SOGS E-JOURNAL

VOL.1, AUGUST 2021

PASSION... VISION... SUCCESS...

Topic: Recurrent Pregnancy Loss (RPL)

President's Message
Dr. Jagruti Desai

Respected Seniors & Dear Colleagues,

It gives me immense pleasure to witness the release of E-journal on the topic of Recurrent Pregnancy Loss (RPL).

“Success needs vision to see, passion to transcend, patience to withstand- develop a passion for learning- if you do, you will never cease to grow.”

“Leadership and learning are indispensable to each other.” – John F Kennedy.

RPL is one of the most distressing conditions for both- clinicians and the patient. The pathophysiology underpinning RPL is incredibly diverse, involving areas such as hematology, endocrinology, immunology and genetics. It is challenging for us to look for advances and updates amidst our busy schedules. This E-journal will serve this very purpose as a quick, easy and efficient way to update you with the most recent advances in the field of RPL.

I congratulate and thank Dr. Jitesh Shah for taking this huge responsibility as the Chief Editor for the creation of this masterpiece; Dr Noopur Chhasatia, Librarian; Dr. Ruta Vekariya and Dr. Hitanshu Bhatt. I thank all of them for their hard work and sincere efforts in compiling the material in such a beautiful way. I also thank all the experts who have reviewed all the articles extensively to make it genuine and precise.

I am sure that all these humongous efforts gone into the creation of this journal will be truly appreciated and will turn out to be an extremely useful tool in your everyday practice.

To wrap it up, “Learning never exhausts the mind.” Happy Learning, Happy Reading!

Dr. Jagruti Desai

Dr. Kajal Mangukiya

Message from Secretary Desk

“A person’s most useful asset is not a head full of knowledge but a heart full of love, an ear ready to listen and a hand willing to help others”

Ages ago, when I became the member of Surat Obgy Society with the aim of getting updated about recent studies and learning from the seniors. I have come a long way from being the EC member to librarian to secretary and the journey has been tremendous.
I have tried to justify my each and every roll for our society. I remember very clearly that last year I got the opportunity as a librarian to spread the knowledge and Inspite of all the ups and downs of the pandemic, my passion for my profession and for our society has never gone down. We found a way through e-magazine. With god’s grace and all your support, we have successfully published two editions.

Today As a secretary, it gives me immense pleasure that we are releasing our 3rd issue with the theme of “Recurrent pregnancy loss”
I’d like to say this to all the readers that we come up with this in depth Clinical discussion about common causes of Recurring pregnancy loss , Recent advances and few common case scenario that we see on regular basis.

And as they quote “Coming together is a beginning, keeping together is a progress and working together is success”
I’d like to
Thank the authors and the scrutinisers from the bottom of my heart
Thanks to the president Dr. Jagruti Desai, librarian Dr Nupoor Chattasiya, chief editor Dr Jitesh shah for his enthusiastic commitment as an editor, great efforts taken by him is seen on every page
Thank you Dr. Ruta Vekarya and Dr. Hitanshu Bhatt for all the support
Speacial thanks to all the sponsors
And Thank you surat obstretrics and gynaecology society members for making this issue successful
And Hope for this support in future too

Together we achieve
Regards
Dr. Kajal Mangukiya
Hon. Secretary SOGS

Message from Librarian
Dr. Noopur Chhasatia

Dear Members

It is time for the new edition of the SOGS journal and it is my pleasure to bring it to you as the librarian of the SOGS. This year's theme, recurrent pregnancy loss, is an age-old puzzle, and in this issue, an encouraging number of our members have attempted to decode it.

This volume includes articles on the etiopathogenesis and genetics in RPL. In addition, we have also included management aspects with a focus on ultrasound, hysteroscopy, ART, and endocrinological management of RPL.

Since the case-based approach is the standard of academic discourse, we have included four clinical cases. There is also a tiny MCQ section included with the hope that it will provide an opportunity to self-assess the understanding of the topics discussed.

I take this opportunity to thank all the authors and the faculty who dedicated their valuable time to preparing and reviewing the material. Our editor, Dr. Jitesh Shah, has been an astute help throughout, and I thank him for his guidance. I also extend my gratitude to president Dr. Jagruti Desai and secretory Dr. Kajal Mangukia for extending this opportunity to me.

Progress is one incremental step at a time, and we have come far with this step; I wish to keep improving our efforts in the years to come.

Sincerely,
Dr. Noopur Chhasatia
Librarian SOGS 2021-2022

Letter from the Editor
Dr. Jitesh Shah

Dear SOGS members,

It is my privilege to take the opportunity as an editor of E- journal on Recurrent pregnancy loss. During the current pandemic situation, our aim is to update SOGS members by sharing knowledge and interesting cases by this platform.

We all know that RPL is a relatively common but tricky situation. Many a times it is difficult to answer the patients' question "why this has happened?" Authors have tried their best to update our knowledge so that we can solve the problem.

This task will continue in future with recent and evidence based articles.

It is rightly said "when there is a will, there is a way"

Best wishes,

Dr. Jitesh Shah

Editor, SOGS

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CLINICAL DISCUSSION

Recurrent Pregnancy Loss

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INTRODUCTION:

A pregnancy loss (miscarriage) is defined as the spontaneous demise of a pregnancy before the fetus reaches viability. The term therefore includes all pregnancy losses from the time of conception until 24 weeks of gestation (20 weeks in few countries with advanced neonatal care infrastructure). Majority of these sporadic losses are due to random numeric chromosomal errors.

Recurrent pregnancy loss (RPL) is one of the most emotionally traumatic, disconcerting and challenging areas in reproductive medicine because the etiology is often unidentified and research on the etiology, evaluation, and management of RPL is often erroneous. Typical methodologic shortcomings include failure to adhere to generally accepted criteria for RPL, improper selection of controls, ascertainment bias ,disparate monitoring of cohorts, no exclusion of aneuploid fetuses, lack of stratification for important factors such as number of previous losses, premature termination of study after interim analysis, and excessive postrandomization patient withdrawal [1].

DEFINITION —

The definition of RPL is varied which makes research on evaluation, management and counseling more challenging.

- Two or more failed clinical pregnancies as documented by ultrasonography or histopathologic examination [2].

- Three consecutive pregnancy losses, which are not required to be intrauterine [3-6].

Non-visualized pregnancy losses (biochemical pregnancy losses and/or pregnancies of unknown location) had the same negative impact on future live birth as an intrauterine pregnancy losses. These definitions also do not take into account the effect of maternal age or the gestational age at which the miscarriage occurred.

RPL can be further divided into primary or secondary. Primary RPL refers to pregnancy loss in women who have never carried to viability. Secondary RPL refers to pregnancy loss in a woman who has had a previous live birth. The prognosis for successful pregnancy is better with secondary RPL [7,8]

Further classification can be Pre-embryonic (<4 weeks), Embryonic (5-9 weeks), and Fetal (>10 weeks)

INCIDENCE —

Approximately 15 percent of pregnant women experience sporadic loss of a clinically recognized pregnancy. Just 2 percent of pregnant women experience two consecutive pregnancy losses and only 0.4 to 1 percent have three consecutive pregnancy losses [9)

At very early gestational ages (eg, at less than 6 weeks of gestation) the risk of miscarriage is 22 to 57 percent versus 15 percent at 6 to 10 weeks and 2 to 3 percent after 10 weeks [10,11]).

The prevalence of miscarriage is higher with increasing maternal age, most likely due to poor oocyte quality (12)

Age(Yrs)	Spontaneous Miscarriage risk (%)
Overall	11 %
20-30 yrs	9-17%
35 yrs	20%
40 yrs	40%
45 yrs	80%

RISK FACTORS AND ETIOLOGY —

RPL is a heterogeneous condition, with numerous causes, diverse treatment options and enormous psychological implications. It is multidisciplinary, involving gynecology, genetics, endocrinology, immunology, pediatrics and internal medicine.

Two major concerns for the physician and the couple are: the cause and the risk of recurrence. The etiology can be identified in less than 50 percent of patients. General etiological categories of RPL include anatomic, immunological, genetic, endocrine, infectious, thrombophilic, male factors and environmental factors. Prognosis is based on the number of preceding pregnancy losses and female age.

Previous pregnancy loss —(13)

Number of previous losses	Risk of miscarriage(%)
Zero	11-13
1	14-21
2	24-29
3	31-33

Genetic factors

- **Parental chromosomal rearrangements** [14,15] In 2–5% of couples with recurrent miscarriage, one of the partners carries a balanced structural chromosomal anomaly,most commonly ***a balanced reciprocal or robertsonian translocation*** less commonly, an inversion. Although carriers of a balanced translocation are usually phenotypically normal, their pregnancies are at increased risk of miscarriage or congenital malformations and/or mental disability secondary to an unbalanced chromosomal arrangement. The risk of miscarriage is influenced by the size and the genetic content of the rearranged chromosomal segments. Balanced translocations are more common in the females likely to result in pregnancy loss if the translocation is of maternal origin.

- **Chromosomal abnormalities of the embryo**[16-17].— In couples with RPL aneuploidies account for 30–57% of further miscarriages. The risk of aneuploidy and association with the number of previous miscarriages is contradictory in several studies but mostly as the number of miscarriages increases, the risk of euploid pregnancy loss increases. The relationship between the karyotype of the abortus and risk of RPL requires further study to better define which abnormalities are likely to be recurrent.Recurrent aneuploid losses may be associated, in part, with the older age of the mothers.

The likelihood that RPL is related to parental karyotypic abnormality is higher when:

- Young maternal age at second miscarriage.
- History of three or more miscarriages.
- History of two or more miscarriages in a sibling or the parents of either partner
- A family history of stillbirth or an abnormal liveborn.

Immunologic factors —

Antiphospholipid syndrome (APS)(18,19) — Several autoimmune diseases have been linked to poor obstetric outcome, but APS is the only immune condition in which pregnancy loss is a diagnostic criteria for the disease. Five to 15 percent of patients with RPL may have APS compared to 2% in normal obstetric patients. APS is the most important treatable cause of recurrent miscarriage

The mechanisms by which antiphospholipid antibodies cause pregnancy morbidity include inhibition of trophoblastic function and differentiation, activation of complement pathways at the maternal–fetal interface resulting in a local inflammatory response and thrombosis of the uteroplacental vasculature.

Other immunological factors (20-23) — Allogeneic factors may cause RPL by a mechanism similar to that of graft rejection in transplant recipients. If the blastocyst is developmentally normal and intact, the embryo is entirely protected by trophoblast cells. In some pregnancies, the blastocyst is genetically deformed and not fully intact so paternally-derived antigens are exposed to the maternal immune system, leading to a rejection response. A secondary immune response would be expected to cause early rejection in cases of RPL.

Alternatively, some mothers with RPL may lack essential components of the networks that provide immunological protection to the embryos, such as appropriate expression of complement regulatory proteins. Dysregulation of the normal immune mechanism, although not well defined, probably operates at the maternal-fetal interface and may involve increased activity of uterine natural killer (uNK) cells, which appear to regulate placental and trophoblast growth, local immunomodulation, and control of trophoblast invasion.

Thrombophilia and fibrinolytic factors [24-25] — Thrombosis of spiral arteries and the intervillous space on the maternal side of the placenta can impair adequate placental perfusion leading to late fetal loss, FGR, placental abruption, or preeclampsia. A relationship to early pregnancy loss is conflicting and may be restricted to specific thrombophilic defects that have not been fully defined, or the presence of multiple defects.

A systematic review of the association between fibrinolytic defects and RPL found a significant association for factor XII deficiency

Procoagulant microparticles can also contribute to the hypercoagulable state and likely to interfere with successful implantation and fetal growth. These were shown to be associated with early and late unexplained pregnancy loss.

Uterine factors [26-33]. — Acquired and congenital uterine abnormalities are responsible for 10 to 50 percent of RPL.

- **Anomalies** — Congenital uterine anomalies are present in 10 to 15 percent of women with RPL versus 7 percent of all women but there is a wide variability in diagnosis and inclusion criteria (first/second trimester) Pregnancy loss may be related to impaired uterine distention or abnormal implantation due to decreased vascularity in a septum, increased inflammation, or reduction in sensitivity to steroid hormones with possibly coexisting cervical weakness. The septate uterus is the most common uterine abnormality associated with RPL and the poorest reproductive outcome due mechanisms not clearly understood The miscarriage rate in women with untreated septum in small observational studies is greater than 60 percent. The longer the septum, the worse the prognosis possibly due to poor blood supply and implantation. Arcuate uteri are more associated with second trimester losses.
- **Leiomyoma** — Submucous leiomyomas that protrude into the endometrial cavity can impede normal implantation as a result of their position, poor endometrial receptivity of the decidua overlying the myoma, or degeneration with increasing cytokine production but no clear association has been proven.
- **Endometrial polyps** — There have been no data showing a relationship between endometrial polyps and RPL.

- **Intrauterine adhesions** — Intrauterine adhesions or synechiae lead to pregnancy loss because there is insufficient endometrium to support fetoplacental growth. The main cause of intrauterine adhesions is intrauterine interventions traumatizing the basalis layer, leading to menstrual irregularities (hypomenorrhea, amenorrhea), cyclic pelvic pain, infertility, and RPL.
- **Cervical insufficiency** — Cervical insufficiency could lead to recurrent midtrimester, but not early pregnancy loss. True incidence remains unknown due to essentially a clinical diagnosis and no specific inter-pregnancy test
- **Defective endometrial receptivity** — Estrogen and progesterone prepare the endometrium for pregnancy. Normal endometrial receptivity allows embryo attachment, implantation, invasion, and development of the placenta. These processes are likely to be disturbed when endometrial receptivity is defective, resulting in unexplained infertility and RPL. Causes of defective endometrial receptivity and biomarkers for evaluation of endometrial receptivity are under research. RPL may be associated with primary receptor defect, uterine stem cell deficiency and enhanced cellular senescence, which then results in abnormal endometrial preparation leading to RPL.

Environmental chemicals and stress (21,34)—There is no high-quality evidence showing a relationship between RPL and occupational factors, stress, or low level exposure to most environmental chemicals. Chemicals that have been associated with sporadic spontaneous pregnancy loss include anesthetic gases, arsenic, aniline dyes, benzene, ethylene oxide, formaldehyde, pesticides, lead, mercury, and cadmium.

Other

Personal habits [1,35]— The association between RPL and obesity, smoking, alcohol use, and caffeine consumption is unclear. These factors may act in a dose-dependent fashion or synergistically to increase the rate of sporadic pregnancy loss.

Male factor [36-38]. — There is a trend toward repeated miscarriages in women whose male partner has abnormal sperms (eg, poor morphology, sperm chromosome aneuploidy, high DFI) Advanced paternal age may be a risk factor for miscarriage.

Infection [39-41] — Some infections, such as Toxoplasma gondii, cytomegalovirus, and primary genital herpes, are known to cause sporadic pregnancy loss, but no infectious agent has been proven to cause RPL. The presence of bacterial vaginosis in the first trimester of pregnancy has been reported as a risk factor for second-trimester miscarriage and preterm delivery, but the evidence for an association with first trimester miscarriage is inconsistent.

Decreased ovarian reserve [42] — Women with unexplained RPL have a higher incidence of abnormal ovarian reserve tests, than women with a known cause of RPL Women with RPL and elevated day 3 FSH or low AMH may have poor quality oocytes that fail to develop after fertilization.

Future research [43-45] — A meta-analysis of studies evaluating whether there is an association between cytokine polymorphisms and RPL concluded there was no more than a

mild non significant association. Progesterone receptor gene polymorphisms, as well as other gene polymorphisms, may play a role in RPL.

HISTORY AND PHYSICAL EXAMINATION — The minimum diagnostic workup of couples with RPL consists of a complete medical, surgical, genetic, and family history and a physical examination.

History — The history should include

- The gestational age and characteristics (eg, anembryonic pregnancy, live embryo) of all previous pregnancies. Gestational age is important because RPL typically occurs at a similar gestational age in consecutive pregnancies and the most common causes of RPL vary by trimester. Eg. Miscarriages related to chromosomal and endocrine defects tends to occur earlier in gestation than losses due to anatomic or immunological abnormalities; however, there is significant overlap.
- Abnormalities in menstrual cycle length may be due to endocrine dysfunction. Presence of galactorrhea, which also suggests endocrine dysfunction (hyperprolactinemia)
- Does the family history display patterns of disease consistent with a strong genetic influence? Is consanguinity present?
- Was embryonic/fetal cardiac activity ever detected? RPL prior to detection of embryonic cardiac activity also suggests a chromosomal abnormality
- Is there exposure to environmental toxins, which may be lethal to developing embryos?
- Is there a personal/family history of venous or arterial thrombosis suggestive of antiphospholipid syndrome?
- History of uterine instrumentation, which may have caused intrauterine adhesions.
- What information is available from previous laboratory, pathology, and imaging studies?

Physical examination — Physical assessment should include signs of endocrinopathy (eg, hirsutism, galactorrhea) and pelvic organ abnormalities (eg, uterine malformation, cervical laceration).

Mental health evaluation — Screening for severe stress and depression should be an integral part of the RPL work-up.

EVALUATION

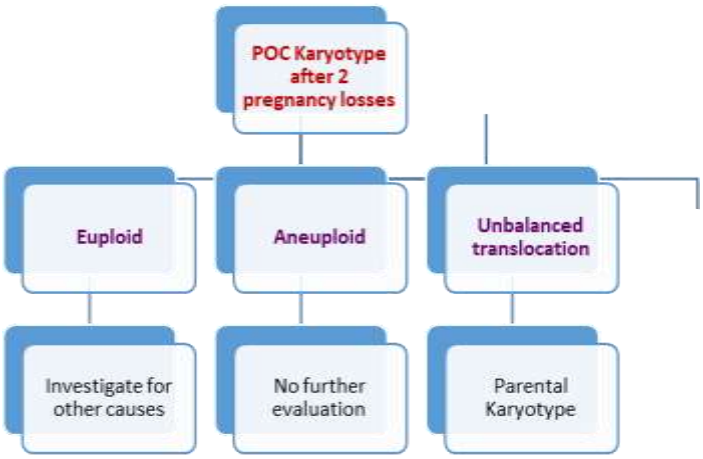
Parental Karyotype [16,21,46]— Karyotyping of couples is part of the evaluation of RPL, despite the low yield of abnormality, cost, and limited prognostic value. The purpose is to detect balanced reciprocal or Robertsonian translocations or mosaicism that could be passed to the fetus unbalanced.

Karyotype of the abortus or products of conception[16,47] **--** Chromosomal abnormalities detectable in parental peripheral blood preparations are an indirect and limited indicator of fetal karyotype vis a vis the fetal karyotype.

- Knowledge of the karyotype of the products of conception allows an *informed prognosis* for a future pregnancy outcome
- To differentiate whether it was sporadic due to abnormal embryo or treatment failure per se and need for further evaluation
- A normal karyotype suggests (but does not prove) a maternal factor as the cause of pregnancy loss, while an abnormal karyotype is usually a sufficient explanation for a nonviable pregnancy
- If the karyotype of the miscarried pregnancy is abnormal (Aneuploidy), there is a better prognosis for the next pregnancy (except unbalanced translocations)

Pitfalls of conventional POC karyotype are:- [48-50].

- Failure to cultivate: Cells from chromosomally abnormal abortuses, are less likely to grow in culture, thereby skewing the results of cohort studies Maternal tissue contamination. Array comparative genomic hybridization (aCGH) does not require dividing cells, and therefore can be useful in fetal demise with culture failure.
- Failure to seek other coexisting causes if cytogenetic study reveals chromosomal abnormality.
- Occurance of noncytogenetic embryonal abnormalities.
- Type of laboratory and analysis: In some cases, karyotype analysis of the abortus indicates a normal chromosomal pattern, but more detailed Acgh demonstrates major abnormalities.
- Need for surgical evacuation.



Uterine assessment [51-57] — Anatomic causes of RPL are usually diagnosed using hysterosalpingography (HSG), sonohysterography. (SHG) or 3D ultrasound(USG) .3D USG and SHG are more accurate than HSG in delineating internal contours along with outer. Utrasound (especially 3D) is useful for making the diagnosis of a septate/arcuate/bicornuate uterus, renal abnormalities associated, the presence and location of uterine myomas and, in pregnancy, the possibility of cervical insufficiency and assessment of fetal viability. Hysteroscopy, laparoscopy, or magnetic resonance imaging (MRI) due to invasiveness and /or cost ,are used as second-line tests when additional information or therapeutic intervention is required .

