FOGSI FOCUS
Emerging Trends in Infertility

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Editor-in-Chief

Jaideep Malhotra
MD FRCOG FRCPI FICS (Obs & Gyn) (FICMCH FIAJAGO FMAS FICOG MASRM FICMU FIUMB)

Professor
Dubrovnik International University
Dubrovnik, Croatia

Managing Director
ART-Rainbow IVF
Agra, Uttar Pradesh, India
President FOGSI–2018

Co-Editors

Narendra Malhotra MD FICMCH FICOG FICS FMAS FRCOG AFIAPM
Vice President, World Association of Perinatal Medicine
President, ISPAT
Agra, Uttar Pradesh, India

Diksha G Sharma MD DNB MRCOG FNB
IVF Consultant
Rainbow Hospital
Agra, Uttar Pradesh, India

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Contributors

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IVF Consultant
Rainbow Hospital
Agra, Uttar Pradesh, India

Contributing Authors

Radhika T Bahl LLM LLB
Head
Legal (Medico-Legal) Services
Fertility Law Care
Gurgaon, Haryana, India

Rishabh Bora MD
Radiologist
Rainbow 4-D Life (Radiology)
Global Rainbow Healthcare
Agra, Uttar Pradesh, India

Neharika M Bora MD FMAS
Infertility Consultant
Rainbow IVF
Global Rainbow Healthcare
Agra, Uttar Pradesh, India

Priya B Chittawar MD DNB FRM

Head
Department of Reproductive Medicine
Bansal Hospital
Bhopal, Madhya Pradesh, India

Susan S George FRANZCOG MD DGO
MRMed

Lead Specialist
Primary IVF
Brisbane, Australia

Shally Gupta DGO DNB MRCOG

IVF Consultant
Rainbow Hospital
Agra, Uttar Pradesh, India

Kirthika KS MD DNB FNB

Consultant in Reproductive Medicine
Apollo Hospitals
Chennai, Tamil Nadu, India

Sudesh A Kamat MSc

Senior Embryologist
Bloom IVF
Lilavati Hospital and Research Centre
Mumbai, Maharashtra, India

Mandeep Kaur MD FNB

Consultant
Department of Reproductive Medicine
Nova IVI
Jalandhar, Punjab, India

Keshav Malhotra MCE

Director
Rainbow IVF
Agra, Uttar Pradesh, India

Chaitanya Nagori MD DGO

Director and Infertility Consultant
Dr Nagori's Institute for Infertility and IVF
Ahmedabad, Gujarat, India

Raju Nair MS MNAMS

Head
Department of Reproductive Medicine
Mitera Hospital
Kottayam, Kerala, India

Sonal Panchal MD

Consultant
Department of Ultrasound
Dr Nagori's Institute for Infertility and IVF
Ahmedabad, Gujarat, India

Sabahat Rasool MD MRCOG DNB
MNAMS FMAS

Reproductive Medicine and Infertility
Consultant
Gynaecworld, The Center for Fertility and
Women's Health
Mumbai, Maharashtra, India

Roshi Satija MS PGDHM PGDMLS

Fertility and IVF Specialist
Director
Department of Reproductive Medicine
Dr Sunderlal Memorial Hospital
IVF Consultant
Max Hospital
New Delhi, India

Duru S Shah MD FRCOG FCPS FICS
FICOG FICMCH DGO DFP

Director
Gynaecworld, The Center for Fertility and
Women's Health
Mumbai, Maharashtra, India

Message

Jaideep Malhotra MBBS MD FRCOG
FRCPI FICS (Obs & Gyn) (FICMCH FIAJAGO FMAS
FICOG MASRM FICMU FIUMB)

Professor
Dubrovnik International University
Dubrovnik, Croatia

Managing Director
ART-Rainbow IVF
Agra, Uttar Pradesh, India
President, FOGSI–2018

June is our month for infertility and environment. FOGSI 2018 is focussing hard on going "Green" and "Paper Free" and we all should take initiatives for this cause. Infertility is seen on the rise and they are no longer private sorrows. Our focus has to start from adolescent itself by propagating healthy lifestyle practices and also working towards a better environment.

Almost 80% causes of infertility can be managed if treated in a proper systematic way. Our aim as doctor is not just to offer treatment but also to add quality, ethics, and dignity in our practice.

This FOGSI Focus has been taken out with a lot of hard work by infertility specialists all over and focuses on many important topics like barriers to infertility care, individualized ovarian stimulation, approach to couples with unexplained infertility, endometrial receptivity, luteal phase support, as well as recent advances of fertility preservation, poor responders, and gadgets in the embryology lab along with new drugs like atosiban. We hope that all of you read this book and update yourself with the new advancements in the rapidly evolving field of infertility and assisted reproductive technology.

Jaideep Malhotra

Preface

"FOGSI Focus" series has been a FOGSI tradition for many years which all the members enthusiastically look forward to. We have dedicated this issue to emerging trends in infertility practice. India faces a high burden of infertility with 22–33 million couples in the reproductive age group suffering from lifetime infertility and which is on the rise. Many of these couples are seen by the general gynecologist in the OPD daily who must be abreast of how to proceed.

Soliciting FOGSI theme of the year "Health Empowerment Respect – Quality Ethics Dignity," we endeavor through this focus to present an evidence-based review of the recent advances in the field of infertility and assisted reproductive technology (ART).

We have tried to cover diverse topics in a simplified manner so that this book is a handy tool for gynecologists to gain more insight into specialized aspects of infertility and ART.

Ranging from commentary on barriers to infertility care, advanced diagnostic modalities like 3D scan, controversial topics like adjuvants, newer advances like fertility preservation, new tools in the lab, and latest evidence on poor responders have been covered by our experts ably.

This book will help our readers to enhance their knowledge and address the needs of infertile couples better. So, happy reading to all of you.

Narendra Malhotra
Diksha G Sharma

Acknowledgments

Contents

Barriers to Infertility Care in Low-resource Settings

Duru S Shah, Sabahat Rasool

With an estimated prevalence of over 80 million worldwide, infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months of regular unprotected intercourse.[1] Parenthood is an important milestone of life, though the urgency and sensitivity may vary. While India tops the list in the need for parenthood, the awareness of fertility problems is the lowest. In societies like ours where parenthood is mandatory, infertility is a devastating experience with serious far-reaching consequences. Social isolation, stigmatization, domestic violence, separations, and divorces are exceedingly common with infertile women. It is a known fact that male factor is responsible for one-third of total infertility cases; however, the burden falls disproportionately on women. In countries with limited resources, despite significant implications on quality of life, the resources allocated for infertility prevention and treatment are negligible.[2,3]

Global access to infertility care is a human right and a case of reproductive equity and social justice. India has a burden of more than 23 million infertile couples. Assisted reproductive techniques (ART) is used by <1% of them. This may be attributed to low awareness and accessibility issues, high cost, and unacceptability. Even in the metropolitans like Mumbai and Delhi, the demand for fertility treatment is 9–12 times higher than the current market.

Understanding the causes of infertility and sociocultural factors is a critical step for developing treatment strategies and resource allocation to address the huge burden. Developing countries owe their infertility burden largely to reproductive infections (RIs) and tuberculosis which damage the tubes.[4,5] Nearly half of men and two-thirds of infertile women in Sub-Saharan Africa have infertility as a sequel of untreated RIs. The number of women with damaged tubes and endometrium as a result of tuberculosis in India is very high. In such a scenario, it is critical to prevent postpartum, postabortal and sexually transmitted infections, tuberculosis and treat them in time.

Male infertility contributes to more than half of all infertility cases. While severe male-factor infertility cases are genetically determined and incurable, they are amenable to treatment through ART. However in developing countries, ART is culturally and religiously unacceptable to many. ART is haunted by social and religious taboos despite a fascinating success story lasting >30 years. Besides, ART for the low resource settings is a catastrophic expenditure. People have to travel from villages to urban areas to access infertility care. Travel away from home, the costs attached with that, expensive treatment and absenteeism from work, loans or credits taken for such treatment make a huge economic impact. Besides, many infertile couples waste a lot of time going to quacks and underqualified healthcare providers. Early referrals to trained specialists would save time and a lot of money. Since there are none or limited services available in the public sector, couples seek ineffective and incorrect therapies. Private sector provides infertility services at a high cost, which is beyond the scope of lower socioeconomic strata. It is this stratum that is most vulnerable to infertility through early sexual debut, reproductive infections, unsafe abortions, tuberculosis, and no access to health care.[6]

Some positive steps have been taken by international bodies to address the unmet need. The World Health Organization (WHO) recommended that infertility must be considered a global health problem and also recommended development of initiatives like low-cost ART. The United Nations (UN) program of action recommends the need to incorporate the prevention and treatment of RIs as well as infertility as part of reproductive health services.[7] The European Society for Human Reproduction and Embryology (ESHRE) has created a task force for developing countries to explore useful, effective and low-cost approaches to ART.

The Walking Egg Project is yet another example to realize affordable infertility care through effective and cheap laboratory procedures like fertilization and culture.

With the support of local policy makers and international community, global access to fertility care can be achieved. Media, nonprofit organizations, insurance companies, and public-private partnerships have to work hand in hand to change the dismal penetration of infertility treatment in general populations in resource poor countries. Adequate training of health professionals and early referrals should be promoted. Regular audit of accreditations, continuing medical education and quality control will ensure high-quality ethical treatments. In India only about 1,000 gynecologists are actually performing ART procedures for a huge figure of more than 23 million infertile couples. From the present number of around one lakh *in vitro* fertilization (IVF) cycles countrywide, the number is estimated to rise to 250,000 and above by the year 2020. The gap has to be bridged.

Affordable ART can be achieved by using simpler protocols, clinical acumen rather than expensive lab testing, reducing the burden of pre-ART investigations, and government subsidies on infertility drugs and lab media without necessarily compromising on pregnancy rates.[8]

With a huge burden of infertility, there is an unsurmountable gap in access to care, more so in countries with limited resources. While a large chunk of resources are channelized towards family planning, reducing maternal, infant mortality and morbidity, vaccinations, and drugs for people with human immunodeficiency virus (HIV), it is important to incorporate ART into comprehensive reproductive health. Spreading awareness about infertility and its treatment, and educating healthcare providers will go a long way in reducing the burden. The policy makers have to be made aware of the profound mental, physical, and social consequences of infertility and also the brilliant success rates of ART. Governments should take initiatives like public-private partnerships and insurance covers for infertility care to improve the penetration of ART. The gravity of the problem is severe and it is high time for action.

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2

Approach to a Couple with Unexplained Infertility

Kirthika KS

INTRODUCTION

"Absence of evidence is not evidence of absence," best explains unexplained infertility.

- Infertility is described as 1 year of unwanted non-conception with unprotected intercourse in the fertile phase of the menstrual cycle[1]
- Unexplained infertility usually refers to a diagnosis made in couples in whom all the standard investigations such as tests of ovulation (basal body temperature, cervical mucus changes, serum luteinizing hormone surge or mid-luteal progesterone), tubal patency (hysterosalpingogram and/or laparoscopy) and semen analysis are normal[1]
- Unexplained subfertility affects up to a quarter of all couples
- The potential causes are disturbances in immunology, genetic, reproductive physiology and endocrinological balance. Mild endometriosis, tubal dysfunction, poor/decreased ovarian reserve, subtle defects in sperm oocyte interaction, cervical function, implantation, and sperm function are the likely etiologies.

TREATMENT OPTIONS (TABLE 1)

- The fertility enhancing treatment plan for any couple with unexplained infertility is based on a rough estimate of

future chance of spontaneous pregnancy in a reasonable time frame
- The availability of resources, age of the female partner, duration of the infertility and presence of previous pregnancy are factors to be considered while planning the treatment[2]
- Treatment is indicated if duration is more than 2 years or the female is more than 35 years.[2]

EXPECTANT MANAGEMENT

- The National Institute for Health and Clinical Excellence (NICE) guidelines advise women with unexplained infertility to have regular unprotected sexual intercourse for a total of 2 years (this can include up to 1 year before their fertility investigations) before IVF will be considered
- It is advocated to personalize the period of expectant management based on a woman's age and the ovarian reserve testing results, including longer periods for younger women.[3]

Ovulation-inducing Agents

- Any role of ovulation induction in women with regular cycle is debatable

TABLE 1: Comparison of success rates with various treatment options

Treatments compared	Clinical pregnancy rates	Live birth rate	Number needed to treat
Expectant management vs. CC+TI	1.03 (0.64–1.66)	0.79 (0.45–1.38)	40
Expectant management vs. natural IUI	1.53 (0.88–2.64)	1.60 (0.92–2.8)	37
Expectant management vs. stimulated IUI	0.61(0.25–1.47)	0.82 (0.45–1.49)	15
Stimulated IUI vs. natural IUI	2.14 (1.26–3.61)	2.7 (1.22 vs. 3.55)	–
Expectant management vs. IVF	NA	22.0 (2.56–189.33)	4
IVF vs. IUI	NA	1.96 (0.88–4.36)	–

CC, clomiphene citrate; TI, timed intercourse; IUI, intrauterine insemination; IVF, *in vitro* fertilization.

TABLE 2: Guide for treating an couple with unexplained infertility

Age <35 years with good reserve	Age 35–39 years with good reserve	Any age with poor reserve/age >40
• Expectant management upto 2 years • Followed by 4 cycles of stimulated IUI • Followed by IVF	• Expectant management upto 1 year • Followed by 3–4 cycles of stimulated IUI • Followed by IVF	• Timed intercourse 6 months • Followed by 2–3 cycles of IUI • Followed by IVF

IUI, intrauterine insemination; IVF, in vitro fertilization.

- Do not offer oral ovarian stimulation agents [such as clomiphene citrate (CC), anastrozole or letrozole] to women with unexplained infertility[2]
- Standalone ovulation induction does not increase the chances of a pregnancy or a live birth. Cochrane systematic review by Hughes et al. in 2010 showed CC was not more effective than no treatment or placebo for live pregnancy rate (LBR) and clinical pregnancy rate (CPR)[4]
- Potential side effects of multiple pregnancy and three-fold risk of ovarian cancer (use >12 cycles) should be discussed.[4]

Gonadotrophins

- A review of a Cochrane database by Athaullah et al. 2009 stated that there is no significant difference in the odds of live birth rates (OR 0.06, 95% CI 0.00–1.15) and pregnancy rates (OR 0.33, 95% CI 0.09–1.20) between oral and injectable agents in unexplained infertility
- The cost of treatment and patient's convenience should be considered before choosing this method of treatment.[5]

Intrauterine Insemination

- There is a hypothesis that increasing the density of the motile spermatozoa available to the eggs during ovulation increases the monthly probability of pregnancy
- The Cochrane review by Veltman-Verhulst et al. in 2016 did not find conclusive evidence of difference in live birth or multiple pregnancy rates in couples with unexplained subfertility when intrauterine insemination (IUI) was compared to timed intercourse both with and without ovarian hyperstimulation[6]
- In studies for optimum time interval between human chorionic gonadotropin (hCG) and IUI (24–48 h), Cochrane review by Cantineau et al. 2014 did not find any significant difference between the groups[7]
- A meta-analysis of randomized controlled trial (RCT) by Polyzos et al. in 2010, concluded that double IUI offers no clear benefit in the overall CPR in couples with unexplained infertility (Table 2).[8]

In Vitro Fertilization/Intracytoplasmic Sperm Injection

- Unlike the empirical nature of other treatment methods, *in vitro* fertilization (IVF) could provide therapeutic solution to couple with unexplained infertility when the reason for their subfertility is ovulatory or tubal dysfunction, sperm, and egg interaction and implantation
- In unexplained infertility, do not routinely offer IUI, with or without stimulation (exceptional circumstances include when people have social, cultural or religious objections to IVF. Advise them to try to conceive for a total of 2 years (this can include up to 1 year before their fertility investigations) before IVF will be considered (NICE 2016)[2]
- A Cochrane review by Pandian et al. in 2012 concluded that IVF may be more effective than IUI + SO. Adverse events and costs associated with the intervention have not been adequately assessed.[9]

The Fast Track and Standard Treatment Trial

The Fast Track and Standard Treatment (FAST) trial demonstrated a shorter time to pregnancy (TTP), higher per cycle prevalence ratios (PRs) (HR 1.25; CI 1.00–1.56) and cost-effectiveness for IVF when compared with oral agents or gonadotropins in patients with unexplained infertility.

▮ TIPS FOR SUCCESS

- Prognosticate the spontaneous pregnancy rates in each couple. Policy of treatment is progression from low- to high-technology treatment
- Three to four cycles of stimulated IUI followed by IVF in women with good reserve
- Early resort to IVF in women with poor ovarian reserve.

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CHAPTER

3D/4D Ultrasound in Endocrinology Evaluation

Sonal Panchal, Chaitanya Nagori

INTRODUCTION

Management of infertility in females needs a close observation of the highly dynamic hormonal status. This can be done by laboratory investigations. Ultrasound, though is a much more patient friendly investigation. Doppler and three-dimensional (3D) ultrasound have a huge potential to explain the female fertility related endocrinology.

Commonly done laboratory investigations in patients of infertility for diagnosis or management are:

- Androgen
- Anti-Müllerian hormone (AMH)
- Follicle stimulating hormone
- Luteinizing hormone
- Estrogen
- Progesterone
- Thyroid
- Prolactin
- Insulin.

ANDROGEN

- It is responsible for recruitment of preantral follicles, to grow to antral. Follicular growth till 6–7 mm is dependent on androgen. More antral follicles, therefore, indicate high androgen
- The follicle number is best calculated by 3D ultrasound. Sono AVC is the software on 3D ultrasound scanners, that not only shows fluid filled follicles as solid structures but also color codes these and assess their size automatically (Fig. 1)
- The size that it assesses is also useful to decide on the commencement of follicle-stimulating hormone (FSH) for ovulation induction
- Increased androgen also leads to increased uterine artery resistance.

ANTIMULLERIAN HORMONE

- The AMH is produced by granulosa cells of preantral and small antral follicles of between 2–6 mm
- The number of small antral follicles (2–6 mm) is represents the functional ovarian reserve[1]
- Antral follicle count (AFC) and ovarian volume showed significant correlation with AMH
- Ovarian volume also can be more accurately calculated by 3D ultrasound, using a software called Virtual Organ Calculation (VOCAL)
- Androgen is the cause and AMH is the effect of recruited antral follicles
- AFC and ovarian volume provide direct measurements of ovarian reserve, while AMH, inhibin B and estradiol are released from growing follicles and so their levels reflect the size of developing follicle cohort.[2]

FOLLICLE STIMULATING HORMONE

- It is secreted by pituitary and its secretion is controlled by gonadotropin-releasing hormone from hypothalamus and negative feedback from estrogen
- Follicular growth beyond 6–7 mm represents the action of FSH
- Early follicular recruitment, fast growth of the follicle in early follicular phase-follicle of larger than 10 mm on the baseline scan indicates high basal FSH levels (Fig. 2)
- In stimulated cycle the normal follicular growth rate of 2–3 mm a day suggests normal doses of FSH. The doses are considered to be low in case of slower growth and high in cases of faster growth.
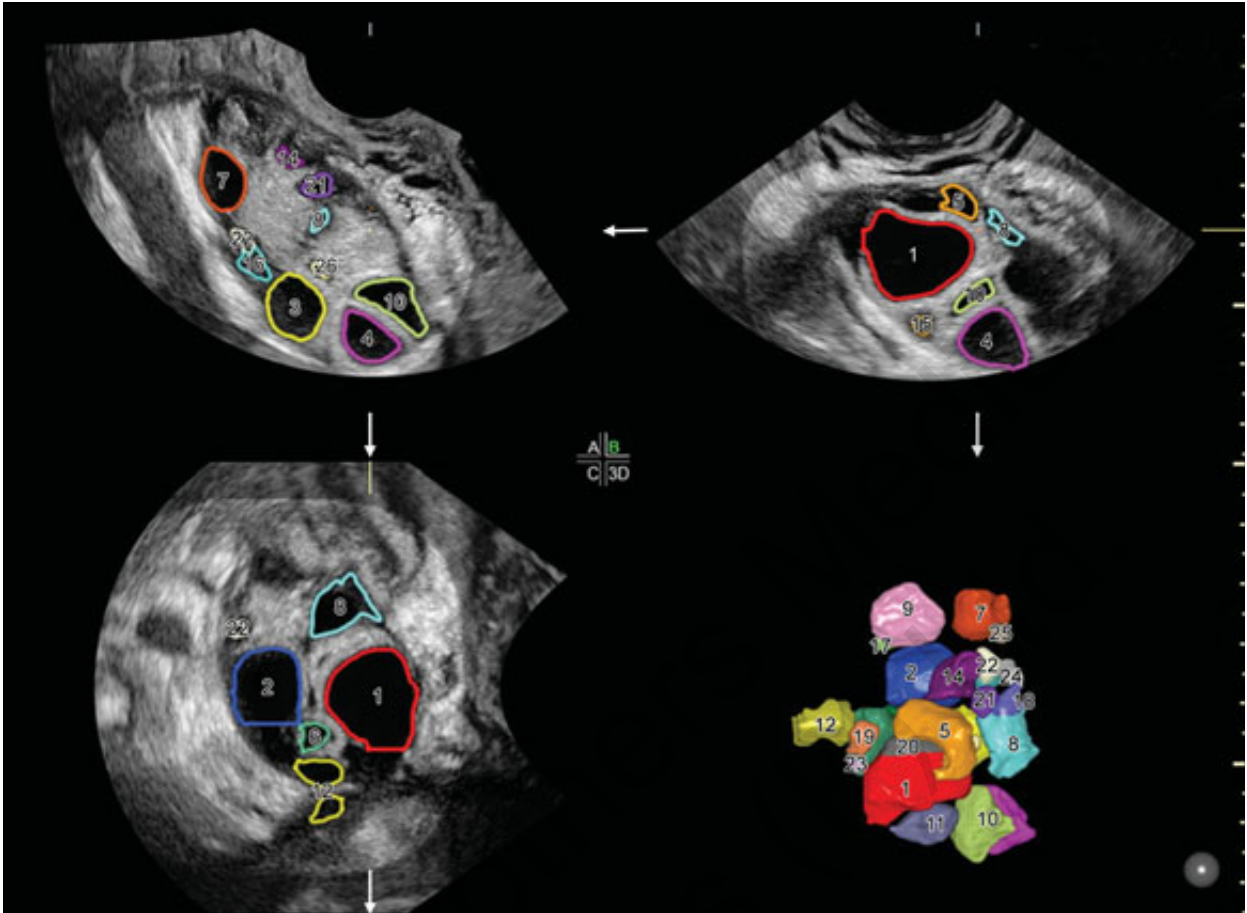
Figure 1: Antral follicle count done by sono AVC on 3D ultrasound.
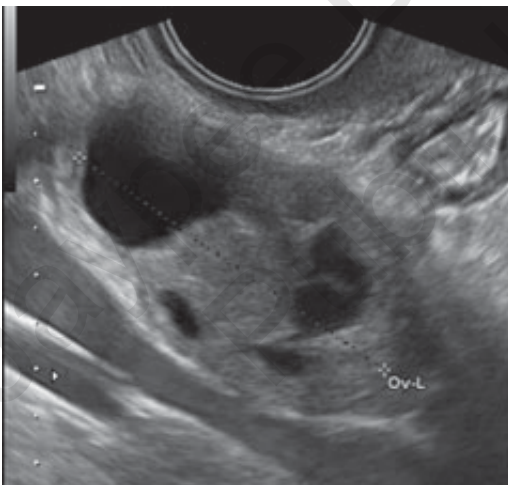
Figure 2: Early proliferative phase—ovary showing early recruitment of follicle.
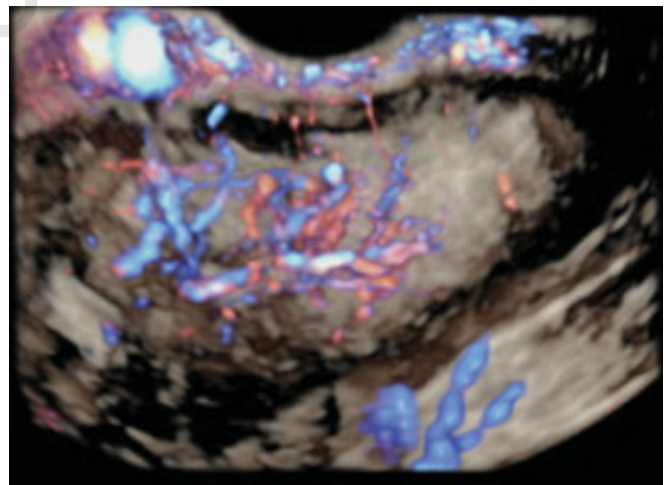
Figure 3: Stromal vascularity in polycystic ovary syndrome.

LUTEINIZING HORMONE

- Luteinizing hormone (LH) is also produced by pituitary. Its secretion is controlled by GnRH from hypothalamus and by positive feedback of estrogen

- Elevated LH levels may be responsible for increased stromal vascularization due to neoangiogenesis, catecholaminergic stimulation, leukocyte and cytokine activation[3] (Fig. 3)
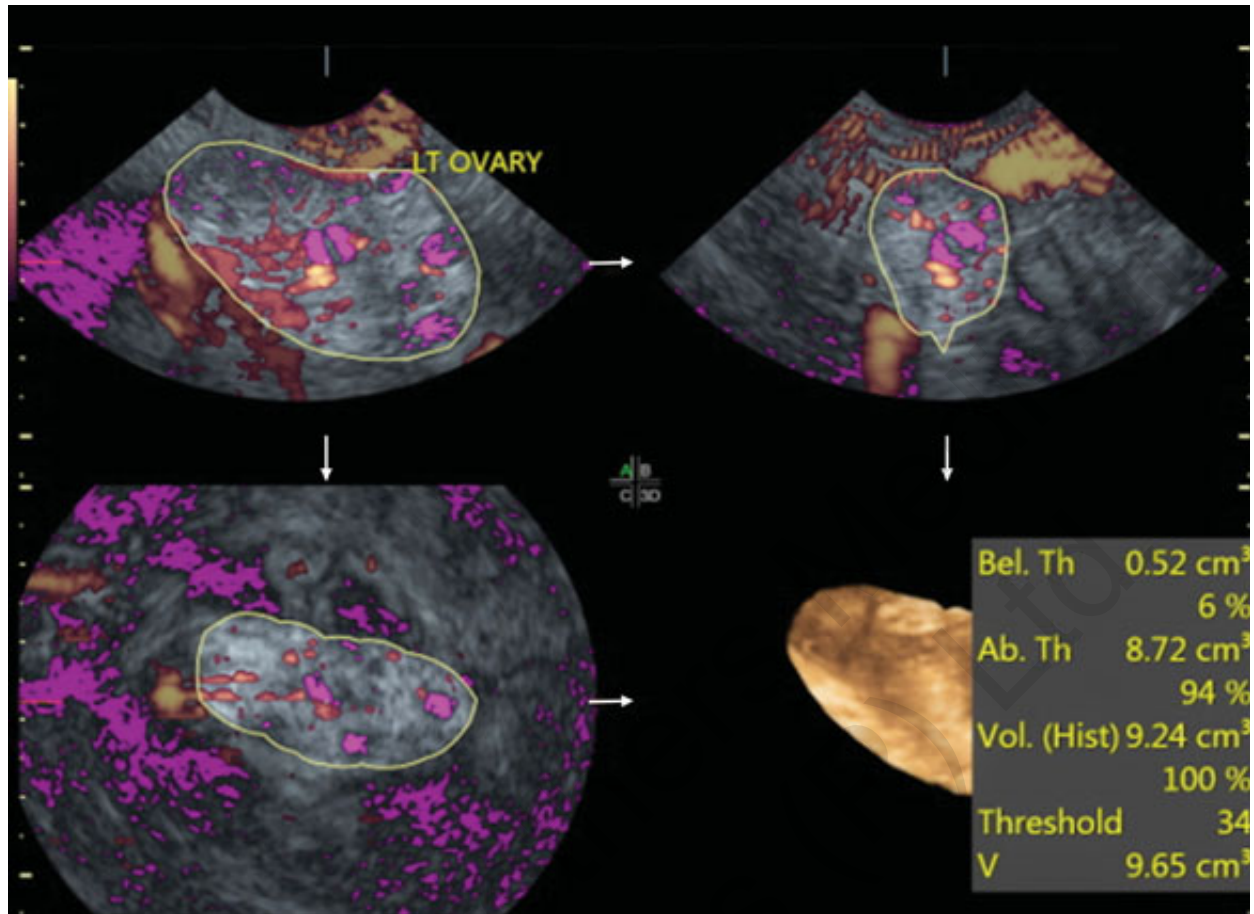
Figure 4: Threshold volume on 3D ultrasound acquired ovarian volume to assess the stromal volume.

