infection (~0.04%).[138]
  - Hemoperitoneum management postretrieval
    - 230 mL = normal estimated blood loss 24 hours postretrieval[139]
    - Almost always resolves with conservative management (if no underlying coagulopathy); 0.07% require surgical intervention.[138,140]
    - Symptoms typically arise 0 to 28 hours after retrieval (abdominal pain, nausea, vomiting, dizziness, weakness, and tachycardia).[141]
    - Evaluation and treatment:
      - ➔ Transabdominal US
      - ➔ Computed tomography (CT) angiogram of the abdomen/pelvis to detect any active bleeding
      - ➔ Serial complete blood count (CBC)/vital signs/abdominal examinations, pain control, comprehensive metabolic panel (CMP) prothrombin time/partial thromboplastin time (PT/PTT), type and screen (type and cross × 2U if unstable vital signs)
      - ➔ If active bleeding and hemodynamically stable, call interventional radiology for possible embolization.
      - ➔ If active bleeding and hemodynamically unstable, consider surgical intervention.
- Higher order gestations
  - The percentage of IVF cycles that resulted in twins decreased from 40.2% in 2012 to 10.4% in 2021, and the percentage that resulted in triplets or more decreased from 2.0% in 2012 to 0.3% in 2021.[142]
  - Multiple pregnancies ultimately depend on the number of embryos transferred. Transferring two high-quality embryos in patients younger than 35 years of age: twins → 40% to 50%, and triplets → 2% to 5%.[143]
  - Ectopic pregnancy: The uterus at the time of ET contracts from the cervix to the fundus. The rate may be 4-fold higher in women with an endometrial lining of less than 9 mm compared to greater than 12 mm. The risk of ectopic pregnancy following ART varies widely: 1.6% to 8.6%.[144]
  - OHSS: This typically affects less than 5% of IVF cycles. Less than 1% of IVF patients require hospitalization for severe OHSS (see Chapter 28).

- Low birth weight: Singleton babies born through fresh ETs may have an increased risk for low birth weight and/or preterm delivery.[145-148]
- FETs and risk for hypertensive disorders in pregnancy (HDP) and large-for-gestational-age (LGA): FETs are associated with a higher risk of HDP and LGA babies compared to fresh ETs and natural conception. This may be due to a lack of corpus luteum and its factors related to angiogenesis, vasoactivity, and the immune system important for implantation.[149-155]
  - Natural or modified natural FET (and associated corpus luteum formation) may be associated with a lower risk of HDP and LGA compared to programmed FET.[156]

Birth Defects

- Subfertile women should be aware that there is an increased risk of congenital anomalies in their offspring, regardless of whether they undergo fertility treatment.[157]
- Birth defects through IVF have been difficult to assess due to inaccurate national birth registries, not controlling for maternal age/high-order multiples/severe male factor.
- Birth defect risks for IVF or IVF/ICSI may be moderately increased compared to natural conception, with specific risks varying by the type of ART and underlying parental factors.[158-160]
- A large Nordic cohort study found that the risk of major congenital malformations in live-born singletons conceived using fresh ICSI was 6.0%, compared to 5.3% for those conceived using fresh IVF, and 4.2% for those conceived without medical assistance.[160]
- A population-based cohort design, linking ART cycles reported to the SART CORS found that children conceived with IVF, particularly those using autologous oocytes, had increased risks for nonchromosomal defects, including cardiovascular, gastrointestinal, and genitourinary defects. The adjusted odds ratios (aORs) for these defects ranged from 1.22 to 1.85.[161]
- A meta-analysis reported that children conceived by IVF and/or ICSI have a significantly increased risk of birth defects compared to those conceived naturally, with an overall pooled risk ratio of 1.37.[162] Specific malformations such as musculoskeletal, cardiovascular, and urogenital defects were more prevalent in ART-conceived children.[159-163]
- Another study highlighted that the increased risk of birth defects associated with IVF was not significant after adjusting for parental factors, whereas the risk associated with ICSI remained elevated even after such adjustments (Table 23-10[158]).

