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GOOD CLINICAL PRACTICE RECOMMENDATIONS

Dear Doctors,

In the ever-evolving field of reproductive medicine, where ground-breaking advances and innovative techniques are constantly reshaping the landscape, it is imperative to have a comprehensive resource that encompasses the latest developments in assisted reproductive technology (ART). These recommendations serve as a testament to the extraordinary progress made in the field and brings together four vital aspects of infertility management, each contributing significantly to the pursuit of parenthood that are:

- *Luteal phase support in ART*
- *The role of tubal surgery in fertility enhancement*
- *Optimizing vitrification technique for cryopreservation success in assisted reproduction*
- *The application of ART in managing infertility*

By bringing together these four crucial areas of infertility management, these guidelines serve as a comprehensive guide for clinicians, researchers, and patients navigating the intricate world of ART. It reflects the collective expertise and dedication of the contributing authors, who are at the forefront of their respective fields, ensuring that the information presented is current, evidence-based, and relevant to the evolving landscape of reproductive medicine. We hope this book inspires and enlightens readers, empowering them with the knowledge to make informed decisions during treatment. May it serve as a catalyst for further advancements, research, and compassionate care in the field of ART.

Best wishes!

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LUTEAL PHASE SUPPORT IN INTRAUTERINE INSEMINATION AND ASSISTED REPRODUCTIVE TECHNOLOGY:

GOOD CLINICAL PRACTICE RECOMMENDATION

Background

The normal luteal function helps in the maintenance of pregnancy. Natural ovulatory cycles involve the production of progesterone after ovulation until the placental function begins at seven weeks gestation. The defective luteal phase can occur due to disturbed progesterone secretion in the secretory phase. Luteal-phase deficiency is a condition wherein insufficient endogenous progesterone is produced for embryo implantation and is associated with infertility and pregnancy loss. Endocrine defects are often induced in the luteal phase with controlled ovarian stimulation techniques that can disturb embryo implantation and lower pregnancy rates. Therefore, luteal-phase support (LPS) is a well-known intervention for almost all stimulated assisted reproductive technology (ART) cycles.[1, 2]

The guideline includes recommendations on the LPS in ART cycles, timing of starting LPS, agents and route for LPS, timespan for LPS administration, and efficacy and safety of LPS protocols. These recommendations are developed to inform clinical decision-making in the management of LPS in patients undergoing ART. While in some cases individualization of treatment is a necessity, these recommendations can provide standards of optimal care for patients. Furthermore, there is also a need for an expert group to develop recommendations suitable for a diverse resource situation as in India.

Scope

The guideline provides clinicians with clear advice on LPS in ART cycles, based on the best evidence available.

Methodology

Based on the collected evidence, recommendations were formulated and discussed until a consensus was reached within the guideline group. The task force consisted of experts in the field of Obstetrics and Gynecology, as well as IVF specialists. Various published data and guidelines were explored to address the role of LPS in ART. This document provides much-required insights and useful, practical, and accurate guidance that aids a practicing clinician. These practice points were developed through a series of e-mails, conference calls, and face-to-face meetings. The task force prepared the initial draft with the help of a medical writer and was reviewed and commented on by members of the Indian Society for Assisted Reproduction.

Level of evidence	Description
Level A	Data derived from multiple randomized trials or meta-analyses or evidence-based clinical practice guidelines
Level B	Data derived from a single randomized trials or large non-randomized trial
Level C	Consensus of opinion of experts or small studies, retrospective studies or registries or narrative/literature reviews
Level D	Data derived from Clinical experience
Class of recommendations	
Class I	Evidence and or general agreement that a given treatment or procedure is beneficial, useful or effective. It is recommended
Class IIa	Evidence is in favor of efficacy/usefulness and should be considered
Class IIb	Efficacy/usefulness is less well established and recommendation may be considered
Class III	Evidence and or general agreement that a given treatment or procedure is not beneficial, useful or effective and in some cases may cause harm. Not recommended

I. Introduction

1. What is luteal phase deficiency and conditions altering it?

Luteal phase deficiency (LPD) may result from insufficient secretory activity of the corpus luteum or, in cases of normal corpus luteum function, a defective endometrial response to normal progesterone levels.[3] LPD refers to a condition characterized by insufficient levels of endogenous progesterone to sustain functional secretory endometrium, proper embryonic implantation, and growth. This inadequacy in ovarian progesterone production may result in early pregnancy loss, recurrent pregnancy loss, or infertility.[4,5] LPD diagnosed through clinical means is defined as a luteal phase of 10 days or less, although alternative definitions include a luteal phase of 11 days or less and 9 days or less.[5]

Pathologic conditions disrupting the normal gonadotropin-releasing hormone (GnRH) and LH pulsatility may cause LPD.[5] Several pathological conditions, including thyroid disorders, hyperprolactinemia, obesity, polycystic ovary syndrome, endometriosis, aging, stress, anorexia nervosa, and other eating disorders, excessive exercise, weight loss, ovulation induction with or without GnRH agonist, and ART have been identified as risk factors for LPD. The most frequently performed ART procedures that may contribute to LPD include in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI), frozen embryo transfer, and donor oocyte cycle.[4]

2. LPS

RECOMMENDATIONS

- LPS in women undergoing ART can be administered through various forms such as progesterone with or without estrogen, hCG, or GnRH agonist (Level C/ Class IIa).
- LPS in IVF and ICSI cycles can be recommended to increase implantation and live births or ongoing pregnancy rates (Level A/ Class I).
- Administration of progesterone should be routinely followed as LPS for controlled ovarian stimulation (COS) or HRT frozen embryo transfer (FET) cycles. (Level C/ Class I).
- Individualized LPS should be applied, according to the treatment protocol, the patients' specific characteristics, and desires. LPS should be initiated on the day of oocyte retrieval or one day after oocyte retrieval and continued at least until the hCG test is positive. (Level C/ Class I).

Discussion

LPS can aid in the early development of the fertilized ovum in cases of infertility, recurrent pregnancy loss, and women undergoing ART.[4] Adequate LPS is essential during IVF and ICSI for improving implantation and pregnancy rates, which can be achieved by substituting deficient LH with GnRH agonists or human chorionic gonadotropin (hCG), which has a longer half-life, or directly by using progesterone with or without estrogen. Although the ideal method of luteal phase supplementation remains a matter of debate.[2]

A review has suggested that LPS may be administered in various forms, including progesterone with or without estrogen, hCG, or GnRH agonist, with women undergoing ART being the most appropriate candidates for this type of support.[4]

A 2015 Cochrane review of 94 randomized controlled trials compared different LPS regimens in a total of 26,198 women, which suggested that utilizing LPS in IVF and ICSI cycles can increase pregnancy and implantation rates. This can be accomplished by the administration of progesterone or the use of GnRH agonist/hCG, which increases the activity of the corpus luteum and, thus, enhances progesterone production. The administration of hCG or progesterone during the luteal phase may be associated with higher rates of live birth or ongoing pregnancy than placebo or no treatment.[2]

In frozen embryo transfer (FET) or donor oocyte cycles, where the corpus luteum is absent, the administration of LPS can be crucial to sustain pregnancy until the placenta can produce enough progesterone. The available evidence does not support the use of LPS in natural, unstimulated cycles. Furthermore, studies have not demonstrated any benefit from LPS in women undergoing ovulation induction with clomiphene citrate with or without gonadotropins. In contrast, LPS using progesterone has been found to be beneficial in women undergoing ovulation induction with gonadotropins followed by intrauterine insemination (IUI).[4]

LPS does not have so many choices as the individualized COS protocols and endometrium preparation protocols. It is the clinicians' responsibility to provide individualized LPS for infertile women based on their specific characteristics, desires, and the treatment protocol. Initiating LPS between 24 and 72 h after oocyte retrieval can be considered and is suggested to be continued at least until the hCG test is positive. The addition of E_2 and the route of progesterone administration appear to be independent of the improvement in outcomes.[6]

II. Optimal LPS in the stimulation protocols

3. When to start the LPS in IVF/ICSI cycles?

RECOMMENDATIONS

- LPS is recommended to be started the day after or the day of oocyte retrieval to day 3 post-retrieval, as per the ESHRE guideline. (Level A/ Class I).
- In FET cycles, embryo transfer for cleavage stage embryo should be done after 3 days of progesterone administration, and blastocyst stage embryo can be transferred after 5 days of progesterone administration. (Level C/ Class I).

Discussion

A systematic review was conducted to provide qualitative evidence-based data regarding the efficacy of LPS on fertility outcome in women undergoing IVF. A study involving 130 patients showed no significant difference in pregnancy

rates when starting luteal support on the day of hCG, the day of egg aspiration, or the day of embryo transfer. Another study on 1111 IVF/ICSI cycles found identical results regardless of when luteal support was started, and regardless of the ovulation stimulation protocol used. Additionally, two studies have also shown that there was no difference in pregnancy rates whether luteal support was started on the day of egg aspiration or on the second or third day after transfer. This systematic review concluded that optimal period to start with LPS would be between 24–72 h after oocyte retrieval, and should continue atleast until a positive pregnancy test.[7]

The European Society of Human Reproduction and Embryology (ESHRE) 2019 recommends starting LPS in the window between the evening of the day of oocyte retrieval and day 3 post-retrieval.[8]

There is a paucity of data on the impact of the length of the progesterone exposure on the reproductive outcome.[9]

An RCT has shown that when cleavage stage day 3 embryos were warmed and cultured overnight to day 4, and transferred on the 5th or 3rd day of progesterone administration, similar CPR`s were noted 27.0% vs. 18.8% respectively.[10]

An RCT compared the outcomes of blastocyst transfer on the 5th or 7th day of progesterone administration, and it was found that the LBRs were in favor of the 5th day of progesterone administration, although not reaching statistical significance (31.1% vs 25.7%, OR=0.76, 95% CI 0.46 – 1.26).[11]

Therefore, there is limited evidence for the optimal length of progesterone exposure before FET, day 3 embryos should be transferred on the 3rd or 4th day of progesterone administration and day 5/6 blastocysts on the 5th or 6th day of progesterone administration.[9]

4. Which agent/route should be used for LPS?

Progesterone for LPS

RECOMMENDATIONS

- The use of progesterone is recommended for LPS in ART cycles for increasing live births and ongoing pregnancy rates. (Level A/ Class I).
- Micronized progesterone and dydrogesterone are recommended to be suitable options for LPS. (Level A/ Class I).
- The dose of natural progesterone recommended are 50 mg daily for intramuscular route, 25 mg daily for subcutaneous route, 90 mg daily for vaginal progesterone gel, and 600 mg daily for vaginal progesterone capsules. (Level A/ Class I).
- Dydrogesterone (30 mg) is recommended to be a viable alternative to micronized vaginal progesterone (MVP) gel in fresh ART cycles due to its comparable efficacy and tolerability, as per the ESHRE. (Level A/ Class I).

Discussion

Progesterone is the mainstay treatment for LPS.[12] Progesterone directly affects the endometrium's secretory transformation for implantation and early development of the fertilized ovum. Progesterone should be provided until the luteo-placental shift. It is available as a pill, capsule, pessary, vaginal gel, and injectable, among other formulations. Oral, vaginal, rectal, intramuscular, and subcutaneous progesterone supplements are mentioned in the literature.[4]

The Cochrane Review of 2015 found that LPS with progesterone is associated with a high rate of live births or ongoing pregnancies. The rates of live births, ongoing pregnancies, and miscarriages are similar regardless of the method of progesterone administration.[2]

The ESHRE recommends the use of progesterone for LPS in ART cycles. It should be started between the day of oocyte retrieval to day 3 post oocyte retrieval and to be continued till the day of the pregnancy test at least.[8]

The ESHRE 2020 guideline recommends any of the non-oral administration routes for natural progesterone as LPS. The usual dosages used include:[13]

- 50 mg once daily for IM progesterone
- 25 mg once daily for SC progesterone
- 90 mg once daily for vaginal progesterone gel
- 200 mg three times daily for MVP in-oil capsules
- 100 mg two or three times daily for MVP in starch suppositories
- 400 mg two times daily for the vaginal pessary.

In a recent study by Griesinger et al, dydrogesterone (30 mg) was demonstrated to be a viable alternative to MVP gel in fresh ART cycles, exhibiting comparable efficacy and tolerability. Despite its oral administration feasibility and comparable efficacy, dydrogesterone is not yet regularly utilized in ART cycles.[4] Dydrogesterone is probably recommended for LPS by ESHRE.[8]

Estrogen for LPS

RECOMMENDATIONS

- The use of estradiol is not recommended along with progesterone supplementation as LPS in GnRH agonist triggered fresh embryo cycles to increase clinical pregnancy and ongoing pregnancy rates. (Level A/ Class IIb).
- Use of estradiol can be individualized based on patients' specific characteristics and decision of the IVF specialist. (Level D/ Class I).

Discussion

A Cochrane Review Study by van der Linden aimed to assess the effectiveness and safety of various LPS methods for subfertile women undergoing ART. The analysis included a total of 94 RCTs involving 26,198 women who received LPS using progesterone, hCG, or GnRH agonist supplementation in ART cycles. In sub analysis researchers included 16 RCTs (2577 women) comparing progesterone vs. progesterone with estrogen. The analysis revealed no significant differences between the two groups in terms of live birth or ongoing pregnancy rates (OR 1.12, 95% CI 0.91 to 1.38, 9 RCTs, 1,651 women, I^2=0%, low-quality evidence). Similarly, there was no significant difference in the incidence of OHSS between the two groups (OR 0.56, 95% CI 0.2 to 1.63, 2 RCTs, 461 women, I^2 =0%, low-quality evidence). Overall, the study suggests that both progesterone alone and progesterone with estrogen are comparable in terms of their effectiveness and safety as LPS for subfertile women undergoing ART.[14] Hence, the addition of estrogen does not seem to improve outcomes.

In a randomized control trial, Ismail Madkour et al. determined the pregnancy outcomes in 220 patients undergoing antagonist ICSI cycles protocol. The patients were randomly divided into two groups, Group 1 received vaginal progesterone alone (90 mg once daily) starting on the day of oocyte retrieval for up to 12 weeks if pregnancy occurred and Group 2 received vaginal progesterone (90 mg once daily) along with estradiol addition (2 mg twice daily) starting on the same day and continuing up to seven weeks, which included the fetal viability scan. The primary outcomes measured were pregnancy and ongoing pregnancy rates per embryo transfer. Secondary outcomes included implantation and early pregnancy loss rates. The results showed no significant difference in pregnancy rates between group 1 (39.09%) and group 2 (43.63%), p= 0.3. Similarly, both groups had comparable ongoing pregnancy rates, with

32.7% in group 1 and 36.3% in group 2, p=0.1. Implantation rates also showed no significant difference between group 1 (19.25%) and group 2 (23.44%), p=0.2. Additionally, early pregnancy loss rates were similar, with 6.3% in group 1 and 7.2% in group 2, p=0.4.[15]

Researchers concluded that the addition of 4 mg estrogen daily to progesterone for luteal support in antagonist ICSI cycles did not demonstrate any significant benefits for pregnancy outcomes.

As per the ESHRE, addition of estradiol to progesterone for LPS is probably not recommended.[13]

hCG for LPS

RECOMMENDATIONS

- The use of hCG as LPS in fresh embryo transfer cycles can increase the rate of live births and ongoing pregnancies but has a high risk of OHSS, thus its use should be individualized based on the patient characteristics and decision of the IVF specialist. (Level A/ Class IIb).