- Those who had low stromal peak systolic velocity (PSV) in the early follicular phase were poor responders.[4] This means patients who have more LH, have better vascularity, they are better responders and do not require addition of LH during ovulation induction
- High LH in PCOS patients leads to exposure to follicles to LH when these are still not dominant and leads to atresia of these follicles and conversion of granulosa cells to theca cells in dominant but not mature follicles. Both these may be seen in patients with oligoanovulation and lead to increase in stroma
- Stromal abundance means high LH. This can be evaluated by 3D ultrasound, with threshold volume software (Fig. 4)
- In the preovulatory period, increasing LH level correlates with the rising perifollicular PSV. This means in a mature follicle, high PSV indicates that the surge has already started
- Premature LH surge may lead to hyperechoic follicular wall with increase in resistance index (RI) of the perifollicular vessels (Fig. 5)
- It acts on theca cells to produce androgen and progesterone from cholesterol. Inadequate LH receptors or inadequate LH levels may lead to inadequate progesterone production and luteal phase defect. This is suggested by scanty corpus luteal vascularity with high RI.

Figure 5: Luteinized follicle showing hyperechoic walls.

ESTROGEN

- It is produced by granulosa cells of growing follicles and its rising level can be correlated with the low resistance perifollicular vascularity
- Growing thickness of the endometrium, cervical mucous, and increasing endometrial vascularity are other

7
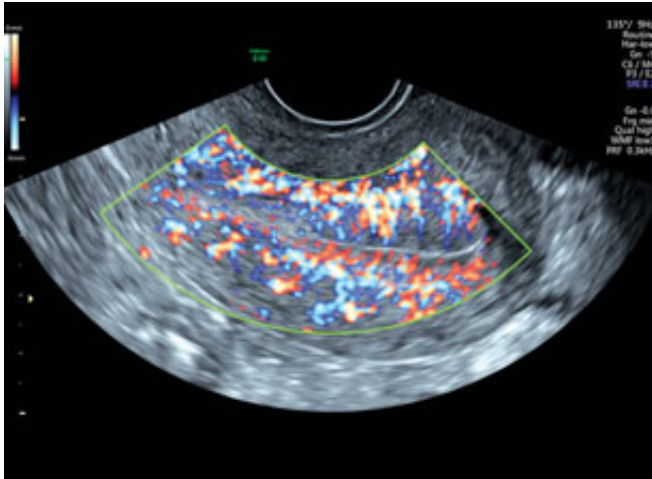
Figure 6: Preovulatory endometrium with intraendometrial flow seen on Doppler.

Figure 7: Early secretory endometrium.

evidences of increasing estrogen in the proliferative phase (Fig. 6)

- Low estrogen due to immature follicle leads to scanty and/or high resistance flow in endometrium.

PROGESTERONE

- In early proliferative phase thick endometrium with residual corpus luteum that shows flows, are indicators of residual active corpus luteum
- Preovulatory progesterone rise is suggested by fluffy outer margins or hyperechogenicity of endometrium in preovulatory phase (Fig. 7)
- It is secreted from the corpus luteum under the influence of LH from theca cells. A clear correlation between RI of corpus luteum and plasma progesterone levels has been seen in natural cycle. RI of the corpus luteum can therefore be used as an adjunct to plasma progesterone assay as an index of luteal function[5]
- High resistance flow in corpus luteum suggests low progesterone levels and luteal phase defect
- Low progesterone also leads to increased resistance on spiral vessels.

CONCLUSION

Hormonal changes occurring day to day during the menstrual cycle reflects as morphological and vascular changes in the ovary and the uterus. Assessing these changes by transvaginal ultrasound, Doppler, volume ultrasound, and correctly interpreting can explain the hormonal basis of these changes.

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Fancy Gadgets in Total Quality Management

Sudesh A Kamat, Keshav Malhotra

INTRODUCTION

We cannot imagine today's world without assisted reproductive technologies (ART), and after decades of innovations and discoveries ART is still going strong. An estimated eighty million people suffer from infertility and this figure is growing, simultaneously the number of clinics offering infertility services in the world are also growing. At present, there a few checks involved in setting up ART clinics and because of this factor the concept of quality management has emerged.

On July 25, 2016, Louise Brown, the first test tube baby born in Oldham General Hospital, Great Britain, turned 38. Her *in vitro* conceived sister Natalie is already 34-years-old. From that time until now, several million children all over the world have been conceived by *in vitro* fertilization. There are an increasing number of couples who are offered ART, IVF above all, as a choice for treating infertility. This is why organizing a quality management system in the ART domain is of high priority and interest. Namely, a quality management system, which provides quality of service, professional attitude, and a positive outcome of the procedure, is of primary interest for the client/patient. A management system that is well organized through a standardized work process and brings appropriate financial reimbursement is a priority for the institution and staff involved in this process.[1]

As we all know quality in an ART clinic is multifactorial, it is hugely dependent of several factors and requires a well-oiled system to produce consistent results in the long run. Temperature, pH, and osmolality are the cardinal factors that affect ART outcomes in the laboratory.

TEMPERATURE

Temperature control for oocytes is crucial. Meiotic spindles are highly sensitive to temperature fluctuations. Abnormal spindles may result in aneuploidy, disruption of embryo development, and implantation; decreased pregnancy rates,

thus making temperature control one of the most crucial elements in lab quality assurance. One of the most crucial areas where temperature loss can occur is the incubator. Conventional box incubators have been researched in detail and studies have reported wide variations that occur in the incubator even when it is closed.

When the lid is opened the temperature drops further and recovery time can be quite a tricky situation to handle when the number of cases being done in the lab are high and the equipment is not adequate enough to support it. Large incubators with multiple door openings will affect temperature—recovery may take up to 30 minutes. Increased door openings—temperature and loss—increased miscarriage rates.[2]

To counter this newer bench top incubators have emerged which provide a faster recovery and a more uniform heating.

Another place where temperature fluctuations are quite common is the micromanipulator. Studies conducted by Cambridge IVF have shown that there is quite significant temperature loss which occurs during Intracytoplasmic sperm injection (ICSI), if caution is not observed. Spindle imaging (Fig. 1) has shown that when ICSI is done on a stage without the objective heater the spindle was only observed 61.4% of the times and clinical pregnancy rates were significantly different form the eggs which were injected over an objective heater.

They also compared outcomes from various heating stages available, i.e., the heated stage with aperture, the heated glass stage and the heated stage with aperture and an air warming system.

It was observed that the stage with the air warming system had the most stable temperature and, thus, it maybe concluded that this system is more suited for manipulation. Still further studies are need to come to a definitive conclusion.

pH

pH is vital for embryo development (Fig. 2) more so, it is even more vital for oocyte viability. The oocytes have a negligible
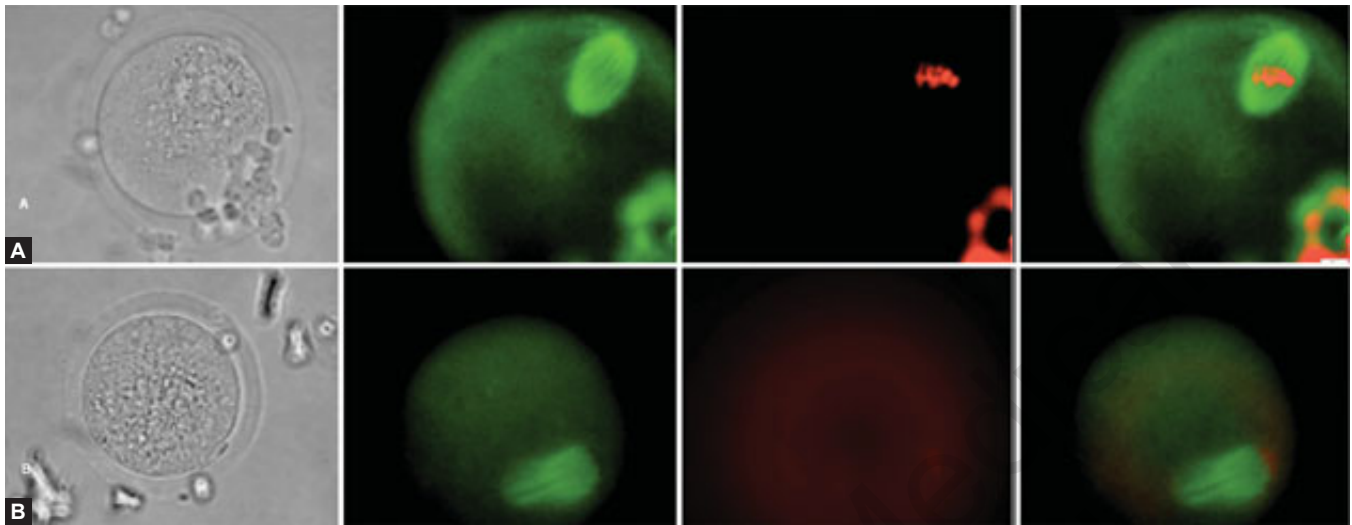
Figure 1: Spindle imaging. **A,** While using a heated stage; **B,** Without a heated stage.
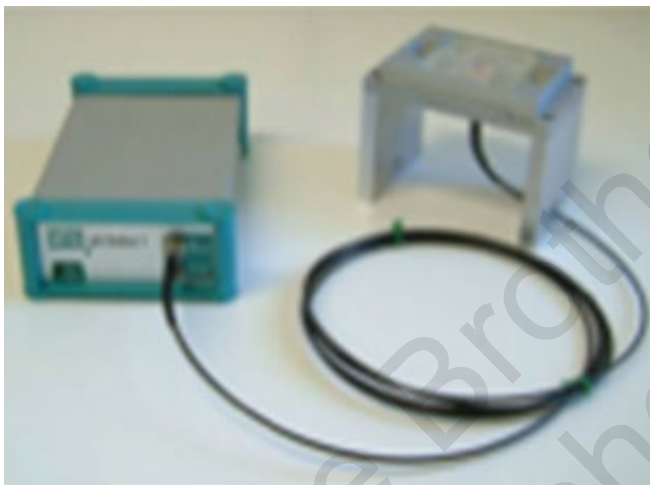
Figure 2: Online ph monitoring system.

TABLE 1: Ph range for embryo development

Developmental stage	pH Range–Lower	pH Range–Higher
Fertilization	7.3	7.4
Cleavage stage	7.2	7.3
Blastocyst	7.3	7.4

margin of tolerating pH fluctuations (Table 1) which gives monitoring pH paramount importance. Various devices are available for measuring pH ranging from pH strips to digital pH meters and the new online pH monitoring systems. Most of the new incubators come with an option of online pH monitoring which is an accurate reliable time efficient way of monitoring pH in your laboratory.

OSMOLALITY

The third cardinal parameter in the list carries equal importance. The solute concentration in the media can exert osmotic pressure impacting on cell volume and stressing the developing embryo. Negative impact of osmolality increase

(>310 mOsm/kg) on embryo development. But with certain precautions osmotic changes can be avoided, like proper dish preparation techniques, humidified incubators, oil overlay etc. Osmometers are available to monitor osmolality but it is a tedious process and implementation is a busy laboratory is quite difficult. It is a great tool to monitor osmolality when preparing media in-house but when buying standardized medias proper precautions eliminate the need for regular osmolality monitoring.

ENVIRONMENT

Culture environment and lab environment are the next big things to check. Hyslop et al.[6] compared environmental conditions between a unique chain of enclosed workstations with incubators (temperature and CO_2 controlled) set at air temperature 35.0°C and hot plate at 37.2°C and open stereomicroscope in a conventional class II cabinet with heated surface set at 37.4°C.

To avoid these fluctuations closed chambers are available which provide incubator like conditions in your work station. Imagine doing ICSI in your incubator. Apart from these newer time-lapse incubators are also capable of providing constant culture environment eliminating the need for daily exposure.

Air Quality

Legro et al. 2010 noted the effects of declining air quality on reproductive outcomes after IVF are variable but increased nitrogen dioxide (NO_2) is consistently associated with lower

TABLE 2: Temperature and pH fluctuations in an open and closed system

Time	Open system		Closed system	
	Temperature	pH	Temperature	pH
0 mins	36.0°C ± 0.1	7.31 ± 0.01	36.5°C ± 0.1	7.3 ± 0.01
3 mins	35.2°C ± 0.3	7.38 ± 0.02	36.6°C ± 0.1	7.33 ± 0.04
20 mins	35.2°C ± 0.3	7.55 ± 0.02	36.6°C ± 0.1	7.33 ± 0.04

live birth rates.[5] Fine particulate matter (PM 2.5) in the IVF laboratory during embryo culture was associated with decreased conception rates. United States Environmental Protection Agency (US EPA) considers significant risk to humans and fertility following exposure to certain chemicals, volatile organic compounds (VOCs) and PM.

We know that VOCs are the main culprits in the Embryology Lab, but that shouldn't deviate us from ignoring presence of other PM like dirt, dust, smoke, etc. A common modality found in most IVF laboratories is high efficiency particle air (HEPA) filtration, but one must understand the HEPA can only remove infectious agents and particles above 0.3 μm. To cover VOCs an added security blanket of an activated carbon filter and a potassium permanganate filter is required.

Media and Consumables

The quality gadgets don't just cover the ART laboratory but also extend to the supplies department. The most important aspect in supply management is ensuring cold chain is maintained throughout. Most companies nowadays supply data logging devices which help us in ensuring that quality products are delivered to our laboratories without fail.

CLEANING

Cleaning is another aspect where new or even older technologies are now resurfacing.We have pretty much moved away from alcohol being used in the laboratory and are now mostly using quaternary ammonium compounds (Fig. 3) which are VOC free and quite reliable. But some of us are going back to using steam cleaners which are quite an effective VOC free, safe and easily available tech to use in the lab.

Safety and Security

There are numerous risks that an Embryology lab has to deal with. Potential fatal ones are storage risks, mixups and staff/

Figure 3: VOC free compounds.

patient safety. The age of automation is upon us and the practice of using the double witnessing system which still has the risk of human error is now being replaced. Automatic witnessing devices are available which use radio-frequency identification technology and can help in preventing fatal mishaps from occurring in the laboratory. Also integrated alarm systems are available for the incubators and liquid nitrogen Dewars which ensures that all incidents are documented and reported to the concerned staff immediately.

DATA MANAGEMENT

In this age of computers and internet, age old method of documentation by hand have lost their worth. Newer data management solutions ensure that each step is documented without wasting time and storage space in the clinic. The software also manage appointments and staff scheduling and have digital signatures which makes accountability very easy. And apart from being ecofriendly they also have options of cloud based record storage which can eliminate the need for creating large storage units for filing.

CONCLUSION

Thus to conclude, we have definitely entered the age of automation and there are a variety of gadget available to us which can ensure better quality of care. The only problem that arises is the cost effectiveness of such solutions. In government funded setups buying such equipment is easy but in private clinics such equipment can be a huge financial investment burden. Also utilizing these will directly as well as indirectly increase the cost for the patients which in a country like ours is not feasible. Unless the government steps in and offers funding to infertile patients, the access to such technologies will be quite difficult. In this age of optimization, we need to find a cost effective way if offering the best quality without compromising on the standard of patient care and still running a viable clinic.

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Absolute Asthenozoospermia

Diksha G Sharma, Rishabh Bora

DEFINITION

Asthenozoospermia is a common cause of male infertility characterized by reduced motility or absent sperm motility in the fresh ejaculate. Absolute asthenozoospermia means 100% immotile spermatozoa.

- Frequency of 1 in 5,000 men
- Low fertilization rate after intracytoplasmic sperm injection (ICSI) with randomly selected immotile spermatozoa especially when using ejaculated spermatozoa.[1]

ETIOLOGY

- Associated with higher follicle-stimulating hormone levels indicative of certain degree of testicular failure
- Ultrastructural defects in the sperm flagellum: Immotile but viable sperms
- Immotile cilia syndrome: Uncommon autosomal recessive disorder with immotile microtubules of ciliated cells and spermatozoa. Absent dynein arms or abnormal microtubules
- Immotile sperms with situs inversus: Kartagener's syndrome
- Necrozoospermia: 100% dead sperms; reactive oxygen species can penetrate the cell and induce degenerative processes in the nucleus resulting in DNA damage[2]
- Genital infections, oxidative stress, cryopreservation, metabolic disorders, environmental pollutants exposure, and delayed epididymal transport
- Hypogonadotropic hypogonadism
- Prolonged periods of an ejaculation
- Antisperm antibodies.

DIAGNOSIS

Care should be taken to examine the semen samples at 37°C and proper abstinence because lower temperatures may affect motility and rarely may cause immobilization. Important to assess a second semen sample and to confirm the diagnosis.

- Immotile cilia syndrome: Family history and transmission electron microscope shows absent dynein arms
- Necrozoospermia: Vitality testing
- Antisperm antibodies: Mixed antiglobulin reaction test, immunobead test
- Infections (chronic prostatitis): Urogenital examination, cultures and transrectal ultrasonography
- Exposure of ejaculate to cold, spermicides: Medical history
- Hypogonadotropic hypogonadism: Hormonal analysis.

TREATMENT

Treat underlying cause. ICSI after sperm selection is treatment of choice.

Testicular sperm extraction (TESE) may also be considered in men with a normal testicular function whenever only a limited proportion of ejaculated sperm are alive because TESE will yield more viable sperm.

Host Test

Live spermatozoa with normal membrane function show swelling of the cytoplasm and curling of the tail due to water influx when exposed to hypo-osmotic conditions. Solution composed of 50% isotonic medium and 50% distilled water was used by Verheyen et al. for selecting sperms.[3]

Mechanical Touch Technique

Evaluating the flexibility of sperm tail by touching it with ICSI pipette; reliability depends on expertise of embryologist.

Figure 1: Electron microscopy of sperm axonemes. **A,** A normal axoneme; **B,** Electron microscopy showing absence of outer and inner dynein arms in a patient with 100% immotile spermotoza; **C,** Electron microscopy showing a 9 + 0 syndrome.
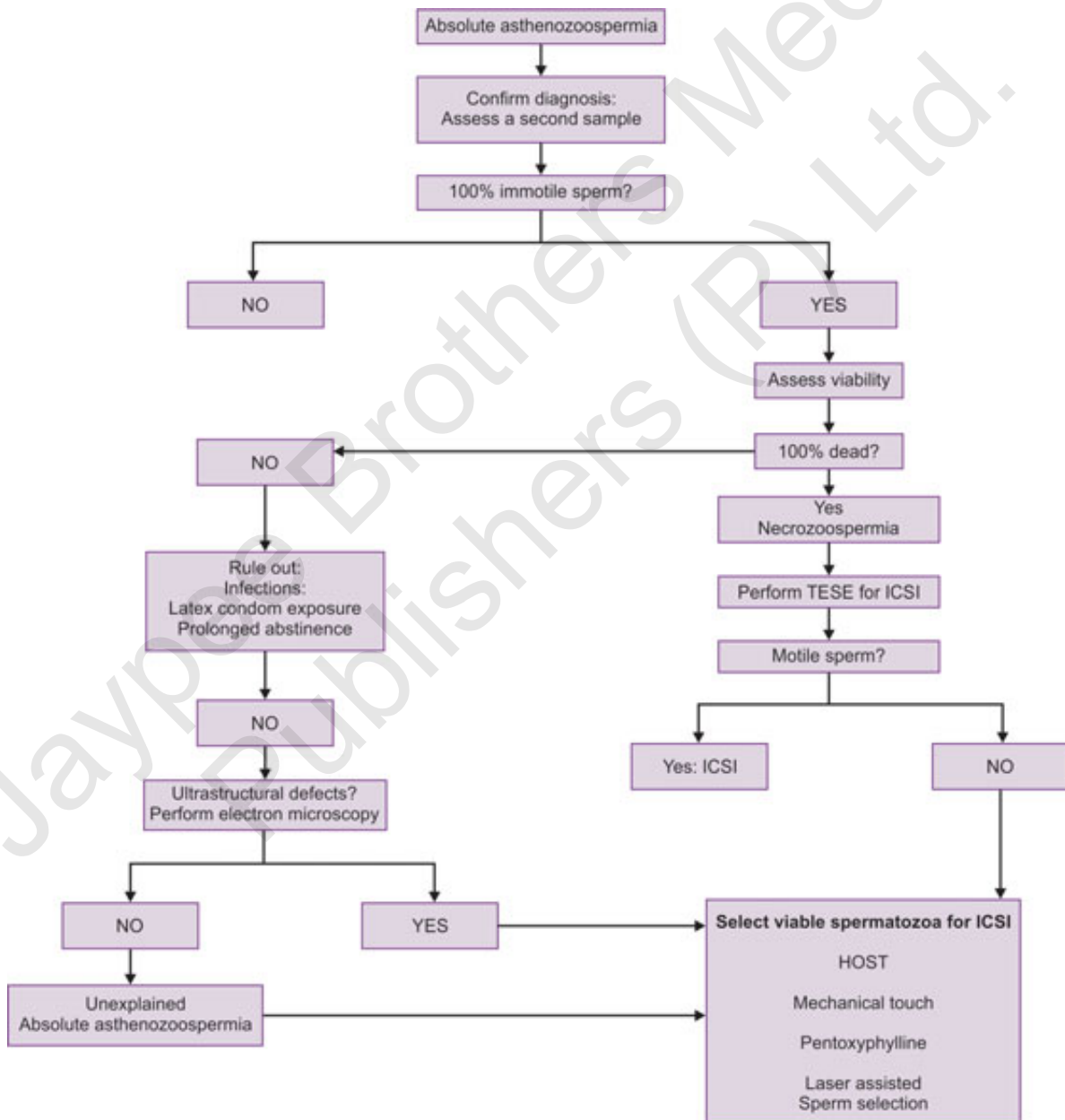
TESE, testicular sperm extraction; ICSI, intracytoplasmic sperm injection.

Flowchart 1: Workup in absolute asthenozoospermia.

Pentoxyphylline

- Improves sperm motility by increasing intracellular cyclic adenosine monophosphate (cAMP)
- Short exposure of sperm to pentoxifylline (PTX) followed by sperm washing prevents embryotoxicity.

Laser-assisted Sperm Selection

Viable spermatozoa show curling reaction of the tail to application of laser shot close to the tip of the sperm tail using a noncontact 1.48 mm diode laser system.

Birefringence-polarization Microscopy

Polarization microscopy shows viable sperms as having birefringent head and mid piece, and using these improves pregnancy rates.[4]

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6

CHAPTER

Advances in Sperm Selection

Keshav Malhotra

INTRODUCTION

In the year 1992, a landmark paper by Williams et al. showed the world that there was a dramatic reduction in the number of sperms recovered from the fallopian tube when artificially inseminated. This reduction clearly brought about a change in the thought process at that time and researchers were now focusing on the physiological barrier or the natural selection that the sperm has to undergo in order to fertilize the oocyte.[1] These physiological barriers include the acidic pH of the vagina, cervical mucus, response to changes in the uterine environment going from the uterus to the tubes, the binding of the sperms to the zona pellucida, etc. These mechanisms selectively reduce the amount of sperms reaching the egg and fertilizing it, but the question now is how to make the sperm selection equivalent to the natural, and how to improve the existing modalities, so as to select the single best sperm from the millions that are ejaculated.

Even though in vitro fertilization in now 4 decades old, we still do not understand the how effective these sperms really are and most judgments are basically based on microscopic evaluation which is not the best way to judge the potential of the sperm. Also we know for a fact that all motile sperms do not have the potential to fertilize the oocytes and judging the sperms based on motility alone again wouldn't be the best way to select.

Now logically if we try to mimic the natural selection in the IVF laboratory our sperm selection would definitely improve and hopefully would give us better outcomes. In this chapter we will try and cover the newer modalities for sperm selection and how we can implement them in the laboratory.

Sperms can by far be considered as the most specialized cells in the body. They are programmed to do the most important task which is to create life. Different parts of the sperm have different functions during the process of fertilization. The head carries the DNA content which has to be delivered to the egg, the mid piece stores the energy
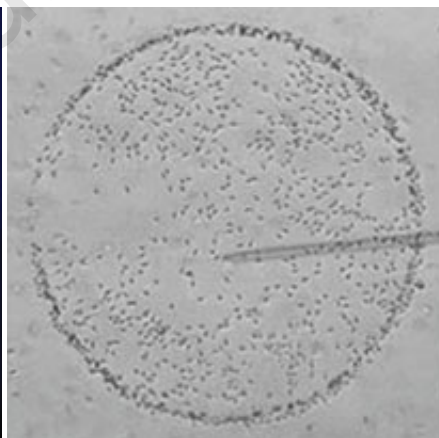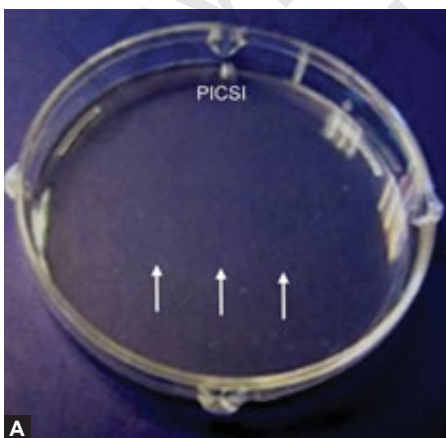
HA-bound sperm:
- Are mature
- Undergo maturation of the sperm plasma membrane
- Have no cytoplasmic residue
- Undergo histone-protarnine-exchange in the nucleus
- Show no DNA-degradation
- Show no acrosomal reaction
- Have normal morphology
- Have low frequency of chromosomal aneuploidies

Figure 1: A, Sperm bound to hyaluronan on physiological intracytoplasmic sperm injection dish; **B,** Properties of hyaluronan bound sperm.

