WHY IUI FAILS?

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Artificial insemination (AI) had been performed for many decades. Almost 200 years ago, John Hunter advised a man with hypospadias, to inject his own semen into his wife’s vagina with a syringe, which produced a normal pregnancy [1]. Sims in the 19th century artificially inseminated 6 women with their husband’s semen obtained from the vagina after intercourse, and one pregnancy was achieved [2]. Intra-uterine Insemination (IUI) is one of the techniques of AI where a bolus of concentrated, motile and morphologically normal spermatozoa is placed inside the uterine cavity close to the oocytes. The frozen sperm for AI in 1953 was first successfully used by Bunge and Sherman [3], but widespread use started in the 1970s, and at present, is a must for donor insemination. The use of washed, prepared sperm cells in IUI has led to significant reduction in the side-effects associated with the use of undiluted semen for IUI, such as painful uterine cramps, collapse and infection [4, 5, 6].

IUI is one of the simple and commonly practiced procedures of Assisted Reproductive Technology (ART). It cannot be called ART under strict criteria, as here eggs are not taken outside the female body for treatment. With the availability of good semen preparation methods as performed in In-vitro Fertilization (IVF) laboratories and IUI catheters, there is a resurgence of interest in IUI in the 1980s; Kolkata being pioneer in this country.

The overall pregnancy rate (PR) in IUI pregnancy is less than 20% per cycle. Maximum success rate is found in ovulatory disorder and the minimum being the male factor infertility. The possible causes of IUI failure appears to be –

1. Man-made sperm selection
2. Poor selection of patients; timed intercourse or IUI; and poor follicular selection
3. Improper egg pick-up
4. Prevalence of empty follicle or poor oocyte quality

Other recognized factors affecting the results of IUI are –

1. Cause of infertility
2. Age
3. Duration of infertility
4. Number of treatment cycle
5. Sperm parameters
6. Use of controlled ovarian hyperstimulation

In spontaneous or natural pregnancy, the sperm passes through cervical mucus, when there is a natural selection of sperm. Eggs are picked up by the fimbrial end of fallopian tubes from Pouch of Douglas (POD), spontaneously and unaided.

IUI involves sperm manipulation during semen preparation, as crude semen can cause shock when put in the uterine cavity. Hence in IUI, the spermatozoa are separated from seminal plasma and treated with nutrients to increase its fertilizability. This manipulation may cause harm to the sperm cells, e.g. production of ROS (Reactive Oxygen Spaces) due to centrifugation. In this process, the spermatozoa those are put inside the uterine cavity come more close to the site of fertilization. The male gamete deposited in uterine cavity as well as the timing of insemination is also artificially selected.

The usual method of semen preparation is mentioned elsewhere [7]. To summarize, this is performed in the usual media with or without addition of protein. Human Serum Albumin (HSA) or Foetal Cord Serum (FCS) is used as additives. In some cases, we have used cervical mucus (CM) as additive. The thought behind it was to mimic the natural process, whereby CM acted as a barrier through which the sperm cells should pass. In this process, the CM collected from ovulating women, when there was at least the cervical score between 8-10 [8]. Some part of the mucus was sent for culture and subsequently, to exclude any infection. The
mucus was preserved in a deep freezer (-80°C), to prevent de-maturation of protein. During sperm preparation, attempt was made to use homologous CM (that is, from the same female partner) mostly. The mucus was either layered over the sperm cells, and the media was poured on it after initial centrifugation. The idea was to isolate the sperm those will swim up through CM. In another group of patients, the CM was mixed with media and layered over the sperm palette. Either of the process produced the same result. Quality, morphology, motility and survival are all included in CM treated group, as compared to the protein treated group (Fig. 1, 2). The PR also improved in all cases using CM. This experiment dictated us to search for sperm-selecting factor in CM. For this, pre-ovulatory CM was collected between days 8-13 serially daily or on alternate days, during ovulation monitoring. This mucus was subjected to cellulose agar media electrophoresis, using paper as a medium. A specific protein band (Fig. 3) appeared in electrophoresis of all ovulating women and the concentration of protein increased gradually and reached maximum, immediately before ovulation. We are in the process to determine the nature of the protein. It appears that this protein is being utilized by the spermatozoa and probably determines fertilizability of male gametes, too. This has been suggested by the absence of protein in CM collected 4-6 hours post-coitally, as compared to similar mucus collected pre-coitally from the same individual, which showed beautiful protein band. In anovulatory CM, this protein band was either absent or was minimal (unpublished data – paper submitted).