Discussion

The administration of hCG externally leads to an increase in the production of progesterone and estradiol by the corpus luteum. In the past, high doses of hCG were used, leading to much higher hormone levels than normal and increasing the risk of ovarian hyperstimulation syndrome (OHSS). However, newer hCG regimens using lower doses or microdoses (100–150 IU) daily have been introduced to mimic a more natural physiological response and reduce the risk of OHSS.[4]

A Cochrane Database of Systematic Reviews 2015 states that hCG as LPS is associated with a high-live birth rate or ongoing pregnancy rate vs. placebo or no treatment, but has high risk of OHSS. The rates of OHSS is also reported to be higher when used with or without progesterone vs. progesterone alone.[2]

In some cases, a GnRH agonist trigger is used instead of hCG, but additional hCG may still be needed for adequate hormone production. Recently, the use of daily microdoses of hCG throughout the luteal phase without exogenous progesterone has been proposed, but its administration can be challenging.[4]

GnRH agonist for LPS

RECOMMENDATIONS

- A single-dose subcutaneous administration of GnRH agonist 6 days after oocyte retrieval can be recommended to improve clinical pregnancy rate, ongoing pregnancy rate, and live birth rates. (Level A/Class I)

Discussion

GnRH agonist increases the secretion of LH from the pituitary gonadotroph cells, strengthens the corpus luteum to produce its own progesterone, and directly affects the endometrium through GnRH receptors.[4]

A RCT has shown that the GnRH agonist support in the luteal phase can result in a significant improvement of pregnancy rates in ICSI cycles following the ovarian stimulation with GnRH antagonist protocol. Women who underwent ICSI cycles with GnRH antagonist ovarian stimulation protocol were randomly assigned to the intervention (GnRH agonist) and placebo groups. The intervention group received a single dose injection of triptorelin (0.1 mg) subcutaneously 6 days after oocyte retrieval. The clinical pregnancy rate was found to be significantly higher in the GnRH agonist group than in the placebo group.[16]

Another study has shown that a single dose of GnRH agonist (triptorelin 0.1 mg SC) administered on day 6 after oocyte pick-up in addition to standard LPS (vaginal progesterone and oral estrogen) lead to a trend toward a higher implantation rate and pregnancy rate in IVF cycles vs. standard LPS group.[17]

In a meta-analysis, administration of GnRH agonist as one dose (0.1 mg of triptorelin 6 days after

oocyte retrieval) increased the implantation, clinical pregnancy rate per transfer, and ongoing pregnancy rate.[18]

A meta-analysis in 2020 included about 3584 cycles from 13 randomized controlled trials concluded that adding of GnRH agonist for luteal support not only improved the clinical pregnancy rate, ongoing pregnancy rate, live birth rate, but also decreased the percentage of abortion.[19]

5. How long the administration of LPS should be continued during early pregnancy?

RECOMMENDATIONS

- LPS should be continued until a positive pregnancy test is confirmed (Level A/ Class I). LPS is commonly used by many clinicians until the 10th week of gestation when the luteo-placental shift occurs. (Level C/Class I)
- When HRT is used for endometrial preparation, LPS should be continued until the placenta can produce enough progesterone to support the pregnancy (Level A/ Class I).

Discussion

Studies show that the placenta begins producing more progesterone after 8 weeks of pregnancy and that LPS is typically given until the 10th week. Evidence suggests that LPS can be discontinued by the 10th week of pregnancy in ART.[6]

A large-scale survey of 84 reproductive centres from 35 countries was conducted encompassing 51,155 cycles. It was found that in 67% of the cycles, progesterone was administered as LPS until 10-12 weeks of gestation, 22% discontinued it when a fetal heartbeat was detected, and 12% discontinued it after a positive hCG test.[20]

A meta-analysis assessed the optimal duration of progesterone supplementation after IVF/ICSI, and concluded that it was unnecessary to continue progesterone supplementation after the first hCG test.[21]

In ART cycles, progesterone supplementation should be continued until placental progesterone production is adequate, around 8–10 weeks of gestation. There is no proven role in adding progesterone or hCG for luteal support once a pregnancy has been established. Use of supplemental progesterone in a non-ART cycle beyond the time of expected menses (2 weeks after ovulation) is not proven to be beneficial.[22]

III. Efficacy and safety of LPS protocols

1. LPS in IUI cycles

RECOMMENDATIONS

- LPS with progesterone should be used following OS-IUI when gonadotropins are used for stimulation. (Level A/ Class I).
- In LPS, oral dydrogesterone has advantages over other progesterone routes due to its lower cost, easy administration, and better patient compliance in patients undergoing IUI. (Level B/ Class I).
- Use of LPS in IUI cycles where mild ovarian stimulation is applied should be based on the decision of fertility specialists. (Level B/ Class IIb).

Discussion

The luteal support protocol is necessary for IUI cycles as deviation in estrogen-progesterone ratios impair endometrial receptivity and reduces implantation and pregnancy rates. Research has shown that LPS increases pregnancy success rates in stimulated cycles. LPS with progesterone or hCG has been observed to improve the endometrial histology of the mid and late luteal phase.[23]

A recently 2022 conducted systematic review and meta-analysis has shown that progesterone administration for LPS following OS-IUI for unexplained or mild male infertility is effective and safe. Progesterone LPS after OS-IUI led to higher live births (RR 1.38, 95% CI [1.09, 1.74];

7 RCTs, n=1748) and clinical pregnancy rates (RR 1.38, 95% CI [1.21, 1.59]; 11 RCTs, n=2163) than no LPS or placebo; and was specifically observed in protocols using gonadotropins for OS-IUI (RR 1.41, 95% CI [1.17, 1.71]; 7 RCTs, n=1114).[24]

A study was conducted to evaluate the effects of different luteal phase protocols on pregnancy success in patients undergoing IUI, 80 cycles which were assigned into 4 groups (20 cycles each) consisting of 2x200 mg/day MVP, 1x250 mg/day hydroxyprogesterone intra-muscularly for 5 days, 2x10 mg oral dydrogesterone and 1x90 mg/day 8% progesterone vaginal gel. The LPS was maintained until gestational week 8 in patients with positive pregnancy test. LPS in patients undergoing OI by gonadotropins and IUI had similar effects on clinical pregnancy and live births with oral dydrogesterone, MVP, vaginal progesterone gel and intramuscular hydroxyprogesterone. The authors opined that amongst all of the agents, dydrogesterone should be preferred due to lower cost and better patient compliance.[23]

When LPS with oral dydrogesterone was compared with MVP capsules in subjects with unexplained subfertility undergoing IUI in conjunction with ovarian stimulation by using rFSH, similar pregnancy outcomes in terms of clinical pregnancy and live birth rates were reported with both treatments. But due to the easy administration, better safety profile and patient tolerability, the authors suggested that oral dydrogesterone should be preferred for LPS in IUI.[23]

Based on conflicting results from various studies and a lack of robust evidence, it is a matter of debate whether LPS in IUI cycles where mild ovarian stimulation is applied would be beneficial. The authors suggest a need for more randomized trials with larger groups to examine the necessity of LPS in IUI cycles.[25]

2. LPS in fresh embryo transfer

RECOMMENDATIONS

- Progesterone is recommended for LPS in fresh embryo transfer cycles. Any of the non-oral administration routes for natural progesterone such as IM and SC progesterone, vaginal progesterone gel, MVP in-oil capsules, MVP in starch suppositories or vaginal pessary can be used. (Level A/ Class I).
- Oral dydrogesterone ranging from 20 mg to 40 mg daily for LPS in women undergoing fresh embryo transfers following IVF can be beneficial and probably recommended. (Level A/ Class I).

Discussion

Progesterone is recommended for LPS after IVF/ICSI. A Cochrane Database of Systematic Reviews has demonstrated that both, progesterone and hCG, during the luteal phase were associated with higher rates of live birth or ongoing pregnancy than placebo in subfertile women undergoing assisted reproduction.[2]

The ESHRE recommends any of the non-oral administration routes for natural progesterone as LPS such as IM and SC progesterone, vaginal progesterone gel, MVP in-oil capsules, MVP in starch suppositories or vaginal pessary.[8]

Starting of progesterone for LPS should be in the window between the evening of the day of oocyte retrieval and Day 3 post oocyte retrieval, and should be administered at least until the day of the pregnancy test.[8]

A systematic review and meta-analysis was conducted to identify, appraise, and summarize the evidence from RCTs to evaluate the efficacy, safety, and tolerability of oral dydrogesterone ranging from 20 mg to 40 mg daily vs. vaginal progesterone capsules ranging from 600 mg to 800 mg/day. for LPS in women

undergoing embryo transfers following IVF. Oral dydrogesterone when used for LPS was observed to provide at least similar results than vaginal progesterone capsules on live birth/ongoing pregnancy (RR=1.08, 95% CI=0.92-1.26, I^2=29%, 8 RCTs, 3,386 women) and clinical pregnancy rates (RR 1.10, 95% CI 0.95 to 1.27; I^2=43%; 9 RCTs; 4,061 women) as per good quality evidence from RCTs. The quality of the evidence was considered to be high for live birth/ongoing pregnancy and clinical pregnancy. Therefore, dydrogesterone can be considered as a reasonable option for LPS in women undergoing IVF, as oral administration is more patient-friendly than the vaginal route.[26] Another systematic review and meta-analysis in 2020 has shown that in the meta analysis of individual participant data (IPD) and aggregate data of all studies, oral dydrogesterone (20 to 40 mg daily) administered for LPS was associated with higher pregnancy rate (odds ratio [OR], 1.16; 95% confidence interval [CI], 1.01 to 1.34; p=0.04), and live birth rate (OR, 1.19; 95% CI, 1.03 to 1.38; p=0.02) vs. MVP capsules (600 to 800 mg daily) .[27] In the meta-analysis of individual participant data, oral dydrogesterone was associated with a significantly higher chance of ongoing pregnancy at 12 weeks of gestation OR 1.32; 95% CI, 1.08 to 1.61; p=0.0075) and live birth (OR, 1.28; 95% CI, 1.04 to 1.57; p=0.0214) compared to MVP.[27]

3. LPS in frozen embryo transfers

RECOMMENDATIONS

- Progesterone supplementation, with either oral dydrogesterone or MVP, is beneficial and can be recommended in HRT frozen embryo transfer cycles. (Level A/ Class I).
- Oral dydrogesterone should be a preferred choice for LPS in HRT frozen embryo transfer cycles over the vaginal route, due to the higher tolerance, better compliance and negligible side-effects. (Level B/ Class I).

Discussion

A recent 2022 published systematic review and meta-analysis based on RCTs has shown that progesterone supplementation is associated with a higher live birth rate (LBR; (RR 1.42, 95% CI 1.15-1.75, I^2 = 0%, moderate-quality evidence) and the clinical pregnancy rate (CPR: RR 1.30, 95% CI 1.07-1.57, I^2 = 0%, moderate-quality evidence) in true natural cycle frozen embryo transfer (tNC-FET) cycles.[28]

The LOTUS I trial has established that oral dydrogesterone (10 mg tid) is noninferior to MVP (200 mg tid), with ongoing pregnancy rates of 37.6% and 33.1% in the oral and vaginal group treatment groups, respectively (difference +4.7% with dydrogesterone). The live birth rates were 34.6% and 29.8% in the oral and vaginal treatment groups, respectively (difference +4.9% with dydrogesterone). Moreover, the satisfaction of patients with the tolerability of oral dydrogesterone for LPS (10 mg bid) was significantly higher compared MVP (200 mg tid). Further, many patients undergoing IVF experienced vaginal discharge or irritation with MVP vs. none with oral dydrogesterone.[29]

Few other studies have shown that LPS with either dydrogesterone or MVP after Fresh ET showed similar live birth rates and miscarriage rates. The use of oral dydrogesterone compared to MVP did not significantly influence the clinical pregnancy occurrence in any women between 18 and 43 years old, who completed an IVF cycle with or without ICSI, followed by fresh embryo transfer. But, oral dydrogesterone is preferred over the vaginal route, due to the higher tolerance and better compliance.[30,31]

Oral dydrogesterone in addition to vaginal progesterone as LPS in frozen embryo transfer cycles has been shown to play a role in reducing the miscarriage rate and improving the live birth rates. Live birth rates were 46.3% in the vaginal progesterone + dydrogesterone

group vs. 41.3% in the vaginal progesterone (p=0.042), with a statistically significant lower rate of miscarriage at <12 weeks in the progesterone + dydrogesterone versus progesterone group (3.4% vs. 6.6%; RR 0.51, 95% CI 0.32-0.83; p=0.009).[32]

4. LPS for third-party reproduction

During third-party embryo transfer, proper LPS should be administered to the third party similar to that of fresh or frozen embryo transfer as per protocol.

SUMMARY OF RECOMMENDATIONS

LPS

- LPS in women undergoing ART can be administered through various forms such as progesterone with or without estrogen, hCG, or GnRH agonist (Level C/ Class IIa).
- LPS in IVF and ICSI cycles can be recommended to increase implantation and live births or ongoing pregnancy rates (Level A/ Class I).
- Administration of progesterone should be routinely followed as LPS for controlled ovarian stimulation (COS) or HRT frozen embryo transfer (FET) cycles. (Level C/ Class I).
- Individualized LPS should be applied, according to the treatment protocol, the patients' specific characteristics, and desires. LPS should be initiated on the day of oocyte retrieval or one day after oocyte retrieval and continued at least until the hCG test is positive. (Level C/ Class I).

Optimal LPS in the stimulation protocols

When to start the LPS in IVF/ICSI cycles?

- LPS is recommended to be started the day after or the day of oocyte retrieval to day 3 post-retrieval, as per the ESHRE guideline. (Level A/ Class I).
- In FET cycles, embryo transfer for cleavage stage embryo should be done after 3 days of progesterone administration, and blastocyst stage embryo can be transferred after 5 days of progesterone administration. (Level C/ Class I).

Which agent/route should be used for LPS?

Progesterone for LPS

- The use of progesterone is recommended for LPS in ART cycles for increasing live births and ongoing pregnancy rates. (Level A/ Class I).
- Micronized progesterone and dydrogesterone are recommended to be suitable options for LPS. (Level A/ Class I).
- The dose of natural progesterone recommended are 50 mg daily for intramuscular route, 25 mg daily for subcutaneous route, 90 mg daily for vaginal progesterone gel, and 600 mg daily for vaginal progesterone capsules. (Level A/ Class I).
- Dydrogesterone (30 mg) is recommended to be a viable alternative to MVP gel in fresh ART cycles due to its comparable efficacy and tolerability, as per the ESHRE. (Level A/ Class I).

Estrogen for LPS

- The use of estradiol is not recommended along with progesterone supplementation as LPS in GnRH agonist triggered fresh embryo cycles to increase clinical pregnancy and ongoing pregnancy rates. (Level A/ Class IIb).
- Use of estradiol can be individualized based on patients' specific characteristics and decision of the IVF specialist. (Level D/ Class I).

hCG for LPS

- The use of hCG as LPS in fresh embryo transfer cycles can increase the rate of live births and ongoing pregnancies but has a high risk of OHSS, thus its use should be individualized based on the patient characteristics and decision of the IVF specialist. (Level A/ Class IIb).