Anticardiolipin antibodies and lupus anticoagulant [58-60]— The minimum immunology work-up for women with RPL is measurement of anticardiolipin antibody (IgG and IgM) and lupus anticoagulant, done twice, six to eight weeks apart, because a low to mid positive level can be due to viral illness and revert to normal. The anticardiolipin antibody titer is considered elevated if medium or high titers of both IgG and IgM isotypes are present in blood. The detection of the lupus anticoagulant is generally based upon an activated partial thromboplastin time, kaolin plasma clotting time, or dilute Russell viper venom test time

Clinical and Laboratory Findings in Reproductive Autoimmune Syndrome

	Antiphospholipid Syndrome (APS)	Reproductive Autoimmune Failure Syndrome (RAFS)
Clinical features	• Thrombosis (≥1 unexplained venous or arterial thrombosis, including stroke) • Autoimmune thrombocytopenia • Adverse Pregnancy outcome ○ Three or more consecutive miscarriages before 10 weeks of gestation ○ One or more morphologically normal fetal losses after the 10th week of gestation ○ One or more preterm births before the 34th week of gestation owing to placental disease.	• FGR (<34 weeks) • Severe Pre eclampsia • Obstetric complications (abruption placenta, chorea gravidarum, HELLP syndrome) • Unexplained infertility • Endometriosis • Recurrent pregnancy loss ○ ≥1 consecutive and otherwise unexplained fetal deaths (≥10 wk) ○ ≥3 consecutive and otherwise unexplained preembryonic or embryonic pregnancy losses
Laboratory findings	• Anticardiolipin antibodies (ACA) (>20 GPL or MPL units) • Lupus anticoagulant (LAC)	• Antiphospholipid antibodies • Lupus anticoagulant • Gammopathy (usually polyclonal, mostly IgM) • Antinuclear antibodies (including antibodies against histones) • Organ-specific autoantibodies (antithyroid antibodies-ATA, anti-smooth muscle antibodies -ASMA)

Thyroid function [61-63] — Thyroid function should be assessed if positive history or symptoms. Screening asymptomatic women for subclinical thyroid dysfunction is controversial but recommended since there is evidence of an increased risk of miscarriage in women with subclinical hypothyroidism and in euthyroid women with thyroid peroxidase (TPO) antibodies.

Hypercoagulable state — Evaluation for an inherited thrombophilia can be considered in rare cases of recurrent, unexplained late fetal loss (after nine weeks of gestation) associated with evidence of placental ischemia and infarction and maternal vessel thrombosis.

Culture and serology [21] — Routine cervical cultures are not useful in the evaluation of RPL among otherwise healthy women.

Autoantibodies and immune function [64-74] — Many studies have reported the presence of autoantibodies in women with RPL. The pregnancy outcome of women with and without antinuclear antibody (ANA) is the same hence routine testing for ANA not recommended.

Peripheral blood NK cells are phenotypically and functionally different from uterine NK (uNK) cells. There is no clear evidence that altered peripheral blood NK cells are related to recurrent miscarriage. Examining the relationship between uNK cell numbers and future pregnancy outcome remains a research field.

Selection of appropriate tests for diagnosis of immune-based RPL (HLA typing, mixed lymphocytotoxic antibody tests, CD56+ cells and cytokine polymorphism) also requires further investigation and validation.

Screening for diabetes — Limited to women with clinical manifestations of the disease.

Progesterone level [75] — Single or multiple serum progesterone levels are not predictive of future pregnancy outcome.

Endometrial biopsy [76] for luteal phase defect is not predictive of fertility status and not recommended.

MANAGEMENT

RPL is an inhomogeneous condition and hence specific guidelines cannot be applicable in general to all. The development of an optimal investigation and management protocol depends on reaching a correct diagnosis of etiology and directing specific treatment. Therapeutic recommendations are largely based upon clinical experience and data from observational studies. The overall live birth rates after normal and abnormal diagnostic evaluations for RPL are 77 and 71 percent, respectively [77]. In all cases, psychological support is vital [78,79].

- **PARENTAL KARYOTYPE ABNORMALITY** [80-85]— Couples in whom chromosomal abnormalities are discovered in one or both partners or the abortus are generally referred for genetic counseling. They should receive information regarding the probability of having a chromosomally normal or abnormal conception in the future.. The magnitude of these risks varies according to the specific chromosomal abnormality and the sex of the carrier parent.

Management options for these couples

- Prenatal genetic studies (amniocentesis or chorionic villus sampling)
- In vitro fertilization (IVF) with preimplantation genetic screening (PGT-A) Gamete donation (egg or sperm)
- Adoption

UTERINE ABNORMALITIES [86-91] — Uterine abnormalities are managed surgically (hysteroscopically) if correctable cause, such as a uterine septum, intrauterine adhesions, or submucosal myoma.

There are no randomized trials evaluating pregnancy outcome after surgical correction of uterine anomalies. In a classic observational series, repair of septate uteri reduced the abortion rate from 84 percent (before surgery) to 12 percent (after surgery) using patients as their own controls The value of prophylactic cervical cerclage in women with a uterine anomaly, but no history of second trimester pregnancy loss, is controversial. Cervical cerclage is associated with potential hazards related to the surgery and the risk of stimulating uterine contractions and should be considered only in women who are likely to benefit. Women with a history of second-trimester miscarriage and suspected cervical weakness should be monitored closely by serial cervical scans. In women with a singleton pregnancy and a history of one second-trimester miscarriage, an ultrasound-indicated cerclage should be offered if a cervical length of 25 mm or less is detected by transvaginal scan before 24 weeks of gestation.

A gestational carrier(surrogate) is an option for women with irreparable uterine defects.

ANTIPHOSPHOLIPID SYNDROME [92] — Aspirin and heparin improve pregnancy outcome in women with APS with RPL. LMWH appear to have additional qualities in preventing adverse pregnancy outcome by their anti-inflammatory and proangiogenic properties.

SUSPECTED IMMUNOLOGIC DYSFUNCTION [93-101] — No alloimmune mechanism has been proven to cause RPL. Immunologic treatments for unexplained RPL are not effective, and may even be harmful as proven in systematic reviews and should used only in the setting of a clinical trial regulated by an Institutional Review Board.

- Paternal cell immunization
- Third party donor cell immunization
- Trophoblast membrane infusion
- Intravenous immune globulin (iv ig)
- Glucocorticoids — Glucocorticoids have several anti-inflammatory effects, including suppression of NK cell activity, but do not appear to be effective for preventing RPL. Steroids for treatment of RPL has been abandoned because of uncertain efficacy and increase in complications, such as preterm premature rupture of membranes, gestational diabetes, and maternal hypertension

THYROID DYSFUNCTION AND DIABETES MELLITUS [102,103] —

- Women with overt thyroid disease or diabetes mellitus should be treated, as medically appropriate, since these disorders can result in serious sequelae.
- Women with elevated serum thyroid peroxidase antibody concentrations are at high risk of developing hypothyroidism in the first trimester and autoimmune thyroiditis postpartum, and should be followed appropriately
- Euthyroid women with high serum thyroid peroxidase antibody concentrations may benefit from treatment with levothyroxine (50 mcg daily) during pregnancy as it may reduce the risk of miscarriage and preterm birth although more trials are required.

POLYCYSTIC OVARY SYNDROME [104] — The miscarriage rate in women with polycystic ovary syndrome (PCOS) is 20 to 40 percent, higher than the baseline rate in the general obstetric population Metformin has been used in women with PCOS to decrease this risk, but the effectiveness of this approach is unproven.

HYPERPROLACTINEMIA [105] — Normal levels of prolactin may play a significant role in maintaining early pregnancy. A study of 64 hyperprolactinemic women with RPL randomly assigned to bromocriptine therapy or no bromocriptine found treatment was associated with a significantly higher rate of successful pregnancy (86 versus 52 percent Prolactin levels during early pregnancy were significantly greater in women who miscarried.

THROMBOPHILIA — Anticoagulation of women with certain inherited thrombophilias may improve maternal outcome (eg, prevention of venous thromboembolism), but controversial in RPL.

TREATMENT OPTIONS FOR UNEXPLAINED RPL — [106]. A significant proportion of cases of RPL remain unexplained despite detailed investigation. These women can be reassured that the prognosis for a successful future pregnancy with supportive care alone is almost 75%.

Several unproven treatments are often offered for unexplained RPL

- Lifestyle modification — Eliminating use of tobacco products, alcohol, and caffeine and reduction in body mass index (for obese women) may improve chances of live birth
- Progesterone[106-110]— Therapeutic effect of progesterone may be related to immune modulation it is possible that earlier initiation of progesterone, such as during the luteal phase, may improve outcome as shown by small studies.

  Older metaaanalysis including smaller heterogenous studies confounded by fetal factors showed a beneficial effect but a large trial comparing first-trimester vaginal progesterone therapy or placebo showed no significant difference

- Human menopausal gonadotropin [111] — An observational study reported that controlled ovarian stimulation with human menopausal gonadotropin (hMG) appeared effective for endometrial defects in women with RPL likely by correction of a luteal phase defect or a thicker endometrium, leading to a better implantation.

- Human chorionic gonadotropin (118) — HCG is critical to early pregnancy, ensuring active maintenance of steroid production from the corpus luteum and for endometrial preparation to facilitate implantation. Although HCG has shown to improve LBR in systematic reviews but there is insufficient evidence to recommend the use of hCG to prevent pregnancy loss in women with a history of unexplained RPL. However hCG also had detrimental effects on decidualization in vitro. There are evidences both for and against its use, so it should be offered to women only within a research trial. Large randomized controlled trials to identify subgroups which are likely to benefit are needed.

- In vitro fertilization and preimplantation genetic diagnosis (PGT-A) [112-116]. — Studies evaluating the value of in vitro fertilization (IVF) in women with RPL have yielded mixed results. Embryos of women with unexplained RPL have a higher incidence of aneuploidy. In a retrospective cohort study of 300 women with RPL, the pregnancy, live birth, and miscarriage rates were similar for women who underwent IVF with preimplantation screening (PGS) and women who elected expectant management Other drawbacks include need for IVF,cost involved and issues related to PGT-A like mosaicism.

- Oocyte donation [117] — Poor quality oocytes may be responsible for 25 percent of pregnancy losses Ovum donation can overcome this problem and has been associated with a live birth rate of 88 percent in women with RPL.

- Surrogacy — A gestational carrier may be considered in RPL not associated with recurrent embryonic aneuploidy or obvious intrinsic gamete factors (eg, single gene defects, diminished oocyte and embryo quality).

FUTURE PREGNANCY PROGNOSIS

- Continued pregnancy loss [119,120]— The greatest risk of recurrent loss occurs during the period up to the time of previous miscarriage. The likelihood of successful pregnancy in women with a history of recurrent pregnancy loss (RPL) was 67-75 percent at 5 years. Increasing maternal age and number of miscarriages are associated with a poorer prognosis.

- Other obstetric issues. [121,122] — Women with a history of RPL who become pregnant may be at higher risk for developing fetal growth restriction and premature delivery, but not for gestational hypertension or diabetes

Comparison of Guidelines for the Investigation and Treatment of Recurrent Pregnancy Loss

Investigation or Treatment	ASRM Guidelines	RCOG Guidelines	ESHRE Guidelines (2017)
Parental karyotyping	Recommended	Recommended only if POC shows unbalanced translocation	Recommended only after individual risk assessment
POC karyotyping	Recommended	Recommended after 2 miscarriages	Trials required aCGH preferable

APS assessment (ACA and LA)	Recommended	Recommended	Recommended
Treatment of APS with heparin and aspirin	Recommended	Recommended	Recommended
Thyroid function	Recommended	Recommended	Recommended
Treatment of Overt Hypothyroidism with levothyroxine		Recommended	Recommended
Treatment of Subclinical hypothyroidism		Recommended	Need more trials,Inconsitent evidence
Glucose intolerance testing in PCO		Insufficient evidence	Not Recommended for RPL prognosis
Metformin in RPL with PCO		Insufficient evidence	Insufficient evidence
Prolactin estimation	Recommended		Not recommended in absence of clinical signs
Bromocriptine for Hyperprolactinemia			Recommended
Ovarian reserve testing			Insufficient evidence
Uterine cavity assessment	Insufficient evidence	Recommended	Recommended (3D US/Sono HG))
Resection of uterine septum	Can be considered	Insufficient evidence	More trials needed
Hysteroscopic polypectomy/Myomectomy	Can be considered		Not recommended
Hysteroscopic adhesiolysis	Can be considered		Insufficient evidence
Serial cervical USG surveillance in suspected incompetence		Recommended	Recommended
Cervical cerclage for second trimester loss		Ultrasound indicated	
Luteal Phase Insufficiency testing	Insufficient evidence	Not recommended	Not recommended
Progesterone supplementation	Insufficient evidence	Insufficient evidence	Insufficient evidence- More RCTs required
hCG supplementation		Insufficient evidence	Insufficient evidence
Bacterial vaginosis	Not recommended	Insufficient evidence	
Hereditary thrombophilias	Not recommended	Recommended for second trimester losses	Recommended in research settings or if additional risk factors
Anticoagulants for hereditary thrombophilia		Insufficient evidence	Insufficient evidence
TORCH Testing	Not recommended	Not recommended	Not recommended

Alloimmune testing (HLA,Perepheral blood NK cells,Cytokine polymorphism)	Not recommended	Not recommended	Insufficient evidence
ANA			For explanatory purpose
Immunotherapy(LIT/IVIg)	Not recommended	Not recommended	Insufficient evidence
Tender loving care	Recommended	Insufficient evidence	Recommended
Obesity, smoking, alcohol			Recommended
Folic acid for hyperhomocysteinemia			Insufficient evidence
Preconceptional Vitamin D supplementation			Recommended based on significant prevalence in RPL
Steroids	Not recommended	Not recommended	Not recommended
G CSF / Intralipids / Heparin/Aspirin/Endometrial scratching for Unexplained RPL			Not recommended
Male partner life style factors.			Recommended
Assessing sperm DNA fragmentation	Insufficient evidence		Considered for explanatory purposes, based on indirect evidence
Antioxidants for men			Insufficient evidence

ASRM: American Society of Reproductive Medicine; RCOG: Royal College of Obstetricians; ESHRE: European Society of Human Reproduction and Embryology –

SUMMARY AND RECOMMENDATIONS

●Recurrent pregnancy loss (RPL) refers to the occurrence of three or more consecutive losses of clinically recognized pregnancies prior to the 20th week of gestation (excluding ectopic, molar, and biochemical pregnancies). It may be primary or secondary.

●0.4 to 1 percent of women have three consecutive pregnancy losses.

● Chromosomal abnormalities are the most common cause of sporadic early pregnancy loss (50 %). 3 to 5 % of couples with RPL have a major chromosomal rearrangement (vs 0.7 percent of the general population); usually a balanced translocation.

●Uterine abnormalities, both acquired and congenital have been reported to be responsible for 10 to 50 percent of RPL in small studies.

●Pregnancy loss is one of the diagnostic criteria for antiphospholipid syndrome.

●Endocrine factors may account for some cases of RPL.

●There is no strong evidence showing a relationship between RPL and occupational factors, stress, or mild exposure to most environmental chemicals.

●RPL typically occurs at a similar gestational age in consecutive pregnancies. The recurrence risk increases as gestational age at the time of loss increases.

●Evaluation of women for RPL may be recommended after two or three consecutive miscarriages depending on other factors like age.

●A detailed history and physical examination should guide the clinician regarding probable etiology and tailor diagnostic investigations and management in RPL.

●The following initial evaluation may be recommended as per need:

•Sonohysterography/3D USG for assessment of uterine abnormalities

•Anticardiolipin antibody (IgG and IgM) titer and lupus anticoagulant performed twice, six to eight weeks apart

•Thyroid stimulating hormone (TSH) and thyroid peroxidase antibodies

•Parental karyotype and karyotype of the abortus if the above examinations are normal.

Additional testing depends upon the diagnosis suggested by the history, physical examination, and laboratory results.

- Couples with chromosomal abnormalities in one or both partners or the abortus are generally referred for genetic counselling.
- Correctable uterine abnormalities such as a uterine septum or intrauterine adhesions may be managed hysteroscopically.
- For women with unexplained RPL, there is not enough evidence that use of vaginal progesterone or HCG improves live birth rates.
- Immunotherapy or glucocorticoids are not effective for RPL and may be harmful.
- Women with hyperprolactinemia and RPL should be treated.
- For unexplained RPL low risk, simple, and less expensive interventions should be preferred over more complex and expensive options.
- Women with a history of RPL who become pregnant may be at higher risk for developing fetal growth restriction and premature delivery.

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Genetics of Recurrent Pregnancy Loss

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Abstract

Human reproduction is remarkably inefficient; with pregnancy loss occurring in 10–30% of clinically recognized pregnancies. Of those, 3%–5% of couples experience recurrent pregnancy loss (RPL). Recurrent early miscarriages (within the first trimester) are most commonly due to genetic or chromosomal problems of the embryo. Knowledge of the genetic background of miscarriages is important for prognosis, as well as the potential planning of prenatal diagnostics in subsequent pregnancies. The aim of this article is to summarize current knowledge on the genetic causes of recurrent miscarriage. It presents the most common parental and fetal genetic disorders (karyotype abnormalities, recessive diseases carrier status, dominant diseases, and thrombophilia) connected with recurrent pregnancy loss.

Introduction

Miscarriage is the most common complication of pregnancy. While the Royal College of Obstetricians and Gynaecologists (RCOG) regard recurrent miscarriages as the loss of three or more successive pregnancies [1, 2], the American Society for Reproductive Medicine defines recurrent miscarriages as at least two successive miscarriages [3]. Genetic abnormalities that may predispose to pregnancy loss include chromosomal aneuploidy, copy number variants, single-gene changes, and others. Chromosomal abnormalities, uterine defects, thrombophilia, immunological factors, endocrine and metabolic factors are the known risk factors involved in the causation of recurrent pregnancy loss in 50% of the cases.

CHANGES IN PARENTAL GENETIC MATERIAL

Changes in parental (paternal and maternal) genetic material that contribute to the increased risk of miscarriage in successive pregnancies include karyotype abnormalities, recessive and dominant disease carrier status, as well as mutations in genes responsible for coagulation and the metabolism of folates.

Karyotype Abnormalities: They are most found as balanced chromosome aberrations, i.e. abnormalities that cause no clinical symptoms in carriers but possibly induce the production of abnormal reproductive cells containing abnormal amounts of genetic material. Among couples with recurrent miscarriages, balanced translocations are confirmed in at least one of the partners in around 3% to 5% of cases [4–6]. Most commonly, these include reciprocal translocations, with inversions and Robertsonian translocations being less common. The status of a balanced chromosome aberration carrier increases the risk of miscarriage in subsequent pregnancies, as well as the risk of the child being born with an unbalanced karyotype. Genetic diagnosis is performed by assessing both parental karyotypes using conventional cytogenetic methods based on light microscopy.

Gene Abnormalities: Recessive gene diseases may be induced by the presence of mutations responsible for single-gene recessive diseases in both parents, particularly when the parents are close of kin. An example of such a disease is congenital methemoglobinemia, as described by Kedar et al. [7]. Other examples include the carrier status of mutations responsible for congenital arthrogryposis or Smith-Lemli-Opitz syndrome. Few autosomal dominant disorders like myotonic dystrophy have also been reported to lead to RPL.

Another cause of recurrent miscarriages is congenital thrombophilia following damage to the maternal factor V gene G1691A (Leiden mutation) and prothrombin gene (G20210A mutation). In the case of factor V, both the Leiden mutation G1691A and the T1328C mutation appear to be important in the pathogenesis of recurrent miscarriages, particularly in cases observed before the 7th week of gestation [16]. However, identification of the polymorphism within factor V gene (Leiden mutation) and prothrombin factor II gene may be an insufficient method of screening for congenital thrombophilia risk factors. Obstetric failures may also be caused by genetically-determined disturbances in the activity of, *inter alia*, factor VII, factor XIII, or beta-fibrinogen [8–10].

