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Institute of Reproductive Medicine

Evaluation of different drugs in Luteal phase support

B N Chakravarty

INTRODUCTION

Edwards and Steptoe were the first to suggest that luteal phase support (LPS) was an essential step for outcome of IVF treatment.[1] Other investigators[2,3] also confirmed that long protocol ovarian stimulation invariably leads to an endocrinological derangement in the luteal phase. A meta-analysis of prospective randomized study[4] showed that LPS is definitely beneficial following long-protocol stimulation in an IVF cycle. The possible mechanisms of luteal phase insufficiency is due to disturbance of pituitary function, possibly in association with an elevated oestradiol concentration after ovarian stimulation resulting in multiple follicular maturation.

Other indications of luteal phase support are – infertility, threatened miscarriage and recurrent miscarriages. Assuming that, in the absence of a definite diagnostic marker available for diagnosis of LPD, use of luteal phase support for these indications is still empirical. But the use of LPS is mandatory in ART treatment cycle. The question is, - which agent(s) should be used for LPS both in terms of efficacy and safety. hCG, progesterone and oestrogen have been used. After nearly 40yrs of extensive trial, undoubtedly **Progesterone** has become the drug of choice. Though in the initial years of ART treatment, hCG was used either alone or in combination and its limitation has now been well realized. Oestrogen as luteal support along-with progesterone has also been used sporadically though its exact efficacy and role is yet to be ascertained.

In this chapter, the current role of hCG will be outlined briefly followed by the choice of progesterone as the main therapeutic agent for LPS. The various pharmacological preparations and routes of administration of progesterone will be discussed in detail. Finally the place of oestrogen in combination with progesterone as LPS will be briefly outlined.

hCG as Luteal Support:

In 1990s, hCG was used either alone or with progesterone for LPS in ART treatment cycles. The advantages claimed are that hCG can produce both E2 and progesterone in addition to some other autocrine and paracrine molecules which add to overall efficacy of corpus luteum function. There are various dose schedules (1000, 2500 or 5000IU) and protocols (every 2 days, every 3days or varying intervals) of hCG administration for LPS. Considering the high-risk of OHSS, very few clinics worldwide are now using hCG as luteal support in ART cycles. A small group of 'low- risk' poorly responding patients (terminal E2 on hCG day is <1500pg/ml) may benefit with hCG combined with progesterone as LPS in ART cycles.[5]

Progesterone as luteal support:

Currently progesterone is the gold standard drug used as a luteal phase support. Progesterone is available in two forms – natural progesterone and synthetic progestin. For luteal support natural progesterone is used in micronized form. Micronization of natural progesterone increases half-life of progesterone with the metabolites including Allo-pregnanolone which has indirect effect on progesterone receptors.

Unlike synthetic progestins, micronized progesterone has no adverse effect on mood changes,[6] high density lipoprotein (HDL) and cholesterol level or on pregnancy outcome. Synthetic progesterone (like Norethindrone, Norgesterol, Levonorgesterol, MedroxyprogesteronE acetate, Norgestimate etc) have variable grades of androgenic side effects and therefore are contraindicated during pregnancy. ***Dydrogesterone or retro-progesterone is the only progestin which is a stereo-isomer of progesterone and has a high affinity for binding with progesterone receptor because of its retro-structure and has been successfully used as LPS.***[7] This molecule of progesterone (dydrogesterone) will be discussed in further details in the subsequent section of this chapter.

Micronized progesterone is the drug of choice for luteal support in ART treatment cycle. There are two important reasons to corroborate this view. ***The first and important reason is that there is usually 2-fold***

high risk of OHSS with use of hCG either alone or in combination with progesterone.[8] ***More importantly, exogenous progesterone provides a few other provisions for endometrial support with regard to embryonic implantation and maintenance of pregnancy.*** The specific benefits are – (see flowchart-1)

- It is well known that progesterone induces release of endometrial nitric oxide (NO) during pregnancy which contributes to improve vascularity by lowering utero-placental vascular resistance
- Nitric oxide (NO) is generated from nitric oxide synthetase (NOS) which is an enzyme that exists in vascular endothelial cells (eNOS)
- Therapeutic agents like progesterone will improve uterine blood flow and therefore may be used to ameliorate embryonic growth retardation and help in stable implantation[9]
- Progesterone up-regulates eNOS expression in uterine and spiral arteries – optimal for implantation
- NO helps in vasodilatation, decidua formation and endometrial remodeling during trophoblastic invasion[10]

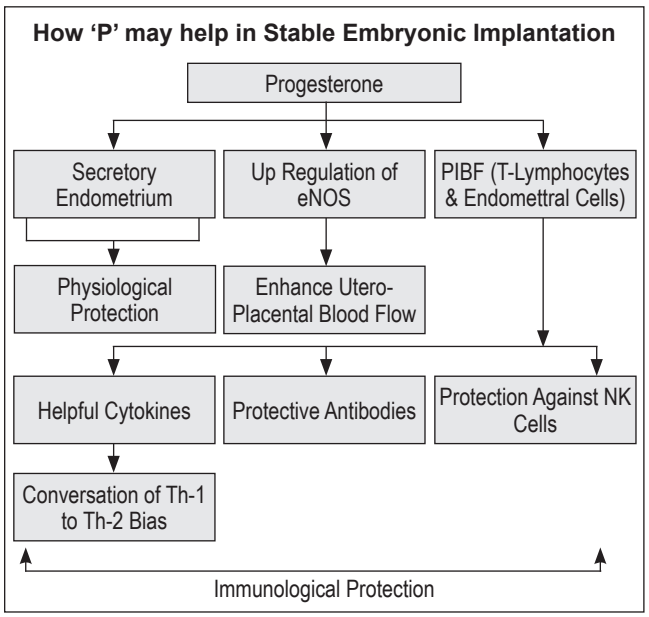
A pilot study was conducted by our group[11]

In idiopathic recurrent miscarriage to assess to sub-endometrial blood flow parameters following administration of two groups of progesterone, - dydrogesterone (Group-A n=51) and Natural micronized Progesterone (NMP - Group-B n=50) compared with 32 women who had no history of recurrent spontaneous miscarriage. It was observed that after progesterone administration (Gr-A & Gr-B) there was significant reduction in resistivity index (RI), pulsatility index (PI) and an increase in end diastolic velocity (EDV) and pregnancy rates. This study confirmed that progesterone in both the pharmacologic formulations helps in improving the endometrial blood flow and thereby helps in achieving viable pregnancy.

Additional supportive function of Progesterone for LPS:

- Maintenance of myometrial quiescence
- This is achieved by stabilizing lysosomal membrane and inhibiting prostaglandin synthesis
- Immunological modulation to favour implantation and maintenance of pregnancy
  - PIBF production
  - Conversion of Th-1 to Th-2 bias
  - Inhibition of natural killer cell activity at feto-maternal interface

Immunophysiologic mechanism of progesterone effect on embryo implantation and maintenance of pregnancy -



Immunomodulatory and anti-inflammatory functions of progesterone are potentiated through hCG and cortisol which inhibit tissue rejection and protect the conceptus. Progesterone positively regulates 'Progesterone induced blocking factor (PIBF)', NK (natural killer) cells, HOX-10, trophoblast HLA gene leading to favourable shift of Th1:Th2 balance in favour of Th-2 type.

Routes of progesterone administration:

There are different routes of progesterone administration in other gynaecological conditions, all of which are not applicable for luteal phase support. This refers to synthetic progesterone formulation other than micronized form of natural progesterone. Routes of administration of progesterone commonly used in practice are presented in following diagram –

Use of micronized progesterone as LPS:

This formulation was first marketed in France in 1980 under the name of Utrogestan and now has been marketed worldwide under various names. It

is obtained from a naturally occurring precursor extracted from soybeans or yam-roots (a Mexican Tree) and is chemically identical to progesterone of ovarian origin. As against synthetic progesterone (except dydrogesterone) micronized formulation provides optimal progesterone bioavailability, which is dependent on both the size of progesterone particle in suspension and the nature of oily excipient.[12] Progesterone can be used; - (a) orally (b) through transdermal route (c) through intra-muscular injection formulation (progesterone in oil) (d) through subcutaneous injection (progesterone in aqueous solution) (e) through vaginal route which is the most accepted and preferred route for administration of micronized progesterone (see fig-2). Each of these formulations will be discussed in following paragraphs.

Oral micronized progesterone:

Micronized progesterone is absorbed orally, but due to its first 'hepatic-pass' effect,[13,14] the absolute bio-availability is only 6% to 8%.[15] This explains why oral administration of micronized progesterone does not help in inducing decidual transformation of E2 primed endometrium,[16] and was demonstrated to be ineffective in providing LPS in ART. Moreover, it has the side effects of inducing drowsiness and gastric irritation. Therefore, oral administration of micronized progesterone is not commonly recommended for LPS.

In spite of these limitations, very recently there has been a renewed interest of oral administration of micronized natural progesterone in a 'sustained release' formulation. If therapeutically effective it will be of benefit since it promises convenience and compliance for the patient.

The formulation shows sustained release pattern over 24hrs with high protein binding of 90 to 99% leading to once a day dosage convenience. The 'smooth' release pattern prevents sudden drug release or loss of bio-availability of the drug due to hepatic metabolism. The dose related central side effects like drowsiness is also minimum. The drug was (oral NMP-SR) developed and was marketed primarily by Madison Pharmacy Associates, Madison Wisconsin Sina 1986. However the drug is on trial, - and its clinical efficacy so far reported has been encouraging. Many more pharmaceutical companies have already started marketing the product. One other synthetic oral preparation of progestin, - dydrogesterone has already been tried as a drug for luteal phase support in ART. Further details of this drug will follow at the subsequent part of this chapter.

Transdermal administration of micronized Progesterone

Use of transdermal application of progesterone for LPS is also not suitable because –

- Unlike transdermal E2 application benefit for LPS cannot be achieved through transdermal application of micronized progesterone
- Poor skin permeability
- Metabolic inactivation of absorbed progesterone by 5α reductase contained in the skin

Intramuscular injectable form of Progesterone

Progesterone in oil for daily intramuscular injection is used to achieve sustained serum concentration of the drug.[17] This route of administration has been used for a long time even before ART came into existence. Intra-muscular injections were used for management of threatened or recurrent miscarriage.[18] Now this

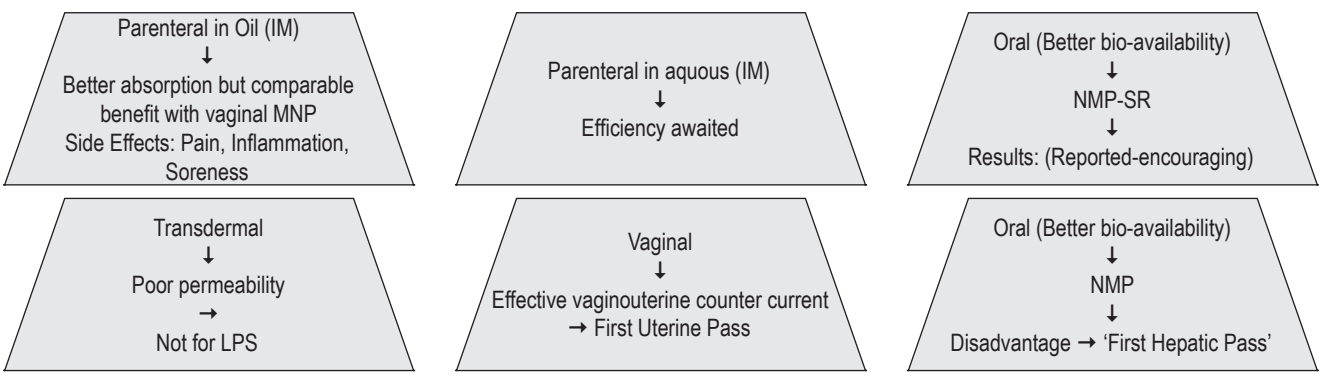


Fig-2

preparation is occasionally used for LPS in ART cycles. The advantages are – (a) Absorption is guaranteed (b) Serum concentration is higher than through vaginal route application (c) But pregnancy rates have been similar both with IM as with vaginal route administration

However there are disadvantages as well. These are –

a) Injectable progesterone is available as Oil based solution
b) Side effects - Pain, inflammation and sometimes abscess formation
c) Moreover inspite of supraphysiologic serum concentration, subphysiologic uterine concentration has been reported

Therefore preference is more for vaginal than for intramuscular route.