GnRH agonist for LPS

- A single-dose subcutaneous administration of GnRH agonist 6 days after oocyte retrieval can be recommended to improve clinical pregnancy rate, ongoing pregnancy rate, and live birth rates. (Level A/Class I).

How long the administration of LPS should be continued during early pregnancy?

- LPS should be continued until a positive pregnancy test is confirmed (Level A/ Class I). LPS is commonly used by many clinicians until the 10th week of gestation when the luteo-placental shift occurs. (Level C/Class I).
- When HRT is used for endometrial preparation, LPS should be continued until the placenta can produce enough progesterone to support the pregnancy. (Level A/ Class I).

Efficacy and safety of LPS protocols

LPS in IUI cycles

- LPS with progesterone should be used following OS-IUI when gonadotropins are used for stimulation. (Level A/ Class I).
- In LPS, oral dydrogesterone has advantages over other progesterone routes due to its lower cost, easy administration, and better patient compliance in patients undergoing IUI. (Level B/ Class I).
- Use of LPS in IUI cycles where mild ovarian stimulation is applied should be based on the decision of IVF specialists. (Level B/ Class IIb).

LPS in fresh embryo transfer

- Progesterone is recommended for LPS after IVF/ICSI. Any of the non-oral administration routes for natural progesterone such as IM and SC progesterone, vaginal progesterone gel, MVP in-oil capsules, MVP in starch suppositories or vaginal pessary can be used. (Level A/ Class I).
- Oral dydrogesterone ranging from 20 mg to 40 mg daily for LPS in women undergoing embryo transfers following IVF can be beneficial and probably recommended. (Level A/ Class I).

LPS in frozen embryo transfers

- Progesterone supplementation, with either oral dydrogesterone or MVP, is beneficial and can be recommended in HRT frozen embryo transfer cycles. (Level A/ Class I).
- Oral dydrogesterone should be a preferred choice for LPS in HRT frozen embryo transfer cycles over the vaginal route, due to the higher tolerance, better compliance and negligible side-effects. (Level B/ Class I).

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ROLE OF TUBAL SURGERY FOR FERTILITY ENHANCEMENT:
GOOD CLINICAL PRACTICE RECOMMENDATION

Background

Tubal factor infertility accounts for 25% to 35% of cases of female infertility.[1] Main causes of tubal factor infertility are tubal obstruction or occlusion (proximal, distal, unilateral or bilateral), periadnexal adhesions, and endosalpingeal destruction. Other causes include presence of pelvic inflammatory disease (PID), endometriosis, ectopic pregnancy, abdomino-pelvic surgery, use of intrauterine devices, and induced surgical abortion.[2] Tubal surgery is a one-time, usually minimally invasive outpatient procedure. Patients opting for tubal surgery can have the advantage of attempting to conceive every month without further intervention and increased chances of conceiving more than once. Reports have indicated young patients to be ideal candidates for tubal surgery, those without any other significant infertility factors, and having a repairable tubal anatomy.[3]

Therefore, tubal surgery can be considered as curative in favorable cases with normal tubal mucosa. Tubal surgery can help to conceive naturally, and it is a preferable option for couples with ethical and religious concerns.[4]

The guideline includes recommendations on the indications for tubal surgery, diagnosis of tubal pathologies using tubal patency tests and other methods, and various tubal surgical approaches based on the type of tubal blockage for fertility enhancement. These recommendations are developed to inform clinical decision-making in patients with tubal pathologies and the possibilities of conceiving in such patients. While in some cases individualization of treatment is a necessity, these recommendations can provide standards of optimal care for patients. Further, there is also a need for an expert group to develop recommendations suitable for a diverse resource situation as in India.

Scope

The guideline provides Gynecologists, as well as IVF specialists with clear advice on tubal surgeries for fertility enhancement, based on the best evidence available.

Methodology

Based on the collected evidence, recommendations were formulated and discussed until a consensus was reached within the guideline group. The task force consisted of experts in the field of Obstetrics and Gynecology, as well as IVF specialists. Various published data and guidelines were explored to address the role of tubal surgery for fertility enhancement. This document provides much-required insights and useful, practical, and accurate guidance that aids a practicing clinician. These practice points were developed through a series of e-mails, conference calls, and face-to-face meetings. The task force prepared the initial draft with the help of a medical writer, and was reviewed and commented on by members of the Indian Society for Assisted Reproduction.

Level of evidence	Description
Level A	Data derived from multiple randomized trials or meta-analyses or evidence-based clinical practice guidelines
Level B	Data derived from a single randomized trials or large non-randomized trial
Level C	Consensus of opinion of experts or small studies, retrospective studies or registries or narrative/literature reviews
Level D	Data derived from Clinical experience
Class of recommendations	
Class I	Evidence and or general agreement that a given treatment or procedure is beneficial, useful or effective. It is recommended
Class IIa	Evidence is in favor of efficacy/usefulness and should be considered
Class IIb	Efficacy/usefulness is less well established and recommendation may be considered
Class III	Evidence and or general agreement that a given treatment or procedure is not beneficial, useful or effective and in some cases may cause harm. Not recommended

Indications for tubal surgery

RECOMMENDATIONS

- Tubal surgery should be indicated in those having proximal tubal occlusion, periadnexal and peritubal adhesions, and for reversal of tubal sterilization. (Level C/Class I).

Discussion

1. Proximal tubal occlusion (PTO)

Proximal tubal obstruction can be caused by amorphous debris and mucus plugs, PID, salpingitis isthmica nodosa, endometriosis, obliterative intraluminal fibrosis, uterine synechiae, fibroids, or polyps situated over the tubal ostium.[4, 5]

- Bilateral PTO
- Unilateral PTO

2. Periadnexal adhesions

Adhesions occur because of tissue trauma caused by sharp, mechanical, or thermal injury. Tissue trauma can also result from infection, radiation, ischemia, desiccation, abrasion, or foreign-body reaction. Adhesions can cause infertility by distorting adnexal anatomy and interfering with the transport of gamete and embryo.[6] Adhesions can be minimized by avoiding unnecessary surgical procedures, using minimally invasive surgical techniques, and by using adhesion barriers.[7]

3. Reversal of tubal sterilization

Tubal sterilization is the most commonly used permanent method of contraception.[8] It is estimated that around 1% of patients undergoing tubal sterilization request reversal surgery later in life.[4] Data obtained from 2253 women who had undergone sterilization has shown a strong correlation between youthful age and regret.[8] The change of mind is also caused by a change in marital status, loss of a child, or change of attitude.[9]

Diagnosis of tubal diseases

History and physical examination

RECOMMENDATIONS

- The patient's history should be considered to evaluate the risk of tubal factor infertility. A history of ectopic pregnancy, PID, endometriosis, or prior pelvic surgery raises suspicion for tubal-factor infertility. (Level A/ Class I).
- The clinician should carry out a PS/PV examination, and look for visible and/or palpable abnormalities of external and internal genitals, along with transvaginal ultrasound. (Level A/Class I).
- Women with a high risk of tubal pathology (previous PID, ectopic pregnancy and/ or endometriosis) should be approached differently from women with low risk (without any co-morbidities). (Level A/Class I).

Discussion

- The patient's history is an important factor to consider in the risk assessment of tubal factor infertility.[10]
- Especially women reporting a history of PID, complicated appendicitis, pelvic surgery, ectopic pregnancy, and endometriosis are at increased risk of having tubal pathology.[11]
- Other workup such as physical examination [PS/PV], determining BMI and looking for visible and/or palpable abnormalities of external and internal genitals is necessary. A transabdominal or preferably transvaginal ultrasound is suggested to investigate the pelvis for, uterine, ovarian or tubal abnormalities. Additional workup depends on the medical history and the abnormalities found during the physical examination and ultrasound.[11]

Tubal patency tests

RECOMMENDATIONS

- Hysterosalpingography (HSG) or sonohysterosalpingography (sonoHSG) is the standard first-line test recommended to evaluate tubal patency. (Level A/Class I).
- HSG or sono-HSG is recommended to screen for tubal occlusion for women not known to have additional risk factors for tubal disease (such as PID, previous ectopic pregnancy or endometriosis)) or those with low risk of tubal pathology. (Level A/Class I).
- HSG with an oil-based contrast should be considered vs. water-based contrast media, as it has been proven to have a therapeutic role (higher rate of pregnancy) through flushing of tubal debris. (Level A/Class I).
- Tubal patency tests (HSG) should ideally be conducted during 7 to 10 day of menstrual cycle (Level A/Class I).
- Women with high risk of tubal pathology should be offered a hystero-laparoscopy with dye to test tubal function and look for other pelvic abnormalities. (Level A/Class I).

Discussion

- Most tubal patency tests can detect other fertility declining pathology, including uterine pathology like polyps, myomas or adenomyosis, ovarian pathology like cysts or endometriomas and pelvic pathology like adhesions or endometrioses along with tubal patency.[11]

Hysterosalpingography (HSG) and Hysterosalpingo-contrast-sonography (sono-HSG)

- HSG is the most commonly used tubal patency test. It has the advantage of evaluating both uterine cavity and tubal patency directly, and has potential therapeutic effect (higher

chance of clinical pregnancy and live birth) when an oil-soluble contrast medium is used.[11]

- Women with low risk of tubal abnormalities can be offered HSG or sono-HSG when the appropriate expertise is available. The Dutch guideline states to offer HSG only to women with high risk of tubal pathology, and laparoscopy only directly to those who have a history of complicated abdominal surgeries, intra-abdominal infections, or endometriosis or when clinical signs of severe endometriosis or hydrosalpinx are visible during ultrasound examination.[12]
- A meta-analysis of 6 randomized controlled trials involving 2,562 patients has shown that women who had undergone HSG with oil contrast had a higher rate of pregnancy than women who used water-based contrast for HSG.[13]
- HSG is the standard first-line test to evaluate tubal patency, especially if reparative surgery is planned.[14]
- Sono-HSG is a non-invasive test, without the risk of radiation, allergy, and anaesthesia.[15] The three-dimensional HyCoSy (3D-sono-HSG) and four-dimensional sono HSG (4D-sono-HSG) have been, and are considered accurate for assessing tubal patency in infertile women.[16,17]
- NICE recommends that for women not known to have additional risk factors for tubal disease, HSG or hysterosalpingo-contrast-ultrasonography (sono-HSG) should be utilized to screen for tubal occlusion. In the absence of pelvic disease, both techniques offer fast, simple and well-tolerated outpatient procedures. HSG is less invasive and makes more efficient use of resources than laparoscopy.[18]
- NICE also recommends that where appropriate expertise is available, screening for tubal occlusion using sono-HSG should be considered because it is an effective alternative to HSG for women who are not known to have comorbidities.[18]
- The guideline of the American College of Obstetricians and Gynecologists (ACOG) recommends using an imaging modality for the detection of tubal patency and/or pelvic abnormalities. Imaging modalities for tubal patency mentioned are HSG and sono-HSG.[19]
- It is best to have HSG done in the first half of the menstrual cycle (days 1 to 14), which reduces the chance of the patient being pregnant.[20]
- Clinicians typically perform the HSG exam during the early follicular phase. Early menstrual cycle timing has certain advantages; the woman cannot be pregnant and the endometrium is thin facilitating visualization of the cavity.[21]

Laparoscopy

- Although laparoscopy has the advantage of directly visualizing the pelvis and all its organs, the need of general anaesthesia with hospitalization as well as the risk of major complications has to be considered.[11]
- Laparoscopy is offered to patients suffering from co-morbidities like PID, previous ectopic pregnancy, endometriosis, or other pathologies instead of HSG.[22]
- The The National Institute for Health and Care Excellence (NICE) guideline suggests offering women with a high risk of tubal pathology a laparoscopy with dye to test tubal function and look for other pelvic abnormalities.[18]

- The advantage of laparoscopy is that the procedure is very suitable for assessing tubal abnormalities such as adhesions due to infections, previous surgery, or endometriosis. In addition, the procedure can be combined with surgical interventions.[12]
- The DUTCH guideline working group is of the opinion that laparoscopy should not be a standard examination in Exploratory fertility research. Laparoscopy is not a better predictor than HSG for natural conception and a randomized trial showed no additional value of a diagnostic laparoscopy after a normal HSG with respect to treatment policy and pregnancy outcome.[12]

Management of tubal diseases

RECOMMENDATIONS

- A patient-centric approach should be followed for the management of tubal diseases and should be based on the decision of the treating fertility specialists. (Level C/Class I).
- Before deciding on a treatment approach, the clinician should consider various factors such as the woman's age, ovarian reserve, disease severity, the number and quality of sperm, safety, the risk of ectopic pregnancy, previous abdominopelvic surgery or disease, and surgical complications. (Level C/Class I).
- Tubal surgery is recommended for women with mild tubal disease; surgery should be performed with appropriate availability of expertise. (Level A/Class I).

Discussion

- Decisions regarding the management of tubal disease are complex and require a patient-specific approach. Active patient involvement and open communication throughout the decision-making process is critical and more likely to ensure satisfaction and avoid conflict should treatment prove unsuccessful.[23]
- Important fertility-related factors to consider include the woman's age and ovarian reserve, disease severity, the number and quality of sperm, and other infertility factors.[23]
- For up to 40% of couples, concomitant disorders are found. Considering patient safety, the risk of ectopic pregnancy, previous abdomino-pelvic surgery or disease, and surgical complications should be assessed. Operator experience and the success rates of the centres ART program are also relevant. Finally, patient factors, including potential treatment costs, religious and cultural beliefs and patient preference are key considerations which shall influence management decisions.[23]
- As per the NICE guideline, for women with mild tubal disease, tubal surgery may be more effective than no treatment. In centres where appropriate expertise is available, it may be considered as a treatment option.[18]

Surgery for tubal pathologies

RECOMMENDATIONS

- Categorizing the severity of tubal damage is suggested as it can help to accurately predict surgical outcomes for women, clinical pregnancy rates as well as live birth rates. (Level C/Class I).

Discussion

The Hull & Rutherford classification (2002) system can be useful to categorize the severity of tubal damage and help to accurately predict surgical outcomes for women with tubal damage, including CPR and live birth rates (LBR).[23,24,25]

The Hull & Rutherford classification (2002) system	
Grade I - Minor disease/favourable surgical prognosis (50%/2y)	• Tubal fibrosis absent even if occluded (proximally) • Tubal distension absent even if occluded (distally) • Mucosal appearances favourable (e.g. folds evident on salpingography) • Adhesions (peritubal-ovarian) flimsy
Grade II - Intermediate/ questionable surgical prognosis	• Unilateral severe tubal damage • With or without contralateral minor disease • 'Limited' dense adhesions of tubes and/or ovaries • (easy surgery), otherwise surgically favourable tubes
Grade III - Severe disease/unfavourable surgical prognosis (10%/2y)	• Bilateral severe tubal damage • Tubal fibrosis extensive • Tubal distension >1.5 cm • Mucosal appearance abnormal (folds absent or honeycomb on salpingography) • Bipolar occlusion • Extensive dense adhesions (i.e. difficult surgery)

Procedures for proximal tubal blockage

RECOMMENDATIONS

- Before deciding any procedure, it is necessary to first sub classify proximal blockage into 'obstruction' which is a time-limited process and may be reversible (tubal spasm or plugging), and 'occlusion' which is permanent (SIN). (Level C/Class I).
- Hysteroscopic tubal cannulation is recommended to treat obstruction for women with suspected proximal tubal blockage. (Level A/Class I).
- Tubal cannulation can distinguish between occlusive and functional obstructive tubal disease, based on which, conservative management or surgical management and/or ART can be recommended. (Level C/Class I).
- Tubal cannulation should be the treatment of choice, if it fails, IVF should be recommended, and not proximal tubal surgery. (Level A/Class I).
- IVF is recommended over resection and microsurgical anastomosis in cases of a true anatomic occlusion (SIN, chronic salpingitis, or obliterative fibrosis). (Level A/Class I).
- IVF is a preferred treatment for proximal tubal blockage in older women and in the presence of a significant male factor. (Level A/Class I).