The impact of mutation within the *MTHFR* gene, a gene encoding a protein involved in the metabolism of folates, on recurrent miscarriages is currently a matter of debate. Reports suggest no relationship between hyperhomocysteinemia and reproductive failures. This may be due to folic acid supplementation, particularly during the first trimester [11].

Changes in Fetal Genetic Material:

It is estimated that about 50% of first-trimester pregnancy losses are associated with chromosome aberrations in the developing embryo/fetus [12, 13]. In most cases, these are *de novo* changes, which means the risk of a similar abnormality occurring during the next pregnancy is low [14, 15]. The largest group of abnormalities in embryonic/fetal genetic material consists of aberrations in the number of chromosomes (86%), mainly autosomal trisomies, monosomy X and polyploidies. The remaining group included structural aberrations (6%) and chromosomal mosaicism (8%) [12]. Fetal autosomal trisomies represent at least 50% of the chromosomal aberrations responsible for pregnancy loss [12, 13]. Trisomies may be generally observed in all autosomal chromosomes, although the incidence of particular trisomies varies

Genetic Assessment of Miscarried Material

Genetic analyses of the miscarried material are usually based on molecular biology techniques. Proper collection of the examined sample is important to avoid contamination with maternal tissue. The miscarried material must first be dried and rinsed of blood with physiological saline before precise isolation of chorionic villi is performed [16]. If the fetal tissues are already well formed and visible, a fragment of the umbilical cord may be used for examination purposes. Miscarried material may also be analyzed in paraffin blocks [17]; such examinations may be carried out several years after the block is prepared [18].

An outline of available classic cytogenetics, molecular cytogenetics, and molecular biology techniques used for the examination of embryonic/fetal material is presented in Table 1

Summary

Recurrent miscarriages may occur due to a diverse range of causes. Knowledge of the genetic background of miscarriage is very important for prognosis, as well as to plan prenatal diagnostics in subsequent pregnancies. However, it is very difficult to make an appropriate diagnosis, particularly during the early stages of pregnancy. Usually, targeted genomic diagnostics are required following a clinical observation by ultrasound or pathological examination of the fetus.

In as many as 50% of cases of recurrent miscarriage, none of the known causes may be determined. Therefore, new potential abnormalities, including genetic abnormalities that lead to pregnancy losses, such as a genetic predisposition to obesity, need to be identified, and the methods used for their diagnosis need to be further expanded and refined.

Table 1: An outline of available classic cytogenetics, molecular cytogenetics, and molecular biology techniques used for the examination of embryonic/fetal material

Method Features	**Karyotype**	**Fluorescent In-Situ Hybridization (FISH)**	**BoBs (BACs on Beads)**	**QF-PCR**	**Chromosomal Microarray**	**Next Generation Sequencing**
Detection	Changes in the number of chromosomes (aneuploidies, polyploidies) Structural aberrations (such as inversions, deletions, additions, translocations) — **balanced and unbalanced** Marker chromosomes Standard cytogenetic examination facilitating the analysis of the entire karyotype to search for chromosome aberrations	Chromosomal aneuploidies Diagnostics of submicroscopic chromosomal aberrations Identification of complex structural aberrations Identification of marker chromosomes Used for the analysis of both metaphase chromosomes (cell culturing required) and interphase nuclei	Most common aneuploidies (13, 18, 21, X, Y) Deletions and duplications of particular regions	Aneuploidies within chromosomes 15, 16, 22 and 13, 18, 21, X and Y Determination of the origin of the additional chromosome	Changes in the number of chromosomes (aneuploidies, triploidies) **Unbalanced structural changes** (such as duplications, deletions, and amplifications)	NGS facilitates sequencing of large genomic regions, high numbers of genes, or a high number of samples within a single test
Limitations	The requirement to culture the cells	Diagnostics of **specific changes** within the genetic material (depends on the probes used)	Diagnostics of changes within the genetic material **as defined in the intended use of the kit**	Diagnostics of changes within the genetic material **as defined in the intended use of the kit**	No detection of translocations and inversions within the genome	Very high sensitivity and ability to detect single nucleotide changes within the genome, leading to excess information uninterpretable for the purposes of diagnosing the genetic causes of miscarriage

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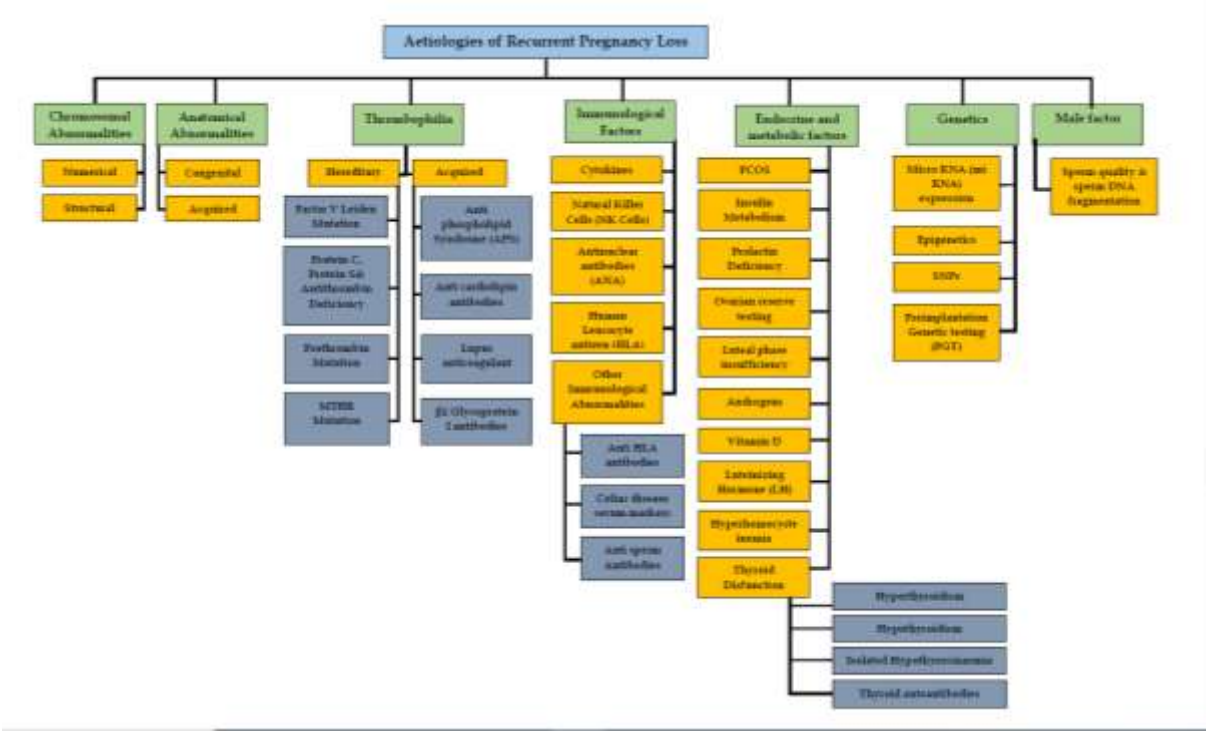

SCREENING FOR HEALTH

Early detection to ensure healthy outcomes for mothers & babies

PRENATAL SCREENING

- Combined First Trimester Screening (hCGß and PAPP-A + NT + Maternal Age)
- Enhanced First Trimester Screening (hCGß, PAPP-A, AFP and PIGF + NT + Maternal Age)
- Pre-eclampsia Screening (PAPP-A, PIGF, UTPI, MAP)
- Second Trimester Screening (hCGß, AFP, uE3 and Inhibin-A)
- NIScreen (cfDNA)
- Second-Tier Tests: Prenatal BoBs™, KaryoLite BoBs™, FISH, Karyotyping

GENETIC TESTS

- Confirmatory Test for Biochemical Screening Tests
- Whole Genome Sequencing
- Whole Exome Sequencing
- Focused Exome Sequencing
- Targeted Disease Panel
- Single Gene Test
- Carrier Testing (launching soon)

NEWBORN SCREENING

Newborn Screening Basic Panels (Screens for 7 disorders):

- CH • CAH • CF • Galactosemia
- G6PD Deficiency • PKU
- Biotinidase Deficiency

Newborn Screening Expanded Panels (Screens for 50+ disorders):

- Fatty Acid Disorders
- Amino Acid Disorders
- Organic Acid Disorders
- Hemoglobinopathies and more...

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Review Article: Role Of Ultrasound In Recurrent Pregnancy Loss

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Background:

Recurrent pregnancy loss (RPL) can be defined as more than two consecutive pregnancy losses at <24 weeks gestation and occurs in 1–2% of fertile women. Known causes or associations of RPL fall into various categories namely Antiphospholipid Syndrome, endocrine, immunological, anatomical, infections, Inherited thrombophilia and genetic. Out of these etiological factors, Ultrasound can be helpful in evaluating anatomical factors in the form of Uterine anomalies and cervical incompetence. It can also be useful in identifying fetal/ embryonic structural defects (Phenotypic features) of genetic origin and give a clue of recurrence which can further be proven on genetic grounds.
It is also to be noted that diagnosing 'pregnancy loss' itself is aided by use of ultrasound, especially in embryonic period when false diagnosis of missed miscarriage can be prevented by following rules laid down by recent criteria.[1]

Ultrasound criteria diagnostic of pregnancy loss:

- CRL of ≥7 mm and no heartbeat
- MSD of ≥25 mm and no embryo
- Absence of embryo with heartbeat ≥2 weeks after a scan that showed a gestational sac without a yolk sac
- Absence of embryo with heartbeat ≥11 days after a scan that showed a gestational sac with a yolk sac

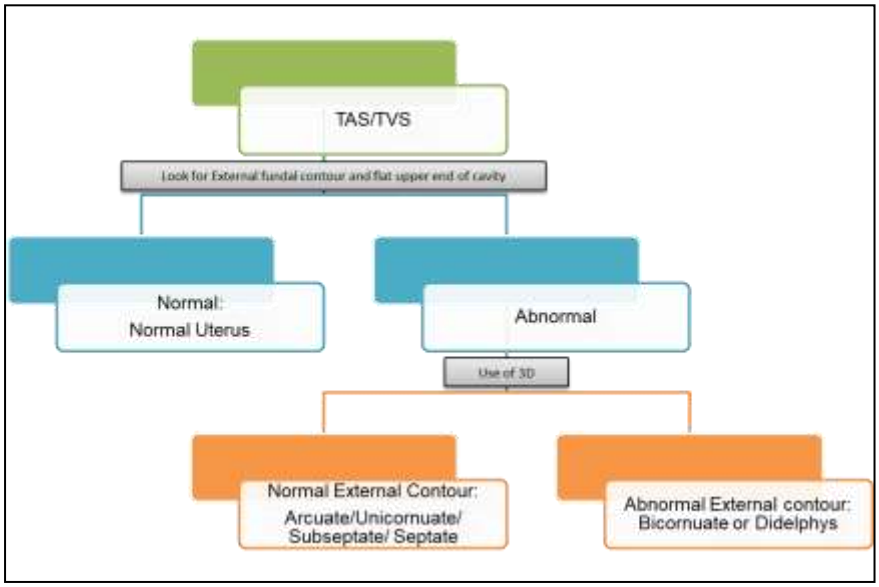
Role of USG in anatomical factors of recurrent pregnancy loss:

A. Congenital uterine malformations:

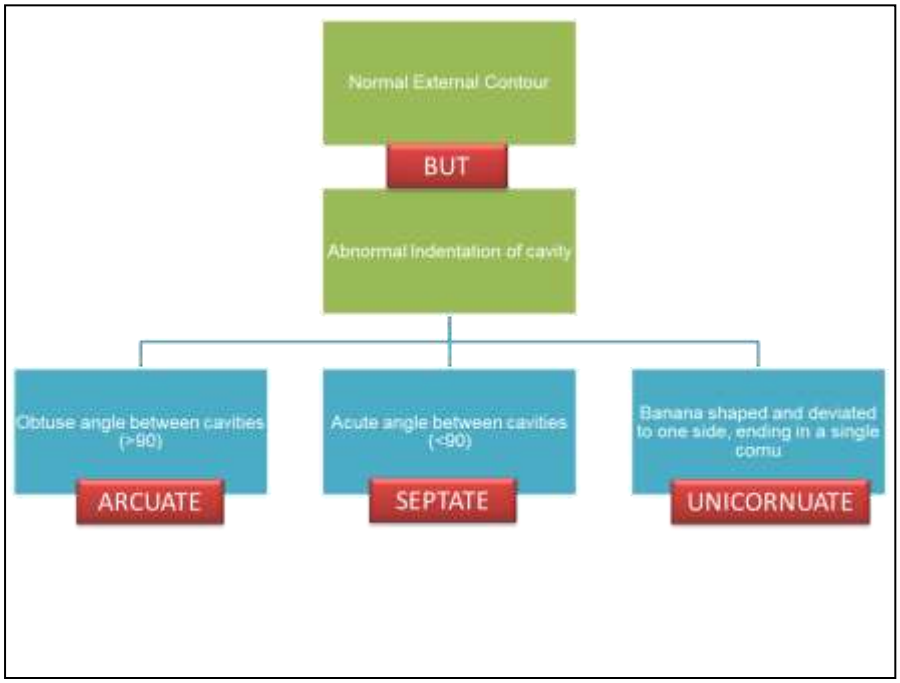
The exact contribution that congenital uterine anomalies make to recurrent miscarriage remains unclear but the reported prevalence of uterine anomalies in recurrent miscarriage populations ranges between 1.8% and 37.6%.[2]
The prevalence of uterine malformations appears to be higher in women with second-trimester miscarriages compared with women who suffer first trimester miscarriages, but this may be related to the cervical weakness that is frequently associated with uterine malformation.[3] It has been reported that women with arcuate uteri tend to miscarry more in the second trimester while women with septate uteri are more likely to miscarry in the first trimester.[4] However, retrospective studies are biased by patient selection and, until well controlled prospective data become available, the role of uterine anomalies in recurrent miscarriage will remain debatable.

Algorithmic approach to diagnosis of uterine anomalies:

Step 1

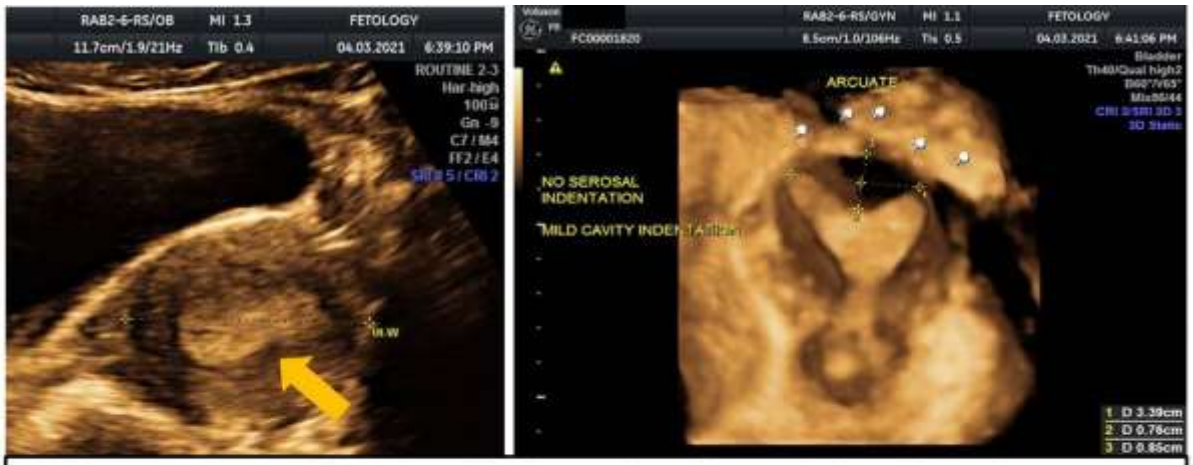


Step 2



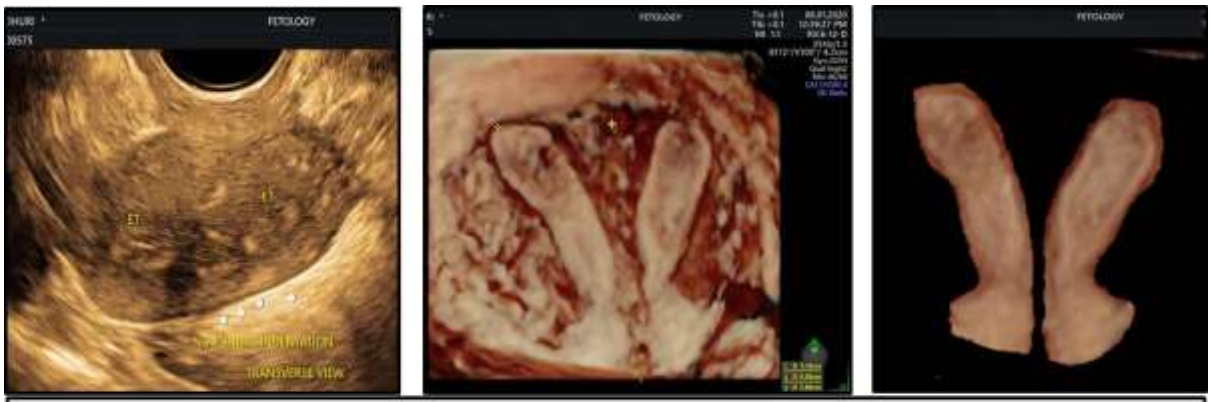
Combination of 2D and 3D findings for diagnosis of uterine anomalies:

1. **Arcuate Uterus:**



2D image shows mild indentation in cavity; 3D rendering showed no serosal indentation; obtuse indentation in cavity

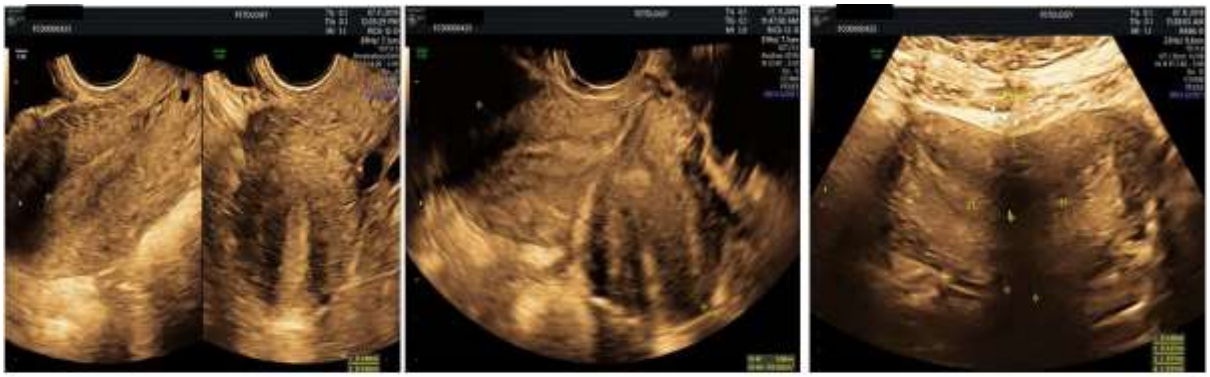
2. **Septate Uterus (U2 ESHRE):**



2D: Indentation at cavity; no serosal indentation; 3D: Angle between the two uterine cavities is < 90; Rendered image after electronic scalpel to document complete septum