Table 23-10 Risk of Birth Defects Following IVF or IVF/ICSI

Singleton Births	aOR (CI)	# Defects[a]	% Defects[a]	Per 100
Spont and fertile	*1.00*	*16,841/293,314*	*5.7*	*6*
Spont and infertility	**1.37** (1.02-1.83)	52/600	**8.7**	**9**
IVF + ICSI	**1.28** (1.14-1.43)	361/4333	**8.3**	**8**
IVF fresh ET	1.05 (0.82-1.35)	71/1005	7.1	7
FET from IVF	1.08 (0.76-1.53)	34/479	7.1	7
ICSI fresh ET	**1.73** (1.35-2.21)	76/713	**10.7**	**11**

Bold signifies statistically significant differences. aOR, adjusted odds ratio; ET, embryo transfer; FET, frozen embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.
[a]Unadjusted.
Source: Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. *N Engl J Med*. 2012;366(19):1803-1813.

Cancer Risk to In Vitro Fertilization Children

- The risk of childhood cancer in IVF offspring is controversial. The evidence is mixed, and further research is needed to clarify these associations.
- A population-based cohort study utilizing SART CORS with state registries found that children conceived via ART, particularly those from autologous oocytes, have an increased risk of certain nonchromosomal birth defects and childhood cancers, including leukemia and central nervous system tumors.[161]
- A British study of over 100,000 IVF children found no overall increase in the risk of cancer; however, they report a very small absolute increased risk for hepatoblastoma and rhabdomyosarcoma.[164]

Cancer Risk in Women Undergoing Assisted Reproductive Technology

- Studies from the 1990s reported an increased risk of breast, endometrial, and ovarian cancer, with more recent studies refuting this.[165]
- Studies of cancer risk from fertility treatment may be confounded by overlapping risk factors for both cancer and infertility, for example, anovulation and endometrial cancer or endometriosis and ovarian cancer.[166]
  - Parity is also inversely correlated with breast and ovarian cancer risk.
- Retrospective cohort study in 87,403 Israeli women undergoing IVF found no significant increase in breast, endometrial, or ovarian cancer in a 7-year or less time span.[167] Note: Borderline ovarian tumors were not included.
- Historical cohort study (with ~15 years of follow-up) in 19,146 women undergoing IVF compared to 6,006 subfertile women not treated with IVF[168]:

Table 23-11 Possible Increased Risks of Ovarian Cancer With IVF

Excluding the First Year of Follow-Up	HR, CI	Overall % Increase
↑ Risk of ALL ovarian cancers	2.14 (1.07-4.25)	1.4 → 3
↑ Risk of borderline tumors	4.23 (1.25-14.33)	0.1 → 0.4

Note: Epithelial cancers account for 60% of all ovarian cancers of which the incidence of all ovarian cancers is 1.4% in a lifetime. Since borderline tumors account for 15% of the epithelial tumors, this means epithelial cancers account for 0.84% of the 1.4% total and borderline would then be 0.13% risk in a woman's lifetime.

ALL, acute lymphoblastic leukemia; CI, confidence interval; HR, hazard ratio; IVF, in vitro fertilization.

- ASRM recommended counseling on cancer risk from IVF[166]:
  - No conclusive evidence of increased risk: colon cancer, thyroid cancer, cervical cancer, endometrial/uterine cancer (although infertility diagnosis may increase risk, ie, PCOS), breast cancer
  - Possible increased risk (that may be due to underlying infertility diagnosis): ovarian cancer (risk is small if present), borderline ovarian tumors
  - Insufficient data: malignant melanoma, non-Hodgkin lymphoma
  - Note: These recommendations are for IVF only. The practice guideline does note increased risk for some of these cancers and prolonged clomiphene risk, although the evidence is not uniformly strong or conclusive.

SUCCESS RATES

- Success rates are IVF center specific and depend on the patient's characteristics, quality of the ovarian stimulation, embryo culture system, and transfer technique. Center-specific pregnancy rates are published by the Centers for Disease Control and Prevention annually and can be found at https://www.cdc.gov/art/success-rates/index.html
- A review article found that 47% of programmed FET patients experience bleeding before the eighth week of gestation, but this bleeding does not impact the reproductive outcome.[169]