Discussion

- Proximal blockage may be subclassified into 'obstruction' which is a time-limited process and may be reversible, such as tubal spasm or plugging, and 'occlusion' which is permanent like Salpingitis isthmica nodosa (SIN).[23]
- Fluoroscopic tubal cannulation, which can be performed at the same time as HSG, carries minimal radiation exposure risk. If the obstruction is not overcome with gentle pressure, a permanent 'occlusive' lesion is presumed, and the procedure terminated. Therefore, tubal cannulation permits distinction of occlusive and functional obstructive tubal disease amenable to conservative management from true occlusive disease requiring surgical management and/or ART.[23]
- In cases of SIN, chronic salpingitis, or obliterative fibrosis, IVF is preferred to resection and microsurgical anastomosis. IVF would also be the preferred treatment for proximal tubal blockage in older women and in the presence of a significant male factor. However, microsurgery may be considered after failed tubal cannulation if IVF is not an option for the patient, but it should be attempted only by those with appropriate training. Tubal implantation has been relegated to historic interest only, as it

is associated with very low success rates and risk of cornual rupture in pregnancy.[14]

Procedures for distal tubal blockage

RECOMMENDATIONS

- IVF is the preferred treatment for distal tubal blockage. Fertility-preserving procedure can be considered for mild distal tubal obstruction such as salpingo-ovariolysis (adhesiolysis) and can be performed particularly in young women with no other significant infertility factors. (Level C/Class I).
- In patients who are not candidates for corrective tubal surgery, laparoscopic salpingectomy or proximal tubal ligation is recommended to overcome the adverse effects of hydrosalpinges prior to undergoing ART to improve the chance of a live birth. (Level A/Class I).

Discussion

- IVF is preferred over salpingostomy for mild hydrosalpinges in older women and for those with male factor infertility or other infertility factors, salpingostomy before IVF may improve the subsequent likelihood of success of IVF while still giving the patient the option to attempt spontaneous conception.[14]
- Patients with a good prognosis have limited filmy adnexal adhesions, mildly dilated tubes (<3 cm) with thin and pliable walls, and a lush endosalpinx with the preservation of the mucosal folds.[14]
- Patients having a poor prognosis have extensive dense peritubal adhesions, largely dilated tubes with thick fibrotic walls, and/or sparse or absent luminal mucosa. Laparoscopic salpingectomy is indicated in patients with hydrosalpinges of poor prognosis as they have a detrimental effect on IVF success rates.[14]
- UK NICE guidance recommends that women with hydrosalpinges should be offered salpingectomy, preferably by laparoscopy, prior to ART to improve the chance of a live birth.[18]
- Careful preoperative and intraoperative assessments are important to identify those patients who are most likely to be benefit from distal tubal surgery. In addition, the overall clinical picture needs to be taken into consideration for example, in the presence of a significant male factor infertility or poor prognostic factors, salpingectomy or proximal tubal occlusion with recourse to IVF is preferred. However, for those who do not wish IVF or have good prognostic factors, consideration should be given to surgical repair.[26]

Surgery for sterilization reversal and microsurgical principles

RECOMMENDATIONS

- Following an individualized approach is recommended regarding tubal anastomosis vs. IVF in women with a prior tubal ligation who wish to conceive and are opting for reversal of sterilization. (Level A/Class I).
- It is necessary to consider the patient age, partner semen quality, surgical technique that was used to perform the sterilization, expense, chance of success, and reproductive preferences in the decision-making. (Level A/Class I).
- Microsurgical anastomosis is the recommended technique for tubal ligation reversal. (Level A/Class I).
- Microsurgical principles should be followed in reconstructive surgery of the fallopian tube, and also considered when gynecological surgery is performed in women of the reproductive age group. (Level B/Class I).

Discussion

- The reversal of tubal ligation is achieved by opening the occluded ends of the proximal and distal segments and anastomosing them with fine monofilament sutures using magnification and microsurgical techniques.[14]
- The main challenge in laparoscopic anastomosis procedures is the technical demands of laparoscopic suturing. Only surgeons who are very facile with laparoscopic suturing and who have experience and training in tubal microsurgery should attempt this procedure.[26]
- Microsurgery is a concept that involves the utilization of a set of technical principles and specially designed micro-instruments to minimize tissue injury and to achieve optimal anatomical reconstruction.[26]
- The use of microsurgical technique should lead to a reduced risk of iatrogenic adhesion formation. Microsurgical principles should be followed not only in reconstructive surgery of the fallopian tube, but whenever gynaecological surgery is performed in women of the reproductive age group.[26]

Is tubal surgery better than IVF and embryo transfer (IVF-ET)?

RECOMMENDATIONS

- For patients with mild tubal diseases, IVF should be considered only if they fail to conceive within 1 year following the tubal surgery.(Class B/Level IIa).

Discussion

- IVF has the advantage of being less surgically invasive and having good per-cycle outcomes. The main disadvantages of IVF include a higher cost and the need for frequent injections and monitoring for several weeks.[3]
- Tubal surgery is minimally invasive, one time, outpatient procedure. Following tubal surgery, patients can attempt conception every month and is associated with more than once chance of conception. To increase the success rate and reduce the risks, surgeons should be well-experienced in laparoscopic and microsurgery techniques. Young patients with no other significant infertility factor and tubal anatomy favorable for repair are considered as ideal candidates for tubal surgery.[3]
- IVF bypasses the problem while tubal surgery corrects the underlying cause of infertility.[4] Patients with mild tubal disease and with adequate ovarian reserve should be approached with tubal surgery initially and should be offered IVF only if they do not conceive within 1 year following the surgery.[8]

SUMMARY OF RECOMMENDATIONS

Indications for tubal surgery

- Tubal surgery should be indicated in those having proximal tubal occlusion, periadnexal and peritubal adhesions, and for reversal of tubal sterilization. (Level C/Class I).

Diagnosis of tubal diseases

History and physical examination

- The patient's history should be considered to evaluate the risk of tubal factor infertility. A history of ectopic pregnancy, PID, endometriosis, or prior pelvic surgery raises suspicion for tubal-factor infertility. (Level A/Class I).
- The clinician should carry out a PS/PV examination, and look for visible and/or palpable abnormalities of external and internal genitals, along with transvaginal ultrasound. (Level A/Class I).
- Women with a high risk of tubal pathology (previous PID, ectopic pregnancy and/or endometriosis) should be approached differently from women with low risk (without any co-morbidities). (Level A/Class I).

Tubal patency tests

- HSG or sonoHSG is the standard first-line test recommended to evaluate tubal patency. (Level A/Class I).
- HSG or sono-HSG is recommended to screen for tubal occlusion for women not known to have additional risk factors for tubal disease (such as PID, previous ectopic pregnancy or endometriosis)) or those with low risk of tubal pathology. (Level A/Class I).
- HSG with an oil-based contrast should be considered vs. water-based contrast media, as it has been proven to have a therapeutic role (higher rate of pregnancy) through flushing of tubal debris. (Level A/Class I).
- Tubal patency tests (HSG) should ideally be conducted during 7 to 10 day of menstrual cycle. (Level A/Class I).
- Women with high risk of tubal pathology should be offered a hystero-laparoscopy with dye to test tubal function and look for other pelvic abnormalities. (Level A/Class I).

Management of tubal diseases

- A patient-centric approach should be followed for the management of tubal diseases and should be based on the decision of the treating fertility specialists. (Level C/Class I).
- Before deciding on a treatment approach, the clinician should consider various factors such as the woman's age, ovarian reserve, disease severity, the number and quality of sperm, safety, the risk of ectopic pregnancy, previous abdominopelvic surgery or disease, and surgical complications. (Level C/Class I).
- Tubal surgery is recommended for women with mild tubal disease; surgery should be performed with appropriate availability of expertise. (Level A/Class I).

Surgery for tubal pathologies

- Categorizing the severity of tubal damage is suggested as it can help to accurately predict surgical outcomes for women, clinical pregnancy rates as well as live birth rates. (Level C/Class I).

Procedures for proximal tubal blockage

- Before deciding any procedure, it is necessary to first sub classify proximal blockage into 'obstruction' which is a time-limited process and may be reversible (tubal spasm or plugging), and 'occlusion' which is permanent (SIN). (Level C/Class I).
- Hysteroscopic tubal cannulation is recommended to treat obstruction for women with suspected proximal tubal blockage. (Level A/Class I).
- Tubal cannulation can distinguish between occlusive and functional obstructive tubal disease, based on which, conservative management or surgical management and/or ART can be recommended. (Level C/Class I).
- Tubal cannulation should be the treatment of choice, if it fails, IVF should be recommended, and not proximal tubal surgery. (Level A/Class I).
- IVF is recommended over resection and microsurgical anastomosis in cases of a true anatomic occlusion (SIN, chronic salpingitis, or obliterative fibrosis). (Level A/Class I).

- IVF is a preferred treatment for proximal tubal blockage in older women and in the presence of a significant male factor. (Level A/Class I).

Procedures for distal tubal blockage

- IVF is the preferred treatment for distal tubal blockage. Fertility-preserving procedure can be considered for mild distal tubal obstruction such as salpingo-ovariolysis (adhesiolysis) and can be performed particularly in young women with no other significant infertility factors. (Level C/Class I).
- In patients who are not candidates for corrective tubal surgery, laparoscopic salpingectomy or proximal tubal ligation is recommended to overcome the adverse effects of hydrosalpinges prior to undergoing ART to improve the chance of a live birth. (Level A/Class I).

Surgery for sterilization reversal and microsurgical principles

- Following an individualized approach is recommended regarding tubal anastomosis vs. IVF in women with a prior tubal ligation who wish to conceive and are opting for reversal of sterilization. (Level A/Class I).
- It is necessary to consider the patient age, partner semen quality, surgical technique that was used to perform the sterilization, expense, chance of success, and reproductive preferences in the decision-making. (Level A/Class I).
- Microsurgical anastomosis is the recommended technique for tubal ligation reversal. (Level A/Class I).
- Microsurgical principles should be followed in reconstructive surgery of the fallopian tube, and also considered when gynecological surgery is performed in women of the reproductive age group. (Level B/Class I).

Is tubal surgery better than IVF and embryo transfer (IVF-ET)?

- For patients with mild tubal diseases, IVF should be considered only if they fail to conceive within 1 year following the tubal surgery. (Class B/Level IIa).

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OPTIMIZING VITRIFICATION TECHNIQUE FOR CRYOPRESERVATION SUCCESS IN ASSISTED REPRODUCTION: GOOD CLINICAL PRACTICE RECOMMENDATION

Introduction

Cryopreservation is vital for preserving reproductive cells like oocytes, sperm, and embryos in assisted reproductive technology (ART). It involves freezing and storing these cells for future use in fertility treatments, offering a lifeline to individuals facing infertility challenges.[1] It benefits individuals at risk of infertility due to medical conditions, those who want to delay parenthood, transgender individuals, and participants in donor programs. It allows for the creation of backup samples, ensuring the availability of reproductive cells when needed.[2] Cryopreservation techniques can be broadly classified into two categories: slow freezing and vitrification.[3] Slow freezing involves controlled cooling rates that can lead to the formation of ice crystals and subsequent damage to the cells.[4] It has several disadvantages, including the formation of ice crystals and cellular damage, lengthy cooling time, and the requirement for expensive equipment to regulate the cooling rate. Studies have shown that slow freezing protocols have lower overall efficiency and cryopreserved oocytes using this method have exhibited higher levels of chromosomal anomalies compared to fresh oocytes.[3,4]

Vitrification, an ultra-rapid method, is now the preferred technique over slow freezing for cryopreserving reproductive cells. It is a technique where the cell (human gametes or embryo) is transitioned from 37 to -196°C in <1 second resulting in extremely fast rates of cooling. Unlike slow freezing, which can cause ice crystal formation and cellular damage due to controlled cooling rates, vitrification utilizes high concentrations of cryoprotective agents (CPAs) and rapid cooling rates to prevent ice crystal formation and minimize cellular damage.[5,6] This innovative approach not only reduces the process time but also mitigates the risks associated with prolonged exposure to CPAs. The application of vitrification in cryopreservation offers significant advantages, including higher survival rates and better post-thaw outcomes for reproductive cells such as oocytes, sperm, and embryos.[3,7,8] By circumventing ice crystal formation, vitrification preserves the structural integrity of reproductive cells, ensuring their viability and functionality upon thawing. These improvements in survival rates and post-thaw outcomes highlight the growing preference for vitrification as the method of choice in cryopreservation. Its ability to minimize cellular damage, along with the shorter process time, makes vitrification a superior and more effective approach in maintaining the integrity and functionality of reproductive cells.

In light of the growing significance of cryopreservation and the superiority of vitrification over slow freezing in preserving reproductive cells, it is crucial to establish

Level of evidence	Description
Level A	Data derived from multiple randomized trials or meta-analyses or evidence-based clinical practice guidelines
Level B	Data derived from a single randomized trials or large non-randomized trial
Level C	Consensus of opinion of experts or small studies, retrospective studies or registries or narrative/literature reviews
Level D	Data derived from clinical experience
Class of recommendations	
Class I	Evidence and or general agreement that a given treatment or procedure is beneficial, useful or effective. It is recommended
Class IIa	Evidence is in favor of efficacy/usefulness and should be considered
Class IIb	Efficacy/usefulness is less well established and recommendation may be considered
Class III	Evidence and or general agreement that a given treatment or procedure is not beneficial, useful or effective and in some cases may cause harm. Not recommended

comprehensive guidelines encompassing the best practices for vitrification techniques. The forthcoming sections of this guideline will delve into various essential aspects of vitrification best practices, covering selection of CPA, the optimal cooling and warming rates, the appropriate storage conditions, and contraindications to vitrification process. Furthermore, considerations for different types of reproductive cells, such as oocytes, sperm, and embryos, will be addressed, including specific recommendations for each cell type.

Scope

The guideline offers fertility specialists precise recommendations regarding the optimal practices to be employed during the vitrification process in ART cycles, drawing upon the most robust and current evidence.

Methodology

The sections and recommendations addressed in the guideline were based on feedback from task force members and previous trials and guidelines. The task force members followed a well-defined grading system (mentioned above) for the critical appraisal of evidence and grading strength of recommendations.

Vitrification is preferred over slow-freezing in cryopreservation

RECOMMENDATIONS

- Vitrification of human gametes and embryos are recommended as it has very high post-thaw survival rates, and improved clinical outcomes. (Level A, Class I)

Discussion

Leading reproductive medicine journal endorses vitrification technique. The European Society of Human Reproduction and Embryology (ESHRE) guideline has suggested that embryo vitrification is safe and using long-stored embryos after vitrification has no negative effect on neonatal health.[7]

This supports the widespread use of embryo vitrification in ARTs, including in vitro fertilization (IVF), and the storage of embryos for future use.