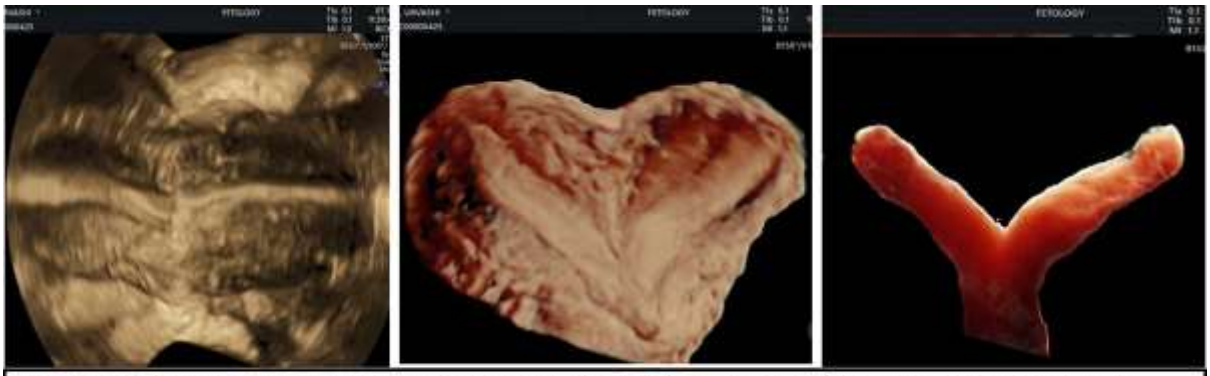
3. Bicornuate Uterus (U3 ESHRE):

2D USG findings:



(a) Two separate cavities; (b) Fundal contour indentation; (c) TAS Transverse section diagnostic of Bicornuate Uterus

3D USG findings:



3D: Indentation at fundal contour; <5 mm from line joining two cornua to serosal surface; 3D rendered image after electronic scalpel to depict bicornuate uterus

B. Cervical weakness:

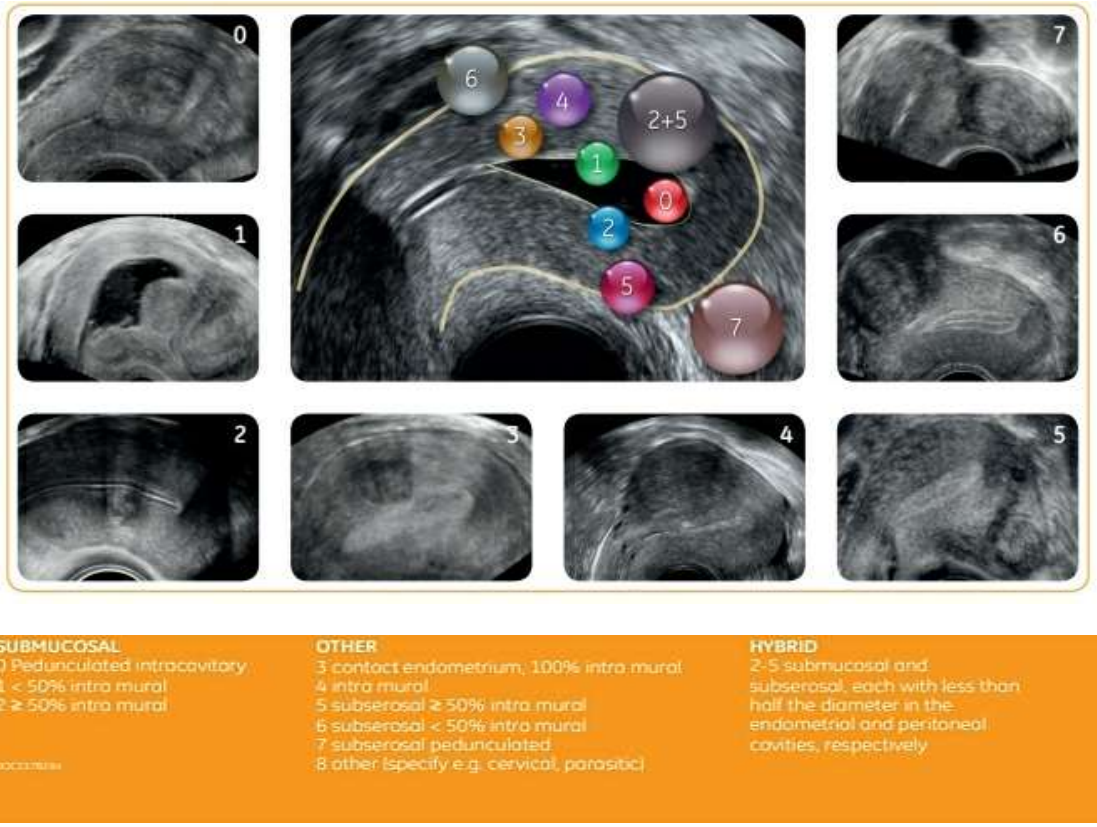
Cervical weakness is a recognized cause of second-trimester miscarriage, but the true incidence is unknown, since the diagnosis is essentially a clinical one. There is currently no satisfactory objective test that can identify women with cervical weakness in the non-pregnant state. The diagnosis is usually based on a history of second-trimester miscarriage preceded by spontaneous rupture of membranes or painless cervical dilatation.

In women with a singleton pregnancy and a history of one second-trimester miscarriage attributable to cervical factors, an ultrasound-indicated cerclage should be offered if a cervical length of 25 mm or less is detected by transvaginal scan before 24 weeks of gestation

C. **Fibroids and other acquired structural defects**:

It is widely acknowledged that the magnitude of effect of fibroids on pregnancy is greatest for submucous, least for subserous and intermediate for intramural fibroids. Robust prospective evidence that myomectomy reduces miscarriage and responsible for improved outcomes is lacking.[5] it is not known whether surgery was responsible for improved outcomes. There is also a lack of evidence regarding management of polyps and intrauterine adhesions in patients with recurrent pregnancy loss. However, until better quality evidence emerges, the ASRM states that it is reasonable to undertake surgical correction in cases of uterine cavity defects associated with fibroids, polyps and adhesions.[5]

Images show classification of uterine fibroids on USG as per the location:



D. **Endometritis:**

The combination of persistent endometrial shreds and/or endometrial focal thickening or echogenicity can significantly predict presence of endometritis as the sensitivity and specificity of the combination were 94.90 and 81.37, respectively. Bi-dimensional ultrasonography done to infertile women at 2 phases of the menstrual period can predict the presence of chronic endometritis as a subtle cause of infertility and might be an indication for hysteroscopic evaluation for these patients. [6]

Conclusion:

All women with recurrent first-trimester miscarriage and all women with one or more second-trimester miscarriages should have a pelvic ultrasound to assess uterine anatomy. Two dimensional Ultrasound with or without sono hystero salpingography for screening and suspected uterine anomalies may require further investigations to confirm the diagnosis using three-dimensional pelvic ultrasound, hysteroscopy, laparoscopy or MRI.

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Hysteroscopy in Recurrent Pregnancy Loss

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Introduction

Recurrent pregnancy loss (RPL) is often a frustrating and confusing clinical challenge for their treating physician. A complete evaluation of couple is not only difficult but yield in the identification of a probable cause in only 40–60% of patients[1,2].

Anatomical causes of RPLs

Uterine anomalies occur in about 19% of women with at least two or more consecutive miscarriages[2]. These include congenital malformations (most commonly bicornuate, didelphic, septate and unicornuate uteri as well as acquired defects (fibroids, adenomas, adhesions and polyps).

Hysteroscopy allows for direct visualization, and sampling of the uterine cavity. Since the introduction, the hysteroscopy has undergone considerable modifications, leading to an increase in patient compliance and tolerance.

Congenital Uterine Anomalies:

Septate uterus: Among women with RPL, septate uteri are the most prevalent of the congenital anomalies, with prevalence ranges between 1 to 2 per 1,000 to 15 per 1,000 women and its association with RPL is high as 76%.

Hysteroscopic resection of a septum is short outpatient basis surgical procedure has low associated morbidity and has been shown to significantly improve reproductive outcomes. Only 20-25% women with septate uteri have spontaneous abortions usually late first or early second trimester miscarriages initiated with mini-labours and bleeding. Patients undergoing successful hysteroscopic septum resection seem to enjoy near normal pregnancy outcomes, with term delivery rates of approximately 75% and live birth rates approximating 85%.

It is important to note that the septate uterus can have associated a double cervix and a double cervix with vaginal septum.

Laparoscopy / Hysteroscopy

The combination of hysteroscopy and laparoscopy is complimentary to each other and considered as the 'gold Standard' for the diagnosis of uterine malformation. Advantage is confirmation of diagnosis and correction in the same sitting i.e. 'See and Treat'.

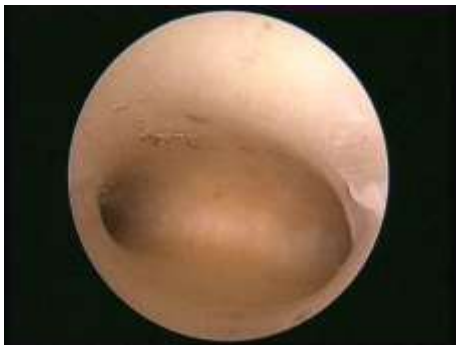


Fig. 1. Normal uterine cavity

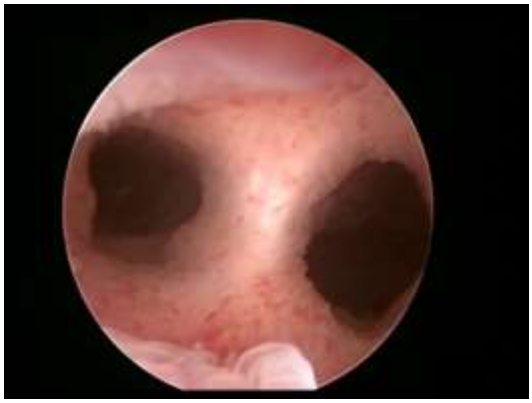


Fig. 2a. Complete Septate Uterus

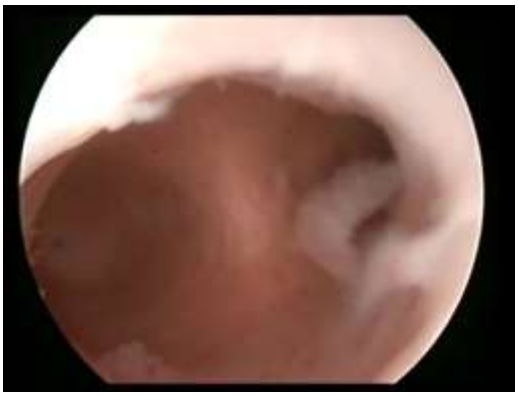


Fig 2b. Subseptate Uterus

Methods of Surgical Treatment

Gold standard method for treatment is hysteroscopic metroplasty which can be done by division or incision of the uterine septum using cold scissors, electrosurgery, resectoscope with an operating loop, laser. Best time to perform metroplasty is early proliferative phase. Misopristol is an effective ripening agent to induce adequate cervical dilatation prior to septum resection[4]. In my opinion there is no need of misopristol for only 4 mm outer diameter of whole assembly.

Office Hysteroscopy: It can be done in office environment with a diameter 2.9 mm or smaller telescope, 4.5 mm inner sheath & 5 mm outer sheath, and 5 fr. Scissors can be introduced from working channel. It can be introduced in the uterine cavity without dilatation of cervix with continuous flow vaginoscopy. Moving the hysteroscope from side to side and visualisation of both the ostia on a panoramic view from the level of internal os verifies completion of resection.

Fig.3- Bettocchi's 2.9mm Hysteroscope

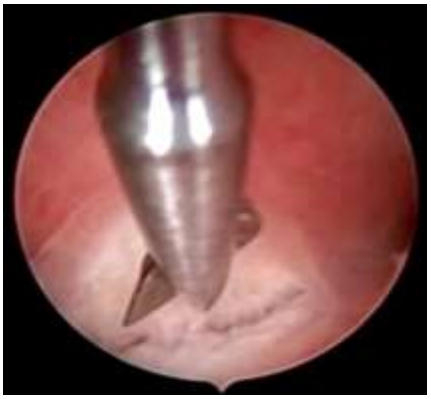


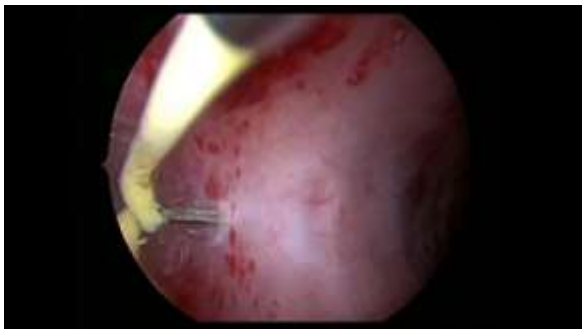
Fig.4 Resection of septum 5f scissors & 5 mm operative sheath

Practical points

- Normal saline is commonly used as distension media for diagnostic as well as with biopolar energy in operative. But when we use monopolar energy then glycine is used. Ideal Light source is Xenon and Camera is high definition.
- Width of the septum is judged by opening jaws of scissors which is 6 mm. if the width more than 10 mm better to use electrodes.

Resectoscope: 4 mm telescope with operative sheath with a diameter of 7-8.5 mm. So cervix needs dilatation of 10 mm. The procedure performed with resectoscope with monopolar or bipolar electrosurgery, using a Collins loop/knife. Recently, miniresectoscopes with small diameter have been developed, avoiding the needed for cervical dilatation, allowing to perform the metroplasty in an office setting. It is advisable to perform diagnostic hysteroscopy first. Once the diagnosis is confirmed, resection is performed using right angled knife. Usually a monopolar underwater cutting current of 80-120 W is sufficient for performing the septum resection. Strict account of intake and output of Glycine should be maintained.

Bipolar and monopolar resectoscopic loops were compared in one randomized study and showed similar efficacy and safety. Once the satisfactory resection of the septum is performed the Hysteroscopic assembly is withdrawn and hemostasis is confirmed. Bleeding following the procedure is self-limiting or may requires uterine bimanual massage.



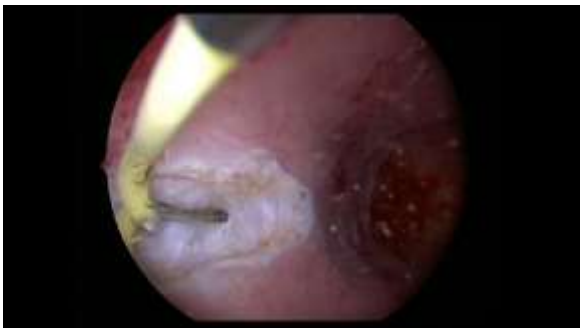


Fig. 5 & 6- Resection of septum with resectoscope

Postoperative care

- Prevention of intra-uterine adhesions: do not need anything for subseptate type. In complete septum one may insert intrauterine Foleys catheter of size 8 with balloon is distended with 3 cc of saline or instil hyaluronic acid or can put IUD.
- Two cycles of Estradiol Valerate 2mg TDS for 21 days along with oral Progesterone 10 mg once a day from day 16-25 are used for rapid epithelisation and endometrial proliferation over the respected raw area to prevent adhesions**.**
- In our experience 7**-**10**%** of patients develop intrauterine adhesions especially at the fundus which may mimic the residual septum. Imaging modality is used for confirming the result after two months. It is well accepted after the study of Fedele that women with a residual septum <1cm shown by ultrasonography after the hysteroscopic metroplasty does not affect the reproductive outcome compared to women with a complete resection[5].
- In selected group of patients second look Hysteroscopy is advocated 8 weeks following the resection of the septum.

Complications: Complication following septum resection is uncommon.

1 Perforation of the uterus
2 Postoperative bleeding
3 Rarely fluid overload

Unicornuate uterus: 1Four possible subtypes can develop: (i) absent rudimentary horn, (ii) non-cavitary (non-functional) rudimentary horn, (iii) cavitary communicating rudimentary horn and (iv) cavitary non-communicating rudimentary horn. The last one may obstruct and present with abdominal pain, subsequently requiring surgical intervention.

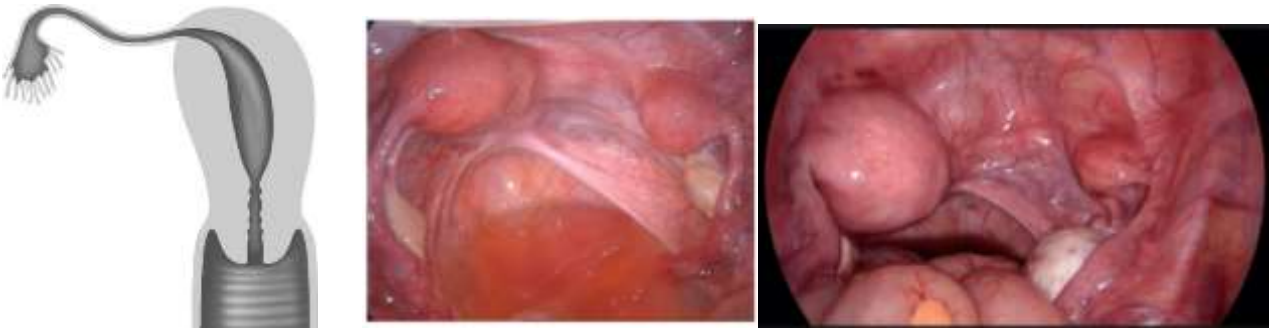


Figure 7. Unicornuate uterus

Although a patient with a unicornuate uterus can have a normal pregnancy, Spontaneous abortion rates reportedly range between 41% and 62% and premature birth rates between 10% and 20%. Other complications include abnormal fetal lie and intrauterine growth restriction[3]. Lateral metroplasty by hysteroscopy to improve neonatal outcome can be tried with guarded response in recurrent preterm births.

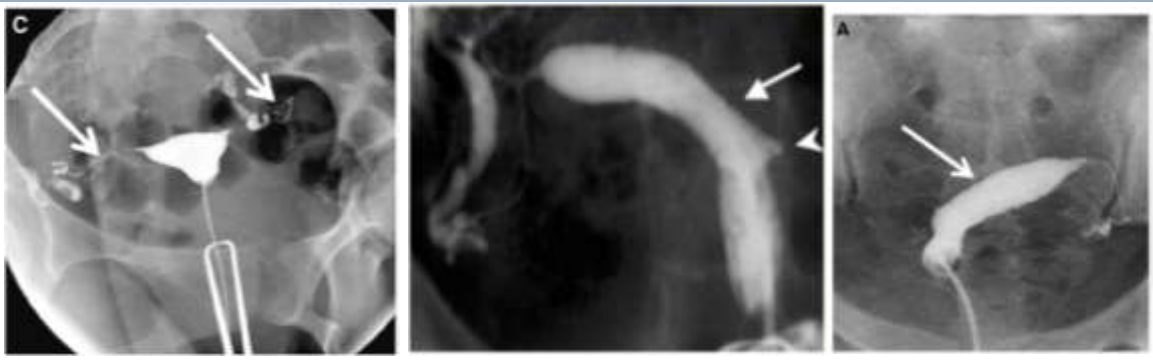


Figure 8. Normal uterine cavity in HSG, unicornuate uterus with normal ipsilateral tube in HSG

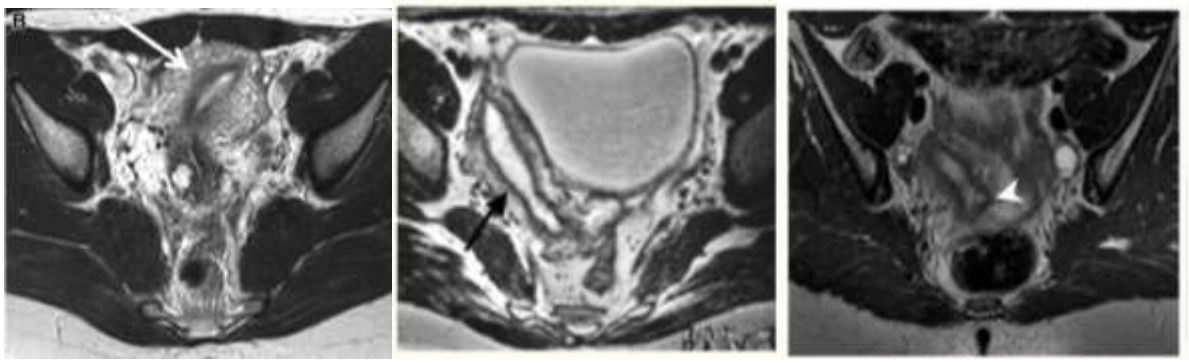


Figure 9. Class II- Unicornuate uterus with no rudimentary horn. Arrow shows classic banana shape appearance of the unicornuate uterus.