Subcutaneous injectable aqueous formulation of progesterone:

This is a new introduction. It has been claimed that with subcutaneous formulation of aqueous progesterone the side effects like pain, local inflammation etc are much less compared to its intramuscular counterpart. But overall benefit of achieving viable pregnancy is yet to be ascertained.

In summary, it appears that oral micronized progesterone through oral administration is not very effective, transdermal application is impractical and injectable route is painful specially when used for longer period of 8-10 weeks. Considering all these evidences it is now clear that vaginal route of application of micronized progesterone is the preferred choice in terms of acceptability and treatment outcome point of view. Moreover, inspite of suboptimal serum progesterone level following vaginal application, tissue concentration of progesterone in the endometrium is high.[19] These arguments are supported by the evidence of possible existence of direct vagina-to-uterine transfer through First-uterine pass effect (FUPE).[20] FUPE results from counter current exchange from vein to artery (from upper vaginal vein to descending cervical artery).

Different pharmacological preparation of micronized vaginal progesterone; their merits and demerits:

Specific advantages of vaginal over other routes of administration have already been outlined in earlier part of this chapter.

In the following paragraphs, primarily specific advantages of intra-vaginal progesterone will be outlined and subsequently the merits and demerits of different manufacturing products of micronized vaginal progesterone will be discussed in more details.

Specific advantages of intravaginal administration of progesterone, these are:- (a) Physiological synchronous secretory endometrial transformation is achieved more with vaginal progesterone than with intramuscular or oral route of administration (b) This is due to 'first uterine pass' effect (c) In addition, this route of administration induces myometrial quiescence – reduces uterine irritability with less risk of embryonic expulsion following embryo transfer (d) Therefore intra-vaginal progesterone supplementation should begin on the day of OPU or one day later

Types of intravaginal progesterone used:

Three types of intra-vaginal progesterone are used -

a) Capsules → e.g. utrogestan orgagest, microgest etc.
b) Gel → e.g. crinone 8.0%, susten 8.0%
c) Pessaries → e.g. cyclogest; not available in India

Pharmacokinetic properties of different vaginal progesterone preparation:

Progesterone **pessaries & capsules** are similar in their pharmacokinetic properties. **Progesterone vaginal gel (8.0%)** which is equivalent to 90mgm natural progesterone (crinone, susten gel) – the specific advantage of gel over pessaries & capsule is, - one application (90 mgm) of gel is equivalent to 600mgm of vaginal capsules or pessaries in divided doses daily

Manufacturing procedure & pharmacological action; - differences between capsules/pessaries and gel:

a) **Progesterone capsules & pessaries are in oil emulsion**; P in the oily phase has to be dissolved in vaginal aqueous secretion for

absorption. Abnormal vaginal discharge will interfere with absorption

b) **Progesterone gel:** oil in water emulsion, on a polycarbophil base; ensures adhesion to vaginal epithelium. Oil in water emulsion guarantees controlled & continuous release. 'P' in aqueous phase is first absorbed in tissues followed by replenishment from the oily phase depot. Polycarbophil maintains a vaginal ph 4.5 resulting in acidity of vaginal flora – acts against infection

Advantages of gel over capsules & pessaries:

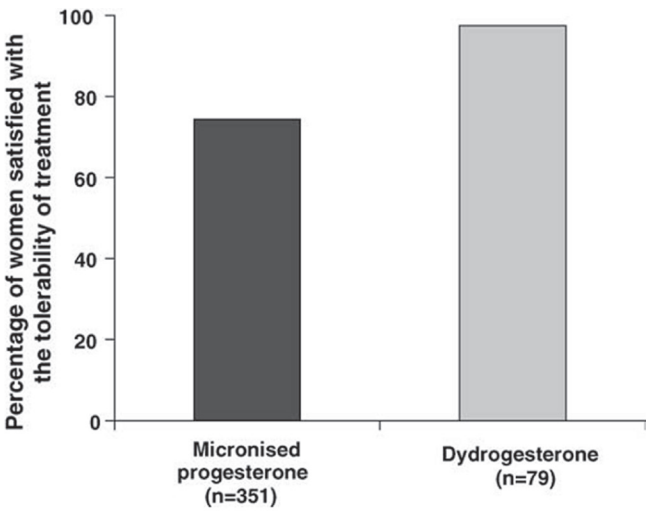
Patient compliance is more with gel than with capsules or pessaries because: (a) It is easier to handle (b) Less time consuming (c) Less pain (d) Single application

Overall advantage of one variety over the other:

Inspite of these differences in all vaginal applications, 'first uterine pass' effect endometrial preparation & pregnancy rates are similar with each preparation.

Use of Dydrogesterone (Duphaston) as Luteal Phase Support:

Dydrogesterone, a retroprogesterone, is a special type of progestogen that can be used for luteal phase support in IVF-embryo transfer programmes.[21–23] It is a stereoisomer of progesterone, wherein the methyl group attached to the 10th carbon is located in the α position (as opposed to the β position in micronized progesterone), the hydrogen attached to the carbon in the 9th position is in the β position (as opposed to the α position in micronized progesterone) and there is an extra double bond between carbons 6 and 7. These changes in configuration make dydrogesterone metabolically stable and orally effective, thereby ensuring that treatment with oral administration is easier than with vaginal micronized progesterone. The gestagenic effect of dydrogesterone is not accompanied by androgenic or anti-androgenic activity, which reduces the risk of influencing gender differentiation of the embryo.[24] Although there are very few published reports regarding the comparative use of dydrogesterone and micronized progesterone as luteal support in ART cycles, our study shows that there are no significant differences in pregnancy rates, miscarriage rates or viable delivery rates with oral dydrogesterone compared to vaginal micronized progesterone. Taking into account parameters such as the patient's age and duration of infertility, pregnancy rates with dydrogesterone were similar to those with micronized progesterone. Interestingly, there was some evidence that, in women whose infertility was due to mild or moderate endometriosis, the pregnancy rate was somewhat higher with dydrogesterone than with micronized progesterone, although the difference was not significant. A previous retrospective study has also demonstrated that dydrogesterone and progesterone are equally effective as luteal phase support in patients undergoing IVF-embryo transfer programmes.[23] In another study, in which oocyte donation was followed by IVF, an ongoing pregnancy rate of 31% was obtained with dydrogesterone.25 In addition to comparable efficacy, a number of advantages have been observed for dydrogesterone over vaginal micronized progesterone, including oral administration, better patient compliance and significantly less bothersome side effects.



Percentage of patients satisfied with the tolerability of micronized progesterone (n = 351) or dydrogesterone (n = 79) ($p < 0.05$).

In our subsequent study,[26] analyzing larger number of patient population with variable patient demographic profile (n=1363) we found comparable results with oral dydrogesterone and the vaginal micronized progesterone application with additional benefit of patient acceptability & compliance in favour of dydrogesterone. Most of these women undergoing IVF have multiple causes of infertility and different demographic profiles. In infertility clinics, very few patients are encountered with a highly favorable profile, and most of the time

clinicians are challenged with infertile women having factors that are unfavorable for IVF. Our aim was to evaluate whether oral dydrogesterone is as effective as micronized P in poor-profile patients. Therefore, these women were classified retrospectively according to the demographic profiles that we commonly encounter in our infertility clinic.

Factors including age (>37 years), FSH (>9–12 IU/mL), dense pelvic adhesions, distorted uterine shape and size, basal antral follicular count (fewer than six), BMI (>25 kg/m2), and duration of infertility(>7 years) were considered unfavorable for IVF outcome. Each factor was given a score of 1 if unfavorable, or 0 otherwise. The subjects were graded depending on the total scores.

The grading criteria showing grade 0 (score 0), grade I (scores 1–3), and grade II (scores 3–5) corresponding to highly favorable, unfavorable, and highly unfavorable categories, respectively, are summarized in Table-1.

TABLE 1: Scoring system adopted for favorable and unfavorable IVF outcome.

Factor	Favorable (score [0)	Unfavorable (score [1)
Age (y)	%37	>37
BMI (kg/m2)	%25	>25
Baseline FSH (mIU/mL)	%9	>9
Aantral follicle count	>6	<6
Antimullerian Hormone	>1ng	<1ng
Pelvic adhesions	Absent	Present
Uterine shape	Normal	Abnormal
Total score	0	6
Note: Grading: Grade 0 (Total Score 0), Highly Favorable; Grade I (Total Score 1–3), Unfavorable; Grade II (Total Score 4–6), Highly Unfavorable.		

These women (23-42 yrs) undergoing controlled ovarian stimulation for IVF treatment (fresh cycle) were analyzed retrospectively. All of them had conventional long protocol stimulation. As per as luteal support is concerned they were classified into three groups: - Group-A (n=422) received 10mg dydrogesterone twice daily (Duphastan; Abbott Pharmaceuticals, India), Group-B (n=482) received micronized vaginal P gel per day (Crinone 8%; Merck Serono, Fleet Laboratories, Watford, United Kingdom), and Group-C (n=459) received 200mg micronized progesterone capsule 3 times daily vaginally (Utrogestan; Basin Pharma, India).

Pregnancy and miscarriage rates for each group were compared among A,B,C (Table-2).

TABLE 2: Demographic data of patients randomly subjected to three different luteal supplementation protocols.

Demographic parameter	Oral dydrogesterone protocol, Group A (n [422)	Micronized vaginal P gel, Group B (n [482)	Vaginal P capsules, Group C (n [459)
Pregnancy rate	28.67 (121/422)[a]	28.63 (138/482)[a]	22.65 (104/459)[a]
Miscarriage rate	11.57 (14/121)[a]	13.04 (18/138)[a]	18.26 (19/104)[a]
Note: Data presented as mean ± SD or percentage (number). [a]Not significant.			

On stratification depending on the grades of patients in each of these groups, the pregnancy and miscarriage rate have been represented in Table-3

No significant differences were observed when comparing the three groups and sub-groups of different grades. It is evident from our study that oral dydrogesterone is effective in infertile women undergoing IVF, irrespective of the cause of infertility or the number of factors contributing to infertility. Our results suggest that oral dydrogesterone is as effective as micronized P for LPS in women undergoing IVF. Several potential benefits of dydrogesterone have led us to believe that this drug

TABLE 3: Comparison of pregnancy rates and miscarriage rates between groups A, B, and C for grade 0, grade I, and grade II.

Parameter		Oral dydrogesterone protocol, group A	Progesterone vaginal gel, group B	Vaginal P capsules, group C	P value
Grade 0	n	73	79	83	
	Pregnancy rate	31.5 (23/73)	34.17 (27/79)	28.91 (24/83)	NS
	Miscarriage rate	13 (3/23)	11.11 (3/27)	16.67 (4/24)	NS
Grade I	n	315	389	357	
	Pregnancy rate	28.25 (89/315)	27.50 (107/389)	21.56 (77/357)	NS
	Miscarriage rate	11.24 (10/89)	13.08 (14/107)	18.18 (14/77)	NS
Grade II	n	34	14	19	
	Pregnancy rate	26.47 (9/34)	28.57 (4/14)	15.78 (3/19)	NS
	Miscarriage rate	11.11 (1/9)	25.0 (1/4)	33.33 (1/3)	NS
Note: Data presented as percentage (number). NS ¼ not significant.					

may be considered as an alternative to vaginal P for LPS. Dydrogesterone, an optical isomer of P, has a high affinity for P receptors, and its conformational structure makes it a metabolically stable and orally effective drug. In patients diagnosed with LPD, dehydrogesterone has been successfully used to induce normal endometrial maturation.[27,28] It is hypothesized that dydrogesterone, through its preferential affinity for P receptor A, has a better potentiality to generate endothelial nitric oxide synthetase and release nitric oxide, thereby enhancing endometrial vascularity.[29] It is also suggested that dydrogesterone restores normal concentration of P-induced blocking factors in T lymphocytes and endometrial cells, which induces a favorable immunologic environment for implantation.[30] To summarize, dydrogesterone is a promising drug for luteal support in woman undergoing IVF and seems to be as effective as micronized P for LPS in women undergoing IVF. Oral dydrogesterone can be considered for routine luteal support because the side effects, such as vaginal irritation and discharge, can be avoided.