The selection of patients is an important factor. On many occasions, poor selection reduces the success rate. It is well understood that IUI helps by increasing the concentration of spermatozoa in the uterine cavity, and they are placed close to the site of fertilization. If there is natural entry of sperm in the uterine cavity, IUI probably does not offer any extra benefit. So, the female partners having poor sperm entry into the uterine cavity are more benefited from IUI. This selection is performed by a simple test called Post-coital Cervical Mucus and Intra-uterine Aspirate Test (PCT-IUA) [9]. In a nutshell, the post-coital vaginal fluid, CM and uterine aspirate are studied. It has been observed that where the sperm entry is
good, the rate of normal pregnancy is 60%. With poor sperm concentration, the normal PR is only 15%. These patients are really benefited from IUI, where the PR is near about 40%.

Another factor is the time of insemination. The oocyte remains fertilizable between 18-24 hours following ovulation. It takes about 6 hours for the oocyte to be picked up and reach ampulla, the site of implantation. Ovulation takes place 36-40 hours following hCG injection. So in semen with good survival, 2 inseminations, one 24 hours and another 40-42 hours after hCG injection is useful. In semen with poor survival of sperm cells, single insemination 40-45 hours after hCG injection is more important (Fig. 4). So before IUI, the sperm survival test is to be performed, to determine the number and timing of insemination.

With proper ultrasound follicular monitoring, the rate of growth and look of follicle should be observed. Maximum pregnancy occurred with the development of 3 or more follicles. Too slow or too rapid growth of follicles indicates poor quality (Fig. 5). Ill-defined or deformed margin or hazy look of the follicles lowers the success rate of IUI. Another success factor for IUI is the poor semen quality. It is an accepted view that the total number of motile sperm of 10 million/ml after sperm preparation gives best success rate of IUI. IUI presents less success rate with 1 million or less sperm concentration per ml. In all forms of IUI, ovulation is induced conventionally or by controlled ovarian hyperstimulation (COH). Mild or moderate seminopathy gives the best success rate. In case of severe seminopathy, pooled or cryopreserved semen samples prepared by sperm wash technique gives better result than single sample, even if it is fresh. This indicates that the total number of motile sperm is the major determining factor for IUI success (Fig. 6).

IUI failure can happen due to improper egg pick-up. Normal egg pick-up depends on the patency of fallopian tubes having adequate lengths, the position being at or towards POD, free and functioning fimbriae, proper tubo-ovarian relation (TOR), and free and clear POD. It has been observed that the fallopian tubes even remaining patent, may face mal-functioning due to kinking, pulled-up portion (C
tube), agglutinated or everted fimbriae. These problems are together termed as Minor Tubal Defects [10]. Sometimes, pedunculated fimbrial cysts or tubal humps may hinder tubal functions. These minor tubal defects may be recent or sub-clinical infections, or mild endometriosis [10]. The author has mentioned in same publication that changes in the TOR as well as adhesions in the POD reduce pregnancy rates, too.

Poor follicular development as well as poor oocyte quality hinders IUI success. One of the main factors for this is endometriosis. It has been observed that in endometriosis with standard ovulation induction (OI), empty follicles may develop and oocyte quality remains poor. Another factor in endometriosis would be phagocytosis of spermatozoa and implantation failure. According to some authorities, in natural attempt of conception, the ejaculated and spontaneously inseminated sperms reach the fallopian tubes and subsequently dip in the peritoneal fluid (PF) for further maturation. The toxic PF in endometriosis containing Prostaglandin (PG) may destroy the sperms [11, 12]. In IUI, the high dose of Gonadotrophin (Gn) use may combat the effect of endometriosis on oocyte development and prepared sperms cells may directly fertilize the egg avoiding the PF, thereby the PR may increase. In a study conducted by the author and his team at “Calcutta Fertility Mission” (02-03 unpublished data) [13], 63 patients with blocked tubes were subjected to ovulation stimulation by Clomiphene Citrate (CC) and hMG (CC at a dose of 10 mg from d3-d7 and hMG 75 IU on d4, d6, d8 and d10). hCG injection was administered when the follicles reached 18 mm diameter. Laparoscopy was performed 34-35 hours following hCG injection. On laparoscopy, 31 patients were found to have endometriosis, in whom developed 110 follicles, which were aspirated laparoscopically. Only 25 oocytes were obtained (25%). From the remaining 32 patients, 131 pre-ovulatory follicles were aspirated and 92 oocytes were obtained (76%). This indicates the prevalence of empty follicles in endometriosis. Among these 25 oocytes obtained from patients with endometriosis, 15 were of poor qualities. The patients were not down-regulated, which improves the results in endometriosis (Fig. 7). During this study, our observation was that the follicular development in endometriosis was faster with
less $E_2$ value as compared to patients without endometriosis. This postulates the prevalence of empty follicles in endometriosis (Fig. 8).