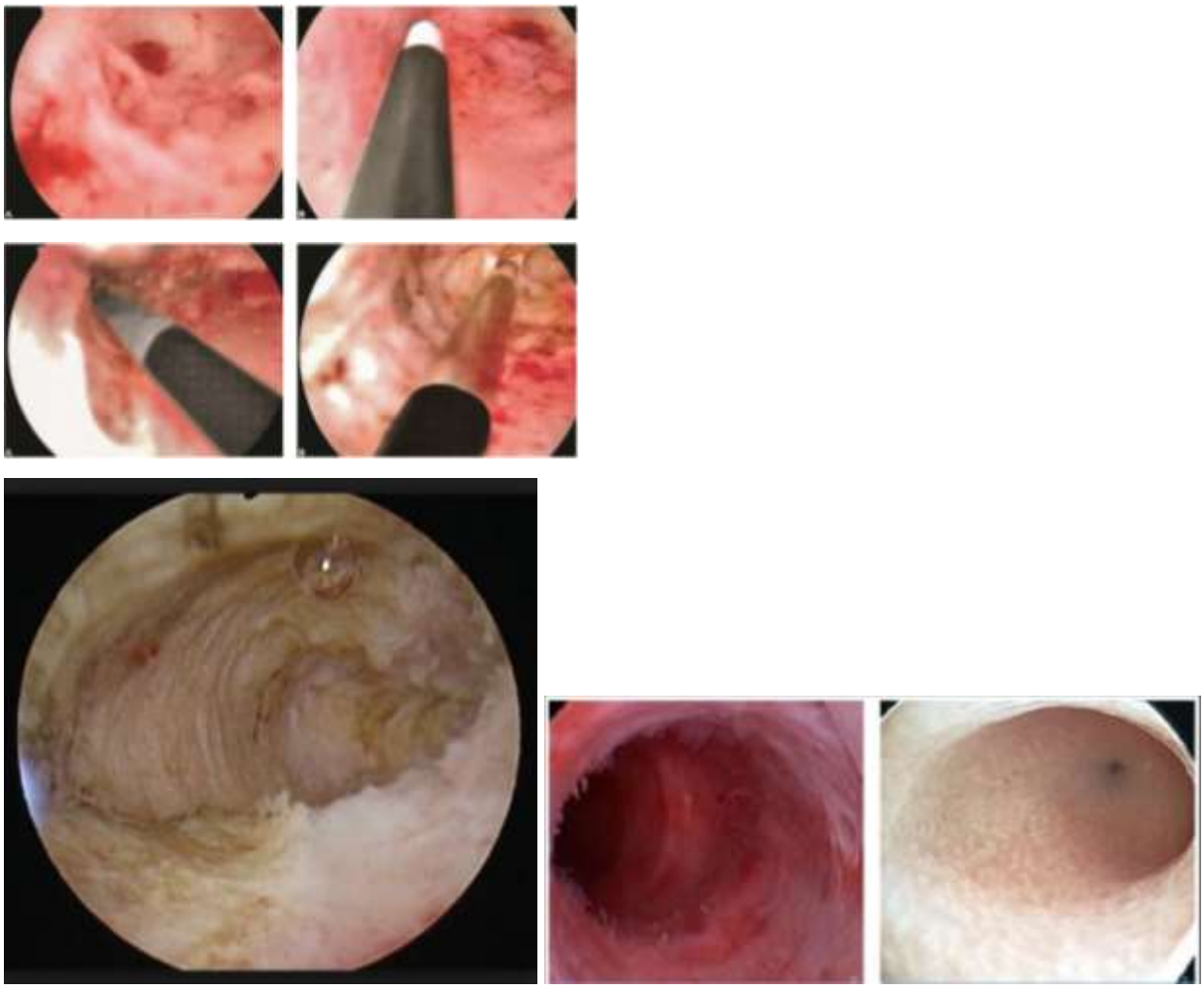


Figure 10. Hysteroscopic view of unicornuate uterus and lateral metroplasty.

Pregnancy implanting in the rudimentary horn usually has a disastrous outcome, with most resulting in uterine rupture[1,3].

Acquired Uterine Anomalies

The acquired uterine anomalies develop in response to hormonal or physical stimuli experienced after puberty; and in women with RPL, they are almost twice as prevalent as congenital anomalies.

Intrauterine adhesions:

Hysteroscopy is now the gold standard of diagnosis and treatment since it provides a good view of the cavity, allowing a precise description of the location and degree of adhesions and concurrent treatment of adhesions. In one review, women with adhesions experienced a high rate of miscarriages (40%) compared with women who had surgical adhesiolysis (25%)[6]. A thin endometrium that fails to respond to hormones leads to implantation failure or early miscarriages due to lack of blood supply.

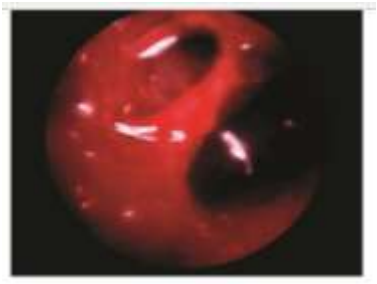


Fig. 11. Fine and Fragile endometrial synechiae

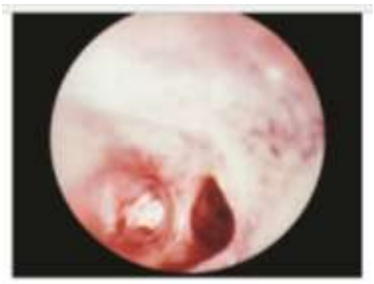


Fig. 12. Myofibrous synechiae near to tubal ostium, remaining endometrium is atrophic.

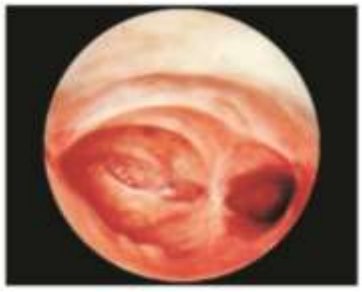


Fig. 13. Connective tissue synechiae with partial debridement

Standard procedure: Either monopolar needle/scissors/versa point / Collin's knife is used to release adhesions. One should withdraw the scope till internal os from time to time, to have a panoramic view of cavity for orientation and to avoid going in the wrong plane. Adhesiolysis should be stopped once the pink myometrium is reached. Good intra-uterine pressure should be maintained. Post-operative estrogen and progesterone treatment for 4-6 weeks is advisable to enhance endometrial growth.

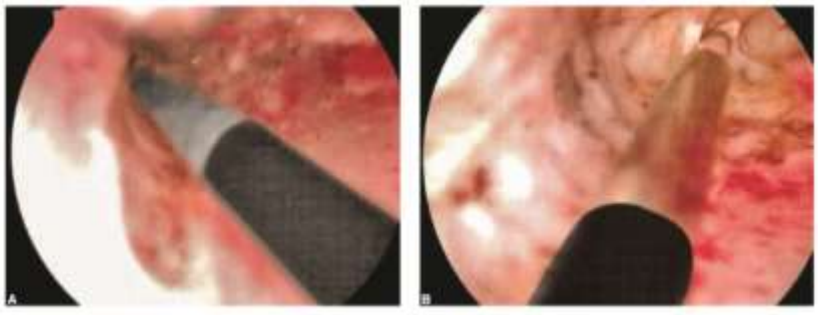


Fig. 14. With versascope lateral and vertical strokes taken so as to release adhesions.

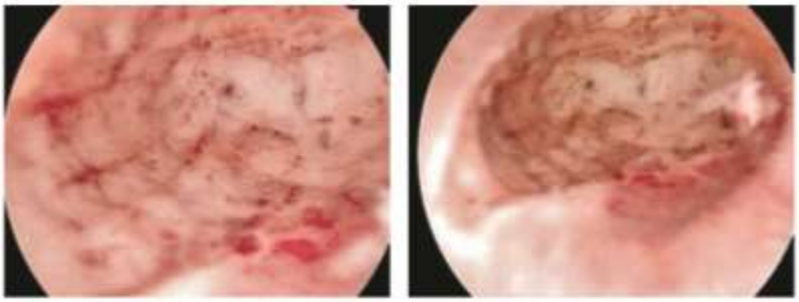


Fig. 15. End result (nearly normal cavity)

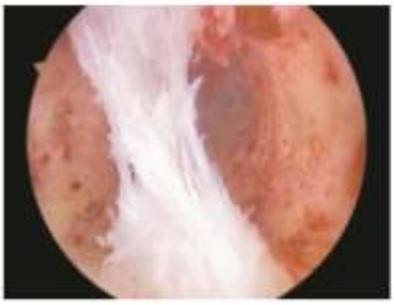


Fig. 16. Band of intrauterine adhesions at isthmus can easily be released with scissors.

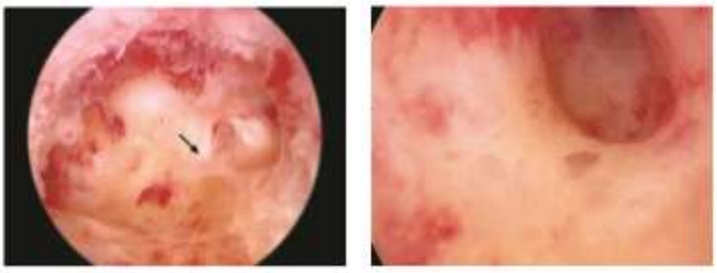


Fig. 17. Filmy adhesions in cavity and near cornu

Fibroids: The prevalence of submucosal and cavity-distorting myomas in women with 2 or more pregnancy losses was found to be 4.08%[7]. The prevalence of uterine myomas was highest in women with 3 or more RPLs (5.91%)[7]. Submucosal fibroids, Type 0 and I fibroids are those most amenable to hysteroscopic resection. Generally, fibroids are more likely to contribute to RPL if they distort the endometrial cavity and/or are >6 cm.

Standard procedure: Either unipolar or bipolar resectoscope can be used with following settings: Flow rate of approximately 250 mL/minute, pressure of 80–100 mmHg, monopolar electricity generator at 60–100 watts and suction pressure of 0.25 bar.

Myoma is shaved down with slicing technique. Type I and type II myoma is shaved down to the level of myometrium till myoma becomes flat. After that the removal of the part nested deep in the myometrial wall can be achieved by 2 techniques:

1. By giving hydromassage by opening and closing the endo-uterine aspiration system, myoma will start protruding in the cavity which can be sliced off. Like this we may be able to remove myoma completely in the same sitting.

2. Cold-knife technique which consists of simple, mechanical passage of the resectoscope loop along the capsule lining of the myoma, detaching if from the fibrous bridges that anchors it to the uterine wall, without any electrocoagulation.

If it is impossible to totally remove the intra-mural fibroid in one sitting, in spite of trying the above techniques, the fibroid can then be treated at a later date (2–3 months later) as over the period intra-mural component of myoma migrates into the uterine cavity.

Meticulous attention to intraoperative fluid balance is imperative, if fluid deficit more than 1 to 1.5 liters is detected, serum sodium is measured and hyponatremia, if present, should be treated. This helps surgeon to determine when to stop a case. If deficit is approximately 1500cc it is advisable to put in a Foley's catheter and give diuretic. It is advisable not to remove big anterior and posterior fibroids in the same sitting to avoid intra-uterine adhesions/ synechie formations.

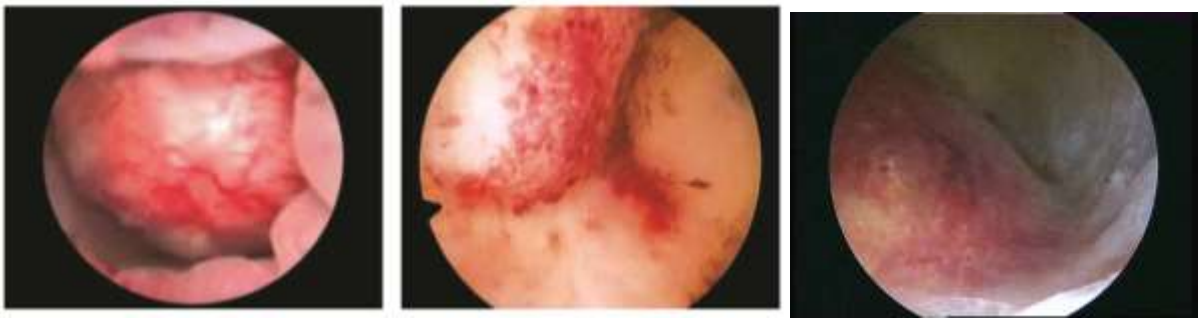


Fig. 18. Type 0, 1 and 2 myoma

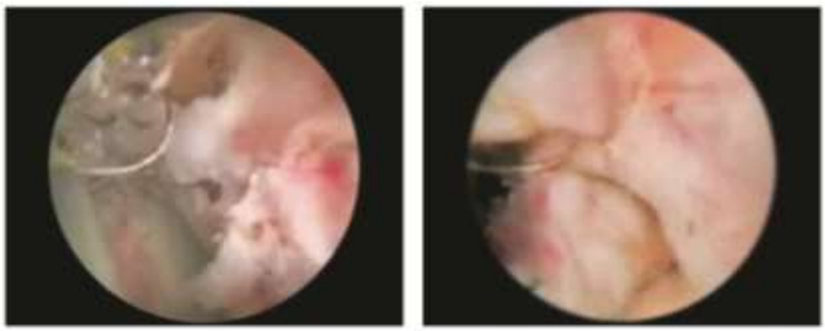


Fig. 19. Resection of type 0 myoma

Fig. 20a. Resection of myoma with loop electrode

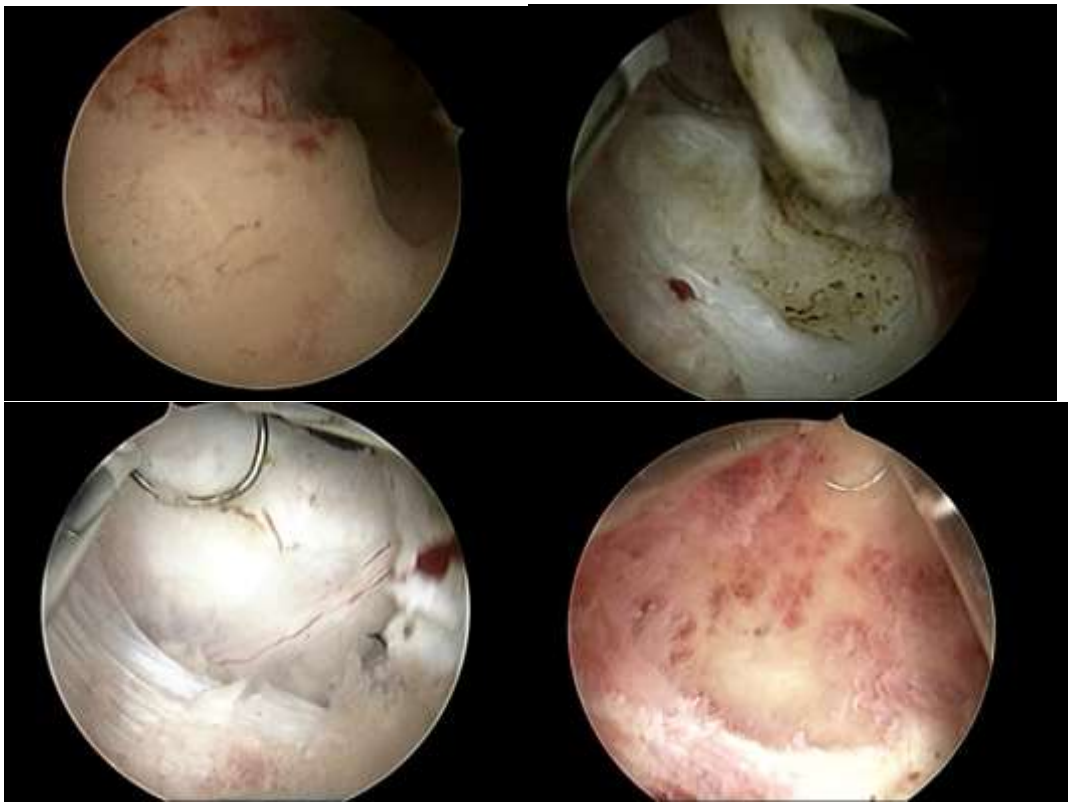


Fig. 20b. Resection of myoma with loop electrode, cold knife technique at base with final picture.

Polyps: Polyps are hormonally induced growths of the endometrium. Although, research is lacking regarding the role of polyps in pregnancy loss. For polyps, surgical removal is often considered for women with RPL if no other causes for pregnancy loss have been found.

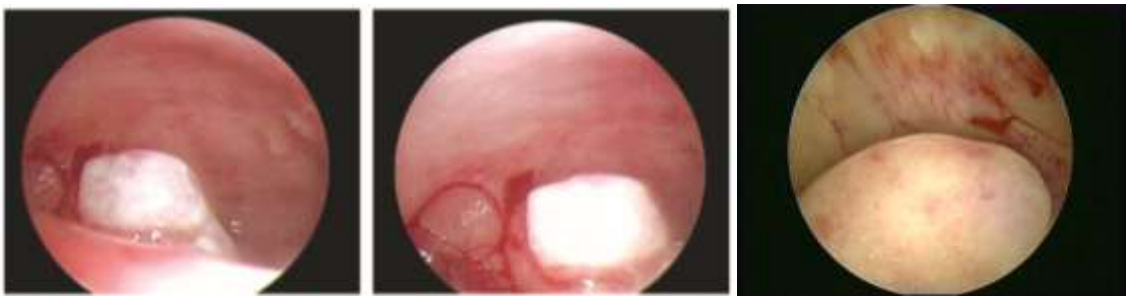


Fig. 21. Endometrial polyp

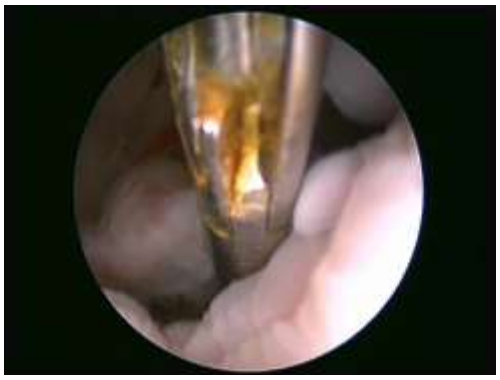


Fig. 22. Hysteroscopic polypectomy using polypectomy forceps

Adenomas: Large adenomas can cause the same distortion of the endometrial cavity seen with fibroids and thus possibly contribute to RPL. The presence of adenomyosis can cause more than double increase in risk of miscarriage and reduction in the likelihood of delivering a viable baby by overall 30%[8]. This rise in miscarriage rate is observed in donor cycles too, i.e. it is independent of oocyte and embryo quality[9].

Hysteroscopy is more specific but less sensitive in diagnosis adenomyosis. One can see variety of presentation with adenomyosis and hence subjected to under/over reporting. There may be Irregular endometrium, endometrial defects, 50% cases have an abnormal hypervascularization, Cystic hemorrhagic lesions with brownish fluid.

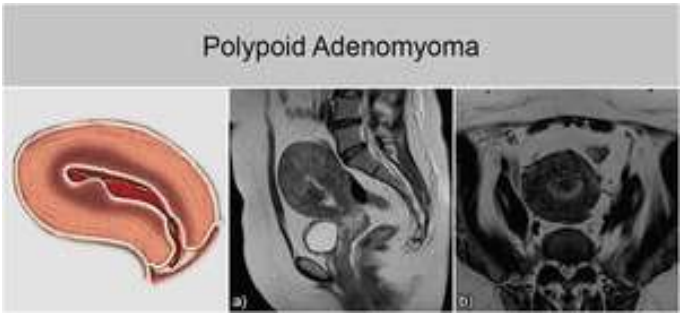


Fig. 23. Pictorial presentation and MRI findings of polypoidal adenomyoma

Fig. 24. Hysteroscopic findings of adenomyosis, punctations, haemorrhagic cysts and blebs.