TABLE 1: Review of literature regarding physiological intracytoplasmic sperm injection[3]

	In agreement	Not in agreement
Hyaluronic acid bound supermatoza		
Mature	–	Nijs 2009
Undergo maturation of the sperm plasma membrane	–	–
Have no cytoplasmic residue	–	–
Undergo histone-protamine exchange in the nucleus	Nasr-Esfahani 2008	Nijs 2009
Show no or less DNA-degradation	Nasr-Esfahani 2008, Parmegiani 2009	–
Show no acrosomal reaction	–	–
Have normal morphology	Nijs 2009, Hong Ye 2006, NasrEsfahani 2008, Prinoso/ova 2009	–
Have low frequency of chromosomal aneuploidies	Sanche z 2005	–

	Increased	Decreased	Similar to conventional insemination procedures
Fertilization rate	Nasr-Esfahani 2008	–	Nijs 2009, Hong Ye 2006 (no predictive value), Sanchez 2005, Janssens 2006, Worrilow 2006
Embryo cleavage	–	–	Janssens 2006, Worrilow 2006
Blastocysts	–	–	Worrilow 2006
Pregnancy rate	Worrilow 2006, 2009	–	Nijs 2009 (no predictive value), Nasr-Esfahani 2008
Implantation rate	–	–	Nasr-Esfahani 2008
Miscarriage rate	–	Worrilow 2006, Sanchez 2005	–
Delivery rate	–	–	Nijs 2009 (no predictive value)

creating mitochondria, the tail carries out the flagellar propulsion. Thus, grading and selecting based on these three important components is something which is generally followed worldwide. The current techniques basically depend on selecting the sperm based on motility (swim up) or on the morphology (density gradient) but these do not really mimic any of the natural processes. The sperms can then be chosen by the embryologist on the intracytoplasmic sperm injection (ICSI) manipulator, thus adding to the selection process, but again these modalities are only based on the judgment of the embryologist which is not standardized and varies heavily between users. The newer techniques which have come up and have started to show a positive impact as far as results are concerned are PICSI or hyaluronan binding capacity, intracytoplasmic morphologically selected sperm injection (IMSI)-intra cytoplasmic morphologically selected sperm injection, microfluidics, magnetic activated sperm sorting and omics.

PHYSIOLOGICAL INTRACYTOPLASMIC SPERM INJECTION

The sperm plasma membrane remodeling is an important step in preparing the sperm to fertilize the egg, during this process specific receptors form like the zone pellucid receptors and the hyaluronic acid (HA) receptors, which aid in cumulus binding. The principle behind the use of PICSI is that, when a mature sperm reaches the HA rich cumulus complex surrounding the human egg, it binds and initiates the final fertilization. Only fully matured sperm have developed HA-receptors and can bind to the zone pellucida. Immature sperm cannot bind to HA and are also found to have higher rates of DNA damage and a higher occurrence of carrying abnormal numbers of chromosomes. Thus, patients that have a high DNA fragmentation index or have suffered from recurrent miscarriages or have poor quality of embryos in multiple cycles can benefit from this technique.[2]

McDowell et al., in 2014, carried out a Cochrane review in 2014 which came to the conclusion that more evidence is required to come to a conclusion to whether or not PICSI is actually beneficial or not.[3]

INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION

Humans ejaculate a considerably large number of morphologically abnormal sperms. We have a strict criteria to grade the morphology of the sperm and also to decide on the treatment options for the patients.

Most of the times patients with poor morphology are directed towards an ICSI cycle, justification being that the embryologist can choose the morphologically normal sperms to fertilize the oocyte. However, recent papers have shown that even the sperms which appear normal in ICSI can have multiple defects as shown in figure 2.

Gross evaluation of sperms on ICSI magnification that is 400x is not sufficient to detect subtle aberrations, which according to some have higher chances of transmitting genetic as well as chromosomal diseases.[4,5]

Sperm vacuoles have also been researched and have mostly been associated with a higher DNA fragmentation index, but this association still needs to be proven.[5,6]

Also there is a need to standardize the scoring system with IMSI (Fig. 3), as of now there are four grades in which the sperms can be classified into and according to the current grading, grade 1 and 2 sperms can be injected but again this needs to be more standardized. Cochrane review on IMSI states that there was no significant difference in live birth between IMSI and ICSI, also no significant difference in pregnancy rates or miscarriage rates. However, we need to study the prognosis of an IMSI cycle versus and ICSI
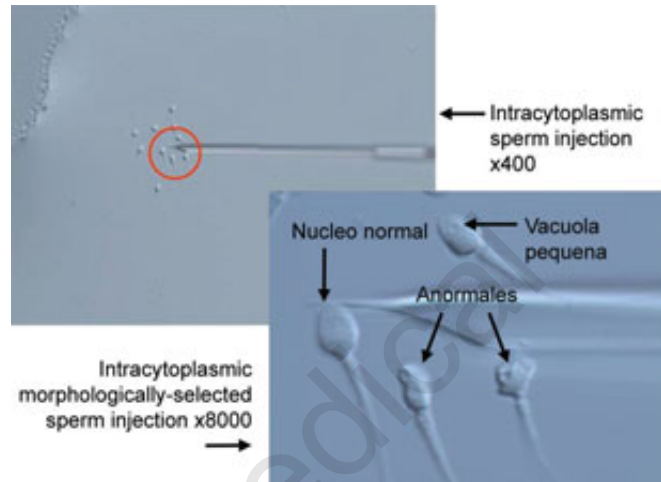
Figure 2: Difference in magnification with intracytoplasmic sperm injection and intracytoplasmic morphological sperm injection.

cycle in patients who actually have poor parameters and then only we would be able to justify the use of IMSI as such (Teixeira DM).[7]

MAGNETIC ACTIVATED CELL SORTING

The spermatogenesis in the adult testes is largely controlled by apoptosis. We already know it for a fact that a large proportion of sperms in the ejaculate are abnormal, now the release of these sperms into the ejaculate is controlled by apoptosis. But numerous studies have shown that multiple sperms show signs of apoptosis once ejaculated. These sperms still have the potential of fertilizing the oocyte but the outcome of an embryo formed by such a sperm is questionable. This is where intracytoplasmic morphological
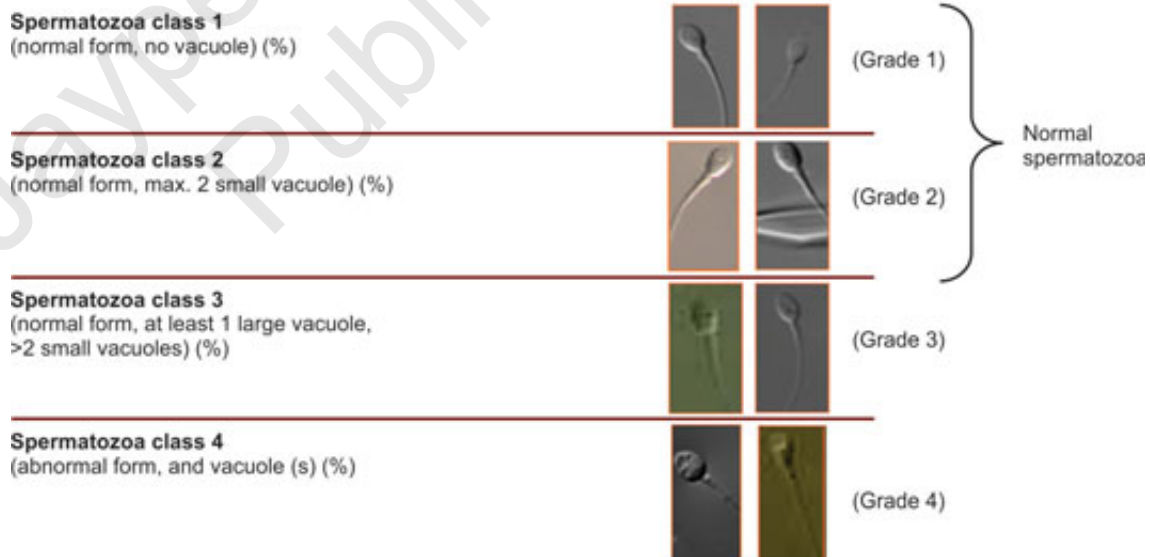
Figure 3: Intracytoplasmic morphologically selected sperm injection sperm scoring.[10]
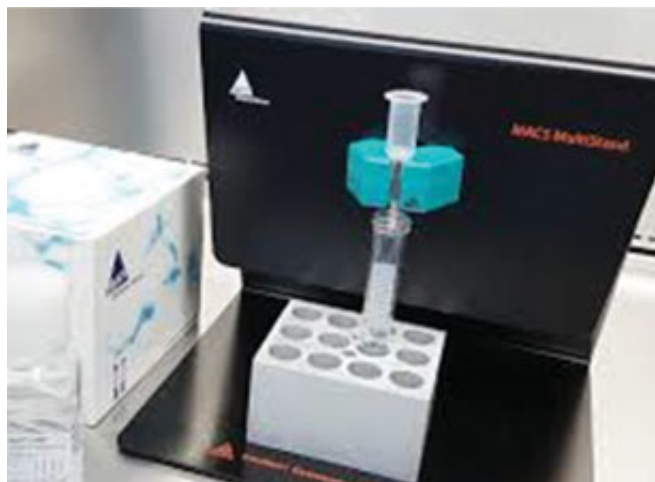
Figure 4: Magnetic activated cell sorting device.

sperm injection (MACS) (Fig. 4) comes into picture. Magnetic activated cell sorting sorts apoptotic sperms from nonapoptotic ones,it uses annexin V which has an affinity to phosphotidyl serine.[8] During apoptosis this phosphotidyl serene is translocated to the outer membrane and thus when mixed in the MACS kit these sperms stick to the magnetic beads which are coated with annexin V. Now these are passed through a magnetic channel, thus removing the apoptotic sperms from nonapoptotic ones.

Application of MACS in IVF:

- Improves fertilization potential
- Increases cleavage and pregnancy rates
- Reduces sperm DNA fragmentation
- Improves live birth rate.

But again more systematic reviews are needed to back these claims.[3]

MICROFLUIDICS

Microfluidics (Fig. 5) is an exciting technology which now takes into consideration the biophysical and biochemical milieu which the sperm encounters in the female reproductive tract. Studies have shown an increase in motile percentage of sperm from below 60–90%, they have also shown better DNA integrity post sorting. Matsuura et al. later showed that a microfluidic sperm sorting system could reduce the treatment time for intracytoplasmic sperm injection.[9,11]

CONCLUSION

Even though the technological advances are occurring at a rapid pace, the biggest challenge that still remains is justifying
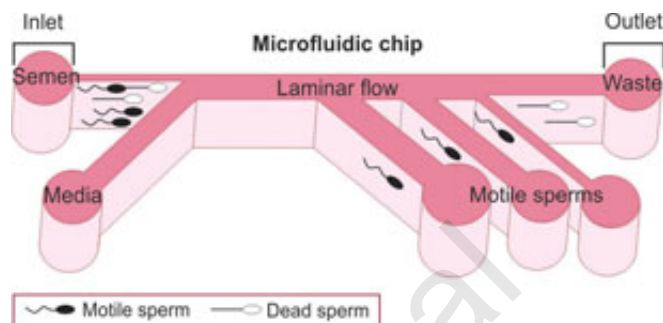
Figure 5: Microfluidic device.

these technologies and proving their efficacy with long term data to support it. Sperm selection brings forth a very exciting new frontier in IVF and it takes us closer to achieving our aim of having a single live birth from a single embryo transfer. It also presents us with various challenges as far as male infertility and its management is concerned.

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7

Moving Toward Individualized Ovarian Stimulation

Susan S George

INTRODUCTION

Ovarian stimulation is an important step in vitro fertilization (IVF). It is aimed at retrieving adequate number of oocytes to develop optimal number of embryos to maximize success. The individualized ovarian stimulation protocol tailored to unique characteristics of a woman is aimed to retrieve optimal number of oocytes and prevent or predict the hyper-response and poor response. This will prevent cancelled cycles caused either by over or poor response. Age was the traditional parameter for predicting ovarian response. The other ovarian reserve markers proved to have prognostic significance in predicting ovarian response are anti-Müllerian hormone (AMH) and antral follicular count (AFC).

Optimum number of oocytes to achieve a pregnancy is suggested to be 8–13 oocytes.[1,2] High and low response are associated with suboptimal outcome due to cancellation, risk of hyperstimulation and poor success. The ovarian reserve markers are useful to plan ovarian stimulation protocols and calculate dose of gonadotropins to get ideal response.

PREDICTION OF RESPONSE AND CLINICAL APPLICATION

The age-related decline in female fecundity and increased risk of miscarriage are related to increased rate of oocyte aneuploidies. The age is a better predictor of quality of oocytes and pregnancy chance compared to other markers, hence it is essential for initial counseling.[3,4]

Considering age as the marker to plan ovarian stimulation protocol is likely to compromise the subset of younger women with accelerated aging and older women with higher ovarian reserve. The Bologna criteria in ESHRE consensus to consider poor response prediction are age, previous low response, abnormal AFC, AMH, and are useful for initial prediction of poor response and counseling.[5]

The AMH produced by the granulosa cells of ultrasonically visible antral follicles correlates with the residual follicular pool and is a good marker of ovarian reserve. AMH is used as a marker for predicting ovarian response and can predict poor and excessive responders.[6]

The AFC measured by transvaginal ultrasound in early follicular phase is shown to predict response to gonadotropins including the poor and over-response.[7]

The low intracycle variability of AMH makes it a more convenient ovarian reserve marker as compared to AFC that needs to be assessed in early part of menstrual cycle. They can be used independently or together to plan ovarian stimulation.

The AMH and AFC are useful to individualize therapeutic strategies of selection of GnRH analogs and fine tuning of gonadotropin dosage.[8] It is suggested to plan agonist cycle in normal responders and antagonist cycles in expected poor and high responders. Algorithms are also derived to calculate gonadotropin dosage by prediction models with variables of age, AMH, AFC, BMI, FSH, etc. and these need more validity before wide application in clinical practice.[8]

Follicle-stimulating hormone (FSH) at high levels may predict poor response and nonpregnancy and is advised to be used as screening test for counseling.[9]

The OPTIMISTIC study reports contradict these data.[10,11] They claimed no improvement in live birth-rates by adjusting dose in poor and hyper-responders. Prevention of severe ovarian hyperstimulation syndrome (OHSS) in hyper-responders is the only advantage in individualized dosing. But in the era of agonist trigger, this advantage is questionable. Individualized dosing based on AFC did not improve live birth-rates.[12]

CONCLUSION

Individualization of ovarian stimulation protocols help to achieve adequate ovarian response and prevent over

response. Predicting over response help to prevent complications such as OHSS. Poor response prediction is important for adequate counseling and planning higher gonadotropin dosages. The individualized treatment regimes help to optimize success, better clinical efficacy and reduce treatment burden. Ovarian reserve markers AMH and AFC can be applied together or independently, depending upon ease of availability and cost involved.

In the light of OIPTIMISTIC study reports, these advantages seem to be questionable. One has to apply the use of individualized stimulation regimes based on clinical setting and aim to optimize success, prevent complications, without additional economic and physical burden on couple. Counseling of poor prognosis women is important. These women can make informed decision to embark on IVF especially then they have financial constraints.

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8
CHAPTER

New Concepts in Tackling Poor Responders

Priya B Chittawar

"Any fool can hope when success lies plainly in view. It wants genuine strength to hope when matters are hopeless."

-Michael F Flynn, Eifelheim

INTRODUCTION

Howard and Georgeanna Jones pioneered the concept of using ovarian stimulation with gonadotropins for couples undergoing *in vitro* fertilization (IVF) in the 1980s. Subsequently, it became the standard practice and the women undergoing controlled ovarian stimulation for IVF were classified into normal responders, hyper responders, and poor responders based on the number of oocytes obtained after administering appropriate dose of gonadotropins. Up to one-third of women fall in category of poor responders (9–24%) and it is vexing problem for the clinicians to optimize their outcome (Box 1).[1,2]

DEFINITION

Historically there have been more than 40 definitions of poor responders in literature. Age, antral follicle count, anti-Müllerian hormone (AMH) levels, number of follicles retrieved and number of oocytes retrieved, have all been used singly or in combination with varying thresholds to define this subgroup. This created a lot of heterogeneity in the data from various trials and meaningful synthesis was not possible.

Spurred by this, European Society of Human Reproduction and Embryology (ESHRE) organized a consensus meeting at Bologna in 2011 to arrive at a standard definition of poor responders (Box 2).[3]

There has been debate on the Bologna criteria for poor responders, as they constitute a heterogeneous group: a 40-year-old with normal AMH is different from a young 25-year-old with low AMH. Also the first criteria "any other risk factor for POR" is not described by the criteria. There is a need to list the various conditions, which puts the patient at high risk of POR.

Another more detailed classification has emerged from the POSIEDON group (Patient-Oriented strategies Encompassing Individualized Oocyte Number)[5] (Boxes 3 and 4).

POSEIDON classification is defines a separate category of suboptimal responders who yield 4–9 oocytes in addition to poor responders (<3 oocytes) and takes into account the age (important marker of oocyte quality) in addition to response to ovarian stimulation and ovarian reserve tests (ORTs). It is

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BOX 1	Response to controlled ovarian stimulation for *in vitro* fertilization

- Poor responders: <3 oocytes retrieved on conventional stimulation
- Suboptimal responders: 4–9 oocytes retrieved on conventional stimulation
- Normal responders: 10–15 oocytes retrieved on conventional stimulation
- Hyperresponders: >15 oocytes retrieved on conventional stimulation

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BOX 2	ESHRE consensus on Definition of Poor Ovarian Response[4]

According to the ESHRE consensus on the definition of POR, at least two of the following three features must be present to label a patient as poor responder:[3]

- Advanced maternal age (≥40 years) or any other risk factor
- A previous poor ovarian response (cycles cancelled or ≤3 oocytes with a conventional protocol)
- An abnormal ovarian reserve test (ORT) [antral follicle count (AFC) <5–7 follicles or anti-Müllerian hormone (AMH) <0.5–1.1 ng/mL]

(In the absence of advanced maternal age or abnormal ORT, two previous episodes of poor ovarian response after maximal stimulation are sufficient to define a patient as a poor responder)

BOX 3	POSIEDON classification

Introduces two new categories of impaired response:

a. A "suboptimal response," defined as the retrieval of four to nine oocytes, which is associated, at any given age, with a significantly lower live birth rate compared with normal responders, i.e., those with 10–15 oocytes

b. A "hyporesponse," in which a higher dose of gonadotropins and more prolonged stimulation are required to obtain an adequate number of oocytes (more than three)

Combines "qualitative" and "quantitative" parameters, namely:

a. The age of the patient and the expected aneuploidy rate

b. Biomarkers and functional markers (i.e., AMH and AFC)

BOX 4	POSIEDON Classification subtypes

- Group 1a: Young patients <35 years with adequate ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/dL) with an unexpected poor or suboptimal ovarian response
- Group 1b: Old patients >35 years with adequate ovarian reserve parameters (AFC ≥5, AMH ≥1.2 ng/dL) with an unexpected poor or suboptimal ovarian response
- Group 2a: Young patients <35 years with poor ovarian reserve parameters prestimulation (AFC<5, AMH <1.2 ng/dL)
- Group 2b: Old patients >35 years with poor ovarian reserve parameters prestimulation (AFC <5, AMH <1.2 ng/dL)

useful as age and oocyte quality are important in terms of quality of oocyte while ORT predict oocyte quantity.[6]

ETIOLOGY OF POOR RESPONSE TO OVARIAN STIMULATION

- Reduced ovarian reserve: Ovarian reserve is the total number of preantral and antral follicles in the ovary and reflects the biological ovarian age of a woman. Ovarian reserve declines with increasing age and about 13 years prior to menopause, there is an accelerated decline in the quantity of remaining oocytes in the ovary (Fig. 1). The best oocytes are ovulated first in the lifetime and with advancing age, the quality and quantity of the oocyte pool reduces.[7] This is reflected in the higher
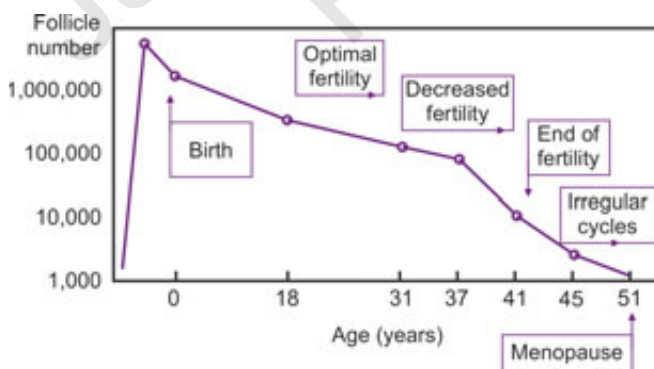
Figure 1: Ovarian reserve and aging.

gonadotrophin requirement, lower peak estradiol values, less number of follicles and oocytes obtained in women with reduced ovarian reserve. The best way to determine ovarian reserve is to quantify the antral follicle count on ultrasound on day 2 or 3 of menstrual cycle and the estimation of AMH levels in the serum

Women who have a poor ovarian reserve are expected to respond poorly to ovarian stimulation in IVF cycles

- Follicle-stimulating hormone (FSH) receptor polymorphism: FSH is the hormone, which is responsible for folliculogenesis. It acts by binding to the FSH receptor at the target organ, which is guanine nucleotide-binding proteins (G proteins) coupled receptor. Different types of mutations in the FSH receptor gene located at chromosome 2, including point mutations, single nucleotide polymorphisms and spliced variants. The inactivating type of mutations result in reduced response to exogenous FSH and result in a poor response to ovarian stimulation. Various types of inactivating point mutations have been described including c.566C>T, c.573A >T and c.662T>G

- Luteinizing hormone (LH) deficiency: LH is a hormone of the luteal phase and is responsible for the LH surge, which leads to final follicular maturation and rupture and in the maintenance of the luteal phase. However, it has a role in the estrogen biosynthesis in the follicular phase. Under the influence of LH, the theca cells convert the cholesterol into androstenedione, which is then aromatized to estrogens in the granulosa cells. Evidence suggests that women with hypogonadotropic hypogonadism definitely benefit from adding LH in the follicular phase. LH deficiency is postulated to reduce androgen levels which in turn reduce granulosa cell sensitivity to FSH which in turn predisposes to poor ovarian response to stimulation. However, the role of LH in poor responders in contentious and more evidence is needed to elucidate the role of LH in ovarian response

- Intraovarian paracrine and autocrine factors.

INTERVENTIONS FOR POOR RESPONDERS

- Precycle adjuvants (androgens/arginine/coenzyme Q): Folliculogenesis in human ovary has two phases, a gonadotropin independent phase and a gonadotropin dependent phase. The gonadotropin independent phase requires almost 300 days and includes the growth from a primordial follicle, till it reaches the stage of antral follicle (2–10 mm size) at which it is visible on ultrasound and acquires sensitivity to FSH

Precycle adjuvants are targeted at this gonadotropin independent phase and aim to optimize the quantity and quality of antral follicles at the beginning of stimulation. The dosage and administration of precycle adjuvants is summarized in table 1. None of the adjuvants have shown improvement in the clinical pregnancy and live birth rates in poor responders
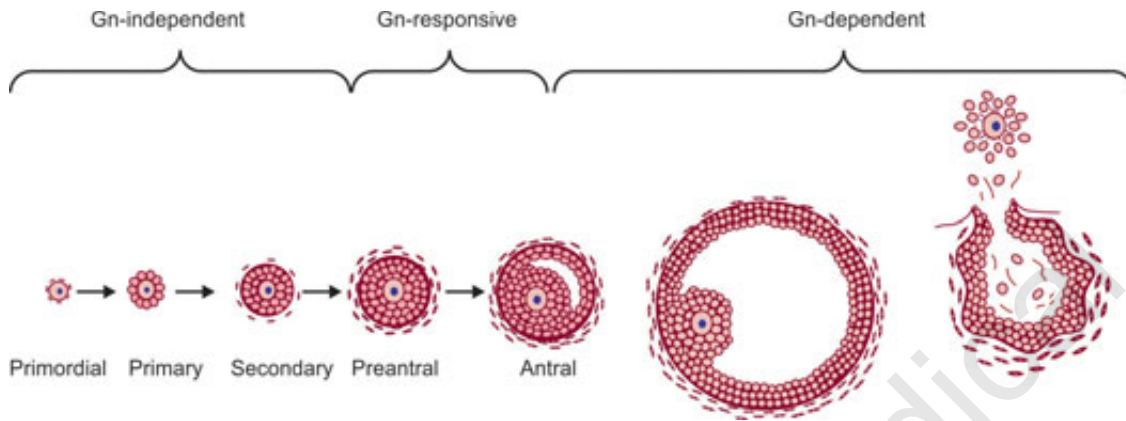
Figure 2: Different stages of ovarian folliculogenesis.[8]

TABLE 1: Dosage and rationale of precycle adjuvants

Type of adjuvant	Duration of administration	Rationale	Evidence
Dehydroepiandrosterone acetate	25 mg three times a day three months prior to start of stimulation	Improves FSH sensitivity of follicles by increasing expression of FSH receptors, increased intraovarian androgens	No benefit in improving clinical pregnancy rates (evidence level 1a)
Testosterone gel	5 mg/day of 1% gel transdermally 2 months prior to start of stimulation	Increased intraovarian androgens leading to increased follicular sensitivity of follicles to FSH	No benefit in improving clinical pregnancy rates (evidence level 1a)
Coenzyme Q	200 mg three times a day for 60 days prior to stimulation	Improves mitochondrial function in oocytes and improves embryo quality	Not enough evidence
Arginine	1 g twice a day	Being nitric oxide donor, postulated to improve follicular microenvironment	Not enough evidence

FSH, follicle-stimulating hormone.

TABLE 2: Alteration in controlled ovarian stimulation cycle

Intervention	Rationale and method	Evidence
Choice of down regulation protocol	1. Use of antagonist protocol 2. Use of short/flare/microdose protocol	No evidence that any particular protocol is superior to other
Using higher dose of gonadotropins	Using 600 IU of FSH versus conventional dose (225–300 IU)	No evidence that increasing dose improves LBR
Oral ovulogens: Letrozole	Using letrozole + gonadotropin protocol (increased local androgens due to blockade of aromatase and improved FSH sensitivity of follicles)	Some evidence to suggest improved LBR
Oral ovulogens: Clomiphene citrate	Using alone or with gonadotropin (LH also rises with FSH rise, this improves steroidogenesis, FSH sensitivity of follicles and better luteal phase)	Not enough evidence
Modified natural cycle	Using MNC to accumulate oocytes or embryos	Not enough evidence
Estradiol pretreatment	2–4 mg estradiol in late luteal phase to synchronize follicular cohort and prevent early selection of dominant follicle	Not enough evidence

LBR, low birth rate; MNC, modified natural cycle.