Comparative clinical trials have shown that vitrification outperforms slow freezing in terms of survival rates (96.9% vs. 82.8%), post-warming embryo morphology (91.8% vs. 56.2%), clinical pregnancy rates (40.5% vs. 21.4%), and implantation rates (16.6% vs. 6.8%).[9]

Vitrification is an efficient and superior method for cryopreserving human gametes and embryos.

It offers a higher survival rate, minimal damage to post-warming embryo morphology, and the potential to improve clinical outcomes.[10]

Indications to perform vitrification

RECOMMENDATIONS

- Vitrification is recommended in various situations where there is a need for cryopreservation of oocytes or embryos, like the storage of surplus embryos of good quality, postponing embryo transfer in special cases like poor endometrium, or severe OHSS, and the preservation of reproductive potential of a woman. It serves as a reliable and effective method for preserving fertility and supporting reproductive treatments. (Level A, Class I).

Discussion

Vitrification is an essential technique in ART for cryopreservation, offering significant advantages in various conditions. Extensive clinical and experimental evidence consistently demonstrates that vitrification provides higher cell survival rates, improved preimplantation development, and superior pregnancy outcomes compared to other methods. Its widespread utilization in cryopreserving oocytes and embryos further underscores its importance in ART.[1]

Fertility preservation is a critical consideration for patients undergoing treatments that may impact their reproductive function and prepubertal individuals at risk of infertility. Vitrification is employed in preserving embryos, oocytes, and ejaculated or testicular sperm. Ovarian tissue cryopreservation is increasingly recognized as a viable option, particularly for prepubertal patients or when there is limited time for ovarian stimulation. However, the cryopreservation of testicular tissue in prepubertal males remains experimental and should be pursued under research protocols when alternative options are not feasible. Patients undergoing fertility-impacting treatments, such as chemotherapy or radiation therapy, may choose oocyte or embryo vitrification to preserve their reproductive potential.[11]

Vitrification is also utilized in cryopreserving donated oocytes for later use in fertility treatments involving donor gametes.[12] Additionally, in cases where a surrogate will carry the pregnancy, vitrification is employed to freeze embryos created through IVF for subsequent transfer into the surrogate's uterus. The advent of excellent vitrification techniques has made surrogacy cycles less challenging for ART clinics with well-equipped embryology laboratories and freezing facilities.[13]

Furthermore, vitrification is commonly used before preimplantation genetic testing (PGT), which analyzes DNA from oocytes or embryos for human leukocyte antigen (HLA)-typing or genetic abnormalities. PGT includes tests for aneuploidies (PGT-A), monogenic/single gene defects (PGT-M), and chromosomal structural rearrangements (PGT-SR). Combining PGT with vitrification enables cryopreservation of embryos for genetic testing, facilitating analysis and selection of healthy embryos. This approach has shown lower miscarriage rates and better cumulative live birth outcomes compared to using unscreened frozen embryos.[14, 15]

Optimizing success rate in vitrification technique: Best practices to follow

The success of vitrification depends on several factors, including the choice of CPAs, the cooling and warming rates, the timing of vitrification, and type of cryopreservation device used.[3,16]

Choice of CPAs

RECOMMENDATIONS

- Choice of appropriate CPA is necessary to protect the cells from ice crystal formation and prevent cryoinjuries. (Level A, Class I).

Discussion

CPAs protect the cells from ice crystal formation and prevent cryo injuries. The selection of CPAs is based on several factors, including their ability to permeate the cell membrane, osmotic properties, toxicity, and their ability to prevent ice crystal formation during freezing and thawing.

CPAs are categorized as either permeating [P-CPAs: glycerol (Gy), ethylene glycol (EG), dimethyl sulfoxide (DMSO), propylene glycol (PG) or 1,2 propanediol, formamide (Fm)] or nonpermeating (NP-CPAs: trehalose, sucrose, glucose, mannitol, galactose), depending on their ability to traverse the cell membrane and enter the cytoplasm. P-CPAs penetrate the cell membrane, stabilize intracellular proteins, reduce the intracellular ice formation temperature, and reduce the impact of intra- and extracellular electrolytes. NP-CPAs create an osmotic gradient and induce dehydration but do not penetrate the cell membrane.[16,17] Both DMSO based and non–DMSO based CPAs are the best viable options for the vitrification of human oocytes and embryos.[3] However, high concentrations of CPAs used in the vitrification procedure can be toxic to cells. The main target of any vitrification protocol must be the suppression of toxicity without any loss of effectiveness by the CPAs. To reduce toxicity while maintaining efficiency, a common practice is to partially load cells with a lower-strength CPA solution before transferring them to a full-strength CPA mixture.[17]

The combination of ethylene glycol and DMSO or ethylene glycol and 1,2-propanediol are effective in oocyte vitrification.[18]

Various techniques to reduce CPA toxicity

RECOMMENDATIONS

- Use of additives such as disaccharides and macromolecules can reduce the toxicity of CPA. (Level A, Class I).

Use of additives can reduce CPAs toxicity

Disaccharides: Large molecular weight additives such as sucrose or trehalose do not penetrate the cell membrane but can significantly reduce the toxicity of CPA by decreasing the concentration required to achieve successful cryopreservation. These additives act as an osmotic buffer, reducing osmotic shock and exposure time to the toxic effects of CPAs.[17]

Macromolecules: High concentrations of CPAs are required for extracellular vitrification. The use of high molecular weight polymers such as polyvinylpyrrolidone (PVP), and polyethylene glycol (PEG) can successfully vitrify extracellularly with the same cryoprotective concentration used intracellularly. Additive polymers can protect embryos against cryoinjury by mitigating the mechanical stresses, influencing the viscosity, reducing CPA toxicity, and creating a viscous matrix for encapsulation of oocytes or embryos. These additives can reduce the CPA concentration by 7% on average and by as much as 24% in combination with increased hydrostatic pressure.[17]

Combination of CPAs can reduce toxicity

Current recommendations emphasize the mixing of different CPAs as a preventive measure against potential toxicity. This approach is rooted in the capacity of CPA combinations to lower the individual components' concentrations below their toxic thresholds while minimizing the exposure time of oocytes/embryos to the solution.[19] A study found that multiple-CPA solutions were significantly less toxic than single-CPA solutions ($p<0.01$). The adverse effects resulting from interactions between CPAs were quantifiable through regression analysis. By employing a combination solution comprising four CPAs (DMSO-EG-Gy-Fm), cell survival rates of approximately 40% were achieved.[19]

Common freezing solutions typically consist of permeating agents (such as EG, Gy, DMSO, PG acetamide) at concentrations above 4 M,

and non-permeating agents (such as sucrose, trehalose) at concentrations above 0.5 M. The widely used protocol for oocytes and embryos involves a combination of 15% DMSO, 15% EG, and 0.5 M sucrose, with a minimal volume of ≤1 µL. Recent research indicates that in this popular CPA combination, while DMSO reduces solute concentration, a higher proportion of sucrose directly increases the glass transition value, allowing for safer storage temperatures. Thus, a deeper understanding of the thermodynamics of each CPA is necessary to identify the optimal combination for effective vitrification.[19]

The concentration of CPA

The CPAs concentration can significantly affect the vitrification process; therefore, their optimal concentration is crucial to achieving successful outcomes. The concentration of CPAs in the vitrification solution can affect the cell's viability during the vitrification process. Higher concentrations of CPAs can be toxic to oocytes, causing damage and reducing their survival rate. On the other hand, lower concentrations CPAs may not provide sufficient protection to the oocyte during the vitrification process, leading to ice crystal formation and damage.[17]

Cooling and warming rates

RECOMMENDATIONS

- Rapid cooling rates and high warming rates improves the survival and viability of vitrified cells. (Level B, Class I).

Embryo vitrification and its outcomes have reached standardization, but for oocytes there is still a need for optimization. The cooling and warming rates used in oocyte vitrification should be optimized to minimize the risk of oocyte damage. The optimal rates vary depending on the vitrification technique, concentration of CPA, and stage of oocyte development. The cell undergoes a temperature transition from room temperature to -196°C in less than two seconds, resulting in extremely fast rates of cooling exceeding 10,000°C per minute. The warming rate is equally, if not more, crucial than the cooling rate; researchers found that a slow warming rate leads to cell death due to the formation and enlargement of small ice crystals inside the cell through recrystallization.[3,21,22] Therefore, rapid cooling rates (at least > 20,000 °C/min) and high warming rates (ΔT from −196 to 37°C = 233°C/3 sec = 4460°C/min) improves the survival and viability of vitrified cells.[16,23]

Choice of cryoprotectant devices

RECOMMENDATIONS

- Open system vitrification is preferred over closed system. (Level A, Class I).
- It is preferable to maintain a separate cryo-tank for seropositive cases, despite the absence of cross-contamination observed in open systems. (Level C, Class IIa).

Cryoprotectant devices are designed to deliver CPAs to biological materials in a controlled manner during the cryopreservation process. Open systems that assure extremely rapid direct contact with liquid nitrogen have been successfully used currently in vitrification technique. However, there are concerns about the possibility of viral contamination in open systems, including liquid nitrogen and vitrification carriers. While no published studies have reported actual cross-contamination of cryopreserved embryos in open systems, alternative approaches can mitigate the risk of contamination.[3,16,24]

These approaches include using sterilized liquid nitrogen via ultraviolet light, storing in the vapor phase of liquid nitrogen to reduce environmental airborne contaminants, and using sterile air.[3,16]

Closed systems are less preferred because of the potential decrease in cooling rates, which may be produced in closed systems due to thermo-isolation and potential increase in ice crystal formation during the cooling process and of recrystallization on warming.[3,16]

A systematic review and meta-analysis including seven studies analyzed the pregnancy outcomes such as clinical pregnancy, or live birth rates after closed or open vitrification of blastocysts. Researchers reported lower pregnancy outcomes with closed vitrification than with open vitrification.[25]

Timing of vitrification

RECOMMENDATIONS

- Vitrification of cleavage-stage embryos should be performed on day 3. (Level A, Class I).
- Blastocysts vitrification should be performed at day 5-7. (Level A, Class I).
- Artificial shrinkage of the large blastocoel (day-5–7 blastocyst) can reduce cryoinjury during both cooling and warming phases. (Level B, Class IIa).
- Post-biopsy blastocyst vitrification should be performed immediately after trophectoderm biopsy and before initiation of re-expansion. (Level B, Class I).
- Oocytes should generally be vitrified before 38 hours of hCG administration. Post warming the oocytes should be cultured for around 2 hours to improve ICSI outcomes. (Level B, Class I)

To achieve optimal cryopreservation, it is vital to carefully consider the timing of vitrifying oocytes and embryos. For oocytes, it is recommended to perform vitrification when they have reached the fully matured state, indicated by the presence of a polar body. This mature metaphase II (M II) stage presents the highest developmental potential, making prompt vitrification essential for preserving oocyte quality effectively.[22,26,27]

In the case of cleavage-stage embryos, it is recommended to perform vitrification on day 3, between the 6-cell and 8-cell stage. This approach has demonstrated superior clinical outcomes compared to vitrification on day 2.[28,29]

Regarding blastocysts, they are typically vitrified when they have reached the expanded or hatching stage, around 5 to 6 days after fertilization. At this stage, blastocysts possess a blastocoel, a fluid-filled cavity, making them suitable for cryopreservation.[3]

To enhance the success rates of vitrification, it is advised to artificially shrink the large blastocoel before rapid-cooling. This technique has shown improved survival rates, resulting in a higher percentage of high-quality and hatching blastocysts.[3,30]

It is worth noting that the timing between trophectoderm biopsy and vitrification impacts the developmental competence of biopsied blastocysts. To maintain their competence, immediate vitrification following the biopsy and before re-expansion initiation is recommended. Vitrifying during the re-expansion process can compromise outgrowth competence.[31,32]

The timing of ICSI is considered to be one of the important factors to determine embryo viability. As per a study, the optimal timing for injection is 37–41 hours after hCG administration. It has been reported that sperm injected immediately after oocyte aspiration (approximately 36 hours after hCG administration) results in a lower implantation rate than after preincubation for at least 2 hours.[33]

Experienced personnel

RECOMMENDATIONS

- Well-trained team is mandatory to succeed and to obtain consistent results in vitrification. (Level C, Class I).

Successful vitrification requires personnel with high skill and experience. Proper training and certification are essential to ensure quality and safety. The Indian Society For Assisted Reproduction (ISAR) Consensus Guidelines on Safety and Ethical Practices in IVF clinics highlight the importance of experienced and well-trained personnel for effective vitrification procedures.[3,34]

Quality control

RECOMMENDATIONS

- Strict quality-control program must be followed in vitrification. (Level C, Class I).

Consistency and reliability of the vitrification process can be ensured by implementing quality control measures, which include regular monitoring of the storage environment's temperature and humidity, controlling learning curves, analysis of the operator's outcomes, as well as routine equipment and CPA checks.[3]

The Alpha consensus meeting aimed to define key performance indicators and benchmarks for cryopreservation techniques, including vitrification, in ART. By adhering to the benchmarks established by the Alpha consensus, ART clinics and laboratories can assess and improve the quality and reliability of vitrification techniques (Table 1).[35]

Table 1. Benchmarks for vitrification techniques in ART[35]

Key performance indicator (KPI)	Competence	Benchmark
Oocyte key performance indicator values		
Morphological survival	70%	85% (95% for donors <30 years)
Embryo development rate	The same as for the comparable population of fresh embryos at the centre	----
Embryo key performance indicator values		
Morphological survival: fully intact	70%	85%
Morphological survival: 50% intact	85%	95%
Blastocyst key performance indicator values		
Survival rate	80%	95%
Transfer rate	80%	95%

Executive summary[3]

- Establish a well-organized and structured program for training and proficiency in vitrification.
- Ensure accurate proficiency and operator evaluations.
- Follow a strict quality-control program that monitors learning curves, evaluates operator outcomes, and verifies vendor solutions used.
- Quality control measures should be implemented to ensure consistency and reliability of the vitrification process.
- Develop a cryopreservation plan in advance, including decisions on which oocytes to cryopreserve and how to distribute them, to manage oocyte quality and quantity and provide patients with more fertility treatment options.
- Use a validated technique with specific cryopreservation and warming solution formulations and devices for vitrification, with the composition of the solutions associated with optimal outcomes.
- Careful handling of oocytes and precise timing of the vitrification and warming procedures is essential for optimal outcomes in cryopreservation and subsequent fertility treatments.
- Assess the technical proficiency of embryologists through competency assessment and quality management system audits.
- Maintain a clear database that tracks and analyzes outcome parameters from the cryopreservation program. Following parameters should be included for oocyte vitrification:
  - » Number of oocytes retrieved
  - » Number of oocytes cryopreserved.
  - » Number of oocytes warmed
  - » Number of oocytes survived and inseminated by intracytoplasmic sperm injection.
  - » Number of oocytes fertilized
  - » Number of embryos acquiring a developmental and quality stage consistent with transfer or cryopreservation
  - » Number of embryos transferred
  - » Number of embryos cryopreserved
  - » Implantation rate
  - » Clinical pregnancy rate
  - » Live birth rate
  - » Number of embryos or blastocysts warmed and transferred for vitrified-warmed embryo transfer (FET) cycles
  - » FET cycle outcome data.
  - » Clinically important information on the pregnancy/delivery/neonates

- Artificial shrinkage of the large blastocoel (day-5–7 blastocyst) can reduce cryoinjury during both cooling and warming phases.
- Manual puncture of the trophectoderm with needle or laser before vitrification improves survival rates of rapid-cooled blastocysts and results in a higher percentage of high-quality and hatching blastocysts.