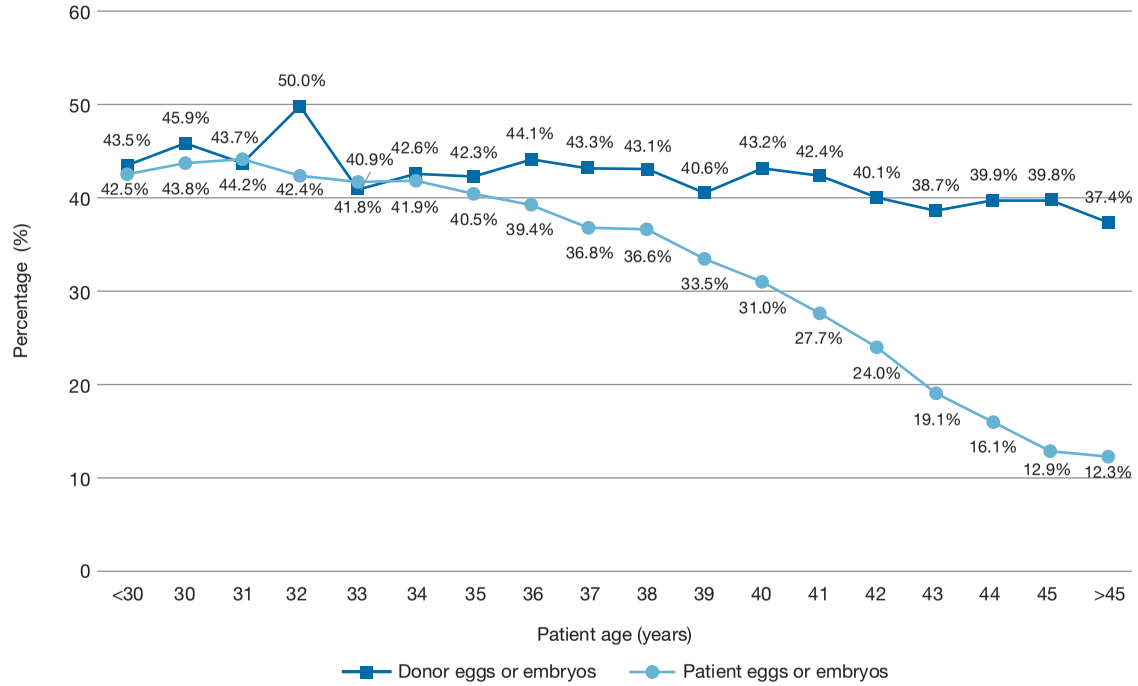

Figure 23-13 Live births per transfer for assisted reproductive technology using fresh embryos from autologous oocytes, by patient age and egg or embryo source, United States, 2021.

Source: Jewett A, Zhang Y, Sunderam M, et al. 2021 CDC assisted reproductive technology fertility clinic and national summary report. Centers for Disease Control and Prevention; 2023. https://stacks.cdc.gov/view/cdc/154438

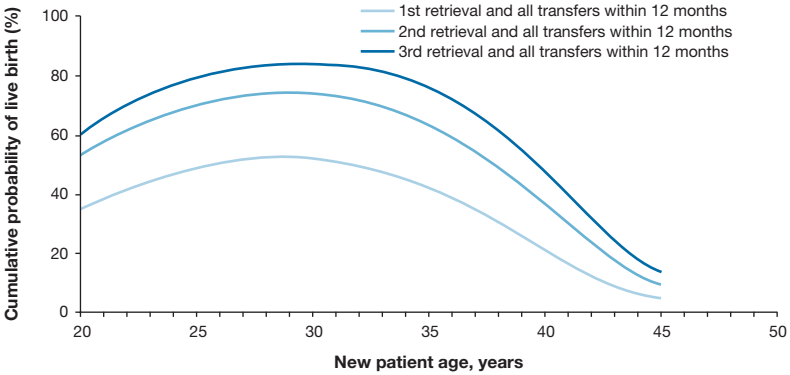

Figure 23-14 Cumulative live birth rates stratified by maternal age.

Source: Reprinted from Gaskins AJ, Zhang Y, Chang J, Kissin DM. Predicted probabilities of live birth following assisted reproductive technology using United States national surveillance data from 2016 to 2018. *Am J Obstet Gynecol*. 2023;228(5):557.e1-557.e10, with permission from Elsevier.

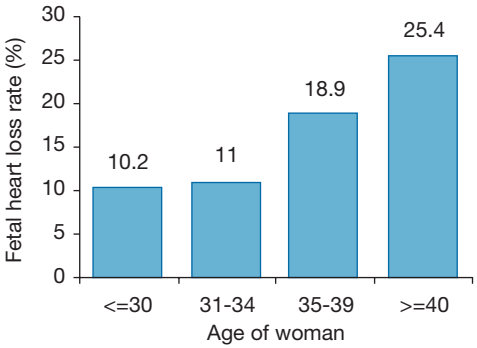

Figure 23-15 Pregnancy loss by age after documentation of fetal cardiac activity (in vitro fertilization—untested blastocysts, retrospective analysis).