Conclusion

Anatomic abnormalities, both acquired and congenital, account for about 20% of the explainable causes of RPL. Minimally invasive surgery is suitable for correction of most of these abnormalities. In general, pregnancy rates are significantly improved after surgical correction.

Picture courtesy: Dr. Sejal Naik, Dr. Sushma Deshmukh, Dr. Paul P.G, Dr. Sunita Tandulwadkar, Dr Ravi Kadasane & Dr Sandeep Mahajan

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RPL – Role of ART

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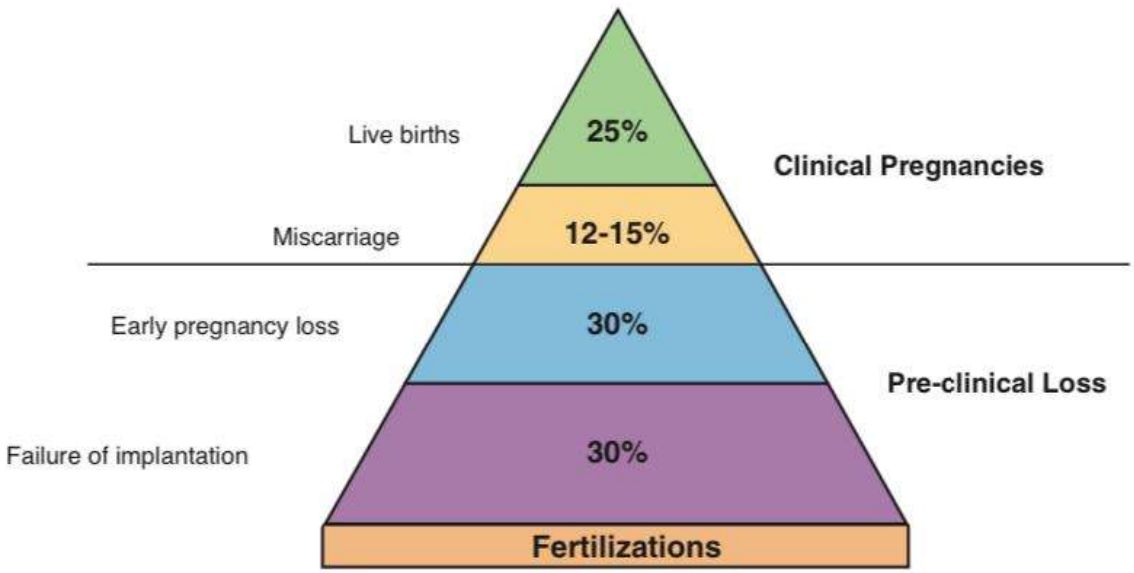
No. Of References:- 6

- Definition of RPL:-
  Recurrent pregnancy loss (RPL) is defined by two or more failed pregnancies and is considered distinct from *infertility*. When the cause is unknown, each pregnancy loss merits careful review to determine whether specific evaluation may be appropriate, and after two or more losses, a thorough evaluation is warranted.

- RPL is a form of infertility:-

  As stated above, RPL is distinct from infertility but socially and to some extent scientifically it is a form of infertility. ART can help these patients

  Humans are very subfertile species. As shown below only 25% of conceptions reaches to the stage of live birth.. ART help in preventing / detecting Preclinical loss and Clinical pregnancies in many cases



- Causes of RPL where ART is helpful:-
  - Life style factors:-
    1. Maternal Age>35 yrs:-
       With increasing maternal age, oocyte number and quality declines. ART helps to counter these by
       - Specific protocols of ovarian stimulation,
       - ovarian rejuvenation medicines/ stem cell therapy.
       - PGS/PGD can help selection of euploid embryo
       - oocyte donation, Cytoplasmic(Mitochondrial) transfer
    2. Maternal Obesity (BMI >30):-
       ART can help obese women as follows:-
       - Correction of anovulation by controlled ovarian stimulation
       - IUI/IVF/ICSI will help alleviate problems of semen deposition in vaginal fornix due to obesity during natural intercourse
    3. Paternal Age:-
       With increasing paternal age, sperm quality deteriorates and chances of aneuploidy increases.
       - IMSI and PICSI help in sperm selection
       - PGS/PGD can help selection of euploid embryo

  - Unexplained Immunological – alloimmune causes:-
    1. Abnormalities in cytokine production – lack of shift of Th1 to Th2 response – Use of High dose Progesterone and Dydrogesterone for luteal support will help shift of Th1 to Th2
    2. Increased levels of Tumour Necrosing Factor(TNF) – alpha in endometrium
    3. Increased uterine NK cells
    4. Lack of endometrial plasticity due to lack of stem cells

    All immunologic endometrial factors are corrected with good endometrial preparation and luteal phase support with the help of estrogen, progesterone and various other agents during ART. Use of TNF – alpha blockers is not associated with congenital anomalies or other pregnancy outcomes

  - Genetic factors:-
    Following genetic abnormalities are responsible for pregnancy loss:-
    1. Fetal aneuploidies or polyploidy
    2. Parental Structural chromosomal anomalies like balanced translocations, deletions, duplications, inversions etc
    3. Single gene disorders – Autosomal recessive/dominant, X- linked etc.
    4. Epigenetic modifications in embryo

    Following ART techniques will help to avoid above genetic factors in embryo:-
    1. PGD/PGS can help selection of euploid embryo
    2. Use of donor gamates/ Donor embryos

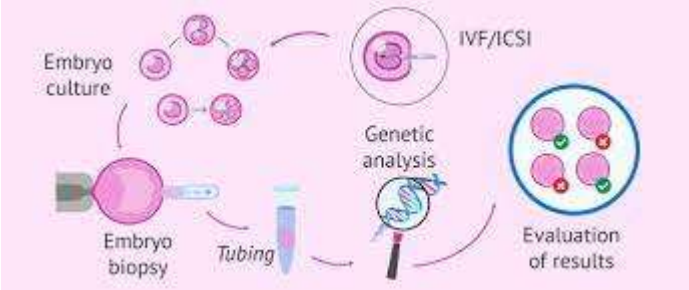
- Hormonal:-
  1. PCOS:-
     ART helps PCOS as follows:-
     - Controlled ovarian hyperstimulation help to ovulate
     - Antagonist protocol with agonist trigger and freeze all technique avoids OHSS
     - Blastocyst culture and PGD helps in selection of good quality embryo
  2. LPD:-
     In all ART procedures, a robust luteal phase support is given
  3. Thyroid disorders:-
  4. Hyperprolactinemia:-

  Correction of thyroid and PRL disorders in RPL improves successful pregnancy outcomes. Addition of ART will still improve successful birth.
  5. Low AMH:-
     DHEA, GH are used in low AMH patients with ART
     IUI, Mild Stimulation protocols, PGD/PGS will help these patients.
     Ovarian PRP, Ovarian Stem cell therapy etc are upcoming treatment modalities to improve ovarian reserve followed by ART
     Oocyte donation is offered as last resort.
- Anatomical:-
  1. Mullerian Anomalies:-
  2. Myomas:-
  3. Uterine Synechiae and Polyps:-
  4. T- Shaped uterus:-

  Surgical correction alone will also improve chances of successful birth but addition of ART after surgery improves success rates a lot.
  Bicornuate uterus or Hemiuterus – where surgery may not be fruitful, ART can be successful
- Semen factors:-
  1. High DFI (DNA Fragmentation index):-
  2. Y- Microdeletions:-
  3. Sperm aneuploidies:-
  4. Male Accessory gland infections (MAGI):-

  In all above conditions, ICSI,IMSI, PICSI will improve better sperm selection to get a successful pregnancy and successful birth
  PGD/PGS will help selection of good quality embryos thereby eliminating aneuploidy sperms

- ART techniques to alleviate RPL:-
  1. Good quality sperm selection:- IVF,ICSI, IMSI, PICSI are useful
  2. Improve Oocyte Quality:- treatment of PCOS, Treatment of endometriosis, In poor ovarian reserve - DHEA, Ovarian PRP/Stem cell therapy
  3. Blastocyst Culture:- Poor quality embryos will not reach blastocyst stage
  4. PGD/PGS:- Trophectoderm biopsy or blastocentesis can be the methods to get genetic material from embryo. FISH, Array CGH or NGS etc are useful and quick techniques



5. Endometrial preparations:- HRT or Stimulation cycles are useful in endometrial preparation in frozen ET. A nicely prepared endometrium supports the pregnancy very well
6. Luteal Phase support:- In ART LPS is given to almost all patients. It helps in supporting and maintaining pregnancy
7. Sperm, Oocyte or Embryo Donation:- In cases with very poor quantity or quality of gamates or embryos, donor gamates or embryos will work nicely.
8. Surrogacy:- In case of severe uterine factors, Surrogacy is a good option for the couples to get their own biological child
9. Use of G-CSF, Intralipid, IM immunoglobulins etc. which are commonly used in ART, will support pregnancy

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Role Of Progesterone In Recurrent Pregnancy Loss

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Progesterone therapy is the ray of hope,
a sliver lining
in the distraught life of
a female with recurrent pregnancy loss

INTRODUCTION:

Progesterone has been implicated as being essential for successful embryo implantation, and for the prevention of miscarriage. Human reproduction is a very inefficient process, the maximum probability of conception in any menstrual cycle is 30%. Only 50-60% of all conceptions advance beyond 20 weeks of gestation.

RECURRENT PREGNANCY LOSS:

Approximately 1% of couples trying to conceive are affected by recurrent miscarriage. The Royal College of Obstetricians and Gynaecologists(RCOG) and the European Society of Human Reproduction and Embryology(ESHRE) define recurrent pregnancy loss as 3 or more consecutive losses before 24 weeks of gestation from single father, while American Society of Reproductive Medicine (ASRM) defines RPL as two previous losses. RPL may result from wide range of aetiology, yet in nearly one third of cases, no cause is identified even after thorough evaluation and such cases are classified as unexplained RPL. Continuation of pregnancy depends on multitude of endocrine events. Various hormones play a critical role in successful growth and development of fetus. Over or underexpression of these hormones may result in failure of pregnancy. These hormones include: Progesterone, Estrogen, Human chorionic gonadotrophin (HCG), Prolactin, Thyroid hormones, Androgens

ROLE OF PROGESTERONE:

During implantation and gestation, progesterone appears to decrease the maternal immune response to allow for the acceptance of the pregnancy. Progesterone decreases contractility of the uterine smooth muscle.

The luteal phase is the later phase of the menstrual cycle, beginning with ovulation and the formation of the corpus luteum and ending in either pregnancy or lysis of the corpus luteum. The main hormone associated with this phase is progesterone, produced in large amounts by the corpus luteum and playing a critical role in increasing endometrium receptiveness for implantation of the blastocyst.

Progesterone induces secretory changes in the endometrium essential for endometrial maturation, endometrial stabilization and embryo implantation and proper regulation of inflammatory mediators to create adequate positive immune response in early pregnancy, preventing pregnancy loss.

Inadequate secretion of endogenous progesterone in early pregnancy has been linked as one of the etiological factors for recurrent miscarriage.

ROLE OF PROGESTERONE IN RPL:

Progesterone plays its role in the following manner :

- It is placentotrophic and so improves trophoblastic proliferation into spiral arteries.
- Improves endometrial maturation by controlling morphological and functional changes in the endometrium and create a favourable environment called the 'implantation/nidation window'
- Luteal phase support: Progesterone stimulates the appearence of pinopodes which are associated with the adhesion of blastocysts to the luminal epithelium.
- Inhibition of uterine contractility: Nitric oxide (NO) generated in the pregnant uterus has been shown to maintain uterine relaxation. Several studies have shown that progesterone enhances NO production in the endometrium.
- Immunomodulating property by shifting the maternal cytokine production towards a Th2 type and decrease the maternal Natural Killer (NK) cells.

Various Cochrane analysis suggested some benefit of progesterone therapy. Initially in 2003, metaanalysis of four trials showed odds ratio (OR) of 0.3 in abortion rate following progesterone therapy. Cochrane 2013 of 14 trials in 2158 cases showed no difference in abortion rate, but sub-analysis on basis of previous history of abortions, miscarriages in the index pregnancy reduced significantly with OR of 0.39 in women with ≥3 abortions. An Indian study on 348 cases has shown reduction on abortion rate with dydrogesterone 20 mg daily with abortion rate of 6.9%vs. 16.8%in control group. Recently, the large multicentric trial with vaginal micronized progesterone in 836 women in first trimester did not find the benefit of therapy in RPL. Live birth rate after 24 weeks of gestation was 65.8% in progesterone group vs. 63.8% in placebo (RR 1.04). It is not known if treatment with intramuscular or oral micronized progesterone would improve the outcome. Our small trial on 90 cases has shown the benefit of oral micronized progesterone in RPL, abortion rate being 3.3% in treatment group vs. 16.7% in nontreated group.

Risk of abortion increases as progesterone level decreases.

Progesterone Levels (in ng/mL)	Risk of Abortion
>25	3%
20-25	7%
15-20	10%
10-15	30%
5-10	80%

At present, a large randomised trial is underway (PROMISE) to assess micronized progesterone in recurrent pregnancy loss. It is a randomised, double blind, placebo controlled

multicentrie trial based in UK and the Netherland studying the effect of progesterone treatment given in the first trimester of pregnancy in the women with a history of unexplained recurrent miscarriages, who conceive spontaneously. Vaginal micronized progesterone 400 mg twice daily is being used starting as soon after a positive pregnancy test as possible and continued upto 12 weeks of gestation.

FORMULATION AND ROUTE:

There are numerous formulations given by various routes such as intramuscular 17 hydroxyprogesterone, oral dydrogesterone, micronized progesterone. Intramuscular 17 hydroxyprogesterone injections are very painful. Vehicle in it may induce labour and clinical trials have shown increased risk of miscarriage . Vaginal micronized progesterone was found effective in initial retrospective trials, but recent large trial has failed to show its efficacy in preventing abortion. In Cochrane review 2011 on 421 cases of four trials, dydrogesterone was found better with reduction in abortion rate. Indian study has also observed that oral dydrogesterone is effective in reducing abortion rate in RPL, 6.9% vs. 16.85. Progesterone treatment has been given for varied duration from 4-24 weeks. In most studies, it is started from diagnosis of pregnancy upto 16-24 weeks of gestation.

SAFETY:

There is concern for pregnancy complications like intrahepatic cholestasis of pregnancy, but no increased maternal complications were found in trials. Virilization in fetus is also not reported in humans. Retrospective study on dydrogesterone showed some increased risk of cardiac defects, but it was a very poorly designed study with bias. Larger trials with vaginal micronized progesterone found no increase in the risk of congenital malformations among offsprings of cases treated with progesterone.

CONCLUSION:

Progesterone is a “pro-gestational” agent that maintains the pregnant state. The paucity of good quality evidence about the efficacy of progesterone in women with history of recurrent early pregnancy loss is responsible for contradictory and ever-changing views amongst clinicians

Even though the studies regarding progesterone supplementation in RPL are scarce and not always statistically significant, the majority of them promote the use of progesterone in women with unexplained RPL. Recommendations based on current evidence state that progesterone supplementation may be of benefit in cases of RPL, especially when the etiology is unexplained.

RPL – Recent Advances

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Recurrent Pregnancy Loss (RPL) is defined as two or more pregnancy losses occurring before 20 weeks of gestation and affecting 1 to 3 % of the couples.

A new algorithm for the evaluation of RPL redefining unexplained miscarriage: Review of current guidelines:

Screening Test:

Parental Karyotyping - Not recommended unless POC CMA reveals unbalanced translocation.

POC Cytogenetic analysis - Recommend: Use CMA for the second and subsequent pregnancy loss

Uterine Anatomy Evaluation - Recommend: 3D ultrasound

Antiphospholipid Antibodies - Recommend: Lupus anticoagulant, Anticardiolpin antibodies, Antiphosphotidyl serine antibodies

Thyroid Function - Recommend: TSH. TPO when TSH more than 2.5 mIU/L

Prolactin - Recommended

HbA1C - Recommended

Hereditary Thrombophilia - Only recommended if a personal or strong family history of thrombosis or thrombophilia

Sperm DNA Fragmentation - Not recommended

PCOS and Insulin Resistance - Not recommended

Luteal Insufficiency - Not recommended

Ovarian Reserve Testing - Not recommended (Use only for explanatory purposes)

Vitamin D Deficiency - Recommend to supplement Vit. D

Currently there is no strong evidence to support routine testing of LH, Androgens, Homocysteine or Prolactin in RPL.

Hormone Therapy: Most of the national guidelines and Cochrane analysis suggests that there is no evidence to support the routine use of Progesterone to prevent miscarriage in early or mid pregnancy. However, the recent paper of Arri Coomarasamy et al has changed the perspectives that progesterone when administered in patients with RPL increased the chances of live birth rates in these patients.
FOGSI Good Clinical Practice:
Oral route Dydrogesterone 10 mg bd till 20 weeks of pregnancy.
Vaginal route: Micronized Progesterone 400 mg bd per day till 20 weeks of pregnancy.
HCG: The current evidence does not support the routine use of HCG.

PCOS :
Despite the persistent controversy about the actual prevalence of PCOS in RPL, a vast number of studies have revealed an amorphous evidence about the association with each other.
Now a days, the correlation between levels of AMH, Homocysteine and Insulin Resistance have become the main subject of interest in PCOS patients in predicting RPL.
HOMA-IR in patients with RPL was significantly higher compared with controls.

Serum Homocysteine level is elevated in PCOS patients with RPL. This elevation is correlated with the degree of obesity, BMI, Insulin Resistance(IR) Status, AMH and androgen levels.

The treatment of Hyperhomocysteinemia and IR in women with PCOS might become the basis for prevention of pregnancy losses and improving reproductive outcome.

Average AMH level in patients with RPL and live births did not differ significantly.

Practice message is if a PCOS patient has RPL and has evidence of Insulin Resistance, it is wise to continue Metformin till at least 14 weeks.

Antiphospholipids in RPL :
APLA Positive patient with pregnancy gives best results when treated with aspirin, heparin and hormonal support in specific situation. Future live births rate is significantly improved when a combination therapy of aspirin plus heparin is prescribed.

Family History of abortions can give leads to Protein S deficiency and Factor V Leiden deficiency (Both Very rare)

❖ **Role of Dehydroepiandrosterone:-**

- Dehydroepiandrosterone supplementation improves pregnancy chances in women with diminished ovarian reserve, by possibly reducing aneuploidy. Since, a large majority of spontaneous miscarriages are associated with aneuploidy, one can speculate that DHEA supplementation may also reduce miscarriage rates.

❖ **Role of Uterine Stem Cells:-**

- "Lack of stem cells to blame for recurrent miscarriages!"
- Above is the title of the paper published by Dr Jan Brosens in "Science daily" and in a journal on stem cells. The researchers examined 183 women, who were being treated at the "Implantation Research Clinic" at the Warwickshire National Health Services trust, by endometrial biopsies. They coined the term "lack of endometrial plasticity" for endometria with depleted HESC (Human Endometrial Stem Cell)

population. This would result in diminished secretion of essential growth factors and cytokines produced by the endometrial cells at the time of trophoblast invasion.

- Autologous platelet rich plasma (PR) has also been injected in the endometrium to improve regeneration.
- These seems to be an emerging role of newer therapies in unexplained recurrent pregnancy loss (uRPL) of growth promoting factors like granulocyte colony stimulating factor (GCSF), PRP, and autologous stem cells to improve the implantation process. They are currently being used with patients with thin endometrium requiring assisted reproductive technology cycles.
- They are also being used in combination e.g. GCSF with stem cells and PRP with stem cells.
- The initial focus is to study its effect in damaged endometrium and document improvement in endometrial thickness and pregnancy rates. However, it has an emerging role in women with uRPL, but only future randomized control trials will prove its worth.