Supplementary administration of oestrogen with progesterone in Luteal Phase Support:

Published reports are controversial regarding supplementary use of oestrogen with progesterone in LPS.[31] reported on the basis of meta-analysis of 3 studies that administration of oestradiol for luteal phase support is unnecessary but at the same time commented that data provided in their published reports are inconsistent. Further studies on endogenous oestradiol values would undoubtedly be worthwhile in order to investigate the approach.

Though generally not approved as a valid drug for luteal phase support but there is a theoretical basis supported by few published reports about this rationality of adding oestrogen to progesterone as luteal support for achieving better pregnancy rates. In this chapter, opinions and controversies through published reports will be briefly discussed.

It is now established that progesterone is the more important hormone in the luteal phase and a direct substitution for its deficiency. But progesterone is ineffective in the absence of oestrogen priming. Only progesterone destroys E2 receptors and may therefore lead to E2 decline in the luteal phase – which ultimately will lead to progesterone receptor depletion. In corroboration of this hypothesis, necessity of E2 for biological expression of progesterone activity in the luteal phase has been suggested through following publications:

i. Garcia et al (1988),[32] → E2 is essential for generating P receptor in endometrial cells. P even present in large quantity cannot express its biological activity without binding with the receptors in endometrial and 'T' lymphocyte cells (PIBF)

ii. Shetty et al, 1997[33] →
- Based on animal experiments using Aromatase Inhibitors to abolish E2 production proved the necessity of E2 for the occurrence of pregnancy in ovulatory cycles
- Pregnancy rate was low, miscarriage rate was high

iii. Sharara FI, Mc Clamrock HD, 2001[34] →
- Demonstrated that magnitude of decrease of E2 level could predict the cycle outcome
- Our study confirmed this observation – minor decline of E2 compared to 'steep' or 'sharp' decline only responded favourably to E2 supplementation

Significance of Range of E2 decline – E2 supplementation in pregnant and non-pregnant group

- <50% decline compared to >50% decline on d7 post ET showed better pregnancy rate with E2 supplementation
- Moreover there was some difference when patients were stratified in favourable and unfavourable categories
- Unfavourable group benefited more than the favourable group

iv. Sharara FI, Mc Clamrock HD, 199935
   a. It has also been demonstrated that under 'only' P supplementation, mid luteal E2 levels decrease in a proportion of cases and they may have a decreased pregnancy rate
   b. They may benefit with E2 supplementation

v. Gruber et al, 2007[36]
   - Higher E:P ratio is observed in the clinically pregnant cases compared to non pregnant and those who had preclinical miscarriages
   - Therefore ratio rather than absolute value of E and P is more significant
   - Higher rates of E:P ratio even in normal, OI & IUI cycles are associated with better pregnancy rates and lower miscarriage rates compared to lower E:P ratio

Route of E_2 administration:- Vaginal or Oral

Experience is limited with vaginal application. However, a few reports37 have suggested that vaginal application is more effective than oral administration. The disadvantage proposed against oral administration is that there is rapid and hepatic inactivation of oestrogen through conversion into oestrone. Whereas specific advantage claimed in favour of vaginal administration is based on possible transport of the drug direct to the endometrium via 'counter current exchange' through vaginal route. Consequently the tissue concentration in endometrium of oestrogen is higher through vaginal route than by oral administration.

However, there is a contradiction against vaginal application. Vaginal delivery of a 'lipophilic steroid' (i.e P) which acts mainly on uterine tissue may not be equivalent to the administration of a hydrophilic steroid (i,e E2) which enters immediately into circulation and acts on different organs of the body. Therefore, vaginal administration of E2 may not be that beneficial as applicable for progesterone. But those who favour and support vaginal oestradiol administration have argued that upper part vaginal application allows better E2 distribution than when E2 is administered in the lower part of the vagina. This concept confirms in favour of vaginal administration.

Our limited experiences on E2 administration with progesterone supplementation for LPS (unpublished):

Plan of study:

Because of controversies published in various studies we undertook a small study of E2 administration along with progesterone as LPS in selected groups of patients. Our plan of study consisted of (a) Long protocol down regulation followed by IVF-ICSI (b) selection of demographically similar types of patients.

For evaluation of efficacy of oestrogen as luteal support, E2 was estimated three times during the later part of treatment.

(i) On the day of hCG trigger
(ii) On the day of ET (do)
(iii) On d7 post transfer. Luteal support with progesterone was initiated from the day of embryo transfer.

Dose and route of exogenous E2 administration:

E2 (oestradiol valerate – Progynova (Zydus Cadila Healthcare Ltd. (German Remedies)); Estrabet, 17-β oestradiol (Abott) was administered orally in a dose of 2-4mg daily (maximum 6mg depending on range of decline of E2). A balanced E2 supplementation as LPS in ART cycle is essential because supraphysiological level of E2 might lead to luteolysis,- whereas sub-optimal level of E2 will indicate either LPD or failing implantation.

Supplementation of oestrogen as additional luteal support was considered in our study on three observations:

(a) Level of endogenous oestrogen on day of transfer (do)
(b) Range of decline of E2 on do (if present) compared to level of E2 observed on hCG day (terminal E2)
(c) Range of decline of E2 (when present) on d7 compared to level of E2 on do

For comparative evaluation nearly 50% of patients in each group received E2 supplementation while the rest continued only with progestogen with additional support of E2. Based on this criteria we started with oestrogen either on do (day of transfer) or from d7 (7 day after transfer)

With this protocol of E2 supplementation, results in terms of miscarriage and pregnancy rates were evaluated between two groups of patients namely those who received the support (n=55) vs those who did not receive the additional support (n=50).

We added oestrogen to progesterone under two circumstances:-

1) Oestrogen supplementation starting From the day of embryo transfer (do):-

When E2 was < 700 pg/ml but not below the level of 500 pg/ml or when E2 decline was between the range of 25-50% compared to that on hCG day. E2 was not started when decline was more than 50%.

Basis of exogenous E2 addition on do: In both the circumstances, we presumed that corpus luteum is not efficient to produce sufficient amount of oestrogen essential for progesterone activity to prepare the endometrium as optimally receptive for blastocyst implantation. Further reduction of do E2 (< 500 pg/ml) or magnitude of reduction (> 50%) compared to hCG day, will indicate an irreversibly defective corpus luteum. Such defective corpus luteum will not be able to produce adequate amount of progesterone to stimulate endometrium to develop and grow even in the presence of adequate amount of oestrogen priming. Table 1 shows no significant change in ongoing pregnancy rate or miscarriage rate with or without exogenous oestrogen support. Failure to achieve a higher-pregnancy rate with E2 support indicates that endometrial priming with oestrogen during follicular phase has not been adequate possibly due to defective follicle formation.

Table 4: Adding E2 on Day of Embryo Transfer (E2 level between 500 to 700pg/ml or <50% decline compared to the level on hCG day)

	With Support	Without Support
Total No.	55	50
Pregnancy	15 (27.27%)	17 (34.01%)
Miscarriage	4 (7.27%)	6 (12.0%)

2) Exogenous oestrogen supplementation starting from d7 (post ET):-

Exogenous E2 supplementation was also advocated when E2 decline on d7 was less than 50%. E2 decline commonly occurs in mid luteal phase (d7 post ovulatory);- indicating commencement of luteal regression. But the decline is not 'sharp' or 'deep'; because regressing corpus luteum which usually recovers through hCG stimulation of corpus luteum in case pregnancy occurs in that cycle. In this situation decline of E2 will never be more than 50%. On the other hand if decline observed on d7 is 'sharp' or 'deep' (> 50%) then it may be presumed that pregnancy has not occurred or even if it has occurred,- implantation is 'shallow' because of failing corpus luteum which cannot be rescued by administration of exogenous oestrogen support (table 4 & 5). This may be because, either corpus luteum is grossly defective or embryo quality was not good to produce adequate amount of hCG for rescuing a failing corpus luteum. In these situations oestrogen supplementation will not be effective.

Of course these presumptions are based on trial with a small number of patients. Further trial with larger patient population is essential which may justify our hypothesis.

Table 5: Adding E2 on Day of Embryo Transfer (E2 level between 500 to 700pg/ml or <50% decline compared to the level on hCG day)

N=105	Total No. of Patients	Pregnancy	Miscarriage	Take Home
With Support	57	22 (38.59%)	3	19 (33.33%)
Without Support	48	7 (14.58%)	1	6 (12.5%)

E2 Support is significant ($p<0.02$) – overall results were better ($p<0.03$)- when E2 decline was <50%

N=84	Total No. of Patients	Pregnancy	Miscarriage	Take Home
With Support	49	13	3	10 (18.36%)
Without Support	35	8 (22.85%)	1	7(20.0%)

E2 Support is non-significant in case of >50% E2 value

Take Home Message:

- Luteal phase defect is controversial in indications like infertility, threatened and recurrent miscarriage; and therefore luteal phase support in these situations is empirical.
- But, in ART treatment cycle, luteal phase support is necessary to optimize outcome of treatment
- Because of risk involved in hCG like OHSS, hCG has not been popular as a drug for LPS
- Moreover hCG as LPS has not been proved to be superior either alone or in combination with progesterone when compared to only progesterone
- Similarly addition of oestrogen as LPS with progesterone has not provided any conclusive advantage, although without further trial with

large number of patients a definite conclusion cannot be reached

- Use of oral micronized progesterone is not desirable because of increased rate of side effect due to unphysiological metabolites
- Recently oral micronized progesterone in slow-release formulation (MNP-SR) has been marketed and is claimed to have better absorption rate with higher bio-availability and less risk of side effects. More trial is essential to validate the effectivity
- Similarly subcutaneous micronized progesterone in place of its intra-muscular co-treatment has been introduced which obviously will be more patient friendly. But the drug's effectivity has to be substantiated by further trial
- Inspite of these innovations at present there is no reason to expect disadvantage with vaginal micronized progesterone
- However oral dydrogesterone because of its specific benefits of preferential affinity to progesterone receptor and relatively better immunomodulation potential and more importantly being orally active may in future prove to be an alternative or even be superior to currently used micronized vaginal progesterone
- Supplementation of oestrogen to progesterone has a sound theoretical background but at present in general in combination with progesterone is not recommended, although there may be a small sub-groups of patient who may benefit with exogenous oestrogen supplementation along with progesterone as LPS

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Volatile Organic Compounds(VOC) control in IVF lab: The important determinant for successful outcome in extended culture

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Purpose: This study aims to describe the role of implementing good laboratory practices to improve in vitro fertilization (IVF) outcomes which are of great interest for practitioners dealing with infertility.

Methods Certain modifications were introduced in May 2015 in our IVF laboratory like high-efficiency particulate air CODA system, steel furniture instead of wooden, use of new disinfectants like oosafe, and restriction of personnel entry along with avoidance of cosmetics like perfume to improve pregnancy rates. Volatile organic compound (VOC) meter reading was monitored at two time points and five different places in the laboratory to compare the embryonic develop- ment parameters before (group A: July 2013–April 2015) and after (group B: July 2015–April 2017) remodeling.

Results The IVF outcomes from 4212 cycles were associated in this study. Reduction in VOC meter readings, enhanced air quality, improvement in blastocyst formation rate, implanta- tion, and clinical pregnancy rate were observed in the labora- tory after implementation of new facilities. Results illustrated that the attention must be focused on potential hazards which expose laboratories to elevated VOC levels. Blastocyst forma- tion rate increased.And also Implantation rate, clinical pregnancy rate, and live birth rate increased by around 12, 10, and 12%, respectively.