Endometrial factors found in adeno-endometriosis \cite{14} or tubercular infestation of endometrium \cite{15} can cause repeated IUI failure. These conditions impair favourable cytokine changes in the endometrium and raises TH1 bias by down-regulating TH2 bias. In a study conducted by the author and his group (unpublished data), it was found that the endometrial fluid in patients with endometriosis collected by IUI catheter contained more macrophages, lymphocytes and polymorphs, as compared to the non-endometriotic patients, who show preponderance of plasma cells, histiocytes and lymphocytic prevalence. Thus, the endometrial fluid from patients with endometriosis inhibited sperm motility to great extent, as compared to the other group. Pre-treatment with GnRH-a reverted this malfunction, indicating the direct effect of endometriosis on the passage of spermatozoa to endometrial cavity.

Tubercular infestation of endometrium can produce unexplained infertility, requiring IUI as a modality of treatment, which might result in a failure. 2-3 attempts of IUI failure demands screening of the endometrium for presence of MTB. This is performed in ways mentioned in the literature \cite{15}. The same author detected that unexplained infertility is due to infestation of endometrium with tubercular bacilli in small numbers. This infestation is treated by removing the MTB by drug therapy, and the PR improves.

Other recognized factors on which the IUI success rate depends are already mentioned. Age is an important factor. Good success rate occurs in patients less than 35 years of age and poor PR in patients at the 4th decade. With an increase in the female age, natural fertility deteriorates. This happens primarily because of the decreasing quality of oocytes and secondarily, endometrial receptivity \cite{16, 17}. The increase in male partner’s age exerts a negative impact on the pregnancy rates \cite{18, 19}. This is perhaps through an increase of non-dysjunction of spermatozoa \cite{20}. Our data shows that in women below 35 years, the success is around 9-10% per cycle.
where women in 4th decade have <1% pregnancy rate. The duration of infertility is another important factor for poor success rate. The chance of conception also declines with duration of infertility \([18, 21]\). Long-standing infertility shows poor success rate with IUI \([22, 23, 24, 25]\), even with standard sperm parameter of \(10 \times 10^6/\text{ml}\) motile sperm. The degree of motility and percentage of morphologically normal spermatozoa are the most important sperm parameters, that affect fertility \([21, 26, 27]\). Infertility of less than 3 cycles gives better success rate below 35 years age group. The results become very poor in patients of nearly 40 years of age, even with COH / IUI. In another study, \([28]\), it was found that the efficacy of COH / IUI cycles significantly decreases with age, and women of 38-39 years of age had success during the first 2 cycles. However, for women aged \(>\approx 40\) years, no benefit was observed after a single cycle of COH / IUI. Women having age of \(>\approx 40\) years should be considered for IVF, after one failed cycle of COH / IUI.

Remohi and associates \([29]\) reported a series of 489 cycles of COH and IUI, where the PR was high in first 4 cycles as compared to cycles 5-10. The retrospective analyses of IUI data \([30, 31, 32]\) showed that using life-table analyses, a relatively constant probability of achieving pregnancy after each IUI was obtained through 6 cycles, followed by almost no increase, even after further continuation of the treatment. So, IVF should be considered in difficult situations of higher age group, long duration of infertility, and prolonged IUI treatment cycles.

In unexplained infertility, IUI / TI in unstimulated cycle show similar poor result \([33]\). When ovulation is stimulated by use of conventional Gonadotrophin (Gn) \([34]\), or by COH, the PR increases \([35]\). This may be due to correction of certain subtle undetected ovulatory defect, causing previous failure.

In case of long-standing infertility caused by reduced sperm quality, the expectant treatment seems to be disappointing, with a spontaneous conception rate of only 2% per cycle \([24]\). Therefore, this strategy is not applicable in clinical practice. For IUI, with or without COH, a PR of 10-18% per cycle has been reported \([36, 37]\). A
Cochrane review showed that IUI is superior to TI, both in natural cycles and in cycles with COH\textsuperscript{[38]}. 