Source: Reprinted from Spandorfer SD, Davis OK, Barmat LI, et al. Relationship between maternal age and aneuploidy in in vitro fertilization pregnancy loss. *Fertil Steril*. 2004;81(5):1265-1269, with permission from Elsevier.

Table 23-12 Number of Untested Good-Quality Blastocysts Needed to be Equivalent to Three Successive Euploid Embryo Transfers and Achieve a 95% Chance of Sustained Implantation

Age	Observed Aneuploidy Rate	# Untested Blastocysts to Achieve a 95% Chance of Sustained Implantation
<35	1	4
35-37	0.97 (0.82-1.14)	5
38-40	0.85 (0.67-1.08)	7
41-42	0.62 (0.49-0.77)	13
≥43		27

Source: Pirtea P, Cedars MI, Devine K, et al. Recurrent implantation failure: reality or a statistical mirage?: Consensus statement from the July 1, 2022 Lugano Workshop on recurrent implantation failure. *Fertil Steril.* 2023;120(1):45-59.

- The number of retrieved mature eggs at which pregnancy rates tend to peak in fresh IVF cycles is generally around **15 eggs,** with some variation depending on the patient's age.[170]

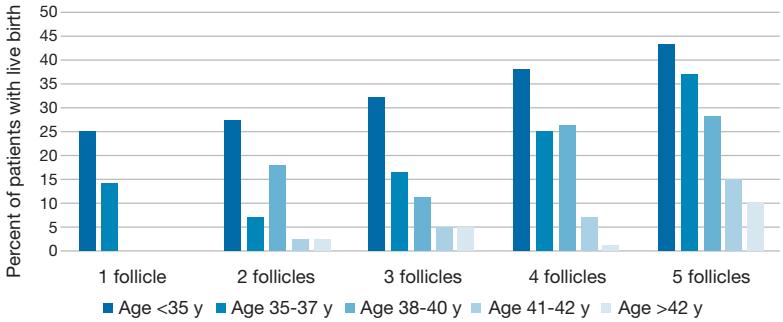

Figure 23-16 Live birth rate by age and follicle number at time of trigger.

Source: Reprinted from Kawwass JF, Kulkarni AD, Hipp HS, Crawford S, Kissin DM, Jamieson DJ. Extremities of body mass index and their association with pregnancy outcomes in women undergoing in vitro fertilization in the United States. *Fertil Steril.* 2016;106(7):1742-1750, with permission from Elsevier.

Table 23-13 Live Birth Rates per Cycle Peak at Different Oocyte Yields Depending on Age

Age	LBR Peaks at *n* Oocytes
<30	6-11
30-34	11-16
35-39	9-17
40-44	15-17

LBR, live birth rate.

Source: Reprinted from Zhang N, Law YJ, Venetis CA, Chambers GM, Harris K. Female age is associated with the optimal number of oocytes to maximize fresh live birth rates: an analysis of 256,643 fresh ART cycles. *Reprod Biomed Online.* 2021;42(3):669-678, with permission from Elsevier.

- **Elevated P_4**
  - Elevated P_4 on the day of trigger may be associated with lower fresh ET pregnancy rates.[171] This negative impact is thought to be due to the advancement of endometrial histologic development, leading to embryo-endometrial asynchrony.[172]
    - There is varying debate in the literature about what P_4 cutoff should be used to determine a "premature rise." Furthermore, it should be noted that elevated P_4 is a continuous variable, not a dichotomous one, that predicts implantation.
    - While some practices utilize a P_4 cutoff of 1.5 ng/mL (~12% of patients), this will likely result in a number of patients (NNT 13) having an unnecessary delay.[173]
    - Practices often utilize a threshold *P* value 1.5 or greater to 2 ng/mL to revert to a freeze only.
    - Elevated P_4 on the day of trigger may be associated with lower fresh ET pregnancy rates but not when transferred in a different cycle as a FET[132]:

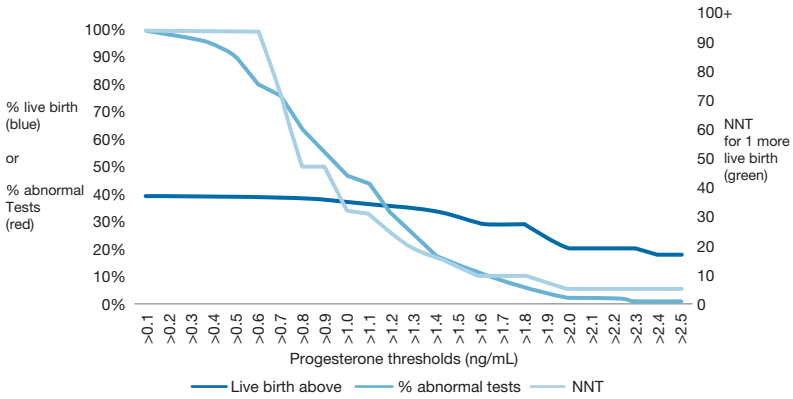

Figure 23-17 Relationship between progesterone (P_4) value and live birth as well as number needed to treat (NNT).

Source: Reprinted from Hill MJ, Healy MW, Richter KS, et al. Defining thresholds for abnormal premature progesterone levels during ovarian stimulation for assisted reproduction technologies. *Fertil Steril*. 2018;110(4):671-679.e2, with permission from Elsevier.

For instance:
P_4 = 1.5 ng/mL, NNT = 15 (15% incidence)
P_4 = 2 ng/mL, NNT = 6 (2% incidence).

- **Cigarette/marijuana use**
  - Smoking cigarettes significantly lowers the LBR per cycle (Figure 23-18[174]).
  - Women smoking marijuana have fewer oocytes retrieved with IVF.[175]

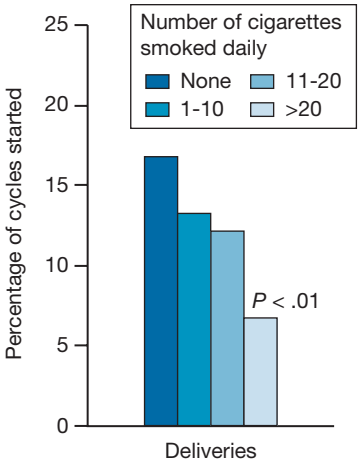

Figure 23-18 IVF live birth rates per cycle initiated based on the magnitude of maternal cigarette utilization.

Source: Reprinted from Pattinson HA, Taylor PJ, Pattinson MH. The effect of cigarette smoking on ovarian function and early pregnancy outcome of in vitro fertilization treatment. *Fertil Steril.* 1991;55(4):780-783, with permission from Elsevier.

- **Obesity**

Table 23-14 IVF Cancellation Rate Based on BMI

BMI	% Cancellation
<40	8-11
≥40	25[a]

BMI, body mass index; IVF, in vitro fertilization.

[a]$P < .01$ compared to <25 group.

Source: Dokras A, Baredziak L, Blaine J, et al. Obstetric outcomes after in vitro fertilization in obese and morbidly obese women. *Obstet Gynecol.* 2006;108(1):61-69.

- **BMI**
  - A retrospective cohort study of 494,097 fresh autologous IVF cycles found that both women who are underweight (BMI <18.5) and those who are obese (BMI ≥30) had significantly lower chances of intrauterine pregnancy and live birth per transfer compared to women with normal BMI (18.5-24.9).[176,177]