Conclusion In conclusion, with proper engineering and mate- rial selection, we have been able to reduce chemical contam- ination and adverse effects on culture with optimized IVF results.

Support None.

Keywords VOC . IVF outcomes. Blastocyst. Air quality. Remodeling.

Introduction

We all know that initial few days of life are nurtured in laboratory in ART procedure.So quality of laboratory environment should be at highest level that can mimic with in vivo environment. Air quality is crucial for the success of in vitro fertilization (IVF), because the presence of volatile organic compounds (VOCs) and particulate materials are harmful for embryo development in vitro.[1] Studies have shown that various factors, such as air quality, temperature, light, and VOCs, in IVF laboratory are known to affect oocytes and embryos and therefore can have detrimental effects on pregnancy rate.[1–3] Embryonic growth and development is affected by direct attachment of VOCs to embryonic DNA and terminating its growth.[1] VOC is also associated with DNA fragmentation in human sperm.[4–6] Many practitioners of assisted reproductive technology (ART) are focusing on VOCs,[2,7–10] many

of which can be detected by simple absorption and gas chromatographic or flame ionization detection and analysis. Construction materials are a major source of VOCs in IVF labs. Wood-based furniture release formaldehyde.[11] PVC flooring materials[12] and carpets[13] also release VOCs. Paints[14,15] and adhesives (especially vinyl floor tile adhesive) release numerous VOCs, including aldehydes. Many cleaning products are also sources of VOCs, for example vinyl floor liquid wax which can contain lead[16] ammonia-based products such as glass cleaners and aerosol propellants such as butane or isobutane. Presently, many IVF laboratories are using ethanol as a disinfectant as it has a broad effect against bacteria, but it is also a known VOC. Autoclaved materials (e.g., drapes, instrument packs) can release VOCs when packs are opened for use. Cosmetics, especially perfumes, colognes, and aftershaves, are highly toxic to embryos in vitro, primarily due to evaporation of their solvent bases.[17] Finally, cigarette smoke contains several hundred volatile compounds including recognized carcinogens and mutagens[18] and high levels can contaminate Bfresh^ air intakes if improperly located.

It is essential for IVF labs to set up an air filtration system that has the ability to filter hydrocarbon pollutants, chemically active compounds, and airborne pathogens. Air filtration system removes small inorganic gaseous molecules commonly known as aerosolized pesticides, like nitrous oxide (N2O) and sulfur dioxide (SO2), as well as heavy metals such as lead, that can adversely affect embryo quality and, hence, clinical outcomes.[3] One of the sources for the transfer of particulate materials is punctuation of walls for plumbing and cables which allows migration of pollution from outside to laboratory area.[19] A carefully controlled sealed environment where the entry of particles and contaminants is significantly minimized may be achieved by highly filtered air being flushed through high-efficiency particle air (HEPA) filters under positive pressure.[1] Recently, oosafe, a quaternary ammonium compound,[20] has been introduced as a disinfectant for cleaning CO2 incubators. Willium Lee[20] reported in his thesis that oosafe is non-toxic for gametes. Many IVF labs—even entire clinics—are now Bperfume-free^ zones.[3]

Our study goal was to evaluate IVF outcomes using extended embryo culture after renovation of IVF suite and implementation of good laboratory practices and volatile organic compound filtration.

Material and methods

Study design: In this observational study, we have evaluated the efficacy of blastocyst formation, clinical pregnancy rate, implantation rate, and live birth rate along with VOC assessment before (group A: July 2013–April 2015) and after (group B: July 2015–April 2017) remodeling of the IVF laboratory of our clinic. One thousand thirty-six IVF and intra-cytoplasmic sperm injection (ICSI) patients were included in our study where our patients had blastocyst transfer. Five hundred fourteen patients were included in group A and 522 in group B. We have also recorded the demographic profile of the patients included in both group A and group B. Long gonadotropin-releasing hormone agonist downregulation was used followed by ovarian stimulation by gonadotropins. Either ICSI or conventional insemination (30,000 to 40,000 motile sperm per oocyte) was done 5 to 6 h of ovum pickup followed by direct swim up technique of sperm preparation. Patients who underwent egg retrieval but had embryo transfer on days 2, 3, and 4 or no embryo transfer at all due to unavoidable reasons were excluded from our study. Frozen cycles were not included as many other factors play a role in embryo quality during freezing. The culture conditions for embryo culture were the same for the groups, that is, temperature, pH, oxygen concentration (5%), humidity, osmolality were maintained. The media used for embryo culture were purchased from Vitrolife Pvt. Ltd. (Göteborg, Sweden). The incubators used were conventional triple gas incubators.

Ethical clearance: Experiments encompassing this study were performed in accordance with the Institutional Human Ethical Committee of Institute of Reproductive Medicine (IRM), HB-36/A/3, Salt Lake City, Sector III, Kolkata 700 106 for conducting the clinical study. The ethics committee operates according to the requirements of Good Clinical practice (GCP), Schedule Y, and Indian Council of Medical Research (ICMR). The protocol number for the clearance is IVFM-11-02 dated 01 Feb 2012.

Remodeling of IVF laboratory: Reconstruction and addition of new facilities were introduced in the areas where gametes and embryos were handled.

This included the embryology laboratory and also the associated areas (oocyte retrieval room, embryo transfer room, sperm process room). The size of the main laboratory is around 120 ft^2. At the end of construction which was completed in 1 month, we waited for another 1 month to allow off-gassing before starting new IVF cycles. Wood-based furniture was replaced with steel. Anodized aluminum was used in doors. Epoxy paints were used on the floor instead of PVC flooring. Double-shield glass on the windows was used to make the room airtight. Aerosol/ammonia-based cleaning of IVF laboratory was strongly discouraged. IVF lab was established as a Bperfume-free^ zone. Cosmetics were also avoided inside the laboratory. Earlier, the incubator was brought in use soon after installation (without burning in); however, later on, it was used after a burn-in period of 10 days. One CODA system was introduced in the main lab away from the incubators. Cleaning of all surfaces and doors, mopping of floors, and emptying of waste were done on a daily basis both in laboratories and adjacent areas. In place of alcohol, a recent disinfecting product named Oosafe® (SparMed, Denmark) was used for cleaning of tables and floors. Sinks were removed and drains were closed in the clean room areas. Separate entry for oocyte retrieval room and laboratory was made with a pass through window between the two. It was used for transfer of gametes and embryos between laboratory and operating room preventing mixture of air between the two. Entry of persons was restricted. Persons were not allowed to smoke in working hours. They had to change their dresses (cotton fabric) before entry and wear cap, mask, and shoe cover. Changing room was just adjacent to the entry door of laboratory. In addition, adhesive-covered mats were used at the entrance to laboratory to remove dirt and dust from the soles of shoes. Care was taken to avoid items which emit VOCs. Powder-free disposable gloves were used to handle gametes and embryos. Our laboratory system design is a stand-alone system that recirculates air.

VOC measurement: VOC levels were measured using a SASWELL VOC meter AQC910.1V-DO. It has an internal mix gas sensor which is VOC sensitive. VOC was measured in various locations within the embryology laboratory and other adjacent areas before and after remodeling. Monitoring was performed in three locations as shown in Table 3 (a.—beside the work station, b. and c.—in areas 1 and 2 between the incubators) inside the embryology laboratory and two locations in the ICSI room adjacent to the laboratory (d.—ICSI room near microscope, e.—ICSI room near incubator) throughout the study period (July 2014 to April 2016) but as an example, we have given data for seven consecutive days at the same time, prior to and after the lab remodeling. We prepared Table 5 considering two time points, one in the morning during ovum pick up and another in the evening. In the morning, 3–4 persons were present in the laboratory whereas only 1–2 persons were present during evening time. We have also measured that the humidity level in the VOC meter was around 40 to 45% throughout the day.

Assessment of fertilization and embryo quality: Following the changes to the laboratory, various embryogenesis parameters were assessed (i.e., blastocyst formation, clinical pregnancy, embryo implantation rates, and live birth rates) in our laboratory and compared with the same parameters before remodeling. Blastocyst was characterized by development of blastocoel in compacted embryo. One to two embryos were transferred on day 5 and other good quality supernumerary embryos were cryopreserved.

Pregnancy testing: Positive urinary β-human chorionic gonadotropin (β-HCG) after 14 days of embryo transfer was defined as biochemical pre gna ncy. Clinical p regn anc ies were con firmed by transvaginal ultrasonography at 6–7 weeks of gestation.

Statistical analysis: Data were presented as the mean ± SEM. Student's t test was done wherever applicable, and significance was expressed as a p value and 95% confidence interval of the difference. Statistical analyses were done using the software GraphPad InStat Version 3.06 (La Jolla, CA, USA).

Results

Out of 4212 patients that were included in our study,2110 underwent IVF/ICSI cycle before remodeling of the laboratory (group A) whereas 2102 had IVF/ICSI cycle after renovation (group B). Baseline demographic parameters like age of the female partners, duration of infertility & basal hormonal profile are mentioned in Table 1.

No significant variation was found in the different baseline parameters between group A and group B.

Table 2 includes cycle-specific details like number of oocytes retrieved, fertilization rate, number of embryos at cleave stage, blastocyst formation rate, and number of frozen embryos whereas Table 3 depicts IVF outcome in terms of clinical pregnancy rate, implantation rate, and live birth rate. Evaluating results over this period, there was demonstrable benefit of operating under these optimum laboratory environmental conditions, which resulted in improvement in all the parameters. Blastocyst formation rate increased significantly (2.25±0.35 vs 3.96±0.82; P value <0.05)) after making changes in the laboratory. Consequently, the implantation rate also improved from 48.91% to 60.73% with $p \leq 0.05$. Clinically successful pregnancies before and after VOC reduction (29.71% vs. 39.42%) varied significantly ($p \leq 0.05$). Simultaneously, the live birth rate also increased from 21.81 to 33.57% with $p \leq 0.05$.

Table 4 represents the variation in VOC meter readings in parts per million (Bppm^) at different locations in the IVF laboratory before and after renovation. Renovation reduced the VOC levels in the IVF as well as the ICSI laboratory significantly. According to the VOC meter readings, workstation was found to be relatively free of organic compounds after remodeling. Figure 1 represents the locations where VOC was measured.

The variations in VOC meter readings (Bppm^) during morning and evening time before and after renovation are shown in Table 5. Reconstruction depicts reduction in VOC meter reading at both the time points for all 7 days. However, in comparison to the evening time, readings are higher in the morning. The panels a, b, c, d, e mentioned in the figure corresponds to the locations mentioned in Table 4.

TABLE 1: Profile of demographic variations in two groups.