- Alterations in controlled ovarian stimulation (COS) protocol: Analogs/higher dose/oral ovulogens like letrozole/modified natural cycle/estradiol pretreatment
  Every month a cohort of antral follicles is recruited which are rescued from atresia by administration of ovarian stimulation. The interventions in the COS protocol aim to maximize the quality and quality of this recruited cohort
  The alterations in COS cycle for poor responders have been summarized in table 2

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TABLE 3: Adjuvants during controlled ovarian stimulation

Adjuvant	Rationale	Evidence
Growth hormone	• 4–24 IU/day from day of start of stimulation to the day of trigger • Acts via IGF-1 pathway and directly to improve follicular microenvironment and oocyte quality	No improvement in live birth rates (Grade 1a)
Pyridostigmine	60 mg twice a day during stimulation. Postulated to increase endogenous growth hormone levels	Does not improve Live birth rates
Luteinizing hormone	• 75–150 IU/day from day 2 of stimulation or from mid follicular phase • Improves intrafollicular androgen levels and expression of FSH receptors on granulosa cells. Improves estradiol synthesis	Not enough evidence in poor responders

IGF-1, insulin-like growth factor-1

- Adjuvants during COS: Pyridostigmine/growth hormone/luteinizing hormone (Table 3)
- Lab interventions: Oocyte and embryo accumulation/PGT/cytoplasmic/autologs mitochondrial transfer. Almost all the adjuvants and alterations in stimulation protocols have not yielded the expected improvement in results in poor responders. It is clear that the problem is in the quantity and quality of oocyte and research has now focused on harvesting oocytes in more than more cycle and then freezing them. The intracytoplasmic sperm injection (ICSI) is then carried out and embryos transferred. There is interest also in mitochondrial transfer to improve the oocyte quality but research is limited due to ethical issues in heterologous mitochondrial transfer

  Preimplantation genetic testing has had limited success in this subgroup as the number of embryos available to test are limited.

CONCLUSION

Poor responders are a challenging group to treat. They are a heterogeneous group and future research should focus on specific subtypes of poor responders based on age/AMH levels/previous ovarian response. The data that emerges could result in meaningful insights in this group of patients.

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Insight into Adjuvant Use In *In Vitro* Fertilization

Diksha G Sharma

INTRODUCTION

Adjuvant therapies are used in *in vitro* fertilization (IVF) as an addition to standard regimes mainly to improve the implantation, optimize the ovarian response or reduce the complications. Clinicians while being faced with distraught couples willing to try anything, must weigh the evidence well and balance the proposed increase in pregnancy outcomes with the associated risks.

These adjuvants can be classified as:

- Adjuvants in poor responders
- Adjuvants in hyper-responders
- Adjuvants to improve implantation
- Adjuvants in the lab.

ADJUVANTS IN POOR RESPONDERS

Dehydroepiandrosterone

Mechanism of Action

- Increase in insulin-like growth factor 1 (IGF-1) which improves follicular response and oocyte quality
- Regulation of luteinizing hormone (LH) stimulated androgen production
- Increases follicle-stimulating hormone (FSH) receptors leading to increase in preantral and antral follicles via intraovarian androgen receptors.

Various authors have reported an improvement in anti-Müllerian hormone (AMH), antral follicle count, mature oocytes and pregnancy rates with dehydroepiandrosterone (DHEA) administration in poor responders. While several authors like Narkwichean et al. 2013 and Bosdou et al 2012 have not found sufficient evidence to support its use in women with diminished ovarian reserve. Recently published meta-analysis by Lin et al. demonstrated significantly increased clinical pregnancy (OR1.45) and live birth rates with DHEA administration.

Adverse Effects

Reports of acne, deepening of voice, excess hair growth.

Growth Hormone

Mechanism of Action

- Work via increased IGF-1 and 2 levels
- Enhances ovarian response to FSH
- Increase DNA repair ability of oocytes.

Cochrane review published in 2010 has shown significant improvement in live birth rates and clinical pregnancy rates with growth hormone (GH) administration in poor responders. Most studies are small and have heterogeneous definitions of poor responders. Recent meta-analysis by Roger Hart et al. did demonstrate a benefit for the use of the adjunct GH, with a reduction in the duration of ovarian stimulation and greater number of oocytes retrieved without evidence of an increased chance of a live birth.

Dose

Varied doses reported in different studies, mostly 4–8 units daily from day of stimulation.

Metformin

It is an insulin sensitizer used as an adjuvant in women with expected hyper-response to stimulation.

Mechanism of Action

- Improves the intraovarian hyperandrogenism and insulin resistance
- Decreases the number of nonperiovulatory follicles
- Decreases the estradiol levels.

Cochrane review by Tso et al. 2009 has shown use of metformin in polycystic ovary syndrome (PCOS) women to be beneficial in reducing the risk of ovarian hyperstimulation syndrome (OHSS) without any increase in pregnancy rates.

Recent systematic review of 10 randomized control trials (RCTs) substantiated these results that metformin decreases the incidence of OHSS by 80 % in PCOS patients. A large multicentric Norwegian RCT did not find any improvement in pregnancy associated complications with the continuous use in pregnancy.

Side Effects

Gastrointestinal side effects are frequently reported.

Aspirin

Mechanism of Action

It inhibits the cyclooxygenase enzyme in platelets and decreases prostaglandin secretion thereby causing vaso-dilatation which is believed to improve uterine blood flow and uterine receptivity.

Various meta-analyses have conflicting views but Cochrane review by Siristatidis et al. 2016 has concluded that the use empirical aspirin in general IVF population cannot be recommended. This meta-analysis of 13 trials did not find any significant difference between aspirin and control group for live birth rate, clinical pregnancy rate and miscarriage rate. These findings are in contrast to the recently published meta-analysis by Lipin Wang et al. stating that aspirin in 100 mg doses may improve pregnancy rates in IVF. There is also some evidence that aspirin administration during controlled ovarian stimulation (COS) can reduce the risk of OHSS based on two RCTs which addressed this question.

Low-molecular-weight Heparin

Mechanism of Action

- Modulates endoetrial receptivity and decidualization of endometrial stromal cells
- Inhibition of production of insulin-like growth-factor-binding protein (IGF-BP)
- Blockage of complement activation and modulation of inflammatory responses
- Reduction in expression of adhesion molecules such as E-cadherin which promotes trophoblast invasion.

Heparin by its anti-thrombolytic activity potentially improves the implantation in women with thrombophilia. However, observational studies have shown conflicting results regarding this effect. The effect of low-molecular-weight heparin (LMWH) in women with recurrent implantation failure was evaluated by Potdar et al. 2013 and found that it significantly improves live birth rate by 79% and reduced the risk of miscarriage by 78% compared with that in the control group. Subsequent studies in unselected population and in women with two previous failures have not demonstrated any beneficial effect in nonthrombophilic women.

Corticosteroids

- Anti-inflammatory and immune suppressive activity
- Reduce endometrial proinflammatory cytokines and uterine natural killer (NK) cell activity.

Recent Cochrane review by Kalampokas et al. 2017 did not find sufficient data on the safety and effectiveness of glucocorticoid administration in women undergoing controlled ovarian hyperstimulation for IVF/ICSI. There is limited evidence that they may improve pregnancy rates in women with autoimmunity (elevated NK cell/ACA/ANA positive) and in women with unexplained implantation failure.

Sildenafil Citrate: Type-5 Phosphodiesterase Inhibitor

Hypothesized to improve the endometrial thickness and blood flow. Some studies like Takasaki et al. 2010, Sher and Fisch 2002 found it to improve endometrial thickness and improve uterine artery RI while others like Check et al. 2004 did not find any improvements.

Not recommended for routine use by British Fertility Society.

Uterine Relaxants

Uterine contractility may be increased in women with endometriosis, fibroids and adenomyosis and recurrent implantation failure leading to a decrease in pregnancy outcomes. Drugs like nitroglycerine, β2-adrenergic antagonists and atosiban have been evaluated but none have been conclusively found to increase pregnancy rates. Randomized study by Huan et al. found improved pregnancy rates in women with endometriosis who were administered atosiban during frozen-thawed embryo transfer cycle.

Immune Therapy

Immune therapy like intravenous immunoglobulin, tumor necrosis factor alpha, intravenous lipids have been suggested as adjuvants in IVF cycles especially in context of recurrent implantation failures. Most studies evaluating their role are poorly designed with methodological flaws. Most of these have risk of major adverse effects like anaphylaxis, infection and immune suppression and cannot be recommended for use.

▮ KEY POINTS

- DHEA: Possibly some benefit in poor responders
- GH: May have a positive effect in poor responders
- Metformin: Decreases risk of OHSS in PCOS women; no improvement in live birth rates
- Aspirin: Lack of proven efficacy in routine IVF cycles
- LMWH: Routine use not warranted in non-thrombophilic women
- Corticosteroids: May improve pregnancy rates in auto-immunity and unexplained implantation failure
- Sildenafil, immunotherapy and uterine relaxants: Not recommended at present.

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Recent Trends in Embryo Transfer

Jaideep Malhotra, Diksha G Sharma

INTRODUCTION

Embryo transfer is the crucial last step among the various procedures involved in an assisted reproductive technology (ART) cycle and probably the most important one in determining its success. It involves placing the embryos in an atraumatic fashion and at the correct position for optimum outcome.

Various factors before during and after the embryo transfer process can have an impact on its outcome.

PRIOR TO EMBRYO TRANSFER

Patient preparation before Embryo Transfer

Acupuncture, analgesics, anesthesia, massage, trans-cutaneous electrical acupoint stimulation, whole-systems traditional Chinese medicine, and prophylactic antibiotics have been suggested to improve the pregnancy rates. There is fair evidence that acupuncture performed around the time of embryo transfer does not improve live-birth rates in *in vitro* fertilization (IVF). American Society for Reproductive Medicine (ASRM) guidelines state that given there is no clear benefit and that there are inherent risks associated with anesthesia, routine anesthesia is not recommended. A single randomized controlled trail (RCT) did not find any benefit of prophylactic antibiotics given at embryo transfer on the pregnancy rates.

Physician Experience and Preparation

According to Kably et al., along with the numerous factors while performing embryo transfer, the most influential factor in the outcome is the operator experience in the system rather than the system itself. Another study found pregnancy rates to decrease when the procedure was performed by less experienced operators.

There are no studies assessing glove use and live birth rates. Therefore, although some physicians may opt to avoid nonsterile, latex, or powdered gloves in hopes of minimizing embryo toxicity, no data support the usage of a particular type of glove to optimize pregnancy rate.

Evaluation of the Uterine Cavity and Position

Routine uterine cavity evaluation before embryo transfer will help identify pathologies that can potentially reduce implantation rates and an opportunity to correct them before embryo transfer. However, according to results of the multicentric inSIGHT trial routine hysteroscopy does not improve live birth rates in infertile women with a normal transvaginal ultrasound of the uterine cavity scheduled for a first IVF treatment.

A trial transfer theoretically provides a sound knowledge about the uterine position (anteverted or retroverted), dimensions, uterocervical angulation, and ease of transfer and aids in an accurate and atraumatic transfer. However, uterine position may vary between mock and real embryo transfer, due to the enlarged stimulated ovaries and there is no consensus about the benefit of a mock transfer.

Removal of Cervical Mucus

Pros:
- Mucus interferes with embryo transfer by blocking the passage of embryos through the tip of the catheter
- It can pull embryos back from the site of expulsion
- Contaminates the intrauterine environment with cervical flora.

Cons:
- Removal can stimulate uterine contractility or cervical bleeding, with a possible negative impact on pregnancy outcomes.

According to ASRM, there is fair evidence based on one RCT by Moini et al. 2011 and one prospective cohort study
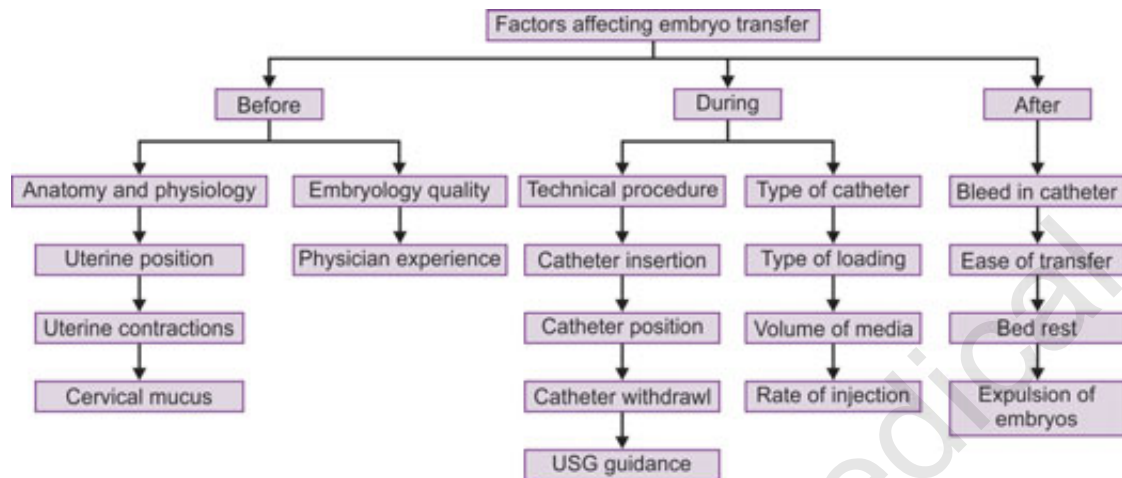
USG, ultrasound.
Flowchart 1: Factors affecting success of embryo transfer.

that there is a benefit to removing cervical mucus at the time of embryo transfer to improve clinical pregnancy and live-birth rates (grade B recommendation).

Application of Seminal Plasma to Female Genital Tract around Time of Embryo Transfer

Recent Cochrane review (2018) concluded that there was low-quality evidence suggesting little or no difference between the groups in rates of live birth or ongoing pregnancy (composite outcome) and low-quality evidence that seminal plasma application may be associated with more clinical pregnancies than standard ART. They concluded that seminal plasma application is worth further investigation.

DURING EMBRYO TRANSFER

Ultrasound Guidance

Advantages:
- Facilitates placement of soft catheters
- Avoids touching the fundus
- Enables confirmation of uterine position
- Estimation of cavity depth
- Confirms placement beyond internal os
- Allows to direct catheter along the cavity and avoid disruption of the cavity
- Decrease incidence of blood in catheters
- Fluid in endometrial cavity can be ruled out
- Helps in straightening of cervix.

Various studies and meta-analyses have demonstrated ultrasound-guided embryo transfer to improve the implantation rate and/or pregnancy rates. Thus, it is recommended by ASRM during embryo transfer.

While selected ultrasound guidance for an anticipated difficult embryo transfer may be an alternative to routine ultrasound guidance, there is insufficient evidence to recommend for or against this practice.

A few studies have compared transabdominal (TA) and transvaginal (TV) ultrasound guidance for embryo transfer performed using Kitazato long embryo transfer catheter. One study found that TV guidance improved patient comfort relative to TA ultrasound due to the lack of bladder filling but increased the duration of the procedure.

Type of Catheter

Various catheters are used for embryo transfer, differing in terms of rigidity, girth and overall shape. The ideal embryo transfer catheter should be soft as it avoids any trauma to endocervix or endometrium and malleable as it find its way through the cervical canal into the uterine cavity.

The ASRM 2017 recommend the use of a soft embryo transfer catheter to improve IVF-embryo transfer pregnancy rates based on grade A evidence. However, it appears that no soft embryo transfer catheter is clearly superior and that commercially available soft catheters perform similarly. Personal choice and cost can guide differential use of one soft catheter over the other.

Syringe and Type of Loading

- Type of syringe and positioning of embryos within fluid column in the catheter can have significant effect on speed of embryo release
- Syringes with conical, or piston like action provide less control for the release of embryos, resulting in abrupt expulsion, which could damage embryos because of ricochet/compression effect
- Air bubble may also result in entrapment of embryos after release from the catheter
- Also dispersion of embryos with air column is in different directions.

Figure 1: Different types of embryo transfer catheters.

Figure 2: Different types of plunger (Flat/conical).

Volume of Media

Recent RCT did not find any difference in pregnancy rates with the use of high volumes of media 40–45 µL as compared to low volume 15–20 µL.

Adherence Compounds in Culture Media

Cochrane review 2014 suggests improved clinical pregnancy and live birth rates with the use of functional concentrations of hyaluronic acid as an adherence compound in ART cycles. However, the evidence obtained is of moderate quality and further studies need to be undertaken.

Placement of the Catheter

It is widely accepted that avoiding touching the uterine fundus is one of the most important factors leading to a successful transfer but there is no consensus on the exact site of transfer. In most studies, pregnancy rates highest when the embryo was placed in the upper or middle area of the uterine cavity, at least 1 cm away from the fundus.

Studies lack consistency in comparative placements, with some studies assessing exact distances from the fundus, and others dividing the uterine cavity into areas. A recent cohort study found better pregnancy rates amongst patients in whom the outer sheath did not go beyond the internal os.

Time Interval before Withdrawing the Catheter

There is fair evidence based on one RCT and one cohort study to recommend immediate withdrawal of the embryo transfer catheter after embryo expulsion (Grade B).

Rate of Injection

Injection velocity of the embryo could impact the trajectory of the placement, and therefore, potentially impact implantation rate and the risk of ectopic pregnancy if a fast speed was used too near the fundus.

- A 2012 simulation study assessed standardization of injection speed by evaluating a pump-regulated embryo transfer device compared with manual injection
- At present there is insufficient evidence to recommend any specific injection speed of the catheter at the time of embryo transfer.

AFTER EMBRYO TRANSFER

Blood in Catheter

There is contrasting evidence about the impact on pregnancy outcomes.

- Various RCT by authors like Meriano et al., 2000, meta-analyses by Philips et al., 2013, and Listijono et al., 2013, conclued that no significant difference on clinical pregnancy rate based on catheter tip contamination with blood

  In contrast various other cohort studies differed in their results

- Currently, insufficient evidence that the presence of blood on the catheter is associated with implantation or pregnancy rates.

Expulsion of Embryos

- Reported incidence <3%
- There is fair evidence that retained embryos in the transfer catheter and immediate retransfer do not affect implantation, clinical pregnancy, or spontaneous abortion rates (Grade B).

Bed Rest after Embryo Transfer

Many studies have been done and none of them demonstrated a benefit of bed rest of any duration in improving the IVF outcomes while one well-designed recent RCT demonstrated possible harm.

EMBRYO TRANSFER PROTOCOL TEMPLATE

For embryo transfer protocol template, see Appendix 2.

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Atosiban in Recurrent Failures

Neharika M Bora

INTRODUCTION

Implantation failure is the main factor affecting the success rate of *in vitro* fertilization (IVF) procedures. Excessive uterine contractions have been described as a potential mechanism for reduced implantation rates in IVF cycles.

The embryo transfer procedure itself increases uterine contractions. Procedure itself increases release of local oxytocin and prostaglandins and any additional manipulation of the vagina or cervix, such as the use of allis tenaculum theoretically provides an additional stimulus for oxytocin and prostaglandin release which further increases uterine contractions.

WHAT IS ATOSIBAN?

Oxytocin antagonist are synthetic analogs that have non-apeptide structure of oxytocin. They act by competing with oxytocin for receptors in myometrium.[1]

Atosiban is an oxytocin/vasopressin V1a receptor antagonist which has a high specificity for the uterus, with limited or no systemic effects. It mimics the normal physiological processes by competing with oxytocin for receptor sites in the myometrium. This results in a dose-dependent inhibition of uterine contractibility. Atosiban has been used successfully in women with preterm labor.[2]

It has also been proven to be as effective as beta-mimetics for treating preterm labor, but with fewer maternal side effects. Because of this specific action on uterine musculature it has been explored for other gynecological treatment.

ATOSIBAN IN DIFFICULT CASES

- Decreasing uterine contractile activity
- Improves uterine receptivity in patients undergoing embryo transfer
- Increases endometrial perfusion (Pierzynski et al., 2007)
- Atosiban also inhibits endometrial prostaglandin F2
- Preferentially relaxes uterine arteries, increases uterine blood flow (Vedernikov et al., 2006)
- It also reduces the number of progesterone peaks (Pei-Yi Chou et al., 2011).

DOSAGE

Commonly employed regimen for Atosiban is intravenous atosiban 30 min before the embryo transfer with a bolus dose of 6.75 mg, and the infusion is continued with an infusion rate of 18 mg/h for ~1 hour. The dose of atosiban is then reduced to 6 mg/h after embryo transfer and the infusion is continued for another 2 hours. Therefore, the total administered dose is 37.5 mg.[3]

TABLE 1: Administer Atosiban IV in 3 successive stages

Single 37.5 mg vial dosage					
Stage	Administration	Route	Dose (mg)	Dose (mL)	Time
1	Initial bolus Over 1 min, 30 min prior to embryo transfer	IV injection	6.75 mg	0.9	Over 1 min
Subsequent infusion with 4 mL of atosiban in 100 mL of NS					
Stage	Administration	Route	Drop rate	Dose (mg)	Time
2	Cont. infusion for 1 h	IV infusion	20 drops/min	18 mg/h	1 h
3	Subsequent infusion for 2 h	IV infusion	6 drops/h	6 mg/h	2 h

IV, intravenous.

PHARMACOKINETICS

The average steady-state plasma concentrations after intravenous atosiban is achieved by 1 hour after the start of the infusion. After the completion of the infusion, plasma concentration declines rapidly. The effective half-life is 18 ± 3 minutes.[2] The plasma clearance of atosiban is relatively high (42 L/h) and the volume of distribution (approximately 18 L) has been found to be consistent with distribution into extracellular fluid. The mean contraction rate decreased by 75% during the third hour of treatment and remained low until treatment termination. The pharmacokinetics of atosiban in pregnant patients are similar to those in non-pregnant women.[4]

MECHANISM OF ACTION

Atosiban, a peptidic compound is used in the management of preterm labor because of its antagonistic activity on uterine contractility.

Piotr Pierzynski in 2007 in their study said that atosiban may have role not only in decreasing uterine contractions but also increasing uterine perfusion and also decreasing production of prostaglandins as told in studies of Vedernikov and Serradeil.

Dr Vuong Thi Ngoc Lan in her study found that atosiban has a very good role in improving implantation and pregnancy rate especially in patients with history of repeated implantation failure. Before atosiban, 14% of her subjects had a high frequency of uterine contractions (≥16 in 4 min), which reduced after atosiban.

This reduction of uterine contractions in all cycles was significant overall (from 6.0 to 2.6/4 min; p <0.01), in cycles with more than equal to 16 uterine contractions/4 min at baseline (from 18.8 to 5.1; p <0.01) and in cycles with less than 16 uterine contractions/4 min (from 3.9 to 2.2; p <0.01). Implantation rate (IR) and clinical pregnancy rate (CPR) improved in all subjects, irrespective of baseline uterine contraction frequency. This was the first prospective study showing that atosiban may benefit subjects with recurrent implantation failure (RIF) undergoing IVF/embryo transfer with cryopreserved embryos who already have been given all and a clinician is left with few little options.[5,6]

This study thus looked at one potential new treatment option for women who experience RIF and also at a potential new role for atosiban.

CLINICAL USES

Although not so commonly employed clinical use in gynecology is in IVF before embryo transfer more so in cases of RIF along with progesterone and sometimes with piroxicam (COX-2 inhibitor).[7]

In a placebo-controlled trial of general IVF patients, Moraloglu et al. (2010) showed that the implantation and clinical pregnancy rates of the atosiban group were significantly higher than the placebo group but this was not a randomized control trial (RCT).

In 2014, a RCT done by Ernest hung Yu Ng on eight hundred subfertile females undergoing IVF stimulation cycle and not donor oocytes found no significant improvement in pregnancy rate, live birth rate, and miscarriage rate in patients with atosiban group and placebo group . They found no difference on the basis of, presence of uterine fibroids, endometriosis, or on the basis of E2 levels.[8]

SIDE EFFECTS

Nausea, vomiting, headache, dizziness, flushes, tachycardia, hypotension, hyperglycemia, injection site reactions, insomnia, pruritis, rash, and pyrexia. Rarely, uterine hemorrhage and atony.

There was a lack of an embryotoxic effect of atosiban in concentrations up to 50-fold therapeutic blood concentrations. Atosiban did not affect the survival of 1-cell rabbit embryos or decrease the percentage of hatched rabbit blastocysts.[9]

FUTURE TRENDS

In 2012 a study on rats were conducted where effect of atosiban was noted on the experimental endometriosis. They found significant therapeutic efficacy of atosiban on endometriosis.

More such studies can be conducted where not only atosiban's effect on uterine contractility be explored but its effect on improving uterine perfusion and decreasing prostaglandins can be put to use.

AUTHORS' EXPERIENCE

A Prospective case control study was conducted, where 60 patients were enrolled, after satisfying a strict inclusion criteria, all of them had a history of one or more implantation failure and then were divided into two groups:

1. Group I: Patients (n = 30) received intravenous atosiban prophylaxis at the time of embryo transfer (ET)
2. Group II: Patients (n = 30) were given nonstimulated (NS) instead of atosiban.

The study was conducted at Rainbow IVF Centre, Agra, which is a tertiary care IVF center, over a period of 3 months.

Patients with RIF fulfilling the following inclusion criteria were prospectively recruited:

- Age 18–40 years
- Baseline follicle-stimulating hormone (FSH) <10 IU/L
- Menstrual cycle of 25–34 days
- Clear information about previous IVF/embryo transfer cycles (including number of embryos transferred, embryo quality, and endometrial thickness)
- One or more good-quality embryo.

The result of this study shows that, in patients with infertility due to various uterine causes, atosiban seems to improve the clinical pregnancy rate up to 33.3%, which was found to be statistically significant.

TABLE 2: Rainbow *in vitro* fertilization demographic

Characterstics	Atosiban GRP	NS GRP
Age	29.1 ± 3.4	29 ± 3.7
Duration of infertility	4.2 ± 2.9	4.8 ± 3
Endo thickness on day of transfer	11.3 ± 2.7	11.2 ± 3
No. of good quality embryos transfered	2–3	2–3
Fresh cycle	7	9
Frozen cycle	23	21
Outcome	Atosiban GRP	NS GRP
No. of embryos	2–3	2–3
Clinical pregnancy	10	4
Single	5	1
Twins	1	1
High order	1	–
Spont abortion	2	2
Ectopic	1	–

CONCLUSION

Embryo transfer is the final step of an IVF cycle, and its success depends on embryo quality, endometrial receptivity and uterine contractions.

Uterine contractions play an important role in embryo implantation as, they may expel the embryos from the uterus and decrease the implantation potential of the embryos.

There are subgroups of patients for whom ET/IVF fails for unknown reasons, atosiban being an oxytocin antagonist, inhibits the increased uterine contractions during ET and is an attractive approach to increase the IVF success in such patients.

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Endometrial Receptivity in *In Vitro* Fertilization

Raju Nair

INTRODUCTION

In spite of much development in the field of reproductive medicine, live birth is still a challenge. The main reason why an embryo failed to implant is still an enigma. The main hypothesis behind this condition is either the problem with embryo and other being issues with endometrial receptivity. The endometrium is a multilayered, dynamic organ overlaying the myometrium. It is one of the most dynamic target tissues in women. It has got cyclic structural and metabolic changes which are related to the ovarian hormones.