Upcoming challenges of vitrification

RECOMMENDATIONS

- The clinicians are advised to consider the effect of storage duration prior to deciding the number of embryos to freeze and store, particularly in cancer patients or those who wants to delay fertility treatment till recovery from the current condition. (Level C, Class IIa).

An upcoming challenge in vitrification is determining the optimal duration for storing vitrified embryos. A recent study showed that long storage of vitrified embryos negatively affected pregnancy outcomes, including clinical pregnancy and live birth rates. However, existing studies are limited in their scope, with retrospective analyses lacking detailed information on the effects of storing embryos for longer than 24 months. As a result, the debate on the ideal duration for storing vitrified cells persists, emphasizing the need for further research to address this issue.[7]

Future insights

In the future, vitrification cryopreservation in assisted reproduction holds promising developments. Rapid-cooling techniques are being explored for ovarian tissue and sperm, particularly benefiting patients with oligospermia or nonobstructive/obstructive azoospermia who require minimal amounts of testicular sperm to be cryopreserved.[3] Prospective studies with long-term follow-up are deemed necessary to ensure the safety of vitrification over extended periods.[7] Crucially, long-term follow-up of infants born from vitrified gametes or embryos will provide essential insights into the safety of the vitrification process.[3] Additionally, advancements in Enhanced Real-time Monitoring and Imaging will aid embryologists in assessing oocyte and embryo quality during vitrification, while integration with Time-lapse Imaging and Automated Vitrification Devices with the incorporation of AI based assessment will enhance precision, minimize human errors, and optimize cooling and warming rates based on real-time data. These future perspectives hold great potential for further improving the success and safety of vitrification in assisted reproduction procedures.[36,37]

Summary

- Vitrification is strongly recommended as the standard of care for the cryopreservation of human oocytes and embryos.
- The optimal timing for vitrification should be based on the stage of embryo development or oocyte maturity. Clinics should aim to vitrify oocytes or embryos at the optimal stage of development to maximize their viability and minimize the risk of damage during the vitrification process.
- Appropriate CPA solutions should be used during the vitrification process to prevent damage to the gametes or embryos. These solutions should be selected based on their ability to protect the cells from ice crystal formation and maintain their viability during the freezing process.
- The selection of optimized protocols, along with operator training, will result in better efficiency, consistency, reliability, and safety.
- High-quality control measures should be implemented to obtain consistent high-quality results with vitrification of oocytes and embryos.

In conclusion, vitrification techniques demonstrate impressive embryo survival rates nearing 100%, and are associated with pregnancy rates that are comparable to, if not surpassing, those of fresh transfer procedures.

SUMMARY OF RECOMMENDATIONS

Vitrification is preferred over slow-freezing in cryopreservation

- Vitrification of human gametes and embryos are recommended as it has very high post-thaw survival rates, and improved clinical outcomes. (Level A, Class I).

Indications to perform vitrification

- Vitrification is recommended in various situations where there is a need for cryopreservation of oocytes or embryos,like the storage of surplus embryos of good quality, postponing embryo transfer in special cases like poor endometrium, or severe OHSS, and the preservation of reproductive potential of a woman. It serves as a reliable and effective method for preserving fertility and supporting reproductive treatments. (Level A, Class I).

Optimizing success rate in vitrification technique: Best practices to follow

Choice of CPAs

- Choice of appropriate CPA is necessary to protect the cells from ice crystal formation and prevent cryoinjuries. (Level A, Class I).

Various techniques to reduce CPA toxicity

- Use of additives such as disaccharides and macromolecules can reduce the toxicity of CPA. (Level A, Class I).

Cooling and warming rates

- Rapid cooling rates and high warming rates improves the survival and viability of vitrified cells. (Level B, Class I).

Choice of cryoprotectant devices

- Open system vitrification is preferred over closed system. (Level A, Class I).
- It is preferable to maintain a separate cryo-tank for seropositive cases, despite the absence of cross-contamination observed in open systems. (Level C, Class IIa).

Timing of vitrification

- Vitrification of cleavage-stage embryos should be performed on day 3. (Level A, Class I).
- Blastocysts vitrification should be performed at day 5-7. (Level A, Class I).
- Artificial shrinkage of the large blastocoel (day-5–7 blastocyst) can reduce cryoinjury during both cooling and warming phases. (Level B, Class IIa).
- Post-biopsy blastocyst vitrification should be performed immediately after trophectoderm biopsy and before initiation of re-expansion. (Level B, Class I).
- Oocytes should generally be vitrified before 38 hours of hCG administration. Post warming the oocytes should be cultured for around 2 hours to improve outcomes. (Level B, Class I).

Experienced personnel

- Well-trained team is mandatory to succeed and to obtain consistent results in vitrification. (Level C, Class I).

Quality control

- Strict quality-control program must be followed in vitrification. (Level C, Class I).

Upcoming challenges of vitrification

- The clinicians are advised to consider the effect of storage duration prior to deciding the number of embryos to freeze and store, particularly in cancer patients or those who wants to delay fertility treatment till recovery from the current condition. (Level C, Class IIa).

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GOOD CLINICAL PRACTICE RECOMMENDATION FOR ASSISTED REPRODUCTIVE TECHNOLOGY IN MANAGING INFERTILITY

Purpose

The primary purpose of these guidelines is to enhance the quality of care and outcomes for individuals undergoing assisted reproductive technology procedures. The guidelines aim to optimize the safety, efficacy, and ethical considerations involved in assisted reproductive technology (ART) by providing evidence-based recommendations. The guidelines also aim to promote standardization and consistency in clinical practices across various healthcare settings.

Scope

These guidelines target a diverse audience, including reproductive specialists, obstetricians, gynecologists, fertility experts, embryologists, nurses, healthcare managers, policymakers, and researchers in the field. By providing evidence-based recommendations, the guidelines aim to improve the quality and safety of ART procedures, enhance patient outcomes, promote ethical practices, and assist healthcare professionals in decision-making. The guidelines also serve as a resource for healthcare managers and policymakers to establish and regulate ART services in line with best practices, ultimately improving the overall delivery of reproductive healthcare services.

Methodology

The sections and recommendations addressed in the guideline were based on feedback from task force members and previous trials and guidelines. The task force members followed a well-defined grading system (mentioned below) for the critical appraisal of evidence and grading strength of recommendations.

Level of evidence	Description
Level A	Data derived from multiple randomized trials or meta-analyses or evidence-based clinical practice guidelines
Level B	Data derived from a single randomized trials or large non-randomized trial
Level C	Consensus of opinion of experts or small studies, retrospective studies or registries or narrative/literature reviews
Level D	Data derived from Clinical experience
Class of recommendations	
Class I	Evidence and or general agreement that a given treatment or procedure is beneficial, useful or effective. It is recommended
Class IIa	Evidence is in favor of efficacy/usefulness and should be considered
Class IIb	Efficacy/usefulness is less well established and recommendation may be considered
Class III	Evidence and or general agreement that a given treatment or procedure is not beneficial, useful or effective and in some cases may cause harm. Not recommended

Introduction

Infertility

Infertility is the inability of a couple of reproductive age to conceive after at least 12 months of regular intercourse without the use of contraception (Table 1).[1] According to the World Health Organization (WHO), infertility is the failure to achieve pregnancy after 12 months of regular unprotected sexual intercourse. In 2022, global infertility prevalence was estimated at 17.5% lifetime and 12.6% period prevalence. Approximately one in six people worldwide experienced infertility at some stage in their lives.[2] Evaluation for infertility can be initiated earlier in individuals with risk factors or if the female partner is over 35 years old. Infertility may be attributed to male factors, ovulatory dysfunction, uterine abnormalities, tubal obstruction, peritoneal factors, or cervical factors. A history, physical examination, semen analysis, and progesterone level measurement can help guide the evaluation. Further diagnostic procedures like hysterosalpingography, hysteroscopy, or laparoscopy may be recommended based on individual circumstances. Treatment options range from clomiphene for ovulation induction to assisted reproductive technologies (ART) like intrauterine insemination (IUI) or in vitro fertilization (IVF). Referral to specialized care may be necessary for the treatment of tubal obstruction. Unexplained infertility in women or men can be addressed by trying to conceive naturally for another year or by considering ART like IUI or IVF.[3]

Table 1. Definition of infertility[4]

Age cut offs	Definition
A woman <35 years	Who has not conceived after 12 months of contraceptive-free intercourse. (Twelve months is the lower reference limit for Time to Pregnancy [TTP] by the World Health Organization)
A woman over 35 years	Who has not conceived after six months of contraceptive-free sexual intercourse.

Investigation of infertility

RECOMMENDATIONS

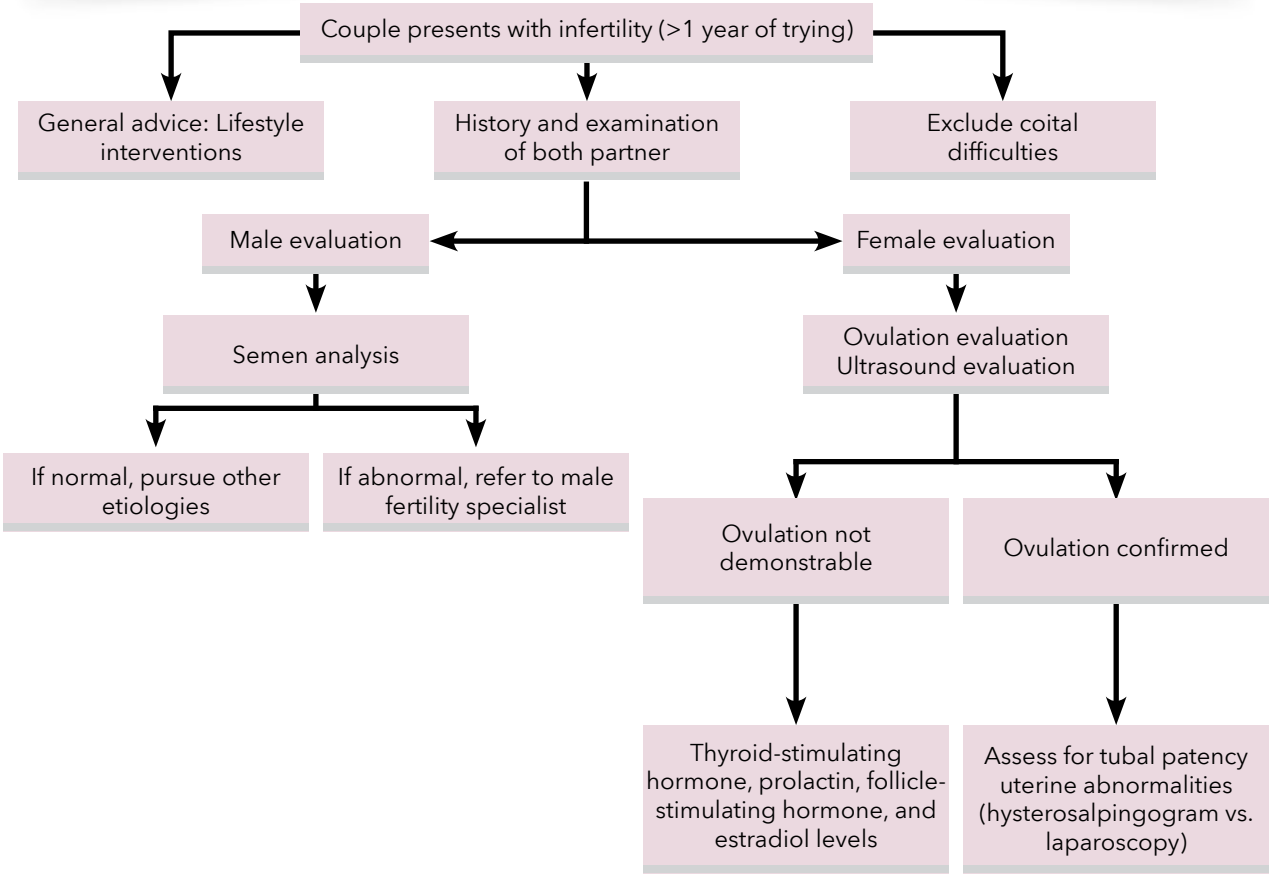
- Clinical investigations are required for those couples who fail to conceive after six months of regular, unprotected sexual intercourse. (Level A, Class I).
- Hormonal investigations for ovulation disorders and semen analysis are the initial fertility investigations required followed by a test for tubal patency. (Level A, Class I).
- Women experiencing infertility and who do not have a history of pelvic infections, endometriosis, or ectopic pregnancy should be provided with the option of undergoing a tubal patency test e.g., hysterosalpingography to screen for abnormalities in the uterus and fallopian tubes. (Level C, Class IIa).
- Results of semen analysis and ovulation assessment should be obtained before testing for tubal patency. (Level A, Class I).
- Immunological testing should not be routinely conducted as part of the infertility evaluation. (Level A, Class I).

Discussion

Couples concerned about fertility should know that about 84% of couples in the general population conceive within one year if they have regular unprotected intercourse. Female fertility declines with age, and the negative effects of alcohol, smoking, and body weight on fertility should be communicated. Preconception care should assess treatment and pregnancy risks on an individual basis.

If pregnancy does not occur after one year of regular unprotected intercourse, further clinical investigation is recommended. This includes semen analysis and ovulation assessment. A hormonal investigation is advised for ovulation disorders. Semen analysis should follow WHO and ESHRE recommendations. Additional tests,

Figure 1. Algorithm for infertility evaluation[1,3]



including clinical andrological investigation, should be done if abnormalities are found.

Results of semen analysis and ovulation assessment should be obtained before testing for tubal patency. Laparoscopy is recommended for women with suspected co-morbidities to investigate and treat tubal and pelvic pathology. Vaginal ultrasound can assess the ovaries, and hysteroscopy may be necessary in some cases. After investigations, each couple should receive information on their chances of spontaneous pregnancy and various treatment options.[5] Figure 1 provides an algorithmic approach to the evaluation of infertility.

Information and counseling

RECOMMENDATIONS

- Patients should be given counseling regarding alternative options and the effect of stress on sexuality and relationship. (Level A, Class I).

Discussion

An integral part of the decision-making process of ART is allowing patients to make informed decisions based on evidence-based information. All information, including information about other options and adoption, should be conveyed

verbally, in writing, or by the audio-visual route. Counseling performed by physicians, nurses, and/or professional counselors at each center should address all medical, psychological, and social questions related to involuntary childlessness. Patients should be aware of the negative impact of stress on relationships and sexuality and counseling should be provided before, during, and after investigation and treatment, irrespective of the outcome of the procedure.[5]

Evidence-based treatments for infertility

Treatment for infertility

Infertility treatments commonly include ovulation induction and ovarian stimulation (OS), which aim to induce ovulation and produce multiple mature follicles. Fertilization can be achieved through timed intercourse or IUI during ovulation. Alternatively, IVF involves retrieving mature oocytes directly from the ovary using an ultrasound-guided needle.[6]

Ovulation induction[5, 7-8]

RECOMMENDATIONS

- Clomiphene citrate, a combination of clomiphene and insulin sensitizers, letrozole, gonadotrophin therapy, and dopamine agonists are the different treatment options available for anovulatory women, depending on etiology. (Level A, Class I).
- Letrozole is recommended as a first line ovulation induction agent in women with PCOS since it promotes mono-follicular development and in view of its non-antiestrogenic properties when compared to clomiphene. (Level A, Class I).