❖ **Are Epigenetic Factors Responsible for Recurrent Pregnancy Loss?:-**

- The term Epigenetics was coined in 1942. It literally means “above genetics” and refers to external influences on DNA that turns gene expression on or off. It is defined as changes in organisms caused by modification of gene expression rather than alteration of the genetic code. Thus it is a change in phenotype without a change in genotype.
- Epigenetic changes may be induced in the mother by a variety of environmental factors like smoking, alcohol, pesticides and other environmental toxins like endocrines disrupters. Smoking alters DNA methylation and affects the expression of 7,000 genes. Interestingly, this effect may persist up to 30 years after cessation of smoking.
  Epigenetic changes encountered in a cell are
  1. DNA methylation – hypo or hyper methylation
  2. Histone protein modification
  3. Transcriptional regulation by presence of noncoding RNAs, like:
     - MicroRNA (miRNA)
     - Small interfering RNA (siRNA)
     - Piwi interacting RNA (piRNA)
     - Long noncoding RNA (lncRNA)
  4. Genomic imprinting where by, one of the two allets of a gene pair is silenced e.g., Angelman and Prader willi syndrome.

  The above changes lead to alteration in cell function by altering the messenger RNA (mRNA) function and protein synthesis in the cell.

- The mechanism by which epigenetic changes influences fetal phenotypes is extremely complex. It is implicated in fetal origin of disease like fetal neurological disorders due to maternal epigenetic modifications in hypothyroidism and gestational diabetes mellitus. In case of unexplained recurrent pregnancy loss (uRPL), the embryo may be epigenetically modified. Epigenetic modifications may cause arrest of cell division and/or growth of the embryo. Analysis of tissue from sporadic miscarriages and women with RPL showed that epimutations of imprinted genes was more frequent in abortuses of women with RPL compared to women with a sporadic miscarriage.

❖ Thromboelastography and Recurrent Pregnancy Loss :-

- Thromboelastography (TEG) is estimation of the tensile strength of the clot by a simple bedside assay. Thromboelastography was proposed as a screening method for detection of hypercoagulability among patients with RPL and selective women are likely to be benefited from anticoagulant therapy.
- Thromboelastography developed by Dr. Hellmut Hartert in 1948 is an efficient method of assessing thrombosis and it overcomes the limitations of conventional methods by providing information on both cellular and plasmatic components. It basically measures the viscoelastic properties of whole blood as it clots. The patients are able to get the relevant information of their hemostatic balance using TEG within 30 to 60 minutes. Meaningful information can be obtained within 10 minutes of initiating the test when maximum clot firmness is reached.
- Although TEG is a simple test to perform, it is difficult to standardize. It is relevant in unexplained RPL but it is not vet widely available.

❖ Recommendation for Immunotherapy in RPL:-

- Immunological Screenings including HLA determination, Anti HLA Antibodies, Anti-HY antibodies, Cytokines, Cytokine polymorphism testing, NK Cell testing are not recommended in women with RPL in clinical practice.
- There is insufficient evidence to recommend LIT(Leucocyte Immunotherapy), IVIG(Intravenous Immunoglobulin), Intra Lipids or G-CSF(Granulocyte Colony Stimulating Factor) as treatment of RPL.

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PICSI and Improved Pregnancy Outcomes in Recurrent Pregnancy Loss due to abnormal Sperm DNA Fragmentation test- A Prospective Clinical Study.

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Abstract

Despite normal semen parameters in male partners of couples who are suffering from recurrent pregnancy loss can have an underlying genetic sperm damage that can be identified. There are couple of tests to identify this DNA damage, the one being DNA fragmentation test. High DNA damage as demonstrated by increased DNA Fragmentation Index (DFI) is associated with recurrent pregnancy loss, recurrent IVF failure, and increased congenital abnormalities. Although intracytoplasmic sperm injection (ICSI) is now a widely-used technique, it is still of interest to improve our knowledge as to which is the best spermatozoon to be selected for ICSI. The selection of a sperm with good genomic integrity is an important consideration for improving intracytoplasmic sperm injection (ICSI) outcome. Current convention selects sperm by vigour and morphology, but preliminary evidence suggests selection based on hyaluronic acid binding may be beneficial. The main clinical aim of this study was to determine the benefits of a hyaluronan (HA)-based sperm selection process for physiological intracytoplasmic sperm injection (PICSI). Secondary aim was to measure confirmed clinical pregnancy (CP), miscarriage following confirmation. Male partners in couples with recurrent pregnancy loss have increased risks of producing aneuploid spermatozoa. Using hyaluronic acid (HA)-binding sperm selection may reduce the genetic risks such as chromosomal aberrations of offspring. In the present study we examined the clinical success of ICSI with HA-selected sperm (‘physiologic’ ICSI, PICSI) as total 66 participants participated in the study over a period of 10 months, Ongoing pregnancy rate (OPR, > 28 weeks of pregnancy) was 48% with missed abortion rate of 23% and failure to conceive in first frozen cycle was 29%. Pregnancy loss was 23% out of which in 14% there was no documentation of fetal pole or cardiac activity and 10 % had absent fetal cardiac activity after initial documentation of Fetal cardiac activity (6-9 weeks of pregnancy). Ongoing

pregnancy rate of 48% is higher than the average plateaued live birth rate of ICSI which is somewhere around 24%. Thus HBA could be considered for sperm selection prior to ICSI because of its success and apparent ability to reduce genetic complications.

Key Words: PICSI (Physiollogical Intracytoplasmic Sperm Injection), ICSI (Intyracytoplasmic Sperm Injection), OPR (Ongoing Pregnancy Rate), RPL(Recurrent Pregnancy Loss), DNA Fragmentation Index.

Introduction

Childlessness in India is estimated around 2.5 percent. It is around 5.5 percent for 30-49 age group and 5.2 percent for 45-49 age group. In absolute terms it is around 4.9 million and if secondary infertility is also added to it then the total number of infertile couples is around 17.9 million. (1)

One in seven couples experience difficulty conceiving a child and rises in the prevalence of infertility and the number of couples seeking help via assisted reproduction technologies (ARTs) is now evident.

Pregnancy loss is common and occurs in approximately 15–25% of clinical pregnancies. Uptill now, recurrent loss of pregnancy was a female centric problem but with advancement in technologies and focusing on sperms which are equally responsible to form a healthy zygote i.e., 50 % responsible for healthy pregnancy outcomes, detailed evaluation of semen parameters is now becoming a cornerstone for management of recurrent pregnancy loss. Recurrent pregnancy loss (RPL) is a distinct disorder defined as two or more failed clinical pregnancies (2). Fewer than 5% of women will experience two consecutive miscarriages and only 1% will experience three or more miscarriages (3). Still our understanding of etiologies behind RPL is not that clear. However, current evaluation of couples with RPL focuses on female factors including endocrine abnormalities such as thyroid disease, hyperprolactinemia and uncontrolled diabetes; uterine factors such as fibroids and Mullerian anomalies, acquired thrombophilia evaluation and karyotyping to evaluate for balanced translocations (4). The man, while important for conception, is investigated only with karyotype.

The semen analysis is generally not a part of the initial assessment of RPL due in part to its limitations as a functional test. However, sperm integrity is essential for sperm—egg interactions, fertilization and early embryonic development (5-7). In addition, paternally expressed genes modulate the proliferation and invasiveness of trophoblast cells and later placental proliferation (8-9). Despite some of the evidence of the effect of sperm on early embryogenesis and placental function, male factors contributory to RPL are largely unexplored.

Fifty percent of couples with RPL will receive the diagnosis of unexplained RPL. This is a frustrating diagnosis that has both physical and psychological implications. There is suspicion that some of unexplained RPL is as a result of an underlying male mechanism that is not currently understood. It is logical that since the male gamete contributes 50% of the genomic material to the embryo and placenta (10-12), the integrity of the sperm genome is essential for the initiation and maintenance of a successful pregnancy (13). Till date, however, compared with egg and embryo quality, relatively little effort has been expended on improving sperm quality beyond processing semen according to WHO guidelines. Such processing may be less effective for ICSI where the egg itself offers no effective barrier to direct insemination by defective sperm, and sperm selection is subjectively dependent on the treating embryologist. What is actually a damaged sperm is really needed to be understood. One excellent test for it is sperm DNA fragmentation test and this test is having relevance in patient with recurrent pregnancy loss with Male factor responsible for it.

Sperm DNA fragmentation- Abnormalities in male genome characterized by damaged sperm DNA may be indicative of male subfertility regardless of normal semen parameters. Sperm chromatin structure evaluation is an independent measure of sperm quality that provides good diagnostic and prognostic values. High sperm DNA fragmentation can compromise fertilization rates, embryo quality, and early embryonic growth and result in pregnancy loss(15). In addition, sperm DNA fragmentation may also compromise the progression of pregnancy and can result in spontaneous miscarriage or loss of biochemical pregnancy.

The susceptibility of male germ cells to DNA damage stems partly from the down regulation of DNA repair systems during late spermatogenesis. In addition,the cellular machinery that allows these cells to undergo complete apoptosis is progressively lost during spermatogenesis. As a result the advanced stages of germ cell differentiation cannot be deleted, even though they may have proceeded some way down the apoptotic pathway. As a consequence the ejected gamete may exhibit genetic damage. Such DNA damage will be carried into the zygote by the fertilizing spermatozoon and must be then repaired, p[preferably prior to the first cleavage division. Several studies have shown that oocyte and early embryos can repair sperm DNA damage. (16, 17). Consequently, the biological effect of abnormal sperm chromatin structure depends on the combined effects of sperm chromatin damage and the capacity of the oocyte to repair it. Any errors that may occur to post fertilization period of DNA repair have the potential to create mutations that can effect fetal development and, ultimately, the health of the child(18,19).

Moreover, a variety of interventions have been demonstrated to decrease sperm DNA fragmentation. Varicoceles are a known cause of sperm DNA damage (20) and many reproductive urologists will evaluate for their presence in couples with RPL. Varicocelectomy decreases sperm DNA fragmentation (21). Indeed, a randomized controlled trial demonstrated higher rates of conception and lower rates of miscarriage in couples with RPL in whom the male underwent varicocele repair (22). Furthermore, Esteves *et al.* demonstrated the effectiveness of using testicular sperm for ICSI over ejaculated sperm during IVF as a strategy to overcome infertility in oligozoospermic men with high sperm DNA fragmentation (23).

Furthermore, we are just beginning to explore the possibility that some men could have an unrecognized inherent genetic predisposition that causes their spermatozoa DNA to become susceptible to fragmentation. This possibility is yet to be thoroughly investigated and would require refined genetic evaluations including assessing epigenetic modifications in the sperm genome.

Despite some of the evidence for DNA fragmentation as a potential etiology of RPL, there are some limitations for its use. The threshold for what is deemed as “abnormal” DNA fragmentation varies in the literature and until there is a standardized method of measuring DNA fragmentation, it may not be widely utilized in the evaluation of couples with unexplained RPL.

Two metanalysis concluded that sperm DNA damage is predictive for reduced pregnancy success using routine IVF but has no significant effect on ICSI outcome. (24,25). Thus assess,ent of sperm chromatin may help predict the success rate of IUI and IVF. It has been also suggested in patients with a high proportion of DNA damaged sperm who are seeking to use ART, ICSI should be method of choice(26). The percentage of spontaneous abortion following IVF/ICSI was increased when sperms with high level of DNA damage were used(23, 24) which highlights the need to assess the sperm DNA damage to predict the possible future miscarriage.

Sperm chromatin structure plays a vital role in protecting paternal DNA integrity by condensing the sperm DNA over 10-fold compared with somatic cell nuclei. Ordinarily, natural selection is effective at screening out defective sperm that have failed to maintain DNA integrity as they

transport through the female reproductive tract. Importantly, as this 'triaging' step is omitted in the direct sperm transfer of ICSI, a greater understanding of the relationship between sperm DNA integrity (and conversely DNA fragmentation) and embryonic developmental potential is needed. Numerous studies have shown clear inverse relationships between sperm DNA fragmentation anomalies in the ejaculate and clinical pregnancy (CPR) or live birth (LBR) rates in in vitro fertilisation (IVF) (23-27). However, the relationship with ICSI outcomes is less clear. We, among others, have reported that miscarriage is a risk factor in ICSI in relation to sperm DNA fragmentation,(27-28) and this may result from an oocyte-mediated DNA repair process(28-31) that adequately supports clinical pregnancy (hence the lack of an association between DNA fragmentation and clinical pregnancy in ICSI compared with IVF), but may be inadequate to sustain it with resulting pregnancy loss (PL). There remains a need to develop more sophisticated techniques to identify functional spermatozoa from those that are immotile, have poor morphology, have poor DNA integrity or are simply incapable of fertilising oocytes. ART sperm preparation including differential density gradient centrifugation has been found to result in enrichment of sperm with intact chromatin, which in turn is likely to improve the chances of a successful clinical outcome.(32-33) While success rates are known to vary widely across clinics, further innovations are needed to improve the plateaued average LBR of 24% for IVF and IVF-ICSI.

Selecting sperm binding to hyaluronic acid (HA) for ICSI is thought to be one such innovation. HA is the natural, non-sulfated glycosaminoglycan secretion of the cervical mucus and the cumulus-öopherus complex.(38) Sperm reaching HA-coated surfaces can bind to and potentially digest the HA, and their subsequent hyperactivation may further facilitate their reaching the egg.(41,42) Immature sperm with excessive cytoplasm appear to have a lower affinity for HA and higher rates of aneuploidy and DNA fragmentation.(43,44) Studies using a HA-selection procedure for ICSI reported higher numbers of grade 1 embryos following ICSI,(45) an increase in clinical pregnancy rate (CPR) with a corresponding drop in miscarriage rate[46] and most recently, a significant reduction in PL and a significantly improved LBR in this group.[47]These outcomes, while encouraging, were drawn from relatively small sample sizes that were insufficiently powered to conclusively test the efficacy of sperm selection by HA-binding for ICSI.(48,49)

Hypothesis

This is designed to test the hypothesis that selection of sperm for injection using HA binding prior to ICSI has beneficial effects in achieving increased on going pregnancy rates and improved clinical outcomes in patients with recurrent pregnancy loss with increased sperm DNA fragmentation index. This study's main strength is its accommodation of clinical and basic science aspects that are fully complementary. Its results will allow us to determine whether HA-binding mitigates for potentially genotoxic levels of DNA fragmentation in patients' sperm.

Objectives

Primary Objective: To determine the efficacy of PICSI in patients with RPL with Ongoing pregnancy rate (OPR)(>28 weeks of gestation) after first frozen embryo transfer.

Secondary Objectives: To determine the impact of PICSI :

- Increasing clinical pregnancy rate based on detection of fetal heartbeat or presence of fetal sac at 6-9 weeks gestation
- Reducing miscarriage rate defined as pregnancy loss after confirmation of clinical pregnancy.

Study design

Inclusion Criteria:

- 1. Couples able to provide informed consent.
- 2. Couples undergoing ICSI procedure.
- 3. Couples with history of recurrent pregnancy loss. (two or more clinically evident missed abortion)
- 3. Female:
    - A. Age: 21–40.
    - B. Body mass index: 19.0–35.0 kg/m^2.
    - C. FSH level 3.0–20.0 miU/mL and/or AMH ≥1.5 pmol/L.
- 4. Male:
    - A. Age: 21–45.
    - B. Able to produce freshly ejaculated sperm for the treatment cycle.
    - C. Should have DNA fragmentation test done prior to the onset of ovarian stimulation. A DFI value of >30% is considered abnormal and inclusion criteria in present study.

Exclusion criteria:

- 1. Couples who have not consented prior to ICSI will be ineligible.
- 2. Couples using non-ejaculated sperm.
- 3. Couples using donor gametes.
- 4. Men with vasectomy reversal; cancer treatment involving any chemotherapy and/or radiotherapy in the past 2 years.
- 5. Split IVF/ICSI procedures.
- 7. If FSH and AMH are tested and either measure falls outside the accepted range.
- 8. Women with PCOD or Poor Ovarian Reserve.

Withdrawal criteria

Participants can withdraw at any time prior to ovum pick up or where, in the opinion of the investigator or the care providing clinical team, it is medically necessary to do so. Study personnel will make every effort to obtain and record information about the reasons for discontinuation, any adverse events and to follow-up the women for all safety and efficacy outcomes, as appropriate.

Methodology

Total 66 patients participated in the study who visited Our hospital, Radha hospital and Candor IVF centre, Surat. The study is conducted between the months of September 2020 to June 2021(10 months).

Women under the age of 40 (mean: 33.18, range: 22–40) with regular (21–35 days) menstrual cycles, with normal baseline follicle stimulating hormone (FSH) level were eligible. Within the

overall studied population, the average male age was 35.8 years (range: 23–45). Patients excluded from the study were as follows: those from whom testicular sperm were taken, who got donor or cryopreserved gametes, received preimplantation genetic diagnosis, underwent sperm sorting procedures, patients whose maternal age was >40 years, and those who demonstrated a sperm count <10,000 motile sperm/mL.

Those participants who satisfied the inclusion criteria will be given ovarian stimulation with short GnRH antagonist protocol and the dose of gonadotropins was not standardised and it was based on clinician's judgement. Male partners gave fresh semen sample and PICSI tray will be used for doing ICSI.

Following ICSI, couples will resume standard care with no further scheduled trial-specific follow-up. However, the couples participating in the study will have their unique ID number allocated on enrolment to the study and linked to the female partner's patient record so that routine fetal/pregnancy outcome data can be captured and recorded.

Semen Analysis:

Semen specimens were collected after abstinence of two to three days on the day of the oocyte retrieval. The sperm sample was maintained at room temperature (18–28 °C) for 30 to 60 min liquefaction. Semen analysis was performed manually according to WHO guidelines and morphology was examined using strict criteria [WHO 2010].

Fertilization:

Gradient centrifugation was used to separate the cellular components of semen. Following centrifugation, the supernatant was removed and the sediment was washed twice. The supernatant was removed again and the sediment was diluted. We placed the final sperm suspension of PICSI patients upon microdots of hyaluronic acid in the PICSI® Sperm Selection Device by cooper surgical USA. After an incubation period of 5 to 10 min, HBA sperm were selected as per the manufacturer's instructions. We selected spermatozoa bound to HA in the junction zone of the two droplets and it was easy to detach then by an injecting pipette and subsequently injected into oocytes.

Embryo culture:

Fertilization was confirmed with the presence of two pronuclei.The embryos were transferred CSCM-NX Medium and blastocyst rate was calculated.Two, or three embryos were transferred following 3 or 5 d of fertilization. Number of embryos to be transferred was based on the couple's decision after consulting with the clinician.

Statistical Analysis:

Primary Analysis: The primary outcome measure is the proportion of women who experience an ongoing pregnancy rate ≥ 28 weeks of gestation. This proportion has as its denominator the total number of women who had undergone intervention of PICSI and as its numerator the number of women who conceive and proceed to have an ongoing pregnancy rate >28 weeks of gestation as a result of their first frozen ICSI cycle. This is because we believe that, if effective, the impact of the intervention will be evident in the first frozen study cycle. Differences in the proportion between ongoing pregnancy rate and other secondary measures will be calculated and studied.

Secondary Analysis: The proportions of each secondary outcome will be measured.

Results

Table 1: Different categories of total couples involved in study over a period of 10 months.

Total number of couples with history of ivf	**66**
Naturally conceived and missed	7
IUI conceived and missed	25
ICSI IVF conceived and missed	34

Figure 1:

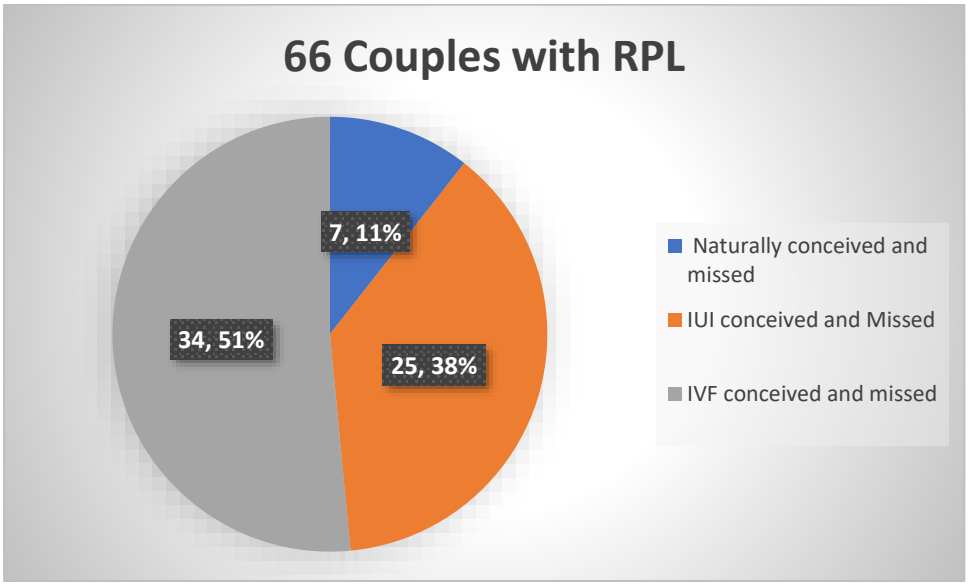


Table 2: Total number of couples conceived in first frozen cycle of PICSI.