ENDOMETRIUM AND INFERTILITY

The primary focus of endometrium in infertility is because of the process of "implantation" which is still an enigma to many scientific bodies. Embryo implantation is the result of a well-structured series of events including cellular adhesion, invasion, and immune regulatory mechanisms. These events are mainly controlled through genetic processes by the ovarian hormones. Before implantation, the embryo approaches the endometrium and once the

blastocyst is oriented correctly (apposition), the zona pellucida is shed. The blastocyst then comes into contact with the epithelial layer and adheres to the endometrial surface (adhesion). Finally, the blastocyst penetrates the epithelial layer and invades the stroma (invasion) (Fig. 1). Successful implantation requires the appropriately timed arrival of a viable blastocyst into a receptive endometrium. The maximal chance of healthy implantation is only 40% per cycle under optimal conditions.

Hertig and Rock in 1956 first suggested the concept of endometrial receptivity and presence of a window of implantation (WOI). The limited period during which the uterus is receptive for implantation of the free-lying blastocyst is called the "implantation window" WOI. In the 1990s, using the ovum donation model, the clinical WOI, which refers to the self-limited period in which the embryo must be transferred back to the receptive endometrium, was demonstrated. Clinical evidence for an endometrial `implantation window' has been described and suggested that in the natural human cycle, blastocyst apposition begins about day luteinizing hormone (LH +6) and is completed by day LH +10 (Fig. 2).
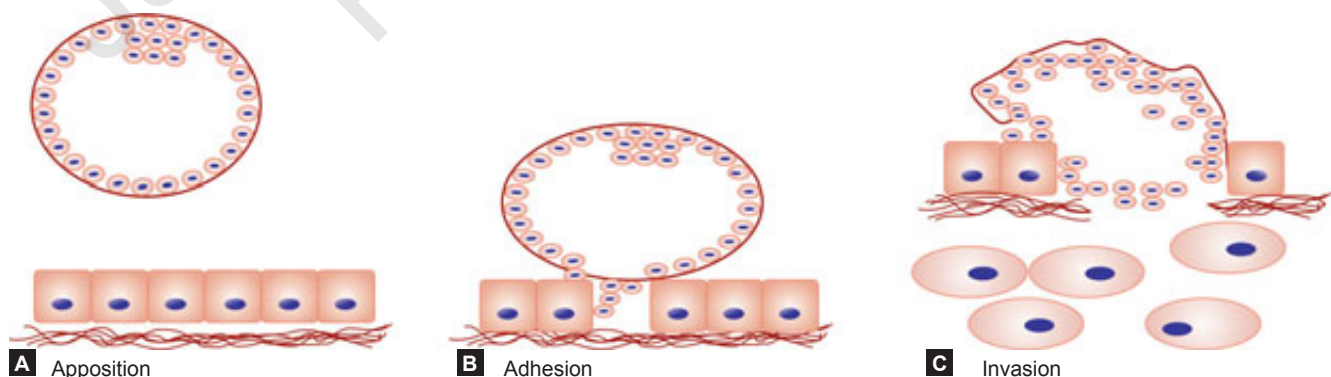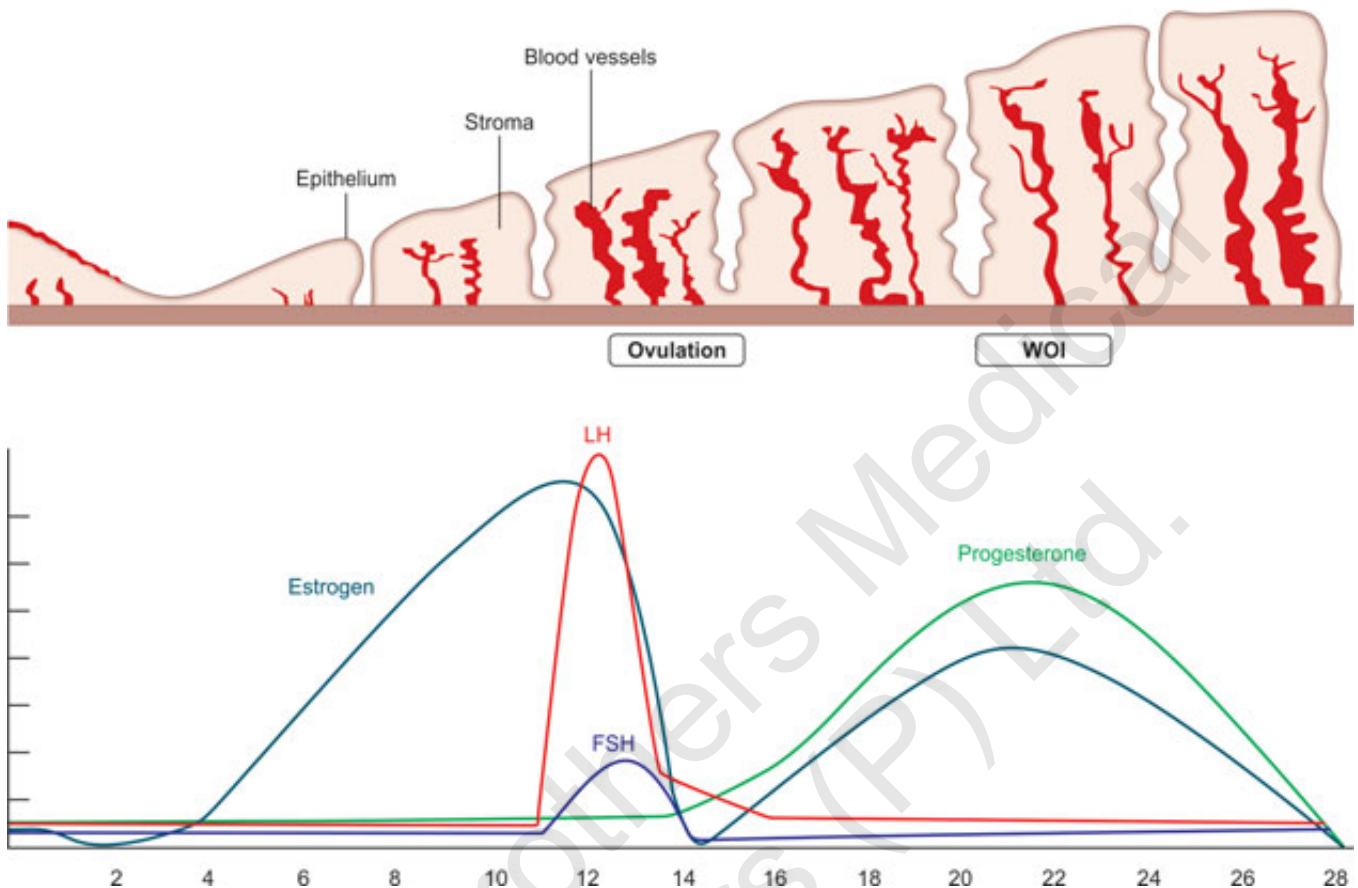

| A Apposition | B Adhesion | C Invasion |

Figure 1: Process of impantation.

WOI, window of implantation; FSH, follicle stimulating hormone; LH, luteinizing hormone.

Figure 2: Hormonal change and endometrial changes.

The specific cellular changes during the implantation window include the transformation of the fibroblast-like endometrial stromal cells into larger and rounded decidual cells, the emergence of large apical protrusions (pinopodes) and microvilli on the luminal epithelium. In parallel, modulations in the expression of different cytokines, growth factors, transcription factors, prostaglandins, and adhesion molecules take place. The slightest imbalance in each of these protein expressions could result in pathological conditions and subsequent infertility.

The most cited factors involved in implantation include the formation of luminal epithelial 'pinopodes' expression of adhesion molecules and cytokines. Pinopodes appear around day 21 and are present only for a few days during implantation, following the peak in progesterone levels and marked by a decrease in progesterone receptor B in the endometrium. In normally fertile women, pinopode formation and regression are closely related to serum progesterone concentrations as well as to the down-regulation of the progesterone receptor B in glandular and luminal cells. Pinopodes were demonstrated at the apical surface of the luminal epithelial cell during the implantation window (day 20 ± 22), therefore claimed strongly as a possible receptivity marker. However, a recent study assessing natural and stimulated cycles within the same patient found no difference in pinopode expression.

WHY ENDOMETRIAL CHANGES HAPPEN IN AN ASSISTED REPRODUCTIVE TECHNOLOGY CYCLE AND WHAT IS THE IMPACT?

The histological changes that an endometrium undergoes during a natural menstrual cycle were described more than 50 years ago. Noyes and co-workers examined the histological features of endometrial biopsies taken during eight thousand natural cycles in three hundred women. By associating histological changes with natural changes in basal body temperature, they were able to link distinct histological patterns to particular time points during the menstrual cycle. The "Noyes criteria" for endometrial dating that resulted from this work have since remained the gold standard approach for evaluating endometrial responsiveness and detecting endometrial abnormalities.

Several factors influence the endometrial development in *in vitro* fertilization (IVF) cycles. Nowadays, most ovarian stimulation protocols include co-treatment with gonadotropin-releasing hormone (GnRH) analogs adjunct to gonadotrophins for prevention of a premature LH

rise. IVF treatment is generally achieved through high-dose gonadotrophin ovarian stimulation. This results in supraphysiological serum concentrations of estradiol (E2) and progesterone (P4). Elevated serum E2 concentrations in stimulated cycles have been associated with more frequent glandular and stromal dyssynchrony. Early and increased exposure to progesterone of the endometrium in stimulated cycles may explain both early secretory transformation and subsequent mid-luteal glandular maturation arrest. The early P4 rise has been implicated as a negative factor for embryo implantation. Human chorionic gonadotropin (hCG) injection to achieve final oocyte maturation is a further possible cause for disrupted endometrial luteal phase morphology. Indeed, a direct effect of hCG regarding advanced endometrial maturation and acquisition of a luteal phase phenotype has been well documented in both *in vitro* experiments and hormone replacement cycles.

The assisted reproductive technology (ART) endometrium presents unique challenges to the transferred embryo. It is well documented that the hormonal environment, which determines endometrial development, is significantly altered during ART cycles, and the window of receptivity may, therefore, be changed (regarding timing) or even absent. A recent study has shown that the clinical pregnancy rates per transfer are significantly higher with cryopreserved embryos than with fresh embryo transfers (ETs). This suggests that a state of impaired endometrial receptivity is induced by ovarian stimulation when compared with the artificial endometrial preparation required for frozen embryo transfer cycles. Histological and immunohistochemical observations using the WOI markers have demonstrated endometrial modifications during the controlled ovarian stimulation compared with natural cycles. It is evident that these high steroid concentrations may influence endometrial development. Histological, immunological, transcriptomic, proteomic, and secretomic studies have demonstrated that the ART endometrium presents a different environment to the blastocyst compared with normally cycling endometrium at the same stage of the menstrual cycle.

Various modes of modification of stimulation protocol have been investigated to overcome the effects of hormonal stimulation. Milder stimulation regimens, in which gonadotrophins were administered at a lower dose, or later in the cycle, or use of gonadotrophins releasing hormone antagonists for pituitary down-regulation, early administration of hCG for final oocyte maturation (as soon as three follicles >17 mm are present) appears beneficial regarding pregnancy rates, especially when day three ETs are performed. However, the critical point to highlight is while stimulation, the derangements of hormones play a very crucial role in desynchronizing the endometrium.

HOW TO MONITOR THE ENDOMETRIAL RECEPTIVITY?

The monitoring of endometrial changes in practical settings is of paramount importance. As the histological changes can be picked up by biopsy which is not practically useful in the actual treatment cycle, a transvaginal ultrasound is a non-invasive, secure, and reliable method to measure the endometrial receptivity. With simple gray-scale ultrasound, we can evaluate endometrial thickness, pattern, or volume as surrogate parameters for endometrial receptivity.

Endometrial Thickness

Endometrial thickness is measured by ultrasound in the midsagittal plane. The measurement is taken from the outer edge of the endometrial-myometrial junction to the outer edge of the thickest part of the endometrium. The triple-line multilayer appearance of the endometrium at the time of ovulation, because of luminal stromal density, has been described as a prognostic factor for pregnancy. It is generally accepted that no pregnancy occurs when the endometrium measures <7 mm. Embryo implantation, clinical and ongoing pregnancy are significantly higher in patients with an endometrial thickness >9–10 mm. It is not clear why a thinner endometrium leads to lower implantation rates. Casper hypothesized that high oxygen concentrations near the basal layer could lead to harmful reactive oxygen species not present at the usual low oxygen tension of the surface endometrium.

Endometrial Volume

Advances in three-dimensional (3D) ultrasound, together with automated software for endometrial measurements, have resulted in more accurate and reproducible measures of endometrial volume which are less operator dependent than standard two-dimensional techniques. Kovachev et al. examined the predictive value of endometrial volume as assessed by 3D ultrasound on the day of ET and found that a volume of < 2 mL resulted in significantly lower implantation rates, whereas an endometrial volume of > 2 mL was a positive predictor for successful ART outcome. Kovachev et al. compared the predictive value of endometrial thickness on the day of ET to that of endometrial volume on the same day. Their results imply that volume is a better predictor of ART outcome.

Endometrial Receptivity Assay

Unlike the embryo, the transition from anatomical to molecular medicine in the diagnosis of the endometrial function just happened a decade ago. Molecular classification of the endometrium using transcriptomic profiling throughout the menstrual cycle has identified that the transcriptomic signature of endometrial receptivity composed of 238 genes. This finding leads to the creation of the endometrial receptivity analysis (ERA). The ERA is now performed by next-generation sequencing that is coupled with a computational predictor and algorithm able to identify the receptivity of an endometrial sample, providing the personalized WOI (pWOI) of a given patient regardless of its histological appearance.

HOW TO IMPROVE THE ENDOMETRIAL RECEPTIVITY?

Medical Methods

- Low-dose aspirin: Studies have shown that low-dose aspirin may increase blood flow to the uterus and improve pregnancy rates. Aspirin therapy is a relatively simple intervention that has potential benefits, but the Cochrane review did not find that it was helpful for endometrial preparation
- Sildenafil vaginal tablets were evaluated in patients undergoing IVF with inadequate endometrium. The use of sildenafil was found to increase uterine blood flow and endometrial thickness. This was a small series and not proved in larger studies
- Acupuncture, Vitamin E, Pentoxifylline, Arginine, etc., has been tried by various scientist but the evidence still lacking.

Endometrial Injury to Enhance Implantation

Recently much debate is happening whether endometrial injury enhances the implantation process. The hypothesis put forth is that by creating an injury we create a micro-environment which favors embryo adhesion. Evidence that endometrial injury performed in the cycle before ART increases the probability of live birth and clinical pregnancy is of moderate quality as reported by a recent Cochrane review.

Intrauterine Therapies

The logic is improving the intrauterine microenvironment. There are many suggestions like low volume hCG, autologous peripheral blood mononuclear cell, granulocyte colony stimulation factor (G-CSF), and autologous stem cell therapy. However, the scientific data so far is inconclusive for the routine use and the clear-cut benefits is not proven with adequately powered studies.

KEY POINTS

- The endometrium is a somewhat enigmatic and dynamic structure in the human body which has got more complex jobs to perform in view of implantation
- The concept of implantation window need to be ascertained before any ART procedures
- The endometrium and its receptivity is different with different ovulation induction protocols
- The strategies to improve endometrial thickness are still in infancy, and more research is needed in this area
- Endometrial injury proves a novel method to enhance endometrial receptivity
- The study of the endometrium is now more complicated with the advent of new biotechnological tools.

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Luteal Phase Support: Current Concepts

Mandeep Kaur, Roshi Satija

PHYSIOLOGY OF THE LUTEAL PHASE IN NATURAL CYCLE

In a normal ovulatory cycle, a successful pregnancy depends on sufficient quantity of estrogen produced from a healthy follicle which causes proliferative changes in the endometrium and adequate, sequential progesterone secreted by the corpus luteum (CL). Progesterone makes the endometrium receptive for implantation and supports early pregnancy. Pulsatile gonadotropin-releasing hormone (GnRH) secretion controls the functioning of this system.

The CL is supported by the human chronic gonadotropin (hCG) of pregnancy and its regression occurs if pregnancy fails. Progesterone is provided by the CL till the seventh week of gestation. At 7–9 weeks gestation the progesterone is supplied by the placenta which is called luteo-placental shift.

There are no definite tests to check for the quality of luteal function. Some studies mention progesterone cut-offs, but it is essential to know that the progesterone production is pulsatile.

WHY IS PROGESTERONE USEFUL FOR IMPLANTATION?

- Secretory transformation of endometrium and mainte-nance of early pregnancy
- Facilitate T- helper-2 lymphocytes useful in implantation
- Improve blood flow and oxygen through action of nitric oxide
- Reduces contractility of the uterus, thus facilitating implantation.

WHAT IS LUTEAL PHASE DEFICIENCY?

Luteal phase deficiency (LPD) is wherein inadequate proges-terone is produced to sustain a secretory endometrium and hence impairs implantation of the embryo.

WHY IS LUTEAL PHASE SUPPORT NEEDED?

Luteal phase is defective in hyper-stimulated cycles especially in assisted reproductive technology (ART) cycles because the pulsatile secretion of luteinizing hormone (LH) is responsible for the function of a normal corpus luteum which is disrupted during controlled ovarian stimulation (COS). Some other reasons for luteal phase insufficiency in ART cycles is described in the table given below.

WHY IS LUTEAL PHASE DEFICIENT IN STIMULATED/ART CYCLES?

- Multifollicular development leads to insufficient luteal phase
- Use of analogues to suppress LH surge
- Removal of large number of granulosa cells during pick up
- Supra-physiological steroid levels lead to negative feedback of the hypothalamic-pituitary-ovarian (HPO) axis and as a result luteal phase insufficiency.

ROUTE OF ADMINISTRATION FOR LUTEAL PHASE SUPPORT

The route of progesterone administration should be efficacious, easy and should have minimum side effects. A Cochrane review in 2011, which reviewed 69 studies, showed that vaginal progesterone, intramuscular progesterone, subcutaneous and supplementation with hCG showed equal pregnancy rates but the chance of developing ovarian hyperstimulation syndrome (OHSS) with hCG is 20 times higher.[1] Thus, progesterone remains the complication free method. Oral preparations lack efficacy as they get metabolized in the liver and fail to reach the target tissue in adequate concentrations. However, dydrogesterone (with limited availability in certain regions and insufficient studies

on safety) is proven to be equally efficacious as vaginal and intramuscular progesterone preparations.[2-4]

Since, vaginal preparations are more patient friendly and have been proven to be equally efficacious compared to intramuscular injections, vaginal preparations are the method of choice.

PROBLEMS WITH INTRAMUSCULAR PROGESTERONE

- Painful, inconvenient
- Risk of allergic reactions
- Risk of infection, abscess
- Need for another person for administration
- Few studies have also reported pulmonary complications requiring admission.

DOSES OF PROGESTERONE SUPPLEMENTATION

Vaginal route preparations are available in the form of vaginal pessaries (600–800 mg/day in divided doses is considered to be optimum dosage), vaginal gel (micronized progesterone bioadhesive gel/8%/90 mg/single use in a day) and even vaginal rings (limited availability and side effects of vaginal discharge).

Parenteral preparations are intramuscular and subcutaneous injections. Intra-muscular injections are oil-based preparations (50–100 mg/day considered to be optimum daily dose in studies). Aqueous preparations are used subcutaneously (use still needs more studies for optimum dosage).

Oral preparations like dydrogesterone to be used solely for luteal phase support still needs more studies (Table 1).

WHEN TO START?

Endogenous production of progesterone starts at the peak of follicular phase, so the initiation of progesterone too early may have detrimental effects, making the endometrium out of phase. Initiation of progesterone on the day of hCG trigger or on the day of oocyte retrieval is considered to be optimum. Starting progesterone very late is also considered to be equally detrimental. In a randomized prospective study by Mochtar et al., no significant difference was found in the pregnancy rates, whether the progesterone was started on the day of trigger, day of oocyte retrieval or on the day of embryo transfer.[5]

WHEN TO STOP?

In *in vitro* fertilization (IVF) cycles with a fresh embryo transfer, few studies suggest that stopping progesterone on the day of positive hCG makes no difference in the outcome.[6] Few other studies recommended the stoppage of progesterone after viability has been confirmed on USG.[7]

Every institution makes their own protocol depending on their past experiences and the courage the clinician has to stop the luteal phase support.

However, in frozen embryo transfer and donor egg cases, it is important to continue luteal phase support till the luteo-placental shift.

IS THERE A NEED TO MONITOR PROGESTERONE LEVELS?

Vaginal preparations compared to intramuscular preparations have lower serum progesterone levels. However, the desired endometrial maturation is achieved. This clearly indicates that the progesterone levels involve unnecessary cost, need for drawing samples, and anxiety related to the levels. There is no evidence to monitor luteal phase support with progesterone levels.

INDIVIDUALIZED LUTEAL PHASE SUPPORT

- Intrauterine insemination (IUI) cycles: Most of the studies prove that luteal phase support in case of IUI cycles is not very beneficial. Pregnancy rates with or without luteal phase support do not show much differences
- ART cycles with GnRH agonist trigger and fresh transfer: In antagonist cycles there is option of agonist or hCG trigger. In agonist trigger, usually there is freeze all and transfer in the next cycle. However, in case a fresh transfer is planned, more aggressive luteal phase regimens are recommended as agonist leads to luteolysis. Low dose hCG (1500 IU) has been recommended on the day of oocyte retrieval but it increases the chance of developing OHSS. Estrogen in combination with progesterone has also been tried. Recombinant LH has also been used for this purpose. More studies are needed before an established protocol can be recommended
- Donor egg/donor embryo/frozen embryo transfer cycles: In these cycles if the lining of the endometrium is prepared with the help of estrogen preparations, priming is done with progesterone, both are continued, if pregnancy occurs till luteoplacental shift

In case of natural cycle transfers, where there is a corpus luteum to support pregnancy only progesterone supplementation is required after embryo transfer.

- Role of adjuvants
  - Aspirin: It is given in low dose and is proposed to improve the blood flow. Few studies showed beneficial effect and others prove that it is not of much use. A meta-analysis on the role of aspirin did not support its beneficial role on pregnancy rates[8]
  - Heparin: Low molecular weight heparin has been known to improve the chances of implantation through the interaction with adhesion molecules and cytokines. Few studies show beneficial effect while others do not.

TABLE 1: Luteal phase support

Progesterone	Route of administration	Dose	Benefits	Side effects
Dydrogesterone	Oral	10 mg BD or TDS per day	Easy, cheap	Metabolized in liver (first pass effect)
Micronized progesterone	Vaginal	600 mg/800 mg per day	Patient friendly, easy, better absorption	Messy, irritation
Parenteral Progesterone	Intramuscular (oil based)	50–100 mg/day	Better bioavailability	Painful, allergic reaction
	Subcutaneously (aqueous based)	25–50 mg/day	Less painful	More studies required

- GnRH agonist: Single dose of GnRH agonist has been shown to improve the chances of implantation. The mechanism of action is still unclear. However, more studies are recommended before routine usage.

KEY POINTS

- Lipopolysaccharide (LPS) in a normal ovulatory cycle occurs by the progesterone secreted by the CL, whereas in COS the CL function is disrupted, hence LPS is required
- The progesterone is started on the day of trigger and continued till 8–10 weeks of gestation or based on the protocol of the IVF set up
- Lipopolysaccharide can be with the use of progesterone, hCG, GnRH agonist. Use of hCG for LPS is not encouraged as there are increase chances of OHSS. Vaginal progesterone is the most common route of administration for LPS.

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Advances in Fertility Preservation Techniques

Shally Gupta

INTRODUCTION

Fertility preservation (FP) refers to methods or techniques of preserving fertility of individuals of reproductive age (both male and female) or prepubescent boys and girls whose future fertility may be compromised for any reason. The need for fertility preservation first arose for cancer patients. But there are other diseases, medical reasons and some nonmedical social reasons also to preserve fertility.

The survival of young cancer patients has improved with latest treatment modalities. And cancer is more common in reproductive age groups. So, fertility preservation techniques give hope to patients with cancers. Chemotherapy and radiotherapy treatments for cancer decrease their fertility if not cause sterility completely. Knowing this is like dying again for a second time.

INDICATIONS[1]

- Some forms cancers in men and women adversely affect fertility
- Recurrent endometriosis causes rapid decline in ovarian reserve
- Treatment options for cancers like chemotherapy and radiotherapy hamper fertility chances
- Gonadotoxic therapies for noncancerous conditions like autoimmune disorders such as systemic lupus erythematosus (SLE), systemic sclerosis, etc.
- Premature ovarian insufficiency due to various reasons such as mosaic turner syndrome, rheumatoid arthritis, glomerulonephritis, inflammatory bowel disease, beta thalassemia and benign ovarian tumors, etc.
- Woman keen to delay pregnancy until after prime reproductive years for nonmedical social reasons
- Transgender surgeries or surgery leading to removal of gonads as in trauma, etc.

FERTILITY PRESERVATION TECHNIQUES

There are many methods of FP, sometime tested and others newer, still experimental. Latter are offered in research institutions and not everyone are skilled in those.[2]

FERTILITY TECHNIQUE IN WOMEN

Cryopreservation of Embryos

Embryo freezing is well established procedure, since its first inception in 1984 and now routinely incorporated as part of any assisted reproductive technology (ART) program for freezing extra embryos and for various other reasons.

Oocyte Freezing

Oocyte cryopreservation is the no longer considered experimental for females. Slow freezing and vitrification are both effective though latter more than former. But freezing of M2 oocytes is preferred over immature follicles needing *In vitro* maturation (IVM). When cancer therapy can be delayed by a month, then controlled ovarian stimulation (COS) followed by cryopreservation of M2 oocyte is done. But when oncological treatment cannot be delayed or hormonal therapy is contraindicated then freezing of immature oocyte and IVM is done. COS can be performed now irrespective of cycle day and hence without delay in cancer treatment too long.

Gonadotropin Releasing Hormone Analog

Gonadotropin releasing hormone (GnRH) analog is given to prevent chemotherapy induced damage to ovarian and testicular tissue. Though exact mechanism is not known but it is believed that it arrests the granulosa cells in early stages and hence less chance of damage to more mature preantral

and antral follicles which are more sensitive to chemotherapy damage. But this effect though seen in animal studies cannot be reliably reproduced in humans. The GnRH agonist protection is not known to be beneficial in breast cancer patients as the tumor cells in breast cancer become resistant to cancer treatment, but it is useful in SLE and ovarian cancer patients. But there is insufficient evidence to support its use.

Cortical or Whole Ovarian Tissue Cryopreservation Later Autotransplantation[3]

It is an experimental procedure and not performed in all centers, but only in few selected ones with relevant expertise. But it is only FP option for young children. The immature follicles are more resistant to damage during vitrification procedure. Ovarian tissue transplantation has a number of advantages over other techniques of FP. They allow for restoration of normal hormones and ovarian function leading to possibility of natural fertility rather than only ART.