Parameters (mean ± SEM)	Group A (n=2110)	Group B (n=2102)	p value
Age of female partner (mean)	32±4	34±5	0.754
Infertility duration(years)	5.4±3.1	5±2.9	0.924
Basal FSH (IU/ml)	6.8±2.1	6.6±1.7	0.941
Days of stimulation	10.3±1.6	10.6±3.3	0.934
Total dose of gonadotropins used (IU)	2250±718	2152±556	0.914
E_2 on hCG day (pg/ml)	3110±1252	2910±1450	0.916
P_4 on hCG day (ng/ml)	0.5±0.3	0.6±0.2	0.781
Mature follicle (>18 mm) on HCG day	7.4±3.5	8±4.1	0.911
Endometrial thickness on the day of hCG (mm)	10.4±2.5	11.1±2.1	0.8303
The p values indicate that the differences between the two groups are insignificant			

TABLE 2: Variations found in the different cycle outcome in two groups

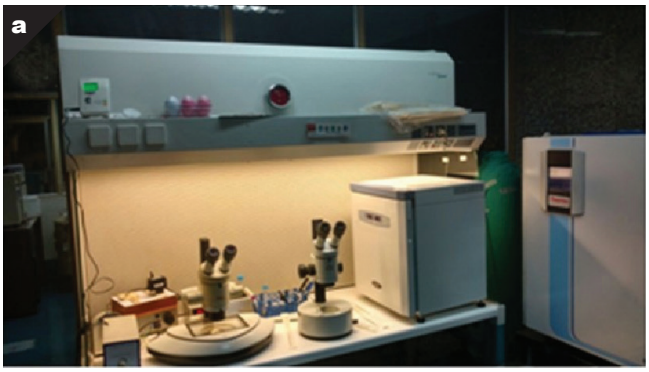
Parameters (mean ± SEM)	Group A (n=2110)	Group B (n=2102)	p value
Oocytes per cycle	12.03±3.1	12.21±3.6	0.091(NS)
Dysmorphic oocytes	1.03±0.04	1.15±0.07	0.709(NS)
Pronuclear stage	7.12±1.2	9.11±1.1	<0.05(S)
Cleavage stage Blastocyst formed	2.25±0.35	3.96±0.82	<0.05(S)
Frozen embryos	0.37±0.04	1.57±0.86	<0.05(S)
NS non-significant, S significant			

TABLE 3: Biochemical rate, clinical pregnancy rate, implantation rate, and live birth rate before and after renovation

Indicators	Group A	Group B	p value
Beta HCG positive (%)	31.63	43.97	<0.05(S)
Implantation rate (%)	48.91	60.73	<0.05(S)
Clinical Pregnancy Rate (%)	29.71	39.42	<0.05(S)
Live birth rate (%)	21.81	33.57	<0.05(S)
The p values indicate that the differences between the two groups are significant			

TABLE 4: Difference in VOC meter readings (Bppm^) at various locations in the IVF laboratory before and after renovation

Locations	VOC reading before remodeling	VOC reading after remodeling	p value
a. Beside work station	19±0.6	4±0.5	<0.05(S)
b. Area 1 in between the incubators	20±2	7±0.4	<0.05(S)
c. Area 2 in between the incubators	21±1	5±0.4	<0.05(S)
d. ICSI room near microscope	23±2	8±2	<0.05(S)
e. ICSI room near incubator	23±1	7±0.5	<0.05(S)
The p values indicate that the differences between the two groups are highly significant. The number of readings per site is 14 (readings have been taken for seven consecutive days at two time points: morning and evening)			



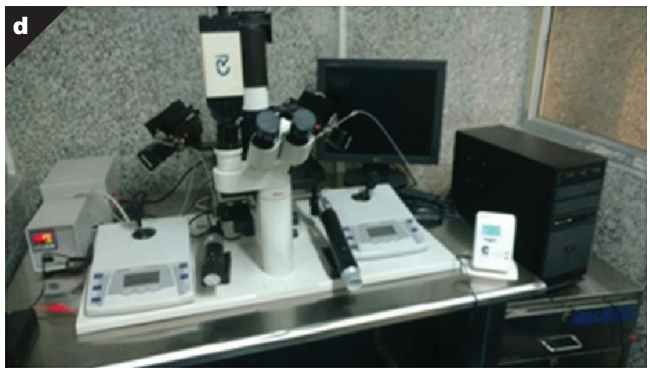
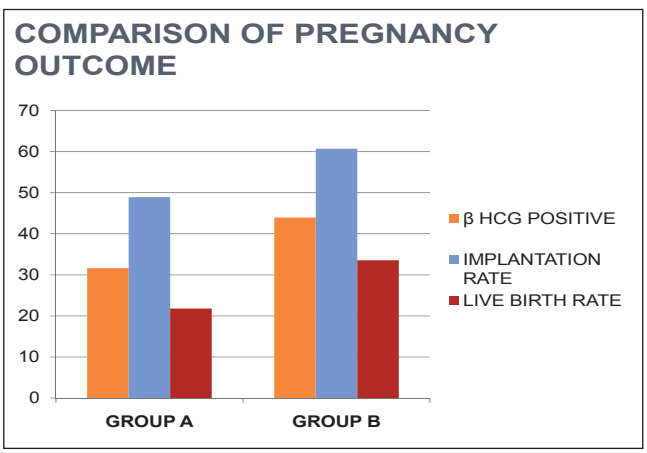


Fig. 1 The variations in VOC meter readings (Bppm^) during morning and evening time before and after renovation are shown in Table 5. Reconstruction depicts reduction in VOC meter reading at both the time points for all 7 days. However, in comparison to the evening time, readings are higher in the morning. The panels a, b, c, d, e mentioned in the figure corresponds to the locations mentioned in Table 4.



There was increase in the number of good quality blastocysts; that is, blastocoels' cavity size was good and it appeared on time, increased cell number in inner cell mass, and increased compactness of trophectoderm cell layer. We have used Gardner classification system for grading the blastocysts.[21]

TABLE 5: The variations in VOC meter readings (Bppm^) during morning and evening time before and after renovation on different days

Day	Morning (11 A.M.) VOC in ppm		Evening (4 P.M.) VOC in ppm	
	Before	After	Before	After
1	19.2	7	14	2.4
2	24	5.4	18.3	4
3	25.4	8	17	3.5
4	22	6	16.5	2.9
5	20	7.3	15	5
6	25.1	6.3	17.4	4
7	24	5.8	18	3

The two sets of data acquired on different days in the morning and evening time were compared separately for before and after renovation. Both the sets were considered extremely significant having $p < 0.0001$. Number of readings per site is 14 (readings have been taken for 7 consecutive days at two time points morning and evening). The mean 95% confidence interval between the two groups during morning is 16.271 and interval of difference between them is 14.106 to18.437. The mean 95% confidence interval between the two groups during evening is 13.057 and interval of difference between them is 11.571 to 14.543

Discussion

This article describes the importance of VOCs on the IVF outcomes in the laboratory. Embryos are sensitive to the environment and VOCs are one of the vital factors affecting embryonic development. VOCs are hydrocarbon-based organic compounds that have a high vapor pressure at room temperature and so are detrimental for IVF outcomes. They are emitted by various laboratory instruments, furniture, perfumes, aftershave, cosmetics, smoking, etc. used by the persons working in the laboratory. According to the present day findings, VOCs (over 1 ppm) are directly toxic to embryos.[22] Ideally, VOC levels should be below 0.5 ppm for acceptable blastocyst development and reasonable pregnancy rates, but preferably zero. So, IVF laboratory should be constructed in such a manner that minimizes the production of VOCs and also where other conditions like temperature and humidity are controlled. Similarly, it is important to have valid monitoring system from time to time.

This IVF laboratory was established in 1999 and renovation was done in May 2015. A detailed study of various parameters was done before and after making changes in the laboratory and found a significant improvement in IVF outcomes after 2015. One of the major challenges was to control air quality inside the laboratory and to ensure that gametes are not in contact with toxic materials. Positive pressure airflow in the laboratory coupled with the use of air purification systems[23] may reduce the concentration of airborne particles and also reduces bacteria and other contaminants, since these microbes attach themselves to the air particles. Removal of these particles requires passing of air using positive pressure through filters having different porosities. High-efficiency particulate air (HEPA) filter is one of such filters which trap most of the microbial contaminants and thus purify air. We have also introduced new CODA filtration unit in our laboratory since the old CODA system filtration was non-functional and the filtration unit was not available. This contributed in improving air quality. According to Munch et al. 2015, poor air quality affects embryo development leading to inferior IVF outcome.[24] Other studies also have demonstrated similar results like ours that is improvements in pregnancy and implantation rates after using the CODA system.[25]

In this embryology laboratory, we have installed one electronic VOC meter that can measure VOC level in ppm concentration accurately in the enclosed laboratory environment. VOC monitoring is a part of our quality control and we are recording it daily. We had measured the VOC values over the entire study period but here we had represented the data for a week as it remained almost similar throughout the study period. There were no drastic fluctuations in levels in either time period. Baseline parameters of the patients included in the study are shown in Table 1. All the parameters were similar in terms of age, hormonal profile, dosage of gonadotropins, etc. The results in Table 3 showed that the number of embryos that developed to the blastocyst stage was significantly higher after changes in IVF laboratory. We had cultured the embryos till blastocyst stage which exposed them for a longer time to the laboratory environment. So, the effect of air quality on the extended culture was reflected more closely. The positive effects were seen not only on blastocyst formation rate but also on clinical pregnancy and live birth rates which increased significantly after renovation. Improvement in all the above parameters was statistically significant cueing towards the deleterious potential of the environmental toxicants. Similar effects were seen by many studies conducted in different parts of the world. Boone et al.[26] observed the increase in highquality embryos after improvement in air quality. Esteves et al.[27] also demonstrated better outcomes after ICSI cycles with strict air quality control. Clinical pregnancy rate after moving into the new laboratory was significantly higher than the old laboratory—42.6 versus 30.6%.[28]

After renovation, visible improvement was observed in the VOC readings at all the places as well as at different time points compared in the table confirming that the changes in the laboratory were effective in reducing contamination in the vicinity of laboratory as depicted in Tables 4 and 5. Human activities in and around the embryology laboratory are one of the major sources of contamination.[15] Various studies have demonstrated that maintaining clean conditions during oocyte retrieval and embryo transfer is one of the critical steps. This holds true for our study also as VOC readings were higher in the morning during ovum pickups when there were more number of persons around and gradually reduced over the day with decrease in number of individuals.

So, after renovation, access was restricted in our laboratory. Separate entry was made for laboratory and operation theater with a pass through window between the two. The present results illustrate that attention must be focused on the construction material, furniture, paints, day to day cleaning, etc. It is recommended to use low odor specialized paints.[29,30] Not only the main IVF laboratory room but also the adjacent areas like ICSI room, operation theater, and changing room play an important role. So, it is important to design laboratory cautiously.[31]

It was observed in a study that oosafe^ had no deleterious effect on gametes and embryos and was better compared to alcohol which itself is a VOC.[20,32] Oosafe disinfectant was found to be effective in cleaning the surfaces of tables, incubators, and floors and eliminating of microorganisms with no effect seen on embryo morphology and survival rate after the use of oosafe.[20] Further studies are required to establish the effectiveness of oosafe.

The overall effect of renovation of laboratory was prominent on the morphology of the blastocysts formed. According to Gardner and Schoolcraft, there were more grade 1 and grade 11 embryos after changing laboratory practices in comparison to before renovation; so, the quality of embryos improved.[21] VOCs may significantly affect the embryo quality[1] and proper design of the laboratory is essential.[31] Air quality conditions were better in the new site and were associated with higher blastocyst formation, implantation, and live birth rates in couples undertaking treatment in our new facility.

Effect of air particulate filtration system on embryo development is debatable. While Heitmann and his colleagues[5] and Morbeck et al. 2015 observed higher embryo development after improving air quality on the other hand, according to Esteves et al.,[33,34] little is known about the benefits of particulate filtration system.[35–37] Other important issues like indoor air quality, equipment/ furniture, construction and disposable materials, cleaning agents used, and personnel are less explored areas. Therefore, in this stud y, we have focused on the abovementioned areas rather than the filtration system.

The strength of this study was the large sample size that had helped to draw an accurate conclusion. One of the limitations of this study was its retrospective nature. More of randomized controlled trials are required to assess the impact of air quality on IVF outcomes. Another drawback was the monitoring of VOC in ppm instead of parts per billion (ppb). Use of VOC meter with detection limit of ppb would have made the study more sensitive.

In conclusion, our study confirms that implementation of air quality control systems and acquisition of good laboratory practices improved IVF outcomes significantly. We therefore suggest that any potential hazards which expose laboratories to elevated concentrations of VOCs should be determined and consequently controlled. So, more sensitive and optimized methods for controlling air contaminations are warranted to improve pregnancy outcomes especially in extended culture. Further research for longer time period, use of more sensitive instruments, and optimized methodology are required to improve our understanding of IVF outcomes due to fluctuations in air quality.

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Compliance with ethical standards: Experiments encompassing this study were performed in accordance with the Institutional Human Ethical Committee of Institute of Reproductive Medicine (IRM), HB-36/A/3, Salt Lake City, Sector III, Kolkata 700 106 for conducting the clinical study. The ethics committee operates according to the requirements of Good Clinical practice (GCP), Schedule Y, and Indian Council of Medical Research (ICMR). The protocol number for the clearance is IVFM-11-02 dated 01 Feb 2012.

Conflict of interest: The authors declare that they have no conflict of interest.