According to the literature, high percentage of motile and morphologically normal spermatozoa is the most valuable sperm parameters to predict IUI outcome\textsuperscript{[39, 40]}. There is a trend towards increasing the conception rates with increasing the inseminating motile count (IMC), but the cut-off value above which IUI seems to be successful, however, varies between 0.3 and 20 x 10\textsuperscript{[41, 38]}\textsuperscript{[41, 38]}. According to this review, IUI should be the treatment of choice in case of male sub-fertility, providing an IMC of more than 1 million that can be obtained after sperm preparation, and in the absence of a triple sperm defect (according to WHO criteria).

A significant improvement in PR was reported when the morphology score was more than 5%, using strict criteria in a meta-analysis by Van Waart et. al. (2001)\textsuperscript{[25]}. For total sperm motility before sperm preparation, the cut-off levels vary between 30 and 50\%\textsuperscript{[42, 43, 44, 45]}.

It seems logical to perform IUI in male infertility cases with inseminated motile sperm (IMSC) count of 1 x 10\textsuperscript{6} – 5 x 10\textsuperscript{6}, and with normal morphology of 4-14\%. However in patients with IUI, the threshold values (<5 x 10\textsuperscript{6}), other prognostic risk factors such as duration of infertility (less than 3 years), the partner’s age (<35) and other additional infertility diagnosis should be taken in consideration\textsuperscript{[46]}.

Many couples are blessed with pregnancy and childbirth following IUI, which is relatively cheap, simple, effective, and non-invasive method of treatment, indicated in certain grade of infertile women. Careful selection of patients is very much important. The female partner who is relatively young with not only patent but structurally and functionally competent fallopian tubes, showing preferably multiple ovulation with OI, and the male partner producing certain number of motile and morphologically normal sperm cells after sperm preparation, get the best result. IUI should be continued for at least 3 cycles, better 6 cycles, after which it probably becomes useless. The couples with repeated IUI failure should be advised with
IVF at the earliest. IUI should preferably be performed at or near centres having facilities of IVF because if hyperstimulation occurs, immediate IVF procedure can be undertaken, and surplus embryos if any, can be kept frozen for future use.

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PROTEIN BAND IN OVULATORY & ANOVULATORY CM

Specific Pr band in preov CM

CM D12 / 13

FIG 3

POOR SELECTION timing

HCG TIMING of INSEMINATION

Insemination timing
42.52 hr. - Poor survival
24 & 42 hr. - good survival

FIG 4

POOR SELECTION follicles

- No of follicles
- Rate of follicular development
- Quality of follicles
  * Ill defined margin
  * Too hazy/ too clear
  * Sudden enlargement

PREG RATE/ FOLLICLE NO IN IUI (OUR DATA)

FIG 5
POOR SELECTION seminal quality
IUI & DIFFERENT SEMINOPATHIES

SEVERE: TC<10mill MOT<10%
MODERATE: TC>10<20n M>10<40%
Motile Sperm Count after wash
Minimum 5 mill/ml Better >10 mill/ml
Excellent 15mill/ml or more
PERIOD OF RETENTION OF ACTIVE
MOTILITY(PRAM)

FIG 6

CC + hMG STIMULATION FOLLICLES &
OOCYTE QUALITY (Our series – '02 – '03)

<table>
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<tr>
<th></th>
<th>Cases</th>
<th>Follicle</th>
<th>Oocyte</th>
<th>%</th>
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<tr>
<td>Endomet</td>
<td>31</td>
<td>110</td>
<td>25</td>
<td>23 %</td>
</tr>
<tr>
<td>Others</td>
<td>32</td>
<td>131</td>
<td>92</td>
<td>71 %</td>
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FIG 7

ENDOMETRIOSIS

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>31</td>
</tr>
<tr>
<td>No oocyte</td>
<td>15</td>
</tr>
<tr>
<td>1 oocyte</td>
<td>12 Poor-8, good-4</td>
</tr>
<tr>
<td>2 oocytes</td>
<td>4 Poor-2, good-2</td>
</tr>
</tbody>
</table>
OUR OBSERVATION IN ENDOMETRIOSIS

- Follicle dev. is faster in endometriotic patients
- E2 value is less in this group on same day.

Published in the book - “Intrauterine Insemination” by Dr. Gita Ganguly Mukherjee & Dr. B. N. Chakravarty; 2012; pg. 358-369