Ovary can be transplanted as a whole (more recently) or as small cortical tissue which are devoid of blood supply. Though former has advantages over latter as it decreases loss of tissue from time since transplantation till revascularization. But it is still a newer technique and not many success stories.

Ovarian tissue cryopreservation has its disadvantage that it requires surgery like laparoscopy, that too twice. The follicular pool of transplanted ovary gets reduced due to ischemic damage and freezing, though it would be still better then not doing any FP procedure. The life of follicles after transplantation will depend on extent of neovascularization.

Ovarian tissue cryopreservation is still more in research setting especially in adult humans, ovary has been preserved in its entirety along with its pedicle in sheep to prevent ischemic damage. Retransplantation is done with vascular reanastomosis, but it is technically difficult in human beings due to large size of ovary and consequent difficulty in freezing and thawing.

Ovarian Transposition

It means transplanting ovary away from the field of radiotherapy (orthotopic in pelvis or heterotopic outside pelvis), so has to have minimum damage to ovaries. Though it cannot eliminate the risk completely due to radiation scatter. The ovaries tend to migrate to original position so this surgery should be done as close to the scheduled date of radiotherapy.

Conservative Gynecological Surgery

To preserve fertility like ovarian cystectomy for borderline ovarian tumors and radical trachelectomy for cancer cervix stage 1A2 and 1B.

Patients with estrogen sensitive breast cancer and other malignancies, there is some risk of recurrence due to increased estrogen associated with ovarian stimulation and pregnancy. But this is not supported by studies.

Fertility Preservation in Men

Cancer itself and its treatment both can independently cause damage to sperm quality and quantity, therefore even tough the sperm quantity and quality may be low but preservation can still be beneficial as intracytoplasmic sperm injection (ICSI) can be performed in such scenarios.

- Sperm cryopreservation is an established technique and should be encouraged for FP
- Hormonal gonadal protection by GnRH agonists etc. is not effective in men and hence not recommended
- Testicular tissue cryopreservation and grafting or reimplantation of testicular tissue is in trials and only experimental.

NEWER IN FERTILITY PRESERVATION[3]

Activation of Follicle[5]

Primordial follicles in cryopreserved ovarian tissues need to be activated by *in vivo* and *in vitro* follicle culture to develop into mature follicles. M2 Oocyte have been prepared using this technology. *In vivo* activated oocytes obtained after transplantation of such ovarian tissue can be used in ART and live birth has been reported from them.

In Vitro Maturation of Oocytes[6]

In vitro culture of primordial oocyte has resulted in development of mature M2 oocyte, but safe use of these mature oocyte derived by *in vitro* culture has not yet been clinically established and we need more research in this direction.

Artificial Ovary[7]

An artificial ovary is created where a 3D framework is present on which preantral and mature ovarian cells are grown to create an ovary like environment. This can transplanted to restore normal ovarian function.

Newer Fertoprotective Agents[8]

Newer fertoprotective agents which reduce follicle loss such as imanitib, T3 hormone, granulocyte colony-stimulating factor (G-CSF) and follicle activation by agents such as AS101, an immune modulator that acts on the PI3K/PTEN/AKT follicle activation pathway.

In Men

In vitro spermatogenesis.

Artificial Gametes in Both Genders[9]

Use of pluripotent stem cell or primordial germ cell to make oocyte and sperms.

TIMELINE

- In 1989, Cha et al.[10] reported the technique of IVM of oocytes and reported their first pregnancy using this technology
- In 2001, first autologous transplantation of frozen-thawed ovarian tissue was conducted by Oktay et al.[11]
- In 2004, Donnez et al.[12] reported the first live birth after orthotopic transfer of frozen-thawed ovarian tissue
- In 2006, first heterotopic transplant live birth has been claimed by Oktay et al.[13]

RESULTS/PROGNOSIS

Pregnancy rates data after fertility preservation are few and studies small due to less number of cancer patients undergoing ART after FP. A similar live birth rate (LBR) per patient among women with cancer undergoing *in vitro* fertilization (IVF) and embryo cryopreservation, and cumulative live birth rate (CLBR) to that achieved with fresh embryos in noncancer patients has been reported.[4] Studies with oocyte cryopreservation in cancer patients are few. There are instances of spontaneous pregnancy after orthotopic and heterotopic ovarian transplantation. More recently live birth has been reported after reimplantation of first cryopreserved ovarian tissue. In men semen cryopreservation is the only successful procedure and others have still not given any results clinically.

ETHICAL CONSIDERATIONS

In case of cancer patients the oncologist should be the first person to initiate the information about FP. It requires collaboration of oncologist and infertility specialist. Under age or minor patients suffering from cancer require guidance and support of family and doctor to explain about the repercussions of treatment and help them make informed choices. Risk of cancer cell transmission along with autotransplantation cannot be completely eliminated.

Long-term storage of gametes is also difficult due to cost and space constraints of ART centers.

Ethical issues arise when surgery for transplantation is performed due to elective reasons rather than gynecological disease or prevention from cancerous therapy.

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CHAPTER

Tricky Ethical Situations in Assisted Reproductive Technology

Narendra Malhotra, Radhika T Bahl

INTRODUCTION

Infertility, is not a life threatening scenario but it causes a lot of mental agony, distress, and trauma of a person or a couple who wish to become a parent and found a family. As per the study carried out by WHO (Diagnosis and treatment of infertility, ed. P Rowe and Vikhalyaeva, 1988) that included India, places of incidence of infertility between 10–15% that means out of population of one thousand million Indians, an estimated 25% (250 million individuals) may be conservatively estimated to be attempting parenthood at any given time; by extrapolating the WHO estimate, approximately 13–19 million couples are likely to be infertile in the country at any given time.[1]

Assisted reproductive technology (ART) means and includes to encompass all techniques that attempt to obtain a pregnancy by manipulating the sperm or oocyte outside the body and transferring the gamete or embryo to the uterus. However, from the past decade there has been huge growth in the ART industry and use of ART techniques to form a family or a medium to attain parenthood.

The very fact that ART can also require the use of sperm, eggs, or wombs from third parties who are not expected to play a role in raising the child and it is a medically assisted procreation (MAP), there are various ethical, moral and legal issues which surmount it and needs to be addressed.
The integral part of medical practice are the following principles:

- Nonmaleficence
- Justice
- Beneficence.

Thus, it is essentially important in the interest of fair and good clinical practice principles and to avoid the legal troubles, that the ethical issues hold a great importance.

ETHICAL ISSUES AND TRICKY ETHICAL SITUATIONS IN ASSISTED REPRODUCTIVE TECHNOLOGY

Ethical issues arise around the creation, selection, transfer of number of embryos and disposal of embryos, as well as around cost, coverage, access, and resource allocation.

Multiple Pregnancy in Assisted Reproductive Technology

The dangers of multiple pregnancies may be documented in our records, but the same are not widely recognized or appreciated.[2] MAP is associated with a significant increase in the incidence of multiple pregnancies (i.e., twin and higher order). High order pregnancy leads to many risks and complications like for the children these are largely due to the complications of intrauterine growth retardation [or small-for-gestational age (SGA)] and preterm delivery, and their long-term consequences.[3]

The medical practitioners in such scenarios have to resort to multifetal pregnancy reduction (MFPR).

The ethical dilemmas of MFPR are closely connected to the problem of abortion. The main difference is that in the case of MFPR it is explicitly the intention not to terminate the pregnancy but to increase the chance of development of the remaining fetuses. Especially for higher order pregnancies, not performing a reduction will increase the risk of losing the pregnancy and all the fetuses. In that sense, the reduction is medically indicated. The first priority lies with the well-being of the children that will be born. In India, it is recommended that not more than three embryos *in vitro* fertilization and embryo transfer (IVF-ET) at one sitting excepting under exceptional circumstances which should be recorded.[4]

Prevention of multiple pregnancies should be preferred to MFPR as there are indications that the original higher order pregnancy has detrimental effects (higher incidence of prematurity) on the development of the remaining fetuses that are carried to term even after the reduction.

The MFPR is morally acceptable if the physician has acted according to the rules of good clinical practice and has tried to minimize the risk of a multiple pregnancy. The benefits for the remaining embryos of reducing a higher order multiple pregnancy exceed the disadvantages of carrying the pregnancy to term or risking miscarriage. With triplets, opinions vary according to personal experience and access to neonatal care. The goal of MAP must be a singleton pregnancy.

Single ET is the simplest and most obvious way of avoiding multiple pregnancies. The best way is to replace good quality embryos one at a time.

However, we need more accurate and simpler methods to identify the embryo most likely to implant. In the transitional period, we accept the risk of twins as a compromise between a strongly reduced pregnancy rate and the increased risk of a multiple pregnancy of a higher order.[5]

There is also a need to improve the efficiency of the cryopreservation protocols. In all cases, priority should be given to the reduction of the multiple pregnancy rates.

Parenting Age

Age Factor: Now that, medical science has made it possible to become parent at any age. The ethical scenario or issue that comes up is till what age one can become a parent?

The World Health Organization defines life expectancy as "the average number of years a person is expected to live on the basis of the current mortality rates and prevalence distribution of health states in a population." Statistics released by the Union ministry of health and family welfare show that life expectancy in India has gone up by five years, from 62.3 years for males and 63.9 years for females in 2001-2005 to 67.3 years and 69.6 years respectively in 2011-2015.

It is also pertinent to mention that at present there is no age limit prescribed under the ART guidelines, however the government is determined to bring an age limit for parenting.[6]

Family Balancing

There is a myth that ART techniques in India could help in family balancing.

For example: A patient is having two boys and now wanted a girl child, she came to IVF center and said to implant with female embryos. Should the clinic proceed?

In India, family balancing in any form is prohibited and taken as a form of sex section and sex determination. Thus, strictly prohibited under law and for the said reason should not be offered ART services.[7]

Ethical Issues Surrounding Embryos

There are Scenarios where embryos are lying cryopreserved for years and years and couple is not able to decide whether to use them or destroy them. Embryos are very first stage of life and in a case of dispute between parties where one wants to bring them to life and the other wants to destroy, what should be the role of clinic as a part of good clinical practice or in such a dilemma.[8]

In such a scenario, it is recommended that at the time of freezing of embryos, as detailed consent be taken covering all the scenarios including release in case of death, divorce, fresh cycle etc.

POSTHUMOUS TRANSFER AND RELEASE OF EMBRYOS TO FAMILY/RELATIVES

After the death of a loved one a family and that too untimely and in the present scenario when the families are already having single or limited number of children but good disposable income and support, the heart longs to get back the child or his child of the lost child. This becomes ethically very challenging for the clinician who sees the pain and agony of the family and at the same time, justifying the acts of not disturbing the established norms of society.

The ART guidelines, do provide reference as a guiding light. The child born to a woman artificially inseminated with the stored sperm of her deceased husband must be considered as the legitimate child, however, the legal heir or nominee cannot use it for having a woman of his choice inseminated by it.[9]

DEOXYRIBONUCLEIC ACID MISMATCH: CHILD'S FUTURE

Due the DNA mismatch, the ethical dilemma is the future of the child? Who will be take the custody of the child? Can the parents refuse to take the baby or the doctor be given the responsibility to raise the child.

In such a scenario, what should be done or is there any specific view under the guidelines or the view adopted by courts of India?

In the view of the authors, it should be the responsibility of the parent irrespective of the DNA mismatch and the doctor can be proceeded for medical negligence and compensation, if there is a error at their end. The courts have in few cases expressed (though not recorded) that no child should suffer. Indian system believes in welfare of the child. However, it is also opined by the courts that the parent should be advised beforehand about the risks associated with ART.

CONCLUSION

The ART procedures are helping to build families but have several associated risks not only in terms of medical risks and questions or challenges the established/classical norms of parenting. The human race and its needs are ever evolving and medical science should be able address them not in progressive way but also giving due regard to ethical issues.

The Practice of a Medical Practitioner in India is governed by the Code of Ethics, 2002 and thus, even in the absence of any law, the ART Specialist are bound by the Guidelines as the ethical code of conduct.[10]

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8. As per the National Guidelines for Accreditation, Supervision & Regulation of ART Clinics in India, 2005, clause 3.11.2. Consent shall need to be taken from the couple for their use of their stored embryos by the other couple or for research, in the event of their embryos not being used by themselves.
9. 3.5.11 of the National Guidelines for Accreditation, Supervision & Regulation of ART Clinics in India, 2005. Collection of Gametes from a dying person will only be permitted if the widow wishes to have a child.
10. Clause 7.21 of Indian Medical Council of (Professional Conduct, Etiquette and Ethics) Regulations, 2002. No act of in vitro fertilization or artificial insemination shall be undertaken without the informed consent of the female patient and her spouse as well as the donor. Such consent shall be obtained in writing only after the patient is provided, at her own level of comprehension, with sufficient information about the purpose, methods, risks, inconveniences, disappointments of the procedure and possible risks and hazards.

Sample Consent Forms

Consent to Destroy Embryos

Section A: ABOUT YOU

Partner 1 Name:

Partner 2 Name:

Partner 1 D.O.B.:

Partner 2 D.O.B.:

ADDRESS:

ADDRESS (If different):

Section B: INSTRUCTIONS
This section MUST be completed

Please state which embryos you wish to be disposed, please tick one option only:

1. All embryos from all cycles

2. Only certain embryos

3. We would like to donate the embryos

Section C: YOUR IDENTIFICATION
You MUST include a copy of photo identification

We have enclosed proof of identification,
e.g., passport or driving license (Please Tick)

Section D: DECLARATION

Signature partner 1:　　　　　　　　　　**Date:**

Signature partner 2:　　　　　　　　　　**Date:**

4.1 Consent Form to be Signed by the Couple

We have requested the center (named above) to provide us with treatment services to help us bear a child.

We understand and accept (as applicable) that:

1. The drugs that are used to stimulate the ovaries to raise oocytes have temporary side effects like nausea, headaches, and abdominal bloating. Only in a small proportion of cases, a condition called ovarian hyperstimulation occurs, where there is an exaggerated ovarian response. Such cases can be identified ahead of time but only to a limited extent. Further, at times the ovarian response is poor or absent, in spite of using a high dose of drugs. Under these circumstances, the treatment cycle will be cancelled.

2. There is no guarantee that:
   a. The oocytes will be retrieved in all cases
   b. The oocytes will be fertilized
   c. Even if there were fertilization, the resulting embryos would be of suitable quality to be transferred.

   All these unforeseen situations will result in the cancellation of any treatment.

3. There is no certainty that a pregnancy will result from these procedures even in cases where good quality embryos are replaced.

4. Medical and scientific staff can give no assurance that any pregnancy will result in the delivery of a normal living child.

5. Endorsement by the ART clinic

I/we have personally explained to _____ and_____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

6. This consent would hold good for all the cycles performed at the clinic.

Name and Signature of the Husband

Name, Address and Signature of the Witness from the Clinic

Name and Signature of the Wife

Name and Signature of the Doctor

Dated:

4.2 Consent for Artificial Insemination with Husband's Semen

_____ and _____, being husband and wife and both of legal age, authorize Dr. _____ to inseminate the wife artificially with the semen of the husband for achieving conception.

We understand that even though the insemination may be repeated as often as recommended by the doctor, there is no guarantee or assurance that pregnancy or a live birth will result.

We have also been told that the outcome of pregnancy may not be the same as those of the general pregnant population, e.g., in respect of abortion, multiple pregnancies, anomalies, or complications of pregnancy or delivery.

The procedure(s) carried out does (do) not ensure a positive result, nor do they guarantee a mentally and physically normal body. This consent holds good for all the cycles performed at the clinic.

Endorsement by the ART clinic
I/we have personally explained to _____ and _____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Name, Address and Signature
of the Witness from the Clinic

Name and Signature of the Doctor

Signed: _____ (Husband)

Dated:

_____ (Wife)

4.3 Consent for Artificial Insemination with Donor Semen

We, _____ and _____, being husband and wife and both of legal age, authorize Dr. _____ to inseminate the wife artificially with semen of a donor (registration no._____ ; obtained from _____ semen bank) for achieving conception.

We understand that even though the insemination may be repeated as often as recommended by the doctor, there is no guarantee or assurance that pregnancy or a live birth will result.

We have also been told that the outcome of pregnancy may not be the same as those of the general pregnant population, e.g., in respect of abortion, multiple pregnancies, anomalies or complications of pregnancy or delivery.

We declare that we shall not attempt to find out the identity of the donor.

I, the husband, also declare that should my wife bear any child or children as a result of such insemination(s), such child or children shall be as my own and shall be my legal heir(s).

The procedure(s) carried out does (do) not ensure a positive result, nor do they guarantee a mentally and physically normal body. This consent holds good for all the cycles performed at the clinic.

Endorsement by the ART clinic

I/we have personally explained to _____ and _____ the details and implications of his/her/ their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Name, Address and Signature
of the Witness from the Clinic

Name and Signature of the Doctor

Signed: _____ (Husband)

Dated:

_____ (Wife)

4.4 Consent for Freezing of Embryos

We _____ and _____ consent to freezing of the embryos that have resulted out of IVF/ICSI with our gametes. We understand that the embryos would be normally kept frozen for 5 years. If we wish to extend this period, we would let you (the ART clinic) know at least 6 months ahead of time. If you do not hear from us before that time, you will be free to: (a) use the embryos for a third party; (b) use them for research purposes; or (c) dispose them off. We also understand that some of the embryos may not survive the subsequent thaw and that frozen embryo-replaced cycles have a lower pregnancy rate than when fresh embryos are transferred.

*Husband

In the unforeseen event of my death, I would like

The embryos to perish

To be donated to my wife

To be donated to a third party

Used for research purposes

Signed: Dated:

*Wife

In the unforeseen event of my death, I would like

The embryos to perish

To be donated to my husband

To be donated to a third party

Used for research purposes

Signed: Dated:

Endorsement by the ART clinic

I/we have personally explained to _____ and _____ the details and implication of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Name, Address and Signature
of the Witness from the Clinic   Name and Signature of the Doctor

Dated:

*The appropriate option may be ticked

4.5 Consent for the Procedure of PESA and TESA

Name of female partner: [   ]

Name of male partner: [   ]

We hereby request and give consent to the procedure of PESA and TESA for ICSI, to be performed on the male partner.

We understand that:

a. There is no guarantee that the sperm will be successfully removed or that sperm will necessarily fertilize our oocytes

b. Should the sperm retrieval fail, the following options will be available for the retrieved oocytes

   (i)   Insemination of all or some oocytes using donor sperm   [   ]

  (ii)   Donation of oocytes to another infertile couple   [   ]

 (iii)   Disposal of oocytes according to the ethical guidelines   [   ]
       (Tick the appropriate option)

Each of the above points has been explained to us by _____ .

The procedure(s) carried out does (do) not ensure a positive result, nor do they guarantee a mentally and physically normal body. This consent holds good for all the cycles performed at the clinic.

Endorsement by the ART clinic

I/we have personally explained to _____ and _____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Signature of Male Partner   Name, Address and Signature of the Witness from the Clinic

Signature of Female Partner   Name and Signature of the Doctor

Dated: [   ]

4.6 Consent for Oocyte Retrieval/Embryo Transfer

Woman's Name:

Woman's Address:

Name of the Clinic:

I have asked the clinic named above to provide me with treatment services to help me bear a child. I consent to:

a. Being prepared for oocyte retrieval by the administration of hormones and other drugs

b. The removal of oocytes from my ovaries under ultrasound guidance/laparoscopy

c. The mixing of the following:

	My oocytes		The sperm of my husband
	Anonymous donor oocyte		Anonymous donor sperm

(Tick the appropriate and strike off the others)

d. The placing in my _____ of

e. 1. _____ (no)   of the oocytes mixed with the sperm

f. 2. _____ (no)   of the resulting embryos

g. 3. _____ (no)   of our cryopreserved embryos

h. 4. _____ (no)   of embryos obtained anonymously

I had a full discussion with _____ about the above procedures and I have been given oral and written information about them.

I have been given a suitable opportunity to take part in counseling about the implications of the proposed treatment.

The type of anesthetic proposed (general/regional/sedation) has been discussed in terms which I have understood.

Endorsement by the ART Clinic

I/we have personally explained to _____ and _____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Signature of Female Partner   Name, Address and Signature of the Witness from the Clinic

Name and Signature of the Doctor

Dated:

4.6.1 Consent of Husband

As the husband, I consent to the course of the treatment outlined above. I understand that I will become the legal father of any resulting child, and that the child will have all the normal legal rights on me.

Name, Address and Signature (Husband)

Name, Address and Signature of the Witness from the Clinic

Name and Signature of the Doctor

Dated:

4.7 Agreement for Surrogacy

I, _____ (the woman), with the consent of my husband (name), of _____ (address) have agreed to act as a host mother for the couple, _____ (wife) and _____ (husband), both of whom are unable (or do not wish to) to have a child by any other means.

I had a full discussion with _____ of the clinic on _____ in regard to the matter of my acting as a surrogate mother for the child of the above couple.

I understand that the methods of treatment may include:
1. Stimulation of the genetic mother for follicular recruitment
2. The recovery of one or more oocytes from the genetic mother by ultrasound-guided oocyte recovery or by laparoscopy
3. The fertilization of the oocytes from the genetic mother with the sperm of her husband or an anonymous donor
4. The fertilization of a donor oocyte by the sperm of the husband
5. The maintenance and storage by cryopreservation of the embryo resulting from such fertilization until, in the view of the medical and scientific staff, it is ready for transfer
6. Implantation of the embryo obtained through any of the above possibilities into my uterus, after the necessary treatment if any.

I have been assured that the genetic mother and the genetic father have been screened for HIV and hepatitis B and C before oocyte recovery and found to be seronegative for all these diseases. I have, however, been also informed that there is a small risk of the mother or/and the father becoming seropositive for HIV during the window period.

I consent to the above procedures and to the administration of such drugs that may be necessary to assist in preparing my uterus for embryos transfer, and for support in the luteal phase.

I understand and accept that there is no certainty that a pregnancy will result from these procedures.

I understand and accept that the medical and scientific staff can give no assurance that any pregnancy will result in the delivery of a normal and living child.

I am unrelated/related (relation) _____ to the couple (the would-be genetic parents).

I have worked out the financial terms and conditions of the surrogacy with the couple in writing and an appropriately authenticated copy of the agreement has been filed with the clinic, which the clinic will keep confidential.

I agree to hand over the child to _____ and _____, the couple (to _____ in case of their separation during my pregnancy, or to the survivor in case of the death of one of them during pregnancy) as soon as I am permitted to do so by the hospital/clinic/nursing home where the child is delivered.

I undertake to inform the ART clinic, _____, of the result of the pregnancy.

I take no responsibility that the child delivered by me will be normal in all respects. I understand that the biological parents of the child have a legal obligation to accept their child that I deliver and that the child would have all the inheritance rights of a child of the biological parents as per the prevailing law.

I will not be asked to go through sex determination tests for the child during the pregnancy and that I have the full right to refuse such tests.

I understand that I would have the right to terminate the pregnancy at my will; I will then refund all certified and documented expenses incurred on the pregnancy by the biological parents or their representative. If, however, the pregnancy has to be terminated on expert medical advice, these expenses will not be refunded.

I have been tested for HIV, hepatitis B and C and shown to be seronegative for these viruses just before embryo transfer.

I certify that: (a) I have not had any drug intravenously administered into me through a shared syringe; (b) I have not undergone blood transfusion; and (c) I and my husband have had no extramarital relationship in the last 6 months.

I also declare that I will not use drugs intravenously, undergo blood transfusion excepting of blood obtained through a certified blood bank, and avoid sexual intercourse during the pregnancy.

I undertake not to disclose the identity of the couple.

In the case of the death of both the husband and wife (the couple) during my pregnancy, I will deliver the child to _____ _____ or _____ in this order; I will be provided, before the embryo transfer into me, a written agreement of the above persons to accept the child in the case of the above-mentioned eventuality.

Endorsement by the ART Clinic

I/we have personally explained to _____ and _____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Signed (Surrogate Mother)

Name, Address and Signature of the Witness from the Clinic

Name and Signature of the Doctor

Dated:

4.8 Consent Form for the Donor of Eggs

I, Ms. _____ consent to donate my eggs to couples who are unable to have a child by other means.

I have had a full discussion with Dr. _____ (name and address of the clinician) on _____
_____.

I have been counseled by _____ (name and address of independent counselor) on _____
_____.

I understand that there will be no direct or indirect contact between me and the recipient, and my personal identity will not be disclosed to the recipient or to the child born through the use of my gamete.

I understand that I shall have no rights whatsoever on the resulting offspring and vice versa.

I understand that the method of treatment may include:
* Stimulating my ovaries for multifollicular development
* The recovery of one or more of my eggs under ultrasound-guidance or by laparoscopy under sedation or general anesthesia
* The fertilization of my oocytes with recipient's husband's or donor sperm and transferring the resulting embryo into the recipient.

Endorsement by the ART Clinic/Oocyte Bank

I/we have personally explained to _____ and _____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Signed   Name, Address and Signature of the Witness from the Clinic

                                                                                                    Name and Signature of the Doctor

Dated:

4.9 Consent Form for the Donor of Sperm

I, Mr. _____ consent to donate my sperm to couples who are unable to have a child by other means.

I have had a full discussion with Dr. _____ (name and address of the clinician) on _____
_____.

I have been counseled by _____ (name and address of independent counselor) on _____
_____.

I understand that there will be no direct or indirect contact between the recipient, and me and my personal identity will not be disclosed to the recipient or to the child born through the use of my gamete.

I understand that I shall have no rights whatsoever on the resulting offspring and vice versa.

Endorsement by the ART Clinic/Semen Bank

I/we have personally explained to _____ and _____ the details and implications of his/her/their signing this consent/approval form, and made sure to the extent humanly possible that he/she/they understand these details and implications.

Signed   Name, Address and Signature of the Witness from the Clinic

| |
| |

| |
| |

Name and Signature of the Doctor

| |
| |

Dated: | |

Standard Operating Procedures

Keshav Malhotra, Diksha Goswami, Neharika Malhotra, Shally Gupta

A. HUMAN SPERM ASSESSMENT

INTRODUCTION

Semen analysis is a routine procedure and most important test for male infertility. Also called sperm count test and is necessary for any couple undertaking the process of assisted reproductive technology (ART). The ejaculate produced by man is assessed for volume, concentration, motility, and should also be tested for vitality and antibodies. Semen is a mixture of secretions from the male genital track. Sperm actually makes up only about 5 % of the volume, the rest comes from the prostate and the seminal vesicles. The first part of the ejaculate has the highest concentration of the sperm with the best motility so it is important that the volunteer retrieve the entire ejaculate prior to analysis.

SEMEN ANALYSIS

Semen analysis is done at the lab. Semen sample is usually collected by masturbation, directing the semen into a sterile container. No lubricants should be used as it may kill the sperms. Two to five days abstinence are recommended before semen analysis, to ensure the reliability of the test. Patient should take a sterile container to the semen collection room. Sample should be collected by masturbation ONLY as condoms and lubricants can kill the sperms. The withdrawal method is not acceptable either as female cells can give false results. Nonspermicidal condoms can be used. Once the semen sample has been collected it should be delivered to the lab as soon as possible and placed in the laminar air flow hood or on a warm stage.