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Role Of Tamoxifen In Women With Thin Endometrium (<7mm) After Clomiphene Use

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INTRODUCTION:

Tamoxifen (TMX) closely resembles Clomiphene citrate (CC) both in structure and mode of action. It appears to have agonistic action on the endometrium.[1] TMX, primarily developed for use in the treatment of breast cancer, is a selective estrogen receptor modulator that closely resembles CC. Published literature reported ovulation rate of 50-90% and pregnancy rate of 30-50% following TMX.[2] Like CC, TMX occupies estradiol-binding sites on the hypothalamic– pituitary axis and prevent the negative feedback effect of estradiol, resulting in increased endogenous gonadotropin secretion.[3] Direct action on the ovary without involving hypothalamic-pituitary axis has also been suggested.[4] TMX unlike CC acts as an agonist on the endometrium and cervical mucus.[2] Simultaneously, its use for ovulation induction for short duration is also not associated with increased risk of ovarian and endometrial cancers.[5]

The increased estrogenic stimulation that has been observed with TMX action on the lower genital tract may be beneficial, especially for those suffering from an adverse response following the administration of CC. It was postulated that, by administration of TMX, it might be possible to mimic the action of CC for the stimulation of ovarian follicles and avoid the adverse effects of CC on the endometrium. All these make TMX a promising alternative to gonadotropins.

Intrauterine insemination (IUI) is commonly used for large group of subfertile patients.[6] It is cheaper, easy to perform, and more acceptable to the couple when compared to in-vitro fertilization (IVF)/intra-cytoplasmic sperm injection (ICSI).

Clomiphene citrate (CC) continues to be the most commonly used drug for ovarian stimulation in IUI cycles. Despite an ovulation rate of 50–75%, pregnancy rate per cycle is in 10%–20% of cases[7,8] due to anti-estrogenic effect of CC at the level of endometrium. Endometrial thinning has been observed in 15%–50% of women who used CC.[9,10]

In order to increase endometrial thickness (ET) various strategies have been tried to minimize the anti-estrogenic actions of CC but with limited success. Addition of systemic or vaginal estrogen along with CC treatment was reported to increase ET.[6,7] Low-dose aspirin[8] and sildenafil used intravaginally[9] may modulate uterine artery blood flow and hence improve ET. Other methods like starting CC earlier in the cycle,[10] use of letrozole[11] and delaying the hCG administration[12] has also been proposed. However, all these options to get better endometrial thickness are controversial.

Treatment with gonadotropins and IUI is found to be highly successful in patients who do not conceive after treatment with CC undergoing IUI. Gonadotropin use raises several concerns like expense, intensive monitoring, multiple pregnancy rate, which is equal to or higher than in IVF.[13]

A prospective study was conducted at our institute to compare the efficacy of low dose CC (50mg), TMX, and gonadotropins in women with thin endometrium (<7mm) following CC (100mg) in IUI cycle.

Materials and Methods:

This was a prospective study carried from December 2011 to June 2013. A total of 502 women between 25 to 38 years undergoing 932 IUI cycles were included for the following indications: male factor, anovulation, and unexplained infertility (Figure 1). All women had endometrial thickness (ET) less than 7mm after 100mg of CC in earlier cycle were recruited. Pelvic ultrasonography was performed and patients with any uterine or adnexal pathology were excluded from the study. All male partners who had total motile sperm count of less than 5×10^6/ ml were also excluded.

A hysterosalpingogram (HSG) was done to rule out tubal block and patients with at least one tube patent were only taken. Moderate to severe endometriosis patients were excluded from the study. Polycystic ovary syndrome (PCOS) was defined according to the modified Rotterdam revised ESHRE/ASRM criteria.[19] The diagnosis of unexplained infertility was done based on normal findings in semen analysis, mid-luteal serum progesterone and tubal patency seen by HSG or laparoscopy.

Total 502 women who had thin endometrium (<7mm) after CC (100mg) in IUI cycles were divided into three groups based on drug of ovarian stimulation. Two months gap was given prior to ovulation induction in all three groups. Group A included 182 patients who had 364 stimulation cycles, received CC 50mg /day from D3 - D7. Group B included 179 patients who had 342 stimulation cycles, received TMX 40mg /day from D3 - D7. Group C included 141 patients who underwent 226 cycles, received u-FSH 75 to 150IU starting from D3 till day of hCG.

Serial transvaginal sonography (TVS) was done from day 10 of the cycle for follicular monitoring. The measurement of the internal diameter of each visible follicle was performed in two planes and the average diameter was taken. In addition, the ET, was measured from the outer to outer edge of the endometrial–myometrial interfaces in the widest part of the endometrial cavity in the mid-sagittal plane. Urinary hCG (5000IU) was given when the leading follicle was ≥18mm and ET ≥ 7mm for ovulation trigger. In patients with ET <7mm ovulation trigger was postponed till ET reached ≥7mm. Women with persistent thin ET(< 7 mm) and/ follicle >24mm were also excluded from the study. Cycle was cancelled in 24 patients who had ≥4 follicles with ≥16 mm diameter. Our main outcome measures were to anayse ET, pregnancy rate and live birth rate.

RESULTS:

Total 277 cycles were cancelled out of 932 cycles. Most of the cancellations in TMX group was due to inadequate response or failure to achieve follicle of ≥16 mm. On the contrary, over response that led to the presence of too many mature follicles (>4 follicles ≥16 mm) was the main cause of cancellation in the gonadotropin group (43.63%). In low dose CC group thin endometrium and luteinised unruptured follicle were the major cause of IUI cancellation. On-demand failure to obtain a semen sample was another reasons for cancellation (Figure 1). In PCOS women response to TMX was inadequate in 55.2% of cycles which were cancelled (Table 1). The clinical profile including age, duration of infertility, BMI, baseline FSH, LH and E2 of patients belonging to Group A, B and C undergoing IUI are comparable. Different cycle parameters of the three groups are shown in Table 2. The ovulation rate was found to be comparable in all groups. Endometrium thickness was found to be significantly higher in both TMX and gonadotropin group than CC group. Follicle number in the TMX group was significantly low ($p<0.001$) compared to CC or gonadotropin group. However, size of the follicle was significantly higher in clomiphene group compared to other two groups on the day of hCG. TMX and gonadotropin group showed similar pregnancy rate (14.52% vs 14.89%) and live birth rate (12.2% vs. 12.7%). But, in low dose CC both pregnancy rate ($p<0.002$) and live birth rate ($p<0.004$) were statistically lower compared to TMX or Gn groups. There were three cases of twin pregnancy in gonadotropin group (Table 3).

DISCUSSION:

The present study showed the role of TMX in ovulation induction compared to gonadotropin and low dose CC in women with thin endometrium following CC. ET as a predictor of success for ART treatment is well established. Studies have shown that pregnancy and implantation rates for the patients with endometrial thickness >7 mm were significantly higher than those of patients who showed a thin endometrium.[2] Furthermore, ET <8 mm on the day of administration of hCG increases preclinical abortions.[22] Thin endometrium, the most common antiestrogenic side effect of CC treatment, has been seen in 15- 50%. This unfavourable effect of CC increases with higher dose.[23] Hence, in our study we have included group A, in which the patients were stimulated with lower dose of CC (50mg) so that the antiestrogenic effect on endometrium will be low. Since thin endometrium is a risk factor for implantation failure, gonadotropin stimulation was used as the next line of management. Gonadotropin therapy, although effective, not only burdens the patient with stress and medical cost but can also cause multiple pregnancy and ovarian hyperstimulation

syndrome (OHSS). Therefore, preventing CC induced thinning of the endometrium by alternative methods like TMX appears promising.

Women who had thin endometrium with CC (<7 mm), exhibited improved endometrial thickness when TMX was used for stimulation in the subsequent cycle.[24,25] In line with the above findings, in our study, we also observed improved ET following TMX similar to the above studies.

The pregnancy rate and live birth rate in TMX group were found to be comparable to gonadotropin group, but significantly higher when compared to CC group (Table 3). Inadequate response leading to cancellation of cycles was significantly higher in PCOS women following TMX. It appears that TMX is not as effective as CC for ovulation induction in PCOS women. This is in contrast to the meta-analysis which concluded that there are no appreciable differences in ovulation or pregnancy rates after treatment with TMX or CC in anovulatory infertility.[26,27] Similar to our findings, a randomized controlled trial (RCT) by Badawy et al reported a significantly lower ovulation rate following TMX compared to CC in PCOS women, which concluded that CC is more successful than tamoxifen in PCOS women.[28]

It has been noted that leading follicle in CC group on the day of trigger was greater ($p<0.001$) compared to the other two groups. This is because many patients in the CC group had ET <7mm when the follicular size reached ≥18mm and hence hCG administration was delayed till endometrial thickness reached ≥7mm, which resulted in greater follicular diameter. The number of cancellations due to over response following gonadotropins was higher probably due to increase in dose of gonadotropin when inadequate response was noted. Though mechanism of action is similar in both CC and TMX, we noted significantly less number of follicles following induction with TMX.

We conclude, TMX appears to be a promising drug in patient with thin endometrium after CC stimulation by increasing live birth. It seems to be less effective in women with PCOS who earlier responded well with CC. Further RCTs are needed to confirm the findings.

Our work on role of tamoxifen in thin endometrium has been published in Journal of Human Reproductive Sciences.

For further reading : Sharma S, Rani G, Bose G, Saha I, Bathwal S, Chakravarty BN. Tamoxifen is better than low-dose clomiphene or gonadotropins in women with thin endometrium (<7 mm) after clomiphene in intrauterine insemination cycles: A prospective study. J Hum Reprod Sci 2018;

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Medical Management Of Male Infertility: Evidence Based

Shovandeb Kalapahar, Sunita Sharma, B N Chakravarty

Approximately 15% of couples of reproductive age are affected by infertility (Sharlip et al., 2002). Male factors alone account for infertility in 20% of cases and is a contributing factor in a further 30% (Comhaire et al., 1987; Nieschlag, 2000; Hurst & Lancaser, 2001). Etiologies of male infertility can be due to identifiable hormonal or anatomical causes (figure- 1) that may be reversible or irreversible. Some of the known causes of male infertility can be treated medically with high success rates. In the majority of patients with male infertility, the patient has unexplained abnormalities in sperm parameters, or unexplained azoospermia (40%-50%), reflecting our poor knowledge of the mechanisms that control the testicular function. While the role of hormone therapy for men with an identified abnormality are well defined, but there is no consensus on the management of idiopathic oligospermia. This chapter will highlight hormone evaluation indicated for male infertility and its management. It also highlights the empirical therapies including antioxidants, estrogen receptor modulators (clomiphene), aromatase inhibitor (anastrozole, letrozole).

HORMONAL CONTROL OF SPERMATOGENESIS:

The spermatogenesis is controlled by genes located on the Y chromosome and takes approximately 70 days to complete from the spermatocyte stage to mature sperm. Another 12-21 days are required for the transport of sperm from the testis through the epididymis to ejaculatory duct.