Discussion

Anovulatory women should be offered ovulation induction after considering male or pelvic issues, weight or eating disorders, stress, or overexercise. If there are no concerns regarding the patient's sperm analysis or pelvic or tubal health, the patient can go through three cycles of ovulation induction before evaluating tubal patency.

Treatments offered for ovulation disorder depend on the etiology:

- Counselling concerning eating habits or stress is offered for women with body mass index (BMI) disorders or for those who have polycystic ovary syndrome (PCOS).[5]
- Letrozole should be considered first line pharmacological treatment for ovulation induction in women with PCOS with anovulatory infertility and no other infertility factors to improve ovulation, pregnancy and live birth rates.[7]
- Recent studies suggest that Letrozole, an aromatase inhibitor, has fewer antiestrogenic effects than clomiphene citrate (CC) and leads to higher pregnancy rates in PCOS patients.[8]
- Health professionals and women need to be aware that the risk of multiple pregnancy appears to be less with letrozole, compared to clomiphene citrate.[7]

Intrauterine insemination[9]

RECOMMENDATIONS

- It is not recommended to perform IUI during natural cycles for the treatment of unexplained infertility. It is not as effective as ovarian stimulation (OS) with IUI, and provides no additional benefit compared to expectant management. (Level A, Class I).
- Using oral ovulogens (clomiphene citrate or letrozole) in combination with IUI is recommended for treating couples with unexplained infertility. (Level A, Class I).
- The use of letrozole or clomiphene citrate in combination with gonadotropins for IUI may be considered due to their potential to improve pregnancy rates. However, it is essential to offer patients thorough counseling about the higher risk of multiple-gestation pregnancies associated with these treatments. (Level A, Class I).
- Couples with unexplained infertility are recommended to begin a course of OS and IUI using oral agents, typically for 3 or 4 cycles. (Level A, Class I).

Discussion

IUI is often the initial treatment for couples with unexplained or mild male-factor infertility due to its less invasive and less costly nature compared to IVF. IUI can be performed in a natural ovulatory cycle or combined with OS to induce multiple follicular developments and improve pregnancy chances. Research indicates that IUI in unstimulated cycles is less effective than OS with IUI and is not significantly more effective than expectant management.

OS with IUI is commonly used for couples with unexplained infertility as an alternative or precursor to IVF. It aims to increase the number of eggs released in a single cycle and position more sperm closer to the site of fertilization, thereby enhancing the chances of conception. Evidence shows that clomiphene citrate with IUI is superior to expectant management and natural-cycle IUI in terms of live-birth rates for couples with unexplained infertility. The multiple gestation pregnancy rates with clomiphene citrate and IUI can range from 0% to 12.5%. Research indicates that letrozole with IUI is as effective as clomiphene citrate with IUI, with similar pregnancy rates and multiple-gestation pregnancy rates.

The use of conventional-dose gonadotropins with IUI treatments has been associated with an increased risk of multiple-gestation pregnancy. Comparatively, there is no significant difference in clinical pregnancy and live birth rates between letrozole and low-dose gonadotropins with IUI versus clomiphene citrate and low-dose gonadotropins with IUI. The evidence is insufficient to determine whether low-dose gonadotropins with IUI offer higher pregnancy rates compared to clomiphene citrate or letrozole with IUI. Similarly, studies have mixed findings regarding the pregnancy outcomes of conventional-dose gonadotropins with IUI, with some showing no difference and others indicating higher pregnancy rates accompanied by a higher rate of multiple-gestation pregnancy.

Regarding the timing of IUI relative to human chorionic gonadotropin (hCG) injection in OS with IUI treatments, research suggests that the timing between 0 and 36 hours does not significantly impact pregnancy rates. Additionally, there is no significant difference in live-birth rates between single IUI and double IUI in treatment cycles using clomiphene citrate. The evidence is insufficient to determine whether ultrasound monitoring for the timing of IUI offers improved pregnancy outcomes compared to urinary luteinizing hormone (LH) monitoring in clomiphene citrate-IUI treatments.

Assisted reproductive technology

RECOMMENDATIONS

- The International Federation of Gynecology and Obstetrics (FIGO) promotes the use of ART to achieve pregnancy and endorses its accessibility in all nations. (Level A, Class I).
- Prior to beginning IVF, other methods, such as expectant management and less invasive interventions, should be considered. (Level A, Class I).
- IVF should be avoided in cases of severe sperm abnormalities or repeated failed fertilization attempts and ICSI may be considered. (Level A, Class I).

Discussion

ART comprises fertility-related clinical and laboratory procedures that are conducted to establish pregnancy immediately or in the future.[10] The International Federation of Gynecology and Obstetrics (FIGO) aligns with the view of the World Health Organization (WHO) that childbearing is an inherent human right that should be universally accessible. The societal stigma attached to infertility can impose social isolation and abandonment upon women. ART encompasses a range of techniques involving the manipulation of gametes outside the body, with IVF and intracytoplasmic sperm injection (ICSI) being the most employed methods.[11] IVF-ET (In vitro Fertilization-Embryo Transfer) is the fertilization of an oocyte in-vitro and the transfer of the fertilized oocyte or embryo to the uterus of a woman. In ICSI, a single sperm (ejaculated or extracted [PESA and TESA]) is injected directly into the cytoplasm of the oocyte to aid in fertilization.[12]

IVF should only be considered when other less invasive methods or spontaneous conceptions have proven unsuccessful.[11]

Factors that will guide the choice of treatment between IVF and ICSI will depend on:

- Semen parameters – sperm count, motility, morphology, DFI
- Other factors which affect the chance to pregnancy
  - » Presence or absence of cervical factor
  - » Endometriosis
  - » Tubal pathology
  - » Uterine pathology – Congenital anomalies, polyp, sub-mucous myoma, IUA
  - » Anti-sperm antibody in male and female partner
  - » Pelvic Factor - History of previous pelvic or abdominal surgeries
  - » Age of the women - above 40 years should consider IVF early in the treatment protocol.
  - » Special situations

Indications of IVF[12]	
IVF –ET	• Irreversible pathology of the fallopian tubes/ blocked tubes » Ovulatory dysfunction who have failed to conceive with conventional methods » Subnormal male factor » Unexplained infertility » Endometriosis » Infertility of immunological origin » Fertility preservation » Candidates for preimplantation genetic diagnosis
ICSI with ejaculated spermatozoa	• Oligoasthenoteratozoospermia • Fertilization failure after standard IVF treatment • The newer indications for ICSI include – poor quality oocytes, low oocyte yield, PGT, IVM, fertilization of cryopreserved oocytes • Poor post-thaw parameters after sperm freezing • Increased DFI • Anejaculation because of spinal cord injury • Retrograde ejaculation
ICSI with testicular sperm (TESA)	• Germ-cell hypoplasia (hypo spermatogenesis) • Germ-cell aplasia with focal spermatogenesis
Use of IVF as a first line versus last resort in certain conditions is debatable and include:	• Unexplained infertility • Mild male factor infertility • Endometriosis without tubal disease • Unilateral tubal blockage • Diminished ovarian reserve • Age > 40 years with good ovarian reserve

Intracytoplasmic sperm injections (ICSI)[13]

RECOMMENDATIONS

- ICSI for unexplained infertility without male factor has shown increased fertilization rates but no improvement in live-birth outcomes. (Level A, Class I).
- ICSI can improve fertilization rates in cases where conventional insemination has resulted in lower-than-expected or failed fertilization. (Level A, Class I).
- The available evidence does not support the routine use of ICSI for all oocytes in cases without male factor infertility or a history of prior fertilization failure. (Level A, Class I).

Discussion

ICSI was developed to enhance fertilization in couples with male factor infertility or prior failed fertilization in IVF cycles. It has also been suggested for unexplained infertility to overcome potential fertilization barriers. While ICSI has been associated with increased fertilization rates in unexplained infertility cases, it does not improve live-birth outcomes. ICSI is recommended in IVF cases where previous cycles have experienced complete fertilization failure despite normal semen analysis. It can reduce the risk of subsequent failed fertilization. ICSI has shown effectiveness in increasing fertilization rates when conventional insemination has resulted in lower-than-expected or failed fertilization.[11]

Personnel[14]

RECOMMENDATIONS

- The practice of ART requires a well-orchestrated teamwork between the gynecologist, the andrologist and the clinical embryologist supported by a counsellor and a program coordinator/director. (Level A, Class I).

Discussion

- The responsibilities of a gynecologist in infertility cases include assessing and diagnosing the causes of infertility, conducting diagnostic tests, recommending appropriate treatments, and performing assisted reproductive techniques based on the diagnostic evidence.
- The responsibilities of the andrologist include diagnosing and treating male infertility, performing semen analysis, interpreting fertility status, collecting sperm for ART, and conducting surgical procedures if needed, while ensuring quality service and maintaining accurate records.
- The clinical embryologist should have extensive knowledge and expertise in various fields including embryology, reproductive endocrinology, genetics, molecular biology, biochemistry, microbiology, and in vitro culture techniques. They are responsible for performing procedures related to gamete and embryo processing, handling, and culturing, maintaining laboratory records, ensuring equipment functionality, and collaborating with the gynecologist in transferring embryos.
- Counsellors in infertility clinics provide support, guidance, and information to patients, addressing psychological stress and managing expectations. They should possess relevant qualifications and report to an independent body for fair practice, ensuring patients are well-informed.
- The program coordinator/director in an ART clinic should be an experienced senior professional, responsible for coordinating team activities, managing administrative tasks, and ensuring compliance, with a post-graduate degree in a relevant field and expertise in ART.

Planning for IVF

RECOMMENDATIONS

- Female evaluation involves hormonal assessments (FSH, E_2, AMH), antral follicle count, and transvaginal ultrasound to evaluate an ovarian reserve and uterine factors affecting fertility. Both partners should undergo infectious disease screening, including syphilis, hepatitis, and HIV. (Level C, Class IIb).

Discussion

Preparing for ART procedures involves evaluating the causes of infertility. Infertility is defined as the inability to achieve pregnancy after at least one year of unprotected intercourse. However, an evaluation can be initiated earlier (at six months) for women over 35 or when specific barriers are known, such as uterine or tubal disease or male infertility. The evaluation includes taking a comprehensive history, covering menstrual and pregnancy history, infertility duration, previous treatments, medical and surgical history, family history, and social and environmental factors. A physical examination is conducted, assessing vital signs, body mass index, thyroid function, androgen levels, and performing a pelvic examination.

For female evaluation, blood tests are performed to determine day 3 follicle-stimulating hormone (FSH), estradiol (E_2), antral follicle count, and Anti-Mullerian Hormone (AMH) levels, which help assess ovarian reserve. Additional tests may include thyroid-stimulating hormone (TSH), prolactin, DHEAS, testosterone, and 17-hydroxyprogesterone to evaluate ovulatory dysfunction. Transvaginal ultrasound is performed to evaluate the uterine and pelvic cavity, often in combination with hysterosalpingogram or sonohysterogram, to identify factors like polyps, fibroids, uterine malformations, hydrosalpinx, or endometriosis.

Male infertility is assessed through semen analysis, and both male and female evaluations typically include infectious disease screening for syphilis, hepatitis, and HIV.[15]

Controlled OS

RECOMMENDATIONS

- Controlled stimulation often involves exogenous gonadotropin injections, such as FSH and LH, to maximize follicle development. (Level C, Class IIb).
- Transvaginal ultrasound monitoring and/or serum estradiol (E_2) testing track follicle growth and ovarian response. (Level C, Class IIb).
- Final maturation is induced with exogenous human chorionic gonadotropin (hCG) or a GnRH agonist before oocyte retrieval. (Level C, Class IIb).
- The GnRH antagonist protocol substantially decreases the incidence of OHSS without influencing the pregnancy rate and live birth rate compared to GnRH agonist long protocol among patients with normal ovarian reserve. (Level A/Class 1).
- Minimal stimulation protocols using selective estrogen receptor modulators (SERMs) offer benefits such as reduced risk of ovarian hyperstimulation syndrome (OHSS) and multifetal gestation, but lower live birth rates compared to conventional IVF. (Level C, Class IIb).
- A segmentation strategy which includes optimization of the ovarian stimulation, including GnRH agonist triggering in a GnRH antagonist cycle, cryopreservation and embryo vitrification followed by embryo replacement in a receptive, non-stimulated endometrium in a natural cycle or with artificial endometrial preparation should form the basis for preventing OHSS in potential hyper-responders. (Level C, Class IIb).

Discussion

The initial cases of IVF relied on natural menstrual cycles with the retrieval of a single oocyte. Although natural cycle IVF is still practiced, controlled OS is now more commonly employed to increase the number of retrieved oocytes per cycle, which enhances the chances of a successful pregnancy. Various agents and regimens are available for controlled OS. Selective estrogen receptor modulators (SERMs) such as clomiphene citrate and tamoxifen are one approach. Utilizing minimal stimulation protocols, known as "mini-IVF," with SERMs offers advantages such as the reduced risk of OHSS and multiple gestations. However, it also results in a lower live birth rate compared to conventional IVF. Another common approach involves the administration of exogenous gonadotropins like FSH and LH to stimulate the development of multiple follicles in a single cycle.[15]

GnRH agonist cycles

Mixed gonadotropins, available as two types of injections (FSH and FSH/LH), are used in ART cycles. To prevent premature LH surges, a GnRH agonist is administered during the luteal phase before starting the gonadotropins. The GnRH agonist dosage is typically reduced when the gonadotropins are initiated, and a trigger is given for final maturation prior to egg retrieval. Pretreatment with oral contraceptives may also be considered. Transvaginal ultrasound and serum estradiol testing are utilized to monitor follicle number and growth as well as ovarian response. Once the ovarian follicles reach maturity (typically when 2-3 follicles reach 18mm in size), final maturation is induced using exogenous hCG or a GnRH agonist.[15]

Gonadotropin-releasing hormone (GnRH) antagonist cycles

Mixed gonadotropin, which includes injections with FSH activity (Recombinant FSH) and those containing both FSH and LH activity, are commonly used in ART cycles. To prevent premature LH surges, a GnRH antagonist is administered. These cycles typically commence with the onset of menses, following confirmation of normal baseline parameters through ultrasound and appropriate levels of FSH and estradiol hormones. In some cases, a cycle may begin after a pretreatment phase of 2-4 weeks using oral contraceptives.[15]

A systematic review and meta-analysis evaluated the effectiveness and safety of gonadotropin-releasing hormone antagonist (GnRH-ant) protocol and gonadotropin-releasing hormone agonist (GnRH-a) long protocol in patients with normal ovarian reserve. In this meta-analysis of 29 randomized controlled trials involving 6,399 patients, the GnRH-ant protocol showed significant advantages over the GnRH-a long protocol. The GnRH-ant protocol resulted in fewer stimulation days, lower gonadotrophin dosage, decreased estradiol levels, fewer oocytes and embryos obtained, and a lower incidence of OHSS. However, there were no significant differences between the two protocols in terms of clinical pregnancy rate, ongoing pregnancy rate, live birth rate, miscarriage rate, and cycle cancellation rate.[16]

The top priority in fertility treatment is the balance between the desire for pregnancy and patient safety. The concept of an OHSS-Free Clinic aims to achieve this by using a segmented approach. This includes optimizing OS with a GnRH antagonist, triggering ovulation with a GnRH agonist, and vitrifying oocytes or embryos. By avoiding OHSS, the physical and psychological health of patients are preserved, increasing their willingness to undergo further fertility treatments. The strategy involves carefully planning OS, cryopreservation, and embryo replacement in a receptive endometrium.[17]

Oocyte retrieval

RECOMMENDATIONS

- Oocyte retrieval should be performed 34-36 hours post-hCG or GnRH agonist administration, regardless of the stimulation protocol. (Level C, Class IIb).