METHODS OF SEMEN COLLECTION

- Masturbation
- Electroejaculation
- Vibrator therapy
- Surgical methods
  - Testicular sperm aspiration
  - Microsurgical epididymal sperm aspiration
  - Percutaneous epididymal sperm aspiration
  - Rete testis aspiration.

INSTRUCTION FOR SEMEN SAMPLE COLLECTION

- See that the sample container is wide mouthed and well packed

- Name and date are written on the container and the lid
- Do not have abstinence of more than 4 days
- Wash your hands and penis with soap and water and dry it with tissue paper
- Collect by masturbation without the use of any oil or lubricants
- See that the semen ejaculate right from the first drop is collected in the container
- Please do not touch the container or the lid from inside
- Close the container and hand it over to the staff on duty
- If you have any difficulty in the process consults the doctor on duty.

COMPONENTS OF SEMEN ANALYSIS

The labaratory equipment and materials used in performing a semen analysis and their storage conditions are enumerated in tables 1 and 2.

Liquefaction

- Take a sample from the laminar flow hood and record its details on your semen analysis sheet
- Liquefaction should occur around 15–30 minutes post-ejaculation but it may take up to 1 hour at room temperature

TABLE 1: Laboratory equipment and materials

Apparatus	*Equipment*
• Laminar air flow hood • pH meter • Centrifuge • Osmometer	• Gloves • Serological pipettes (1 mL) • Transfer pipettes • pH strips • Glass slides • Cover slips • Mackler's chamber/sperm-meter • 10 mL or 20 mL syringes • Falcon test tubes • Semen collecting containers • Four-well Nunc dish

TABLE 2: Reagents

Stored at room temperature	*Stored at 4°C*
• Mineral oil • Stains for vitality tests	• Flushing medium • Culture medium • Percoll 45% and 90% • Sperm freezing media

- Prolonged time to liquefaction (more than 2 hours) may indicate poor prostatic secretions since the prostate gland produces the majority of the liquefying enzymes.

Color

A normal semen sample appears homogenous and opalescent. It may be less opaque if the concentration of sperm is low. It can also appear with a red or yellow tinge depending on the man's health and vitamin intake.

Volume

Measure the volume with the help of serological pipette. An average volume is between 2 and 6 mL per ejaculate. A very low volume may suggest congenital bilateral absence of vas deferens since the seminal vesicles (which produce majority of a normal semen sample) are absent in this condition.

Viscosity

For checking out viscosity we do string test. Normal sample runs in small drops and viscous sample forms smaller or larger threads depending on its viscosity. High viscosity may interfere with analysis of motility, concentration, and antibody coating.

String test:
- <40 mm: Normal viscous
- 40–60 mm: Equivocal
- >60 mm: Hyper viscous.

pH

pH is measured with pH strip. Normal pH range for the semen is 7.0–9.0.

Measure the pH after volume and viscosity—by touching the "emptied" volumetric pipette to the test strip (Table 3).

Motility

- First make 2 smears to air dry and use later. Prepare 2 frosted glass slides and label. Place 10 µL onto one slide and place the second slide on top and smear across both surfaces. Allow to air dry
- Place 10 µL of semen on a clean slide and cover with a cover slip. Allow the sample to settle before viewing, but do not allow the sample to dry out. A phase contrast microscope is best for this purpose
- If >10% of the spermatozoa are involved in clumping, then the motility is assessed on the free spermatozoa and the agglutination is noted in the appropriate section of the form
- Counting—select random fields and avoid selecting fields near the edges of the cover slips. At least two hundred sperm should be counted and then percentage of motile cells calculated. Motility of the sperms should be reported as percentage of sperms demonstrating progressive, non-progressive motility or immotile sperms (Table 4).

TABLE 3: Interpretation of pH

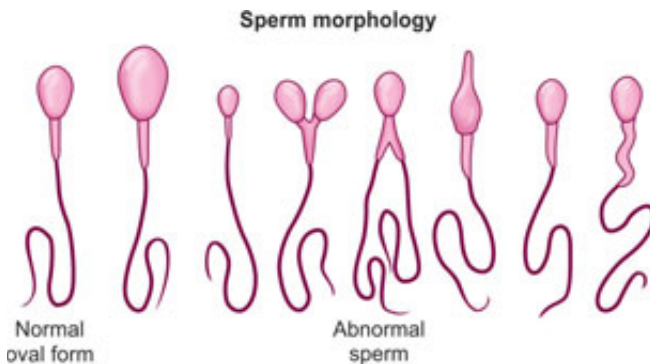| Normal range	7.0–9.0
Low pH	<7.2 indicates seminal vesicle deficiency
High pH	>9.0 indicates acute prostatitis

TABLE 4: Different types of motility

R/L	Rapid and linear progression
NP	No progression but twitching
IM	Immotile

Figure 1: pH strips.

TABLE 5: Degrees of agglutination

Rating	%
Not significant	<10
+	10–30%
++	31–70%
+++	>70%

Figure 2: Sperm morphology.

Agglutination

Agglutination is clumping of sperms into aggregates. Two types of agglutination occur:
1. Nonspecific agglutination: Sperm cells adhere to various cell debris, leukocytes
2. Specific agglutination: Sperm cells adhere to each other in site specific manner like head to head, head to tail, and tail to tail.

Site specific agglutination specifies immunological cause and antisperm antibody test conducted.

Select random field and avoid selecting fields near the edge of the cover slip. Score as given in table 5.

Morphology

Human sperm has a notoriously high concentration of abnormal looking sperm. There are many defects which can occur and some are more detrimental to fertility than others. A figure of around 10% normal forms is often appropriate.

Use the wet motility slide to make a rough approximation of head, mid piece, tail, and cytoplasmic droplet defects (Table 6).

Debris

A high concentration of white blood cells in the semen can indicate infection or inflammation. A white blood cell count of >1 million/mL is cause for concern and should be followed up with a general practitioner.

After counting the sperm concentration, make a note of the debris. Count the number of large round cells (leukocytes and immature germ cells) and the number of small dark cells

TABLE 6: Different morphological defects

Sperm structures	Morphological abnormalities	
Head defects	• Large • Small • Tapering • Pyriform	• Amorphous • Vacuolated • Double head
Neck and mid piece defects	• Absent tail • Bent • Irregular	• Distended • Thin
Tail	• Short • Multiple • Hairpin	• Broken • Irregular width • Coiled
Cytoplasmic droplet defects	>1/3 the size of the head	

TABLE 7: Debris scoring

Not significant	<5% total sperm cell population
+	5–10%
++	>10%

TABLE 8: World Health Organization 5th Edition Criteria

Semen analysis	Criteria
Volume	≥1.5 mL
Total sperm count	40×10^6 million per ejaculate
pH	≥7.2
Sperm concentration	$\geq 15 \times 10^6$ million per mL
Motility	40% total motility 32% forward progression within 60 minutes of ejaculation
Morphology	• Head—oval and smooth • Mid piece—straight and slightly thicker than tail • Tail—single, unbroken, and straight • Normal forms 4% or greater
White blood cells	≤ 1 × 10/mL

(erythrocytes and unidentified cytoplasmic masses). Score as given in table 7.

PROCEDURE OF SEMEN ANALYSIS

• Collect semen sample from the patient and check the patients details on the sample container and the consent form are correct
• Liquefy the semen sample at 37°C on a warmer for 20–30 minutes. If the sample is viscous then keep it for another 30 minutes or pass it from the syringe to liquefy or if the
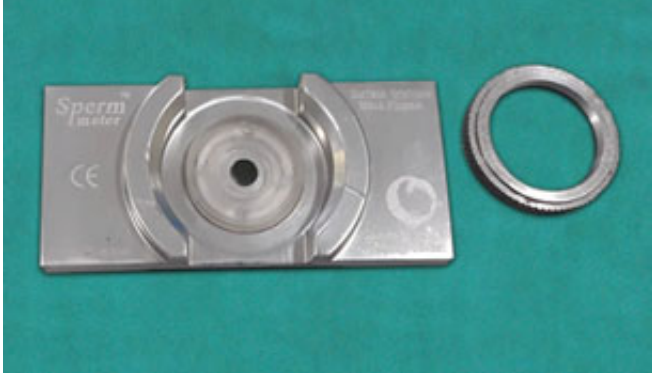
Figure 3: Maklers counting chamber.

sample is very viscous, the antibodies may be working against the sperm, therefore, perform a blood test to confirm

- A 2 mL sample is a standard volume provided by patients
- Measure pH of semen using a pH strip: 7.2–7.4 is the normal range
- Motility estimate is performed from the raw semen sample: 10 µL of semen is placed in Makler chamber and various factors analyzed are:
  - Total count, i.e., sperm concentration as million per ejaculate
  - Motility
    - Number of sperm moving forward and linear (any direction from A to B)
    - Number of sperm static
    - Number of sperm in different direction
  - Presence of pus cells
  - Agglutination
- Morphology of sperm is measured using Papanicolaou (PAP) staining method or Diff. quick method. Abnormal sperm does not mean they will produce abnormal offspring, i.e., 2 heads, 2 tails leads to poor motility but a viable offspring.

Method of Performing Semen Analysis

Mix the specimen well, taking care to avoid bubble formation. With the aid of pipette, place a small drop in the center of the disc area. Grasp the cover glass with your fingers and immediately place the cover glass on the disc area. Press gently, the drop will spread on the entire area of the disc into a thickness of 10 µ.

Once the cover glass is in the place, avoid touch, lifting, and covering again, as this may change the uniform spread of sperm within the chamber.

Lift the chamber and place it on the stage of the microscope.

Note: Never use 40× object with this chamber. The cover glass may be damaged while trying to focus. Even when using the proper 20× objective, take care not to press on the cover glass, the image is usually seen clearly when the tip of the objective is about 1mm, above the surface.

It is recommended to use a 20× objective and 10× eyepiece with this chamber. A 10× objective is not recommended because sperm will be seen too small. A 40× objective cannot be used due to thickness of the cover glass.

MAKLER COUNTING CHAMBER

Description

The Makler counting chamber is a simple to use device for accurate and rapid sperm count, motility, and morphology evaluation, from undiluted specimen.

The chamber is composed of two parts:

1. The lower main part has a metal base, flat glass on which the sample is placed. Around the disc there are four pins. Their tips are 10 µ above the surface level of the disc.
2. The upper part is the cover glass encircled with a metal ring. At the center of its lower surface there is a 1 mm² grid, subdivided into 100 squares, each one of 0.1×0.1 mm. When the cover glass placed on the four tips, the space bounded in a row of 10 squares is exactly one millionth of mL. Therefore, the number of sperms heads in 10 squares indicates their concentration in million/mL.

Accessories

- Cleaning brush
- Tissue paper
- Chamber grip: This device should be placed on the stage of the microscope during sperm analysis. It grips the chamber tightly and smooth shifting of the chamber on the stage. When the sperm analysis is completed, hold the grip and slide the chamber out. Slide the chamber again into the grip for new sperm analysis.

Preparation of the Chamber

Before placing the sample on the disc, make certain that the opposed surfaces are absolutely clean and free of dust, since the size of most particles is larger than the very thin space between the glasses. For this purpose, use the lens paper for wiping both surfaces.

The cleanliness can be tested by placing the cover glass on the four tips and looking for color fringes at the four contact points (Newton's phenomenon). They can be best seen against fluorescent light.

Sperm Count

Place a sample drop on the Makler chamber and place it on the microscope. After the sperms are brought into focus, move the stage of the microscope and locate the grid in the center of the view area. Then, adjust the chamber so that the grid lines will appear in vertical and horizontal position.
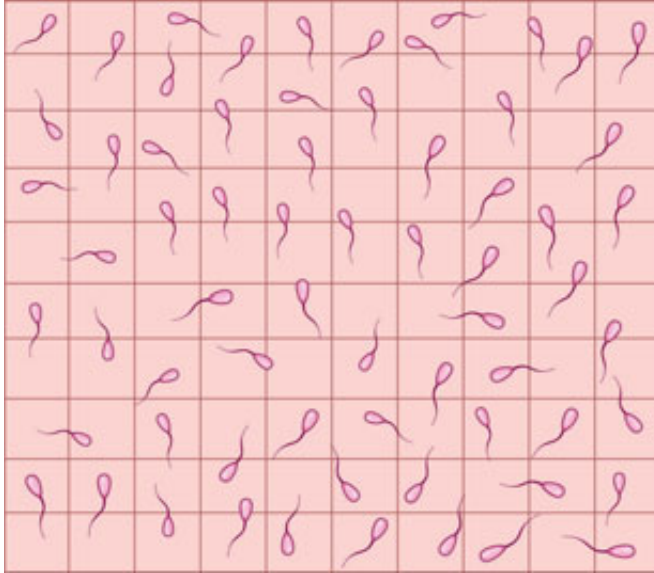
Figure 4: Grid in maklers chamber.

During this search you will have the opportunity to observe the followings:

- Are the sperm spread uniformly? If not, the sample was not mixed well enough
- Are all sperm seen in one focal plane without blurring? If not, perhaps the surfaces were not clean and large particles have intervened between the two surfaces of the chamber.

In either case, repeat this brief procedure from the beginning.

Motility Evaluation

It is suggested to perform motility evaluation within 3–5 minutes after application of the sample to avoid due to tendency of sperm to migrate from the periphery. Count all non-motile sperm within 10 squares. Then count the motile sperm in the same area and estimate the grade of motility. Repeat this procedure in another area of grid, as well as from another 3–4 drops and calculate the average.

This estimation is much more accurate than that performed from ordinary slide where sperm may be compressed by the cover slip and their movement impaired. Makler counting chamber provided standard conditions for all analyzed specimen where sperm can move freely in a frictionless horizontal plane.

Morphology

Rapid evaluation of sperm morphology can be performed out of a wet unstained sample containing immobilized sperm. A phase contrast microscope is performed for this purpose. Count all the normal and abnormal sperm in a certain area of the grid and repeat this procedure from other samples to make a total count of two hundred.

Other staining procedures like PAP staining method and Diff. quick method can also be used for finding out the accurate morphology percentage.

Special Cases

Bubbles: If bubbles appear in the grid area, it is recommended that the drop be replaced by another one, unless the bubbles are too small to interfere with the analysis. Large particles of dust, threads, etc., can also interfere with the count and the drop should be replaced.

Major variations in counts between drops of the same specimen occur when samples were not mixed well, in cases of high viscosity, or when the surface area of the chamber was not clean of particles or dust.

Clumping: Sometimes sperm will clump within the chamber if too much time elapses until they are analyzed or counted. In this case, the drop should be replaced with a properly mixed new specimen.

Cleaning and Preparation for Reuse

- Do not rinse or soak the chamber in tap water. Dip the brush into water or noncorrosive antiseptic solution and simply wipe both sides of the glass
- Then, squeeze the brush and sponge off the remaining water. Finally dry the surface with the lint free lens paper
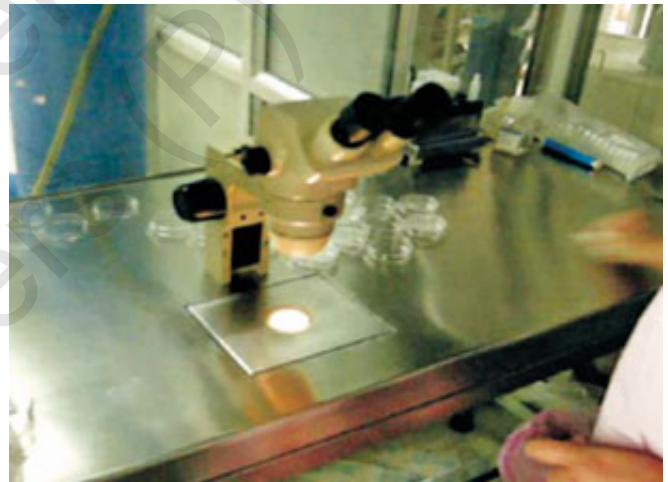

Figure 5: Laminar hood and workstation.

Figure 6: Round bottom tube 14 mL.

Figure 7: BD 1 mL syringe.

Figure 8: Serological pipette.
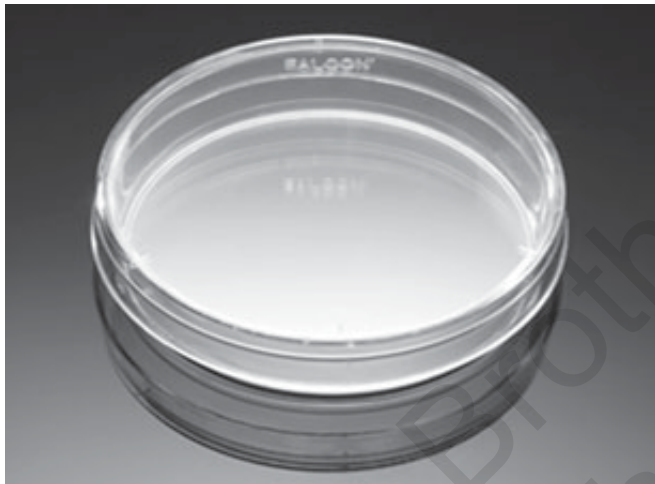

Figure 11: Four well dish.

Figure 9: Falcon 60 mm dish (Petridish).

Figure 10: Centre well dish.

- Avoid touching the tips of the pins as much as possible
- The chamber is now ready for reuse.

In general, there is no need to change the focus once it has been fixed for the examination. Simply slide the Chamber in or out of the chamber–grip without raising the objective.

B. OVUM PICK UP

OVUM PICK UP (OOCYTE RETRIEVAL)

Ova are recovered about 34–36 hours after hCG injection and the procedure is carried out under ultrasound guidance and light general anesthesia.

Equipment required for the procedure:
- Ultrasound scanner with transvaginal probe and needle guide
- Aspiration needle, single lumen on double lumen
- Suction pump with vacuum regulator
- Stereo zoom microscope
- Heating blocks with heating stage
- Sterile working cabinet with laminar air flow and heating stage.

Disposables which are required for this procedure:
- Falcon round bottom test tube (14 mL)
- Falcon 60 mm dish (Pertidish)
- Transfer pipette/serological pipette
- Four well dish
- Falcon center well dish
- Culture media
- Mineral oil
- Compatible ART tips.

Following steps involved in the procedure:
- The patient is placed in a lithotomy position before oocytes recovery
- Patients vulva and vagina are cleaned with sterile dilute betadine
- The transvaginal probe is cleaned with soap solution and milli Q water covered with sterile probe cover and then inserted into the vagina to see the number and location of follicles and ensure that ovulation has not occurred

- The transvaginal probe has to be closed again and it is covered with sterile probe cover. The sterile probe guide to hold needle is attached to the probe
- Light general anesthesia with propofol is administered
- The probe is inserted back into the vagina. Sterile disposable needle double or single lumen is inserted through the guide and pierced through the fornix of the vagina to enter the pelvic cavity
- Transvaginal probe is moved to visualize the follicle and then tip of the needle pushed into the follicle with quick thrust
- When the needle tip is inside the follicle, foot pedal from the aspiration pump is pressed to aspirate the follicular fluid into the pre warmed sterile 10 mL tube. A pressure of less than 100 mmHg is applied
- In case of follicle flushing using double lumen needle, when the follicle is collapsed flushing medium is pushed into the follicle and it is again aspirated back into the tube
- Repeat the procedure till all the follicles are aspirated from both ovaries.

Figure 12: Oocyte pick-up procedure.

Figure 13: Oocytes retrival.

Figure 14: Screening the follicular fluid.

Figure 15: Picking up COC and washing.

Lab procedure:
- Aspirated follicular fluid from patient's ovaries are immediately transferred to laboratory for evaluation of oocytes
- Screen the entire follicular fluid under stereo zoom microscope
- Divide the fluid into two or more petridishes forming a thin layer so that fluid is scanned quickly for the presence of oocytes
- The fluid is rapidly scanned for the presence of the oocytes cumulus complex
- After recovering the oocytes, give 4–5 washes in the flushing medium to remove debris and then shift in incubator.

C. ICSI LAB SET UP AND PROCEDURES

Intra cytoplasmic sperm injection (ICSI) is carried out with the help of ICSI manipulator.

Indications for intra cytoplasmic sperm injection are:
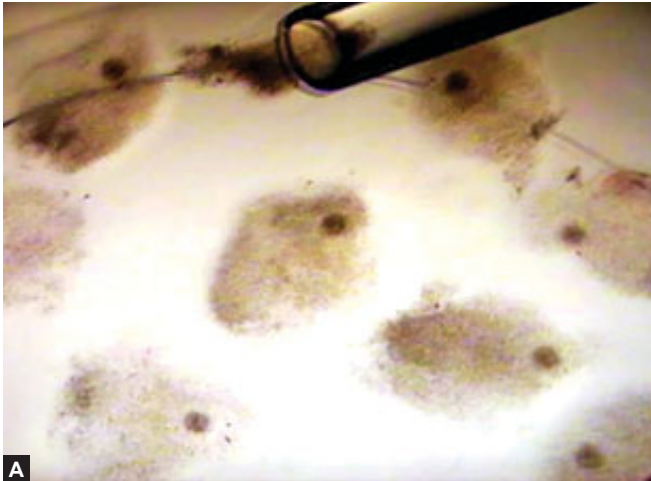- Ejaculated sperm
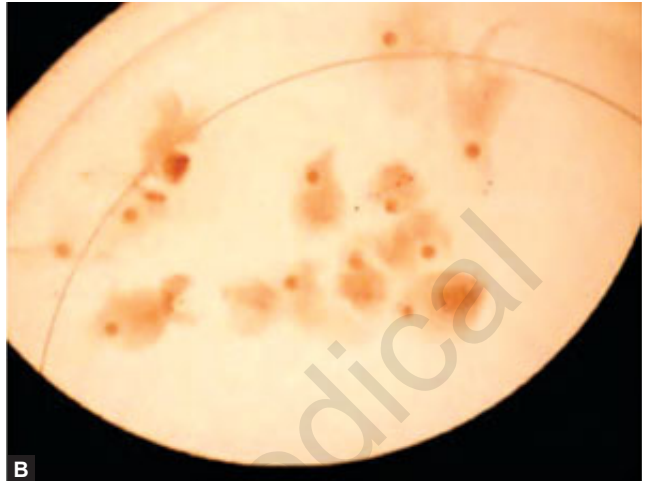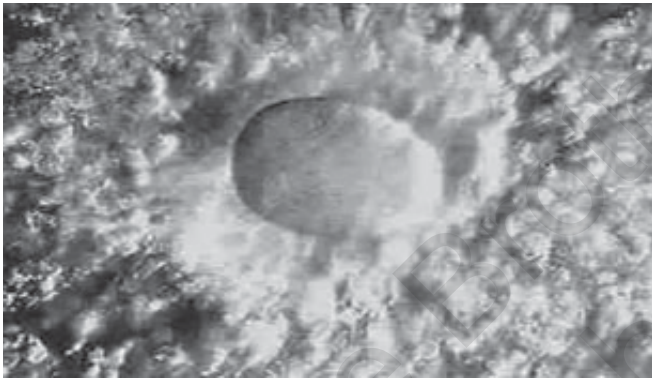
Figure 16: Cumulus oocytes complex

Figure 17: The oocyte in the center, many cumulus cell are seen around the oocyte; this is a low quality oocyte.
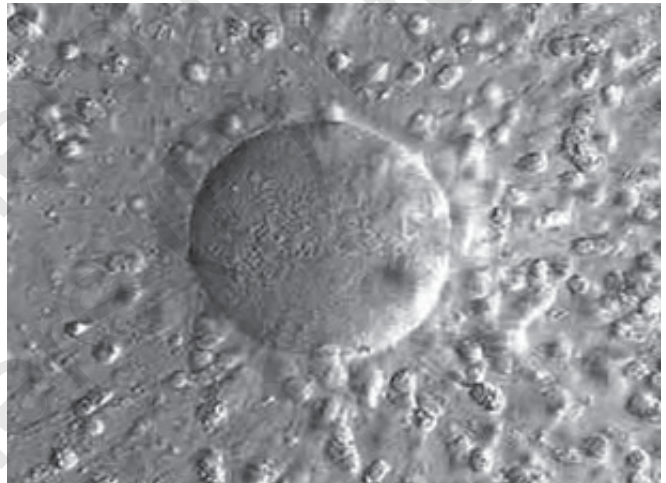

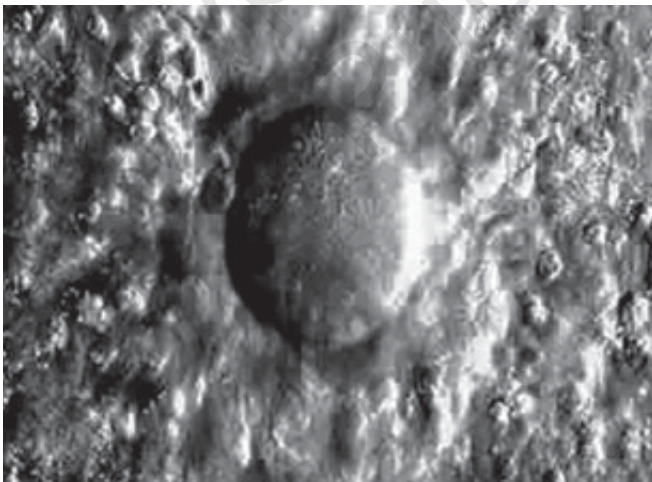**Figure 19:** A good looking oocyte (high quality)

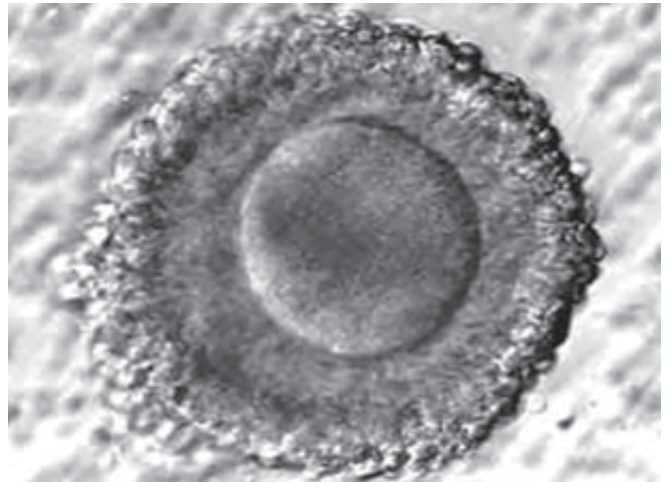**Figure 18:** Another oocyte, Polar body is visible at 10 o'clock.