Testicular function includes the production of testosterone (T) and spermatogenesis. The seminiferous tubules (the site of spermatogenesis) and the Leydig cells (the source of testosterone) of the testis along with sertoli cell are involved in proper testicular functions. Hypothalamic-pituitary-gonadal (HPG) axis controls the function through two pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone(LH). LH stimulates Leydig cells in the testicular interstitial cells

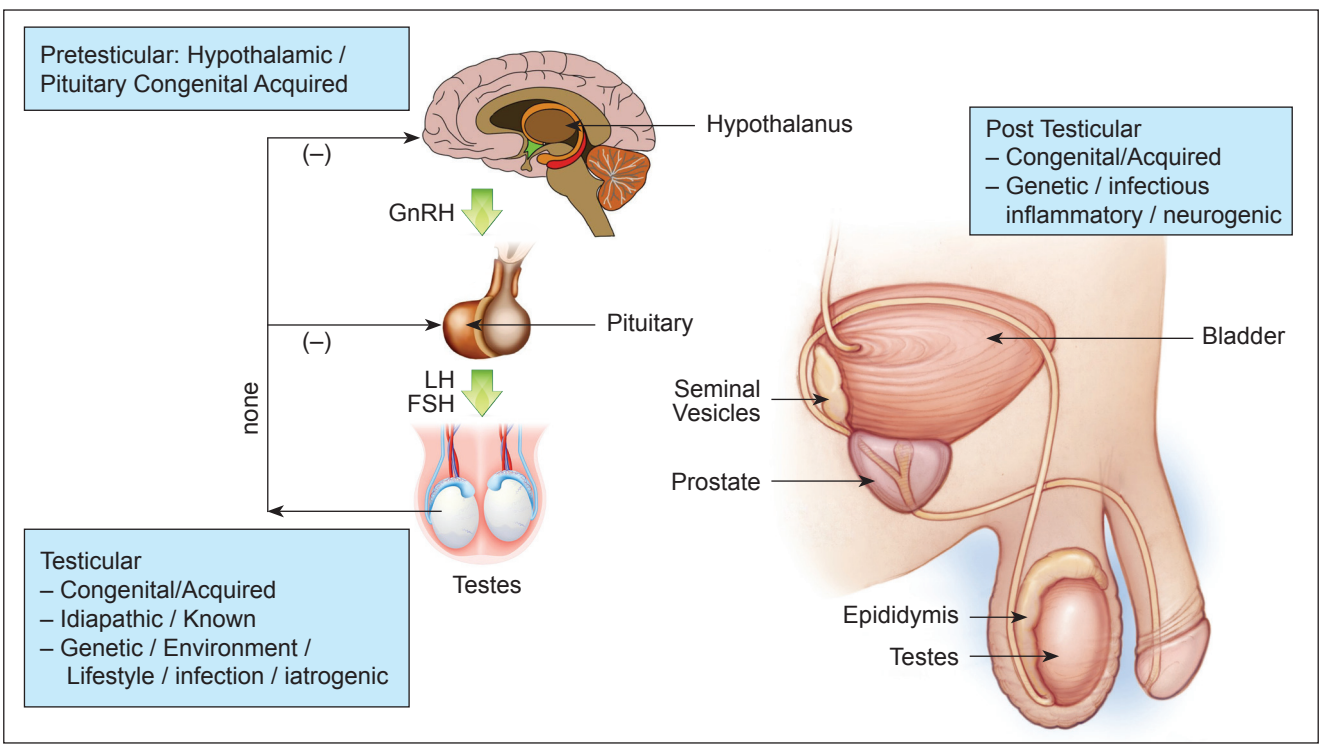

Figure 1: Principal Mechanisms behind Impaired Semen Quality

to synthesize and secrete testosterone. The actions of LH are supported indirectly by FSH, which induces the appearance of LH receptors on testicular leydig cells and stimulates synthesis of androgen binding protein (ABP) in sertoli cells. Spermatogenesis is dependent on high intratesticular T and follicle-stimulating hormone (FSH) stimulation of the Sertoli cells. Although T is essential for spermatogenesis, the administration of T and other androgens inhibit spermatogenesis by exerting a negative feedback on HPG, inhibiting luteinizing hormone (LH) stimulation of intratesticular T production, as well as FSH stimulation of Sertoli cells.(Figure 2)

Normal testicular function requires the actions of Testosterone which is highly concentrated into the lumen of the seminiferous tubules to support spermatogenesis in the germinal epithelium and sperm maturation in the epididymis (concentrations within the testis are 50-100 times higher than in blood).The actions of testosterone in support of spermatogenesis are mediated by the sertoli cells,which line the seminiferous tubules and contain androgen receptors.

High estradiol (E2) level also inhibit LH and thus impairs spermatogenesis The patients with low T and high E2 are good candidate for treatment with aromatase inhibitors.

Male infertility evaluation revolves on three basic parameters:

1. History
2. Clinical examination
3. Investigations

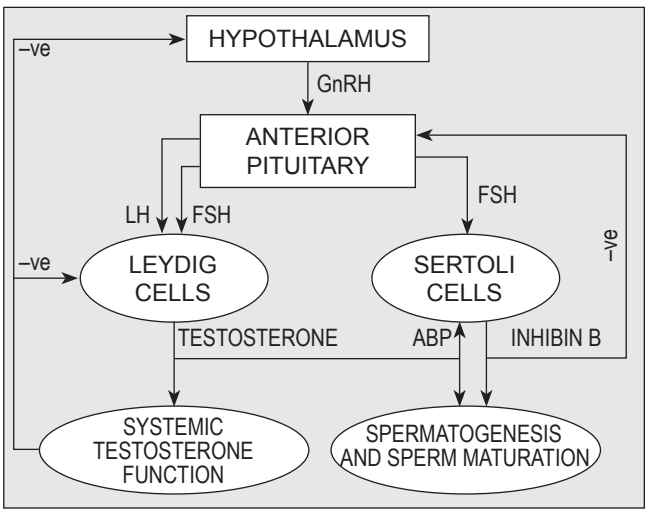

Figure-2

History:

A detailed history can elicit a wide range of information about the cause of male infertility.

- Childhood illness and pubertal development (delayed in primary or secondary hypogonadism), undescended testes
- Past history of trauma, torsion, surgery, treatment by radiotherapy or chemotherapy can affect semen parameters.
- Retroperitoneal lymph node dissection can interrupt the sympathetic chain and can cause erectile dysfunction or retrograde ejaculation.
- Medical History like respiratory tract infection or diabetes. Diabetes can cause erectile dysfunction or retrograde ejaculation (neuropathy or vasculopathy). Other endocrinological causes like hyperprolactinemia causes decrease in libido and congenital adrenal hyperplasia can cause delayed puberty and subfertility.
- Sexually transmitted infections in patients with semen abnormality can due to stricture of urethra, vas deferens or epididymis. Past history of GTB may be related to obstructive azoospermia. Acute viral fever can cause temporary suppression of testicular function but is reversed within 3 months.
- Exposure to gonadotoxins like chemical and environmental toxins, Anabolic steroids, antibiotics like gentamicin, nitrofurantoin, antihypertensives etc.
- Local heat due prolonged occupationl heat exposure or use of tight undergarments or presence of varicocele.

Endocrine Evaluation in Male infertility

Semen analysis undoubtedly, plays a central role in the laboratory evaluation of male factor infertility. An endocrine evaluation is only indicated after an abnormal sperm parameter or clinical finding suggesting an underlying endocrinological cause. Total testosterone and FSH are indicated if total sperm count is < 10 mill/ml or with impaired sexual function. When total T is low (< 300ng/dl),estimations of LH, PRL and free T are indicated. If T value is low and level of FSH is high,this diagnosis is more in favour of Hypergonadotropic hypogonadism,they poorly respond to medical

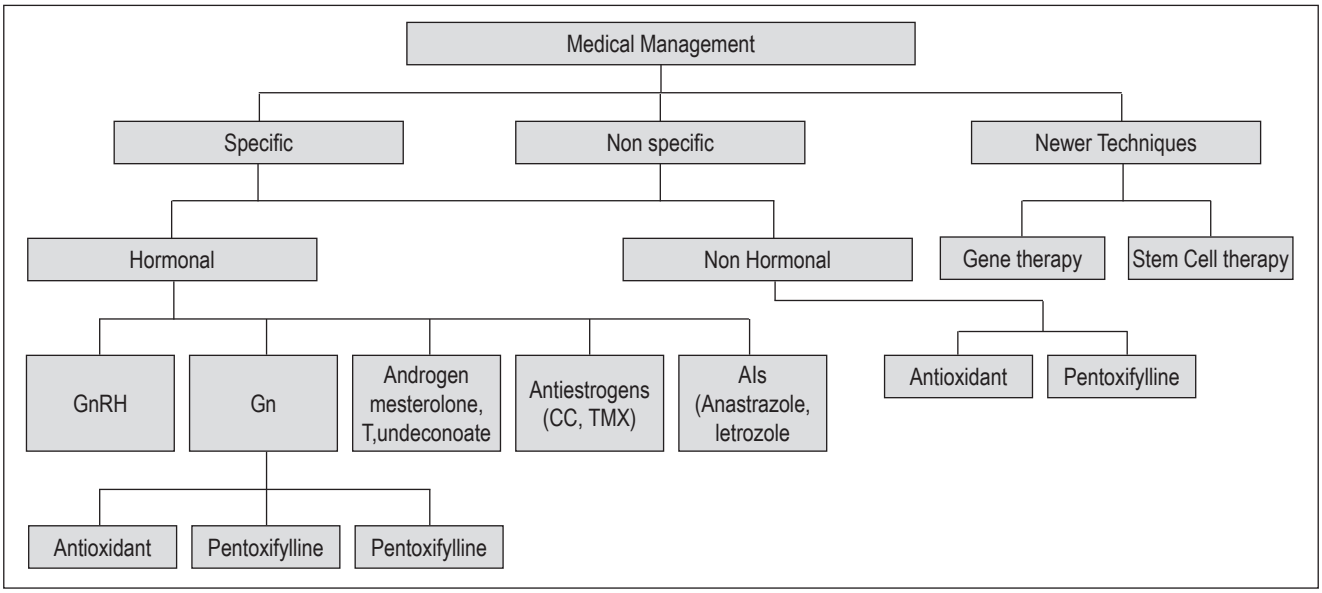

Figure-3

management. Low testosterone with low FSH suggest hypogonadotropic hypogonadism (table-1). Medical management is highly successful in these men. Low testosterone with normal FSH is another group which also responds better with medical management.Altered Testosterone:E2 ratio is an indication of management with aromatase inhibitors. Impaired sperm production was reported in cases with decreased testosterone, increased estrogen, and a decreased serum T/E ratio.

TABLE 1: Serum hormones in different scenario of male infertility

Hypogonadotrophic hypogonadism-	High FSH and LH
Testicular failure	Low testosterone, high FSH, normal LH
Spermatogenic failure	Normal testosterone, high FSH and LH
Androgen insensitivity	high testosterone
Hyperprolactinemia	high PRL
Thyroid dysfunction	Either over or underactive thyroid gland

Indications Of Medical Management

SPECIFIC:

- Hypogonadoptropic hypogonadism.
- Hypothyroidism.
- Hyperprolactinemia.
- Spermatogenesis suppression after testosterone abuse.
- Testosterone deficiency with elevated estrogen.
- Infection.
- ROS.

NONSPECIFIC:

- Emperic management.

Drugs for medical management

Clomiphene Citrate: When there are low testosterone levels with a normal T/E ratio, clomiphene citrate (CC) is the drug of choice. Clomiphene citrate(CC),a nonsteroidal antiestrogen acts by inhibition of estrogen feedback and thus increasing GnRH secretion followed by increment of FSH, LH that leads to raised Testosterone secretion and improved spermatogenesis.It is given in a dose of 12.5-25 mg daily or 50 mg on alternate day. Testosterone after 4 wks of therapy and semen analysis after 3 months will indicate the response of this treatment. Some of its side effects are gynaecomastia, acne, cataract, hypertension, minimal weight gain. Ross et al. observed > 50% patients responded within the first 3 months of treatment and the rest of them responding after 6–15 months.

Aromatase inhibitor (AI) therapy

Aromatase inhibitors are the drug of choice in patients with normal testosterone levels but abnormal T/E ratios (<10:1). High estrogen along with low T levels have been observed to impair semen parameter. Elevated estrogen exerts negative feedback on HPG axis- it decreases LH necessary for the production T, and FSH necessary to optimize spermatogenesis. It also decreases estrogen production by inhibiting aromatase activity in leydig cells and thus elevating

intratesticular T and improves sperm production. Letrozole and anastrazole has been shown in clinical trials to improve spermatogenesis

Gonadotropins

hCG (Human chorionic gonadotropin) as a LH analogue stimulates intratesticular Leydig cells to produce testosterone and improve spermatogenesis by increasing both intratesticular and serum testosterone. This is the only on-label drug for the treatment of male infertility.