Discussion

Oocyte retrieval has evolved from laparotomy to laparoscopy and is now standardly performed vaginally using ultrasound guidance. Irrespective of the stimulation protocol, mature oocytes are collected 34 to 36 hours after the administration of hCG. Oocyte retrieval is carried out under intravenous sedation, using ultrasound guidance and transvaginal aspiration. The ovaries are visualized with a vaginal ultrasound probe, and a needle guide assists the physician in accurately targeting each follicle, aspirating the oocyte, and extracting the follicular fluid. Retrieving a higher number of oocytes (up to 15) is associated with improved live birth outcomes.[15, 18]

Cryopreservation[5, 19]

RECOMMENDATIONS

- Cryopreservation of excess embryos should be offered as a standard part of infertility services, considering the typical abundance of embryos in IVF/ICSI treatments. (Level A, Class I).
- Implementing a successful cryopreservation program can enhance cumulative live birth rates and promote the use of single embryo transfer as an efficient option. (Level A, Class I).
- The availability of more high-quality embryos from elective single-embryo transfer cycles contributes to the success of cryopreservation. (Level A, Class I).
- Cryopreservation not only preserves embryos for future use but also helps mitigate the risks associated with OHSS, while simultaneously reducing the cost of subsequent cycles in fertility treatments. (Level A, Class I).

Discussion

Cryopreservation in ART can be categorized into elective and non-elective indications. Originally, cryopreservation was primarily used for medical indications in women without other fertility options. However, it has now expanded to include elective scenarios such as oocyte donation and social oocyte freezing. Clinical oocyte freezing is another option where multiple OS cycles are performed to collect a larger batch of oocytes, increasing the chances of future IVF success, especially in cases of recurrent implantation failure. The indications for cryopreservation in ART practice are summarized in Table 1.

Oocyte cryopreservation offers a solution to avoid moral objections, legal restrictions, and disputes related to embryo cryopreservation and storage. It provides a feasible option for women who prefer not to freeze embryos.

Table 1. Indications for cryopreservation in ART practice

	Elective	Non-elective
Oocytes	**Oocyte donation, oocyte banking** Avoids the need to match the donor's and recipient's cycles, and addresses the demand for donor oocytes, thereby alleviating waiting lists	**Medical oocyte freezing** In women about to undergo gonadotoxic treatment for cancer or other conditions, or with a medical pathology that impairs fertility, such as severe endometriosis or genetic conditions including Turner's syndrome
	Planned oocyte cryopreservation Allows women wishing to defer childbearing to preserve their fertility in anticipation of age-related fertility decline **Others** Oocyte cryopreservation can provide a feasible alternative where embryo cryopreservation is not an option because of religious, moral, or ethical objections, or restrictive legislation	**Incidental oocyte freezing** Emergency freezing in IVF when sperm is not available on the day of oocyte retrieval Storage of "spare" oocytes during IVF
	Clinical oocyte freezing Accumulation of oocytes to increase the likelihood of future success in cases of poor responders or recurrent implantation failure, or to increase their availability for PGT	
Embryos	**Preimplantation genetic testing (PGT)** PGT is facilitated by the opportunity to use the freeze-all strategy for storing embryos for transfer in subsequent cycles after testing **Patient's or physician's preference** The ability to store surplus embryos can reduce the number of embryos transferred during a fresh cycle and thus minimize the risk of multiple pregnancy, reduce the need for repeated stimulation cycles, and increase cumulative pregnancy rates	**Elevated progesterone** Elevated progesterone in the late follicular phase has a negative impact on pregnancy rate, although the reasons for this are not entirely clear **Avoidance of OHSS** Embryos may be cryopreserved rather than proceeding with a fresh embryo transfer to allow ovarian recovery and thus prevent OHSS when excess follicle development has occurred following ovarian stimulation in the IVF cycle

However, it is worth noting that embryo cryopreservation is a well-established technique, and large observational studies suggest higher implantation and pregnancy rates with frozen-thawed embryos compared to embryos derived from frozen oocytes.[18]

Complications

RECOMMENDATIONS

- Patients with OHSS should be provided with comprehensive supportive care, including anticoagulants and fluid resuscitation. Additionally, plasma expanders, calcium gluconate, and dopamine agonists should be administered, and ascitic tapping may be required. (Level C, Class IIb).
- Treatment progress should be monitored through hematocrit/blood count and routine investigations to ensure appropriate management. Once the ovarian stimulation has subsided, a frozen embryo transfer should be performed for the patient's best possible outcome. (Level C, Class IIb).
- ART is associated with an increased risk of multifetal pregnancies which in turn are associated with increased incidence of stillbirth, neonatal death, and other maternal antenatal complications. (Level C, Class IIb).

Ovarian hyperstimulation syndrome[15]

Exogenous administration of gonadotropins followed by hCG can lead to OHSS, characterized by the overproduction of growth factors like VEGF and the formation of new blood vessels. This causes fluid shifts, resulting in symptoms such as ascites, edema, pleural effusion, renal injury, pericardial effusion, and thromboembolism. Patients with PCOS, multi-follicular development, high oocyte retrieval (>24), and elevated estradiol levels are at a higher risk of OHSS. Treatment involves supportive care, anticoagulants, and fluid resuscitation. Frozen embryo transfer is recommended after the resolution of OS.

Antenatal and neonatal complication[15]

Multifetal pregnancies resulting from ART have maternal and fetal consequences. They are associated with conditions like hyperemesis gravidarum, gestational diabetes, and hypertensive diseases of pregnancy. Compared to singleton pregnancies, multifetal pregnancies have a higher risk of preterm birth, stillbirth, and neonatal death. Singleton IVF pregnancies also carry increased risks compared to naturally conceived pregnancies, including perinatal mortality, preterm delivery, low birth weight, cesarean section, placenta previa, placental abruption, and preeclampsia. Despite the lack of evidence, patients should be informed about the potential risks.

Single embryo transfer (SET) policy

RECOMMENDATIONS

- Single embryo transfer is the optimal strategy in IVF treatment to prevent multiple pregnancies and reduce the risk of preterm birth, ultimately increasing the likelihood of a healthy live birth. (Level A, Class I).
- Transferring a single high-quality embryo (preferably blastocyst) out of multiple available embryos can significantly decrease the occurrence of twin pregnancies. (Level A, Class I).

Discussion

Single embryo transfer (SET) is recommended as an effective method for reducing the rate of twin pregnancies. By transferring one high-quality embryo from a pool of at least two available embryos, the incidence of twin pregnancies can be significantly reduced. Successful implementation of elective SET requires high-quality laboratories and reliable cryopreservation programs. Eligibility guidelines for elective SET should consider factors such as maternal age, number of previous IVF/ICSI cycles, and embryo quality. Observational studies show that outcomes are less favorable when only one embryo is available, but selecting a single embryo for elective transfer leads to better results.[5]

According to a systematic Cochrane review, elective single embryo transfer (SET) in fresh IVF/ICSI cycles is associated with a lower chance of live birth compared to double embryo transfer (DET). However, when combined with a high-quality freezing program and subsequent transfer of a single frozen-thawed embryo, the live birth rate is comparable to DET. It is recommended to discourage the transfer of three or four embryos.[5]

Preimplantation genetic testing (PGT)

RECOMMENDATIONS

- PGT is a technique used to identify genetic defects in embryos created through IVF before pregnancy. (Level A, Class I).
- PGD is performed when one or both genetic parents have a known genetic abnormality, and testing is done on embryos to determine if they also carry the abnormality. (Level A, Class I).
- PGT for aneuploidies (PGT-A) is the most common form of PGT, used for indications such as advanced maternal age, recurrent miscarriage, and repeated implantation failures. (Level A, Class I).
- PGT helps improve the selection of embryos with the highest potential for successful implantation and a viable pregnancy. (Level A, Class I).
- It is recommended that Preimplantation Genetic Testing for Monogenic (PGTM) and Preimplantation Genetic Testing for Structural Rearrangements (PGT-SR) should be offered wherever necessary and indicated. (Level A, Class I).
- A clinic conducting PGT procedures should be duly accredited with PCPNDT licenses, should adhere to the reporting in the prescribed forms and should follow the rules and regulations of the PCPNDT Act. (Level A, Class I).

Discussion

Preimplantation genetic testing (PGT) is a method used in IVF to identify genetic defects in embryos before pregnancy. Preimplantation genetic diagnosis (PGD) focuses on testing embryos from parents with known genetic abnormalities, while preimplantation genetic screening (PGS) screens embryos from parents presumed to be chromosomally normal. PGT and PGS are the only options available to avoid the risk of having a child with a genetic disease before implantation, offering a way to prevent heritable genetic diseases and eliminating the need for pregnancy termination after an unfavorable prenatal diagnosis.[20]

Recently, there has been a change in the terminology for PGT. Previously known as preimplantation genetic screening (PGS) and PGD, these practices are now collectively referred to as PGT. PGT-A, which focuses on testing for aneuploidies, constitutes the majority of PGT procedures, accounting for approximately 90% of cases. PGT-A is commonly utilized in situations such as advanced maternal age, recurrent miscarriage, and repeated implantation failures, with the aim of selecting embryos with higher chances of successful implantation and healthy pregnancy outcomes.[20]

PGT-M recommendations suggest using linked markers for accurate embryo genetic status. However, incorporating multiple markers has raised development time and costs due to complexity. PGT for chromosome structural rearrangements (PGT-SR) is a standard and commonly performed procedure in IVF/PGT centers. It is designed for patients facing challenges in conceiving or at a high risk of pregnancy complications and abnormal births due to inherited unbalanced rearranged chromosomes.[20]

SUMMARY OF RECOMMENDATIONS

Investigation of infertility

- Clinical investigations are required for those couples who fail to conceive after six months of regular, unprotected sexual intercourse. (Level A, Class I) .
- Hormonal investigations for ovulation disorders and semen analysis are the initial fertility investigations required followed by a test for tubal patency. (Level A, Class I).
- Women experiencing infertility and who do not have a history of pelvic infections, endometriosis, or ectopic pregnancy should be provided with the option of undergoing a tubal patency test e.g., hysterosalpingography to screen for abnormalities in the uterus and fallopian tubes. (Level C, Class IIa).
- Results of semen analysis and ovulation assessment should be obtained before testing for tubal patency. (Level A, Class I).
- Immunological testing should not be routinely conducted as part of the infertility evaluation. (Level A, Class I).

Information and counseling

- Patients should be given counseling regarding alternative options and the effect of stress on sexuality and relationship. (Level A, Class I).

Evidence-based treatments for infertility

Ovulation induction

- Clomiphene citrate, a combination of clomiphene and insulin sensitizers, letrozole, gonadotrophin therapy, and dopamine agonists are the different treatment options available for anovulatory women, depending on etiology. (Level A, Class I).
- Letrozole is recommended as a first line ovulation induction agent in women with PCOS since it promotes mono-follicular development and in view of its non-antiestrogenic properties when compared to clomiphene. (Level A, Class I).

Intrauterine insemination

- It is not recommended to perform IUI during natural cycles for the treatment of unexplained infertility. It is not as effective as ovarian stimulation (OS) with IUI, and provides no additional benefit compared to expectant management. (Level A, Class I).

- Using oral ovulogens (clomiphene citrate or letrozole) in combination with IUI is recommended for treating couples with unexplained infertility. (Level A, Class I).
- The use of letrozole or clomiphene citrate in combination with gonadotropins for IUI may be considered due to their potential to improve pregnancy rates. However, it is essential to offer patients thorough counseling about the higher risk of multiple-gestation pregnancies associated with these treatments. (Level A, Class I).
- Couples with unexplained infertility are recommended to begin a course of OS and IUI using oral agents, typically for 3 or 4 cycles. (Level A, Class I).

Assisted reproductive technology

- The International Federation of Gynecology and Obstetrics (FIGO) promotes the use of ART to achieve pregnancy and endorses its accessibility in all nations. (Level A, Class I).
- Prior to beginning IVF, other methods, such as expectant management and less invasive interventions, should be considered. (Level A, Class I).
- IVF should be avoided in cases of severe sperm abnormalities or repeated failed fertilization attempts and ICSI may be considered. (Level A, Class I).

Intracytoplasmic sperm injections (ICSI)

- ICSI for unexplained infertility without male factor has shown increased fertilization rates but no improvement in live-birth outcomes. (Level A, Class I).
- ICSI can improve fertilization rates in cases where conventional insemination has resulted in lower-than-expected or failed fertilization. (Level A, Class I).
- The available evidence does not support the routine use of ICSI for all oocytes in cases without male factor infertility or a history of prior fertilization failure. (Level A, Class I).

Personnel

- The practice of ART requires a well-orchestrated teamwork between the gynecologist, the andrologist and the clinical embryologist supported by a counsellor and a program coordinator/director. (Level A, Class I).

Planning for IVF

- Female evaluation involves hormonal assessments (FSH, E_2, AMH), antral follicle count, and transvaginal ultrasound to evaluate an ovarian reserve and uterine factors affecting fertility. Both partners should undergo infectious disease screening, including syphilis, hepatitis, and HIV. (Level C, Class IIb).

Controlled ovarian stimulation

- Controlled stimulation often involves exogenous gonadotropin injections, such as FSH and LH, to maximize follicle development. (Level C, Class IIb).
- Transvaginal ultrasound monitoring and/or serum estradiol (E_2) testing track follicle growth and ovarian response. (Level C, Class IIb).
- Final maturation is induced with exogenous human chorionic gonadotropin (hCG) or a GnRH agonist before oocyte retrieval. (Level C, Class IIb).
- The GnRH antagonist protocol substantially decreases the incidence of OHSS without influencing the pregnancy rate and live birth rate compared to GnRH agonist long protocol among patients with normal ovarian reserve. (Level A/Class 1).
- Minimal stimulation protocols using selective estrogen receptor modulators (SERMs) offer benefits such as reduced risk of ovarian hyperstimulation syndrome, and multifetal gestation, but lower live birth rates compared to conventional IVF. (Level C, Class IIb).
- A segmentation strategy which includes optimization of the ovarian stimulation, including GnRH agonist triggering in a GnRH antagonist cycle, cryopreservation and embryo vitrification followed by embryo replacement in a receptive, non-stimulated endometrium in a natural cycle or with artificial endometrial preparation should form the basis for preventing OHSS in potential hyper-responders. (Level C, Class IIb).

Oocyte retrieval

- Oocyte retrieval should be performed 34-36 hours post-hCG or GnRH agonist administration, regardless of the stimulation protocol. (Level C, Class IIb).

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