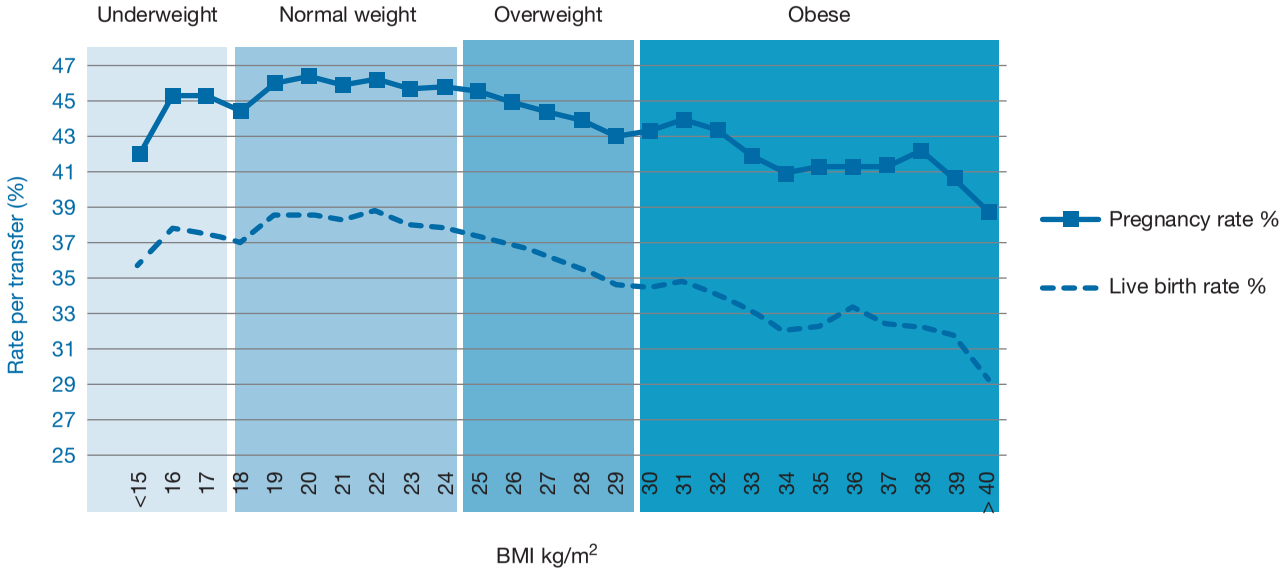

Figure 23-19 Body mass index (BMI) impact on in vitro fertilization live birth rates using fresh, autologous oocytes.

Source: Reprinted from Kawwass JF, Kulkarni AD, Hipp HS, Crawford S, Kissin DM, Jamieson DJ. Extremities of body mass index and their association with pregnancy outcomes in women undergoing in vitro fertilization in the United States. *Fertil Steril.* 2016;106(7):1742-1750, with permission from Elsevier.

- **Alcohol consumption**[178]
  - 16% lower LBR if a woman drank greater than 4 drinks per week compared with less than 4 drinks per week[178]
- **Exercise**
  - Regular exercise before and during an IVF cycle either has no effect on cycle success or may have a small beneficial effect. Further, well-designed studies are needed.[179]
  - A larger study with greater than 2,000 patients analyzed exercise and the initial IVF cycle with conflicting results.[180]

ANTIPHOSPHOLIPID SYNDROME AND THROMBOPROPHYLAXIS

During In Vitro Fertilization

- In women with antiphospholipid antibody syndrome (APS) undergoing IVF, thromboprophylaxis is generally recommended to mitigate the risk of thrombotic events.
- American College of Rheumatology (ACOG)[181]:
  - If **no history of venous thromboembolism (VTE)**: prophylactic low-molecular-weight heparin (LMWH) plus low-dose aspirin (LDA)
  - If **history of VTE**: therapeutic LMWH plus LDA
- LDA should be stopped 3 days before egg retrieval and resumed the following day. Patients taking LMWH should hold on the day of trigger and resume the evening of retrieval until E_2 is less than 50 pg/mL; repeat E_2 4 to 7 days after the retrieval.

During Pregnancy

- ACOG[182] (polar body [PB] 196):
  - If **no history of VTE or history of provoked VTE**: surveillance only (prophylactic LMWH + LDA if additional risk factors such as first-degree relative with thrombotic episode prior to age 50, obesity, prolonged immobility, etc)
  - If **history of VTE**: prophylactic LMWH plus LDA

SUBCHORIONIC HEMORRHAGE

- No intervention is necessary, and no need to restrict activity.
- Larger subchorionic hemorrhage symptom (SCHs) (50% of the size of the gestational sac have been associated with a higher miscarriage risk)[183]
- LDA and heparin have been associated with an increased risk of SCH.[184]
- Risk of early pregnancy loss:
  - Non-IVF patient study with 451 having an SCH revealed no increased risk of pregnancy loss.[185]
  - **IVF patient study**: A systematic review and meta-analysis study concluded that SCH in the first trimester does not significantly increase the risk of early pregnancy loss (OR = 1.39, 95% CI = 0.97-2.01) or LBR (OR = 0.77, 95% CI = 0.55-1.08).[186,187]

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