In hypogonadotroic hypogonadism,hCG can be given intramuscularly or subcutaneously three times per week starting with a dose of 1000-1500 units . If serum testosterone is not increased after two months, hCG is to be increased by 50 percent and targeted T is usually achieved after 3-6months. Treatment with hCG alone has been observed to maintain spermatogenesis for short period of time and maintenance of spermatogenesis need FSH replacement in the form of hMG (75-150IU) or rFSH(recombinant FSH 150IU) thrice in a week. Promising result has been observed after replacing gonadotropins in this manner. Improvement time for spermatogenesis is usually after 6-9months therapy and sometimes 1-2 yrs is required before response.

A long term study on Japanese men observed a correlation between pretreatment testicular size and gonadotropin response (> 4ml testicular size responds better than <4ml). Gonadotropins are safe and well tolerated with relatively few side effects such as gynecomastia, acne, influenza-like symptoms, and weight gain which can be minimized with dose adjustment.

Synthetic analogue of gonadotrophin releasing hormone (GnRH) is an alternative treatment of hypothalamic-induced gonadotrophin deficiency caused by loss of hypothalamic function. Because of short half life these drugs need necessary pulsatile release (frequent injections, nasal spray, implantable pump). The inconvenience of its administration and lack of evidence of strong benefit in hypogonadotropic hypogonadism of this treatment makes this method clinically impracticable.

Dopamine agonist

Hyperprolactinemia inhibits pulsatile secretion of GnRH and can cause hypogonadism and infertility in upto 1 % of infertile men. Dopamine agonists are indicated for the management of infertility in these men. Both bromocriptine and cabergoline can effectively restore normal prolactin and testosterone level and improve libido, potency, semen quality. Increased testosterone levels and potency are observed within approximately 3-6 months after normal prolactin levels are achieved but time to restore semen quality generally takes longer. Bromocriptine (2.5 mg to 10 mg daily) or Cabergoline (0.25 to 1 mg twice in a week) can be used in hyperprolactinemic hypogonadism. Cabergoline is more effective than Bromocriptine in suppressing prolactin level especially in prolactinoma. Dopamine agonist treatment normalizes and maintains normal prolactin levels in approximately 82% of hyperprolactinemic men. Side effects like dizziness, nausea, vomiting, nasal stuffiness and orthostatic hypotension occur less frequently with cabergoline. For men with prolactinoma not responding to dopamine agonist with maximal tolerated dose, surgery is an alternate option.

Androgens

Androgens are an essential hormone in male causing development of reproductive organs, puberty and sexual function. Approximately 20%–30% of infertile male have low T and increased LH.High intratesticular T (25–100 times greater than normal serum levels) is maintained after combining with androgen binding protein, secreted into the seminiferous tubules. Testosterone activate androgen receptors in sertoli cells results in initiation and maintenance of spermatogenesis and inhibition of germ cell apoptosis. Androgen receptor abnormalities may cause abnormal sexual development in male. Oral or parenteral administration of androgens will not allow high levels of intratesticular testosterone but at the same time exogenous T and its metabolite, estrogen, would only suppress HPG axis and hence release of GnRH and gonadotropin (FSH, LH) will also diminish resulting in suppression of testicular testosterone production.

Antioxidant

High levels of reactive oxygen species (ROS) in seminal fluid are is a major contributor to the pathology of infertility in 30–80% of infertile men. The exact mechanism of ROS induced decline

in sperm dysfunction is not completely known, peroxidative damage to axoneme, oxidation of lipid mem¬brane components and fragmentation of both nuclear and mitochondrial DNA leading to decline in sperm functions.

High levels of DNA damage in spermatozoa has been shown to be associated with not only infertility and recurrent pregnancy loss (RPL), but also childhood mortality, complex poly¬genic disorders, dominant genetic disorders and childhood cancers. Conflicting reports exist regarding the antioxidants therapy in infertile men and the required dosage to reduce the ROS induced sperm damage. Cochrane meta-analysis regarding supplementation of oral antioxidants to improve pregnancy outcomes in subfertile men observed increased live birth rate after antioxidants therapy. The antioxidants most commonly associated with increased clinical pregnancy rates were Vitamin E and zinc though this meta analysis studies analysed showed large heterogeneity with low quality of evidence.

Male obesity

Male obesity has been linked to decline in semen parameters and subfertility. Hypogonadotropic hypogonadism seen with obesity like low serum gonadotropin, total testosterone, and free testosterone concentrations due to decrease in serum sex hormone binding globulin (SHBG) and increase in estrogen through aromatization in adipose tissue

Obesity is also associated with insulin resistance, metabolic syndrome, diabetes mellitus, and sleep apnea adds to hypogonadism and infertility.

Life style changes like increased physical activity, dietary modifications, and weight reduction to be advised in male obese patients which have been shown to improve semen quality. Aromatase inhibitors therapy can be useful in optimizing T:E2 ratio and improving testosterone levels.

Surgical sperm extraction and hormonal manipulation

Men with non-obstructive azoospermia(NOA) will have focal spermatogenesis in 60% to 70% . Increasing intratesticular testosterone in certain men with NOA might be useful for surgical sperm recovery. CC, AI, and gonadotropins can be used for this purpose.

Sexual Dysfunction: :

Erectile dysfunction→

Erectile dysfunction is responsible for 1% of causes of male infertility. PDE5 inhibitor considered 1st line therapy. The drug inhibits the action of phosphodiesterase enzyme and helps to build up cGMP with consequent enhancement of erection. They only enhance the natural response of sexual stimulation and cannot stimulate erection in absence of normal stimuli. Sildenafil can be given in dosage like 25,50,100 mg. Effectiveness start from 30 minutes from administration and remain effective for 12 hr. Other PDE 5 inhibitors are Tadalafil, Vardenafil also effective. Sudden fall in blood pressure specially when used along with nitrites or hypotensive therapy or α-blockers may be seen in PDE 5 users .Other side effects are facial flushing and mild headache. Sildenafil does not alter myocardial O_2 consumption so not contraindicated in heart disease.

Use of Oral testosterone is indicated where endogenous serum testosterone level is low. Intracavernous injection of vasoactive agent such as Alprostadil, papaverine, phentolamine can be tried. Topical drugs like Alprostadil, organic nitrates, minoxidil, yohimbine can als be used. Vaccum constriction devices may be helpful in improving erectile dysfunction.

Ejaculatory dysfunction

Retrograde ejaculation: Results from failure to closure of bladder neck during ejaculation. Retroperitoneal lymph node dissection, diabetes mellitus, prostrate or bladder neck surgery, spinal cord injury, multiple sclerosis, α receptor antagonists, antidepressents, antipsychotics, transverse myelitis etc.

In retrograde ejaculation medical treatment is succesful in 50% cases. Spontaneous pregnancy rate have been observed upto 35%. Drugs used are sympathomimetics like Pseudoephidrine : 60 mg qid or 120 bid. Phenylpropanolamine: 75 mg bid, and Imipramine(tofranil) : 25 mg tid. Post orgasmic urine sample can be collected either after voiding or after catheterisation following alkalinization of the urine with oral sodium bicarbonate or increasing fluid intake. This specimen is then centrifuged, checked for count and motility and accordingly used for the selected procedure resuspended before used

by either IUI, IVF, ICSI. $NaHCO_3$ and antibiotic mixture should be used for 7 days prior to the day of collection, to make urine pH 7.3 & osmolarity 280 mosm.

Premature Ejaculation:

Premature ejaculation can be considered when ejaculation occurs within 15 seconds from the beginning of sexual intercourse. Recent studies have shown that serotonin is significantly associated with premature ejaculation. Psychological factors also have a major role in its causation.

Antidepressent, selective serotonin reuptake inhibitors can also delay orgasm in male. The drug should be taken two to six hours before sexual activity (dapoxitin 30mg). Application of anaesthetic cream to the head of penis about 20-30 minutes before intercourse, psychiatric therapy, behavioural therapy, squeeze method, stop start method are some of the treatment modalities suggested in premature ejaculation. In total ejaculation failure, electro-ejaculation or vibratory stimulation are effective. Surgical sperm retrieval like TESA or PESA is the option after failure of above methods.

GENITAL TRACT INFECTION:

Genital tract infection is seen in 10-20% men with infertility (WBC > 1 million/ml).Chlamydia is the most common cause in genital tract infection. Evidence of infection is ascertained by finding of leukocytes in seminal plasma under the microscope or by presence of bacteria revealed by semen culture. Presence of 6-8 lukocytes per high power field require antibiotic treatment because excess leukocyte may induce sperm damage through production of reactive oxygen species. Antibiotic therapy by giving empirical treatment with single dose fluroquinolone or 2-4 wks doxycycline (100mg twice) is most useful. Evidences have shown that antibiotic therapy can improve sperm quality but its effect on pregnancy rate not was not observed in the studies.

IMMUNOLOGICAL INFETILITY:

Testis is an immunologically privileged site where the testis have foreign antigen, but there is no antibody formation due to blood testis barrier. Breaching this blood testis barrier as seen in testicular trauma, inflammation or vasal obstruction as after vasectomy can initiate an immune response, resulting in an inflammatory reaction and anti-sperm antibodies production. Use of steroid did not show significant improvement in pregnancy rate but these therapy found to be associated with side effects.

Emperical Medical Management In Male Infertility

Men with idiopathic infertility when there is no correctable cause, treatment option available is assisted reproduction.

Gonadotropins

Gonadotropins are the only medical therapy approved by the FDA for the medical management of male factor infertility. A Cochrane review observed improvement on live birth and pregnancy rates when men with idiopathic male subfertility were treated with gonadotropins (GnRH, hMG, FSH).

Clomiphene citrate is mainly effective in mild grade hypogonadotropic hypogonadism with Oligospermia. It can be given in the Dose: 12.5-25 mg daily or 50 mg on alternate day. Cochrane review after evaluating ten studies have stated that there is no difference in the pregnancy rates between the anti-estrogen group and the control groups.

Tamoxifen, an antiestrogen, weaker than clomiphene, with the dose of 10 mg twice daily for 3 to 6 months may be used for idiopathic male infertility.

Aromatase inhibitors

Advantage of AI over CC is increase in endogenous testosterone level without associated increase in estrogen level. Randomized trial have shown that after treatment with aromatase inhibitor, FSH,LH & T will increase but significant improvement in semen parameters and pregnancy rates was not observed A recent study have stated that AI can cause spontaneous pregnancy in 20% of oligospermic. There are case reports of azoospermic men treated with AIs and spermatozoa was seen in ejaculate.

Androgen

Androgen use in idiopathic male infertility is not fully proven. Direct stimulatory effect or rebound effect of androgen on spermatogenesis may be beneficial. Low dose oral testosterone such as MESTEROLONE (25 to 50 mg tds) or Testosteron Undecanoate 40 mg may be given in this indication. Side effects of

testosterone therapy are acne, weight gain, water retention was noted. No significant improvement in pregnancy outcome after oral testosterone therapy was seen.

Pentoxifylline

Pentoxifylline (PF) is a methylxanthine derivative inhibit phosphodiesterase and increases cAMP levels. Increased cAMP levels enhances acrosome reactions and improves sperm motility. PF itself improves microcirculation and because of its antioxidant properties it Improves sperm motility enhances acrosome reaction, and increases the proportion of hyperactivated spermatozoa. In vitro treatment of sperm with topically applied PF showed improved fertilization in male factor infertility and poor fertilization. The recommended dose of PF is 400–600 mg 3 times a day for a period of 3–6 months. PF continues to be utilized in vitro. To improve sperm motility PF in vitro but orally administered PF in management of idiopathic male infertility has not been proved to be successful.

Conclusions :

Understanding physiology of spermatogenesis and its hormonl contol is essential for the assessment and management of male infertility. Aim is to control LH levels from pituitary, which will stimulate T production from the Leydig cells and FSH levels to stimulate Sertoli cells and spermatogenesis, and eliminate any estrogen excess which could be detrimental to sperm production. Medical therapy is only beneficial in few known cases of male infertility like hypogonadotropic hypogonadism, hyperprolactinemia, thyroid disorder. Medical treatment is not useful for the treatment of idiopathic male infertility due to doubtful benefit and cost associated with it.

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