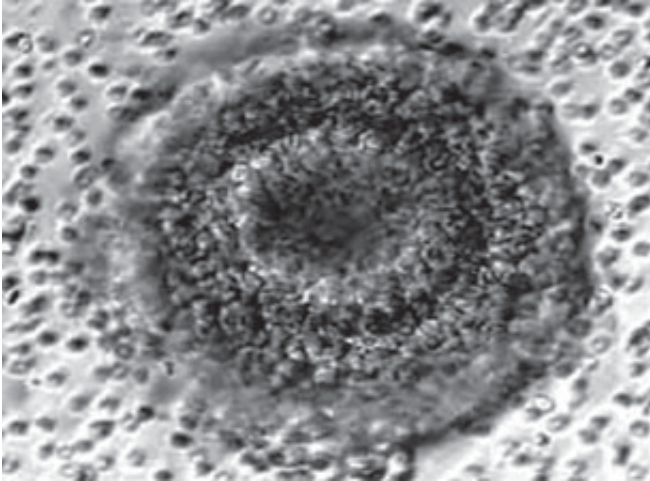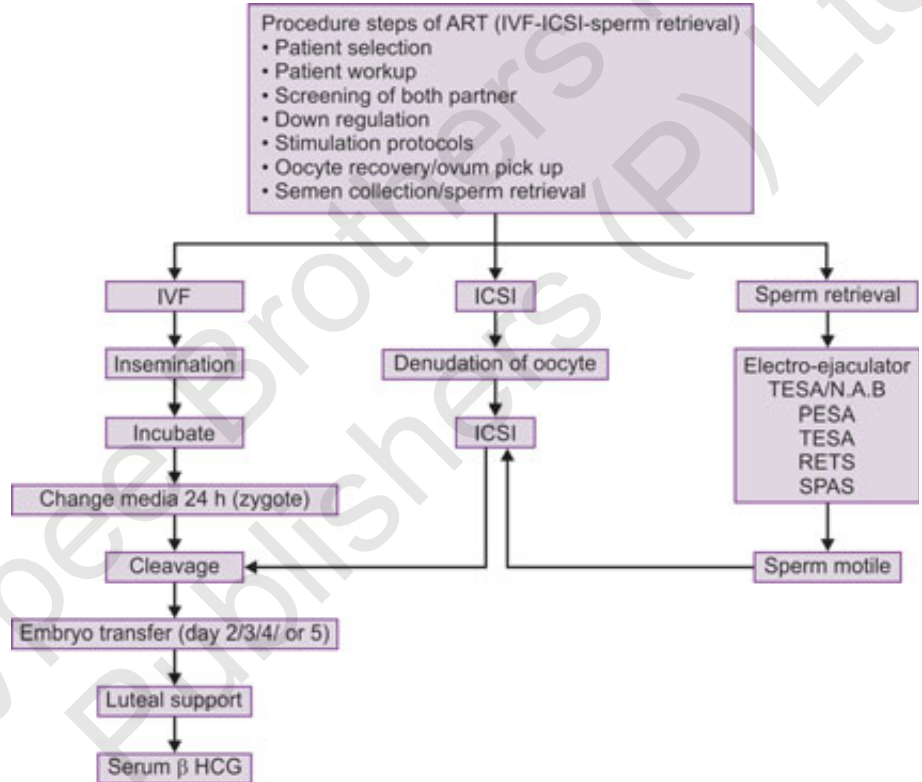**Figure 20:** Very immature egg corona and cumulus cell are tightly packed.

Figure 21: Many RBC are seen around the oocyte.

Figure 22: Placing COC in four well dish.

ART, assisted reproductive technology; IVF, *In vitro* fertilization; ICSI, intracytoplasmic sperm injection; TESA, testicular sperm aspiration; NAB, needle aspiration biopsy; PESA, percutaneous epididymal sperm aspiration; hCG, human chorionic gonadotropin

Flowchart 1: Step of assisted reproductive technology.

- ○ Oligozoospermia, asthenozoospermia, teratozoospermia
- ○ Globozoospermia
- ○ Fertilization failure after IVF-ET
- Epididymal sperm:
  - ○ Congenital absence of the vas deferens
- ○ Failed vaso-epididymostomy
- ○ Failed vasectomy
- ○ Obstruction of both ejaculatory ducts
- Testicular sperm:
  - ○ All indications for epididymal sperm
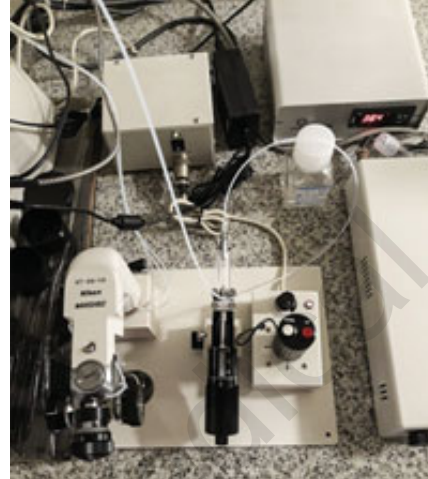 - ○ Azoospermia.

Figure 23: ICSI workstation.

Figure 25: Microinjector.

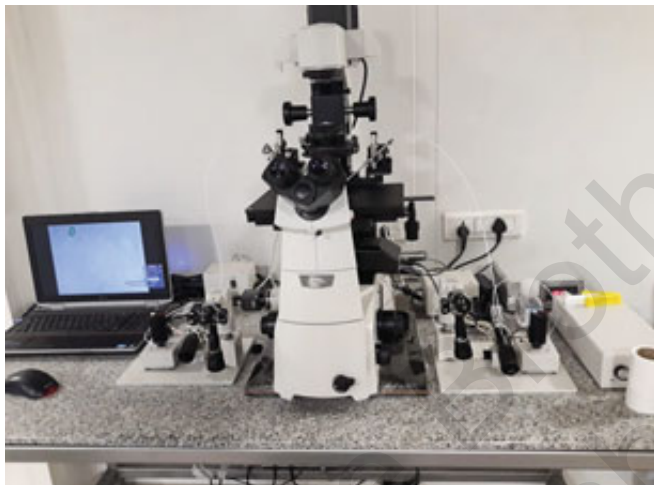- 200×, 400× magnification
- Antivibration table/platform.

Figure 24: ICSI inverted microscope with micromanipulator.

MICROMANIPULATOR SYSTEM

Micromanipulator system consist of three major parts:

1. Inverted microscope
2. Micromanipulator
3. Microinjection-micro tool.

INVERTED MICROSCOPE

Intra cytoplasmic sperm injection procedure is done by using inverted microscopes. Inverted microscopes are used to look very closely at the eggs or embryos. The microscope stage where the dish is placed is heated to 37°C. So inverted microscope consist of:

- Heated stage: Which maintains the optimum temperature for eggs/embryos
- Good optics: Good optics provide high resolution to embryos/eggs. Differential interference contrast, Hoffman modulation contrast are available

MANIPULATOR

Two identical manipulators are mounted on either side of the microscope. Both of these manipulators are capable of x, y, z axis movement. These manipulators provide coarse and fine movement.

MICROINJECTOR

Microinjector consists of syringe attached to a teflon tubing and a steel holder. The steel holder is to hold the micro tool injection needle and egg holder. Microinjectors are available as air microinjectors and oil microinjectors.

Types of manipulators:

- Narishige
- Eppendorf
- Research instrument (RI).

PROCEDURE

Procedure of ICSI involves following steps:

1. Preparation of oocytes for ICSI
2. Micro tool fixing and alignment
3. Sperm immobilization
4. Oocyte manipulation
5. ICSI.

Preparation of Oocytes for ICSI

The oocyte cumulus complexes retrieved are pre incubated in culture medium for 2-4 hours and meanwhile the semen sample is prepared. Following preincubation, the cells surrounding the oocyte are removed this is called denudation to assess the nuclear maturity of the oocytes. Hyaluronidase is the enzyme that is used to disassociate the cumulus cells.

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Exposure of the OCC to hyaluronidase should not be more than 60 seconds. After denudation oocytes are transferred to the ICSI dish and incubated.

Protocol

- Add 2–3 drops of hyaluronidase solution (80 IU) to dish containing OCC for 60 seconds
- Pasteur pipette is used to take the OCC in and out of the pipette, till the granulosa cells are removed. Gently carry out the procedure
- OCC are then transferred to HEPES buffered medium
- Using 300 µm flexipets, the remaining granulosa cells attached are further denuded with 170 and 140 µm flexipets
- All denuded cells are assessed for nuclear maturity by visualizing the polar body
- Only mature oocytes, i.e., MII stage are used for ICSI.

▌ MICRO TOOL FIXING AND ALIGNMENT

- Bring the coarse and fine adjustment to neutral, i.e., zero
- Fit the holding pipette which is larger in diameter, use this as a guide to set the other tool
- Fix the injection tool at the right hand side and bring this in line with the holding pipette
- Release air bubbles if any in the holder and the tubing and re-fill the tubing with oil
- Slowly rotate the injection screw clockwise to release some oil into the pipette, till it fills half of the pipette length seen outside the holder
- Alignment of the micro pipette is done through the inverted microscope and bring the micropipettes above the lens with coarse adjustment
- Use 4x, 10x objective lens and bring the pipette in view with coarse adjustment
- Initially, a faint shadow of the pipette is seen then rotate the holder along its axis till the pipette is seen as straight needle and focus its tip
- Repeat the similar procedure for injecting needle
- When both the needles are aligned at lower magnification, shift the magnification to 20x
- Aspirate some HEPES media in the holding and polyvinylpyrrolidone (PVP) in the injection needle before starting ICSI.

Sperm Immobilization

- Sperm immobilization can be done by slashing method
- Add diluted sperm preparation to PVP drop than load the oocytes into the culture medium drops on the prepared ICSI dish
- Bring the injection pipette into focus under low magnification
- Focus the tip of the injection needle, sperm should be in the same focus
- Select the morphologically good sperms from the periphery of the PVP droplets

- The sperm is immobilized by slashing the tail below the mid piece with the injection tip. Avoid slashing at mid piece
- Adjust the tip above the tail, lower it till it makes contact with the tail and bottom of dish
- Strike the tip from left to right quickly against the bottom of the dish
- The sperm are then aspirated in to the injection needle, tail first by rotating the injector anticlockwise slowly.

Oocyte Manipulation

- Focus the drop containing oocytes under lower magnification that prevents the accidental breakage of the needles
- Focus the oolemma of the oocyte under 20× objective, bring down the injection pipette, focus the tip with fine adjustment, so that the tip and oolemma are in the same focus
- Z axis movement will simultaneously roll the oocyte and the polar body
- Once the polar body is rolled to the desired position (6 or 12 o'clock), bring the holding needle down and focus
- Focus both the oolemma and the inner aperture of the tip of the holding needle, gently aspirate the oocyte by anticlockwise movement and stabilize it
- Aggressive and excessive aspiration can deform the oocyte or even damage its cytoskeleton.

Intra Cytoplasmic Sperm Injection

- Firmly hold the oocyte with holding pipette with gentle suction
- Lift the holding pipette (prevents slashing the oocyte while injecting) a bit and focus bring down the injection needle close to zona pellucida with immobilized sperm in it
- Focus both the oolema and the tip of the injecting needle make sure it is at middle of the egg i.e. at 3 o'clock position
- When the injection needle is positioned, push the sperm toward the tip of the injection needle
- Stabilize the sperm at the tip of the needle
- Slowly advance the injection pipette into the oocyte
- Indention should be seen at the point of penetration
- Aspirate the ooplasm into the injecting needle, initially the flow of the ooplasm into the pipette will be slow followed by a sudden free gush of ooplasm
- Sudden movement of the ooplasm inside the injecting needle confirms the rupture of the oolemma
- Stop the aspiration as soon as possible when you notice the sudden gush of ooplasm. Gently release the ooplasm mixed with the immobilized sperm back into the ooplasm
- Remove the injection pipette out of the oocyte and elevate the injection pipette
- Release the oocyte from holding pipette
- Repeat the procedure till all the oocytes are injected
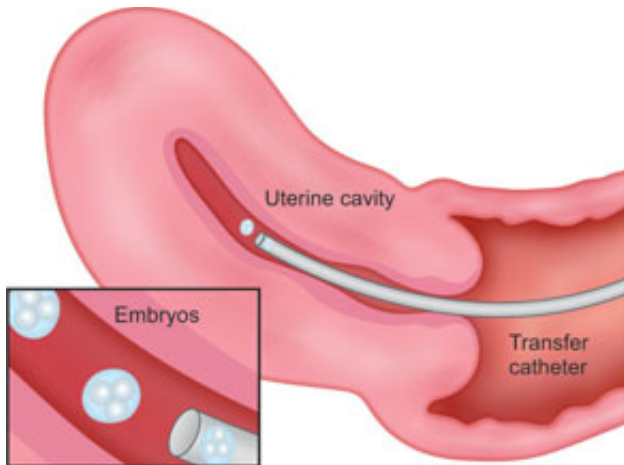- Transfer the injected oocytes to the culture dish.

Figure 26: Embryo transfer.

Figure 27: The cervix is visualized using Cusco's.

Figure 28: Cervix cleaning by gauze

Figure 29: Different type of embryo transfer catheter.

D. EMBRYO TRANSFER

Embryo transfer (ET) is important and crucial parameter of ART procedure. All the success depends on ET. The ultimate goal of a successful ET is to deliver the embryos atraumatically to the uterine fundus, where implantation is maximized. The ET technique may directly influence the outcome of ART. It has been demonstrated that there is a significant difference in the pregnancy rates associated with different individuals performing the ET within the same in-vitro fertilization (IVF) program. The need to standardize the protocol for ET technique was regarded as the most important factor influencing the success rate. Moreover, it is estimated that poor ET technique may account for as much as 30% of all failures in assisted reproduction. Unfortunately, this failure must have affected thousands of infertile couples seeking pregnancy through assisted reproduction every year. Therefore, extra attention and time should be given to the procedure of ET. Meticulous ET technique is essential
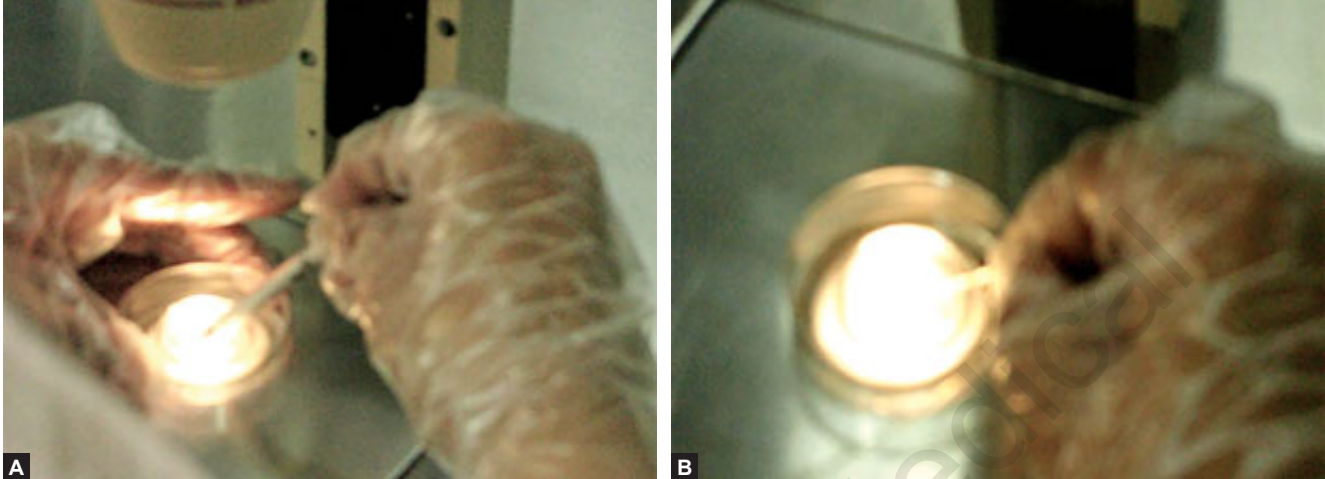
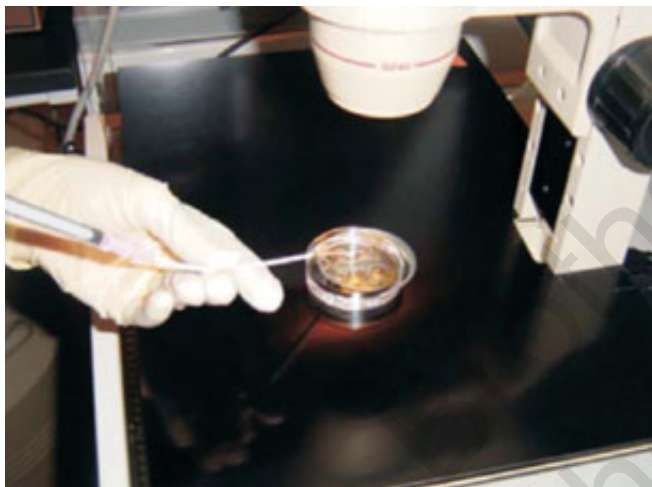**Figure 30:** Preparing embryo transfer dish.

Figure 31: Loading of embryos.

for IVF success. This final step in assisted reproduction will determine the fate of a long process and great effort, from ovulation induction and oocytes retrieval, to the tedious high-technology procedures in the laboratory, not to mention the desperate hope of infertile couples.

EMBRYO TRANSFER PROCEDURE

- The patient is put in the lithotomy position and the cervix is visualized using Cusco's speculum
- The cervix and the vaginal vaults are cleaned of cervical mucus and vaginal secretions using tissue culture media and sterile gauze
- The catheter type is chosen according to the suitable one used for the trial
- The ET catheter is flushed with 3 mL tissue culture medium, and then filled with the transfer medium
- About 15 µL of transfer medium is aspirated first and then the embryos are aspirated next in another 10 µL medium. Finally, 10 µL medium is aspirated to withdraw the embryos away from the catheter tip

- The loaded ET catheter is introduced through the cervix to pass the internal os and then gently advanced in the mid-uterine cavity and stopped from 1–2 cm short of the fundus
- The screw of the vaginal speculum is loosened so that the two valves of the vaginal speculum apply a gentle pressure on the portio vaginalis
- At this moment, some patients experience suprapubic heaviness and discomfort. After 1–2 minutes, when this complaint disappears, the embryos are ejected and pressure is kept on the plunger of the syringe while slowly withdrawing the catheter out
- The speculum is kept in place for an average of seven minutes and then removed
- The catheter is checked for any retained embryos. If found, retransfer is done immediately

In figure 33A, the catheter is checked for any retained embryo and figures 33B and 33C show the outer sheet of catheter which is also checked out for retained embryo after ET.

- For difficult cases, general anesthesia is given in the form of propofol 2 mg/kg as an induction dose and anesthesia is maintained by inhalation of isoflurosane 1.5% and oxygen 100% through a facemask
- The trial ET is repeated and if not successful a tenaculum is used to stabilize the cervix.

FACTORS DETERMINING SUCCESS

Pregnancy success rates depend on many factors
- Some obvious ones
  - Quality and number of embryos
  - Age of woman
  - Sperm quality
  - Infertility cause
- Some not so obvious
  - Technological background of doctors
  - Embryologists
  - Nurses and other scientists implicated in the treatment
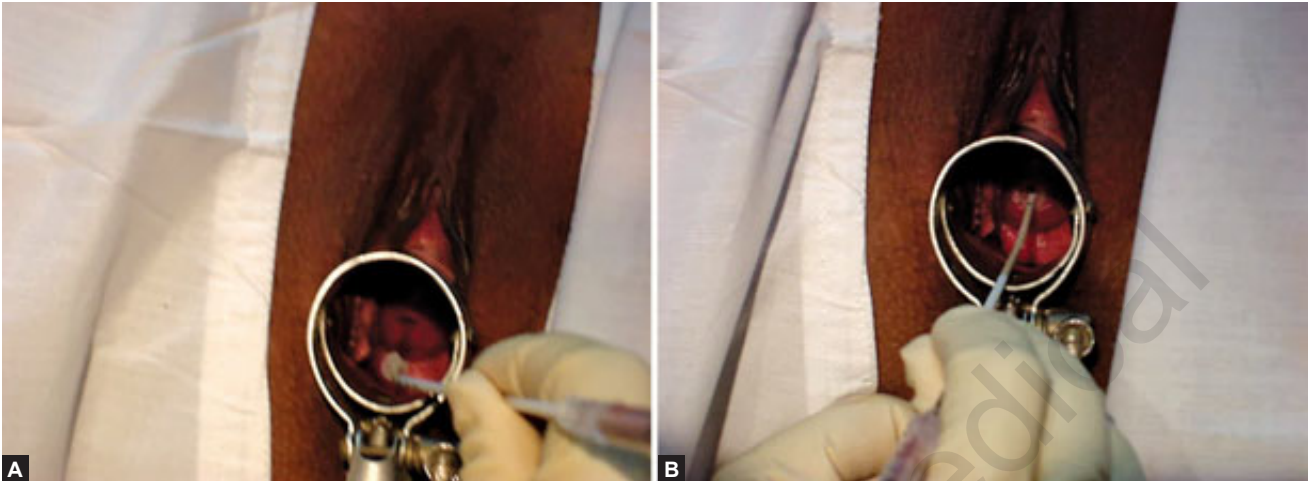 - The set up and the quality control of the laboratory.

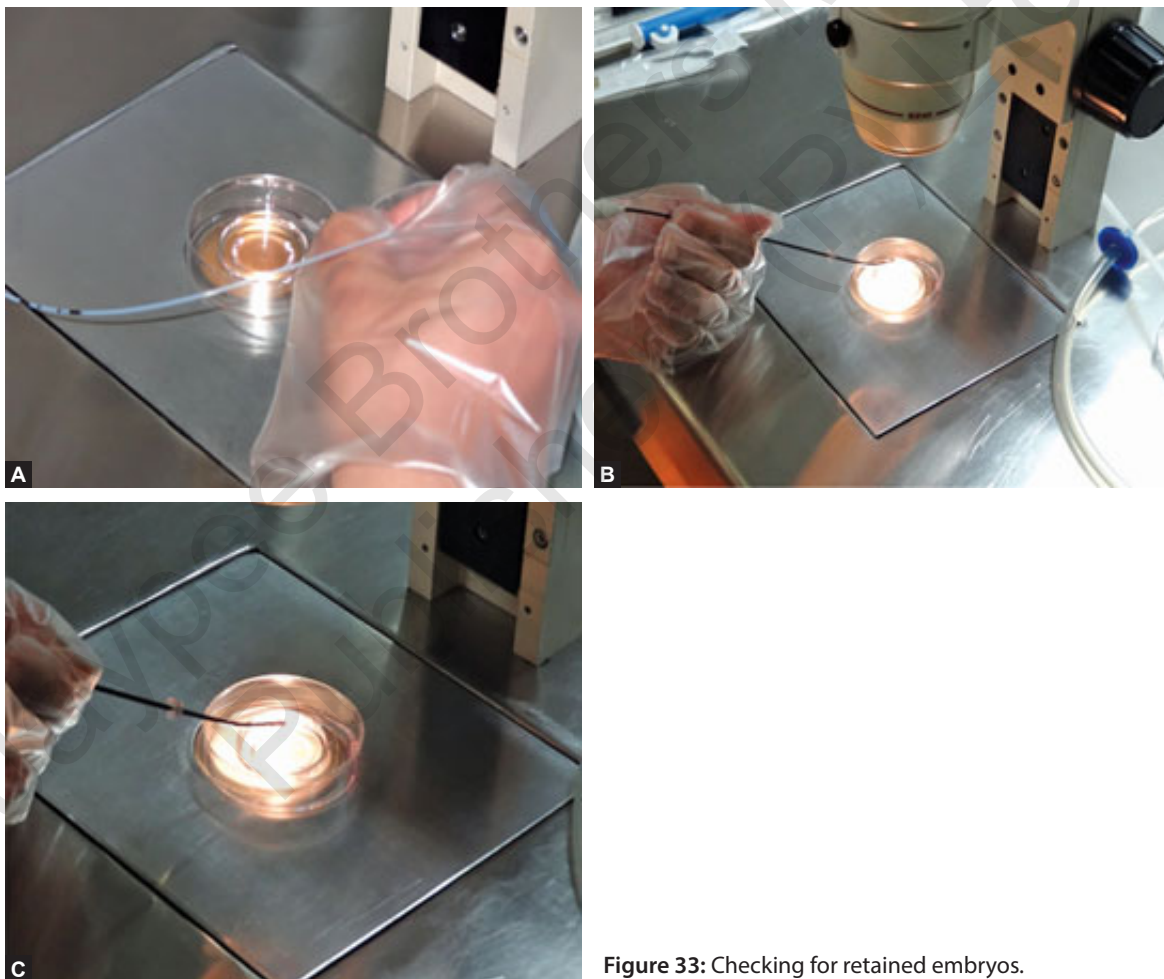**Figure 32:** The loaded embryo transfer catheter is introduce in cervix.

Figure 33: Checking for retained embryos.

EMBRYO TRANSFER

- Often reviewed as an unimportant variable
- Clinicians are reluctant to change habits

- Much of inefficiency of implantation may reside with ET techniques:
  - Uterine contractions
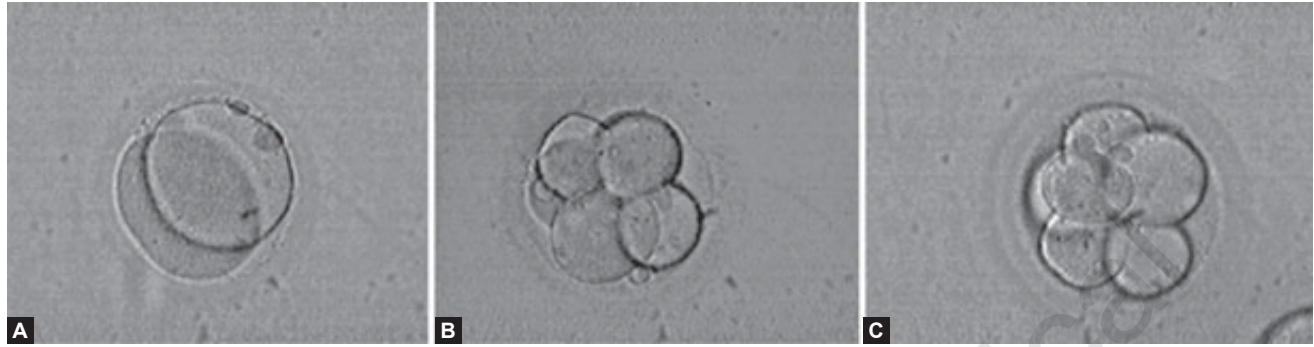 - Expulsion of embryos

Figure 34: Different stages of embryos. **A,** 2 Cell; **B,** 4 Cell; **C,** 8 Cell.

- Blood or mucus on the catheter
- Bacterial contamination of the catheter
- Retained embryos
- Easy transfer; smooth transfer without force or notation of trauma
- Difficult transfer; needed uterine manipulation, multiple attempts force trauma and rarely dilatation
- Difficult transfers negatively associated with ongoing pregnancy rates 42.3% versus 31.1%

- The importance of keeping embryo temperature close to 37°C, oxygen and carbon dioxide concentrations under specific range cannot be overlooked
- It may be difficult to control environmental factors
- Thus the time elapsed between embryo loading and embryo deposition could be a prognostic factor of implantation and pregnancy rates.