Total Couples with RPL	Couples conceived with PICSI in first frozen cycle transfer	Total percentage of positive result.
66	47	71.21%

Figure 2:

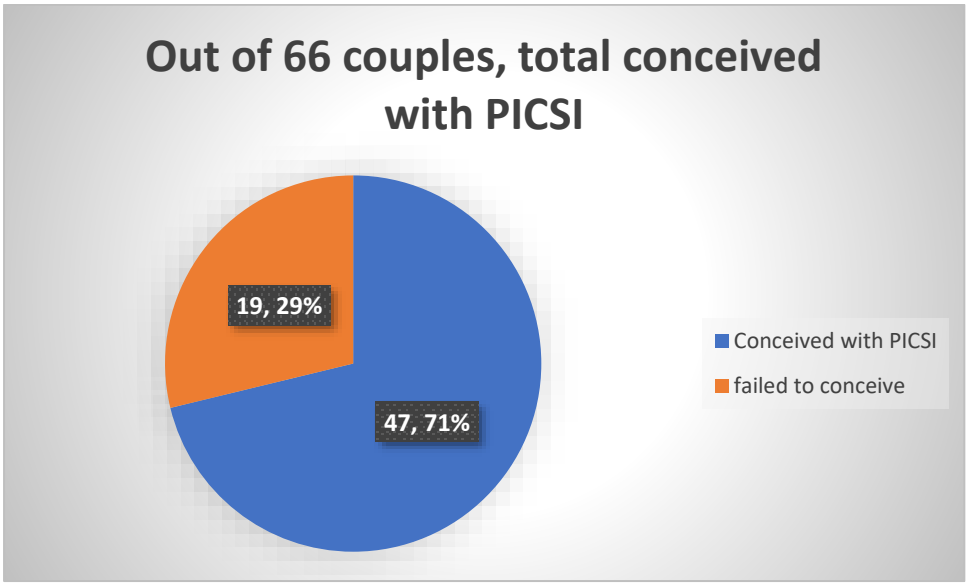


Table 3: Percentage of Conceived Pregnancy in each subcategory with previous history of RPL naturally conceived, with IUI and with IVF.

Previous RPL History	Couples conceived with PICSI in first frozen cycle transfer	Percentage of total conceived. (out of 47)	Percentage of conceived pregnancy out of their respective category of involved participants.
Naturally conceived and missed	4	8.51%	57.14%(4 out of 7)
IUI conceived and missed	15	31.91%	60%(15 out of 25)
ICSI IVF conceived and missed	28	59.57%	82.35%(28 out of 47)
Total conceived	47	100%	

Figure 3:

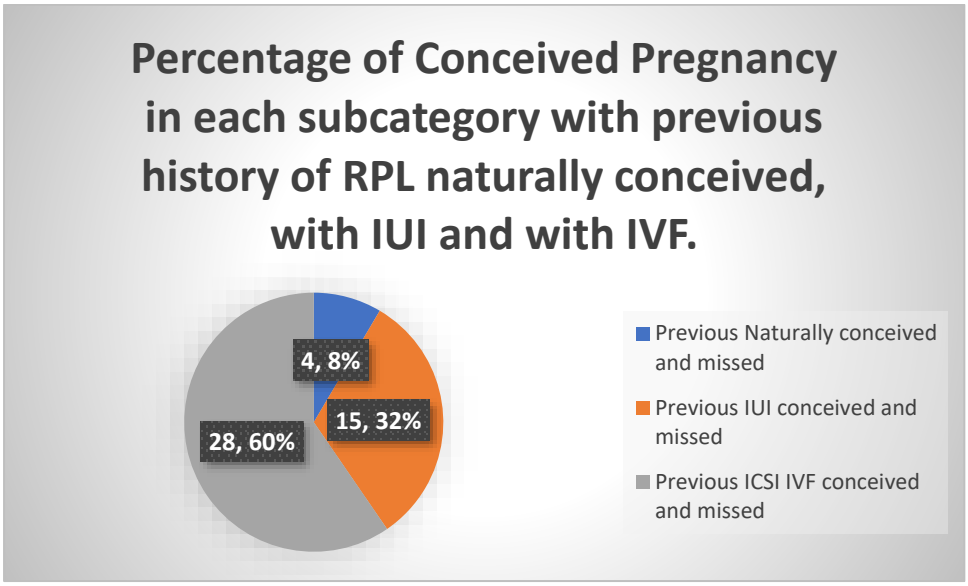


Table 4: Primary Objective measurement. Ongoing Pregnancy rate in total conceived patients.

Parameters	Data	Percentage out of Total conceived(47)	Percentage out of total Participants(66)
Ongoing Pregnancy rate (OPR)	32	68%	48.49%
Missed Abortion	15	32%	22.72%
Total conceived patients	47	-	

Figure 4:

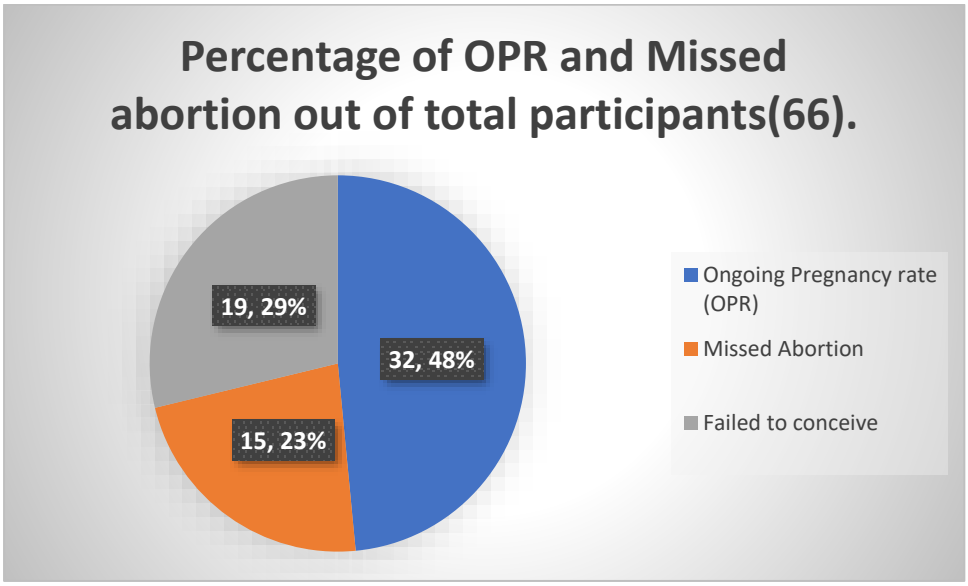


Table 5: Secondary objective measurement.

1)Clinical pregnancy loss before documentation of fetal cardiac activity.

2) Clinical pregnancy loss after documentation of fetal cardiac activity.

Parameters	Data	Percentage out of total conceived(47)	Percentage out of total participants(66)
Clinical pregnancy loss before documentation of fetal cardiac activity.(< 6weeks)	9	19.15%	13.64%
Clinical pregnancy loss after documentation of fetal cardiac activity.(6 to 9 weeks)	6	12.78%	9.1%

Figure 5:

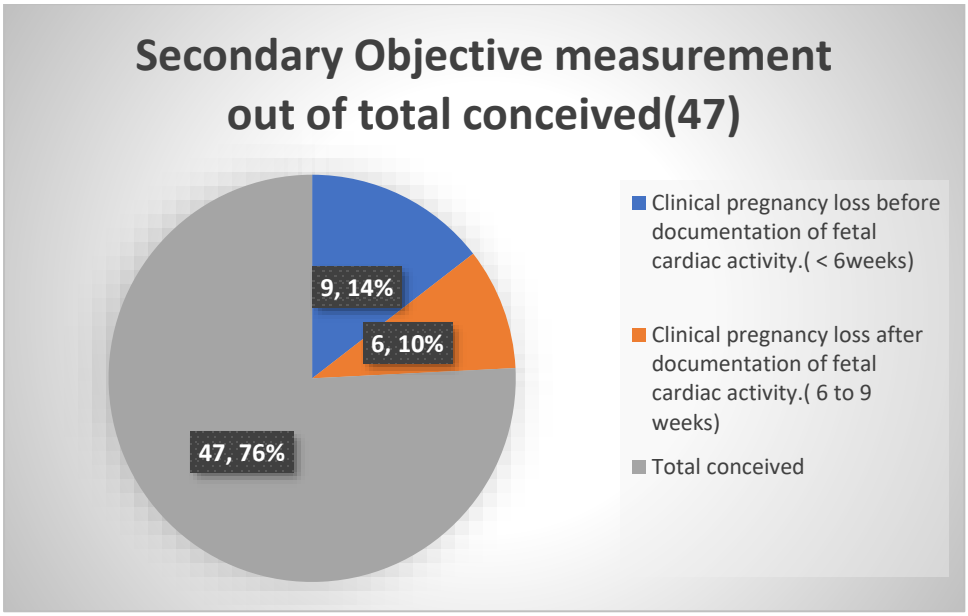
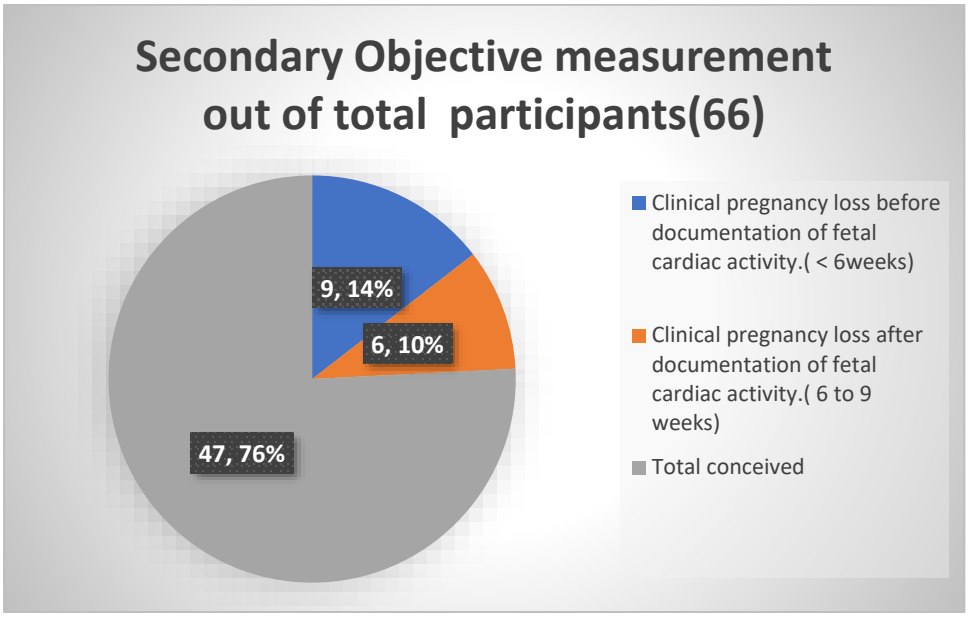


Figure 6:



Final Result table: Percentage out of total participants written in brackets.

Total participants	66				
Participants conceived	47(71%)	OPR(>28 weeks)	32 (48%)		
		Pregnancy loss	15(23%)	<6 weeks	9(14%)
				6-9 weeks	6(10%)
Participants failed to conceive	19(29%)				

Total 66 participants participated in the study over a period of 10 months, average AFC count was 17 with average oocyte rate 13, average M2 rate 61%, with fertilization rate of 93% with blastocyct rate of 61% and average fertilization rate of 71%. Out of total 66 participants Ongoing pregnancy rate (OPR) 48% with missed abortion rate of 23% and failure to conceive in first frozen cycle was 29%. Pregnancy loss was 23% out of which in 14% there was no documentation of fetal pole or cardiac activity and 10 % had absent fetal cardiac activity after initial documentation of Fetal cardiac activity (6-9 weeks of pregnancy).

Discussion:

As per study of Emese Varga Tóthné et all (2014), The FR, IR, CPR, and LBR (live birth rate)of the PICSI group with <50% HBA were significantly higher and the PLR was lower than in the ICSI group with <50% HBA ($p < 0.01$). A statistically significant correlation was found.

Another study Jackson Kirkman-Brown et all, Southampton (UK): NIHR Journals Library; 2019 Feb showed result, A total of 2772 couples were randomised and 2752 couples were included in the primary analysis (PICSI, $n = 1371$; and ICSI, $n = 1381$). Clinical – primary outcome: 379 out of 1381 (27.4% PICSI) and 346 out of 1371 (25.2% ICSI) couples who were randomised (up to 24 hours before treatment) into the trial achieved a term live birth ≥ 37 weeks' gestation [odds ratio (OR) 1.12, 95% confidence interval (CI) 0.94 to 1.34; $p = 0.18$].

Secondary outcomes: CP was achieved for 487 out of 1382 (35.2% PICSI) and 491 out of 1375 (35.7%, ICSI) couples (OR 0.98, 95% CI 0.84 to 1.15; $p = 0.80$). Miscarriage affected 60 out of 1381 (4.3% PICSI) and 96 out of 1371 (7.0% ICSI) of couples (OR 0.61, 95% CI 0.43 to 0.84; $p = 0.003$). Preterm LBRs were 46 out of 1381 (3.3% PICSI) and 45 out of 1371 (3.3% ICSI) (OR 1.02, 95% CI 0.67 to 1.55; $p = 0.94$). Mechanistic: in the subset of samples examined, HBS correlated with sperm motility, concentration, fertilisation rate and DNA fragmentation. Sperm DNA compaction was weakly associated with clinical pregnancy rates (CPRs), but neither HBS nor DNA fragmentation was predictive of any clinical outcome.

It is observed that average live birth rate by ICSI is plateaued at 24 %, in our study the ongoing pregnancy rate i.e., pregnancy continued beyond 28 weeks (as the study was conducted in last 10 months, live birth rate will be studied prospectively by strict follow up of patients) the ongoing pregnancy rate is 48% with PICSI which is higher to that of ICSI.

A relationship between HA selected sperm and increased levels of developmental maturity [Cayli et al. 2004; Huszar et al. 1994, 2003], as well as nuclear [Kovanci et al. 2001; Jakab et al. 2005], and cytoplasmic integrity [Huszar et al. 1997; Sakkas et al. 1999] have been demonstrated.

A similar increase in IR, CPR, and lower PLR values was found by Worrilow and colleagues [Worrilow et al. 2006; Worrilow et al. 2007; Worrilow et al. 2012]. Others compared conventional sperm selection and the use of sperm selected from a liquid source of HA and an increased IR was found [Parmegiani et al. 2010]. The same positive trend was observed comparing polyvinylpyrrolidone-ICSI ($n = 110$) and PICSI ($n = 92$) treatments [Ménézo and Nicollet 2004]. In a study of 50 couples, a higher FR was observed when HA-selected spermatozoa were injected into oocytes [Nasr-Esfahani et al. 2008]. These studies, in accordance with ours, did not demonstrate any negative effect on embryogenesis using HA sperm selection for ICSI, but they all was ‘in-house’ developed HA slides.

Another study reported that the only benefit of injecting HA selected sperm was a lower PLR which consequently translated to a higher LBR, both of which were not statistically significant [Majumdar and Majumdar 2013]. Sperm DNA damage was found to be positively correlated with PLR when 11 studies involving 1,549 *in vitro* fertilization (IVF) and ICSI cycles were systematically reviewed [Zini et al. 2008]. It is well known that the proportion of immature sperm closely correlates with chromosomal disomies [Kovanci et al. 2001]. The relationship between the frequencies of chromosomal aneuploidies and diminished sperm maturity is thought to reflect that cytoplasmic retention and diminished maturity in sperm are associated with a low expression of the HspA2 [Eddy 1999; Huszar et al. 2000]. The relationship between sperm zona pellucida binding competence and maturity has been identified earlier. In the semen samples there were sperm with various degrees of cytoplasmic retention, but all sperm bound to the zona pellucida were mature as characterized with the absence of any cytoplasmic retention. Diminished HspA2 chaperone activity found in developmentally immature sperm is thought to be connected with a diminished presence of DNA repair enzymes, causing DNA chain breaks and fragmentation [Dix et al. 1996; Eddy 1999; Huszar et al. 2000]. There is a correlation between the decreased levels of expression of the HspA2 chaperone and sperm cellular development as well as IVF success [Ergur et al. 2002; Huszar et al. 1992, 2000]. Van Steirteghem et al. [2002] found increased rates of *de novo* numerical and cytogenetically detectable structural chromosomal aberrations following ICSI. The low concentration of HspA2 in the undeveloped spermatozoa likely suggests numerical chromosomal aberrations in sperm of oligozoospermic or severely oligozoospermic men [Huszar et al. 2007]. Selecting individual mature sperm with low levels of chromosomal disomy, diploidy, and sex chromosome disomy is facilitated by HA-binding and might reduce the potential genetic complications in male candidates for ICSI [Jakab et al. 2005]. It has been observed that almost all HA-bound spermatozoa are devoid of persistent histones, which correlated with DNA strand breakage [Sati et al. 2004].

Conclusion

PICSI could be considered for sperm selection because of its success and apparent ability to reduce genetic complications in patients with RPL due to high DNA fragmentation index (>30%), which is one of the causes for inducing genetic abberations in embryo which leads to repeated pregnancy loss mainly in first trimester. However, this must be extended to a larger study.

Conflict of Interest: The authors have no potential conflicts of interest to disclose. The choice of the PICSI dish used for interventions was based on its ready availability, solid construction, careful quality control and relative ease of use. There were no commercial considerations in its adoption. A successful conclusion of the study could help establish a more consistent and objective procedure for sperm selection by ICSI that can be extended to different HA-selection platforms.

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Successful Management Of A Series Of RPL Patients With Antinuclear Antibody Positive Titer

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Introduction

Repeated spontaneous pregnancy loss is a physically and emotionally challenging occurrence for both the expectant parents and the treating clinician. Although repeated pregnancy loss (RPL) is a frustrating clinical condition, fortunately it is amenable to treatment. Immunological factors are one of the most prevalent causes of RPL. The immune diagnosis of pregnancy loss is most often determined as a diagnosis of exclusion. The major causes for the same are Antiphospholipid Antibody Syndrome and Thrombophilia. A subgroup of patients with RPL have positive ANA levels and hence can be successfully treated via low molecular weight heparin.

Case Report

Series of five patients with RPL, positive ANA and other APLA panel negative titers were managed successfully via administration of LMWH and Ecosprin.

S No.	Patient's age (in years)	Previous obstetric history	Current Pregnancy	Result post treatment
1	26	**G3P1L0A1:** 1) 1st pregnancy: 1 missed abortion at 10 weeks with D&E done 2) 2nd pregnancy: - IUFD at 38 weeks - Negative APLA panel apart from ANA which was positive - H/O bleeding history at ~28 weeks of pregnancy - Case history of sudden IUFD	**LMP:** 30/9/2019 **EDD:** 7/7/2020 **Treatment history:** 1) First visit to author was on 11/11/2019 at 6 weeks of live pregnancy 2) Postive result in ANA test conducted on 28/12/2019 (with negative results in other APLA tests) 3) LMWH started on 31/12/2019 and in light of economic factors, 40 mg SC was provided on alternative days. 4) Subsequently, patient had an uneventful antenatal period apart from mild hypertension during last month of pregnancy for which Labetalol was provided. LSCS done	Healthy male child of 2.6 kg was delivered by patient

			on 12/6/2020 due to decreased foetal movements.	
2	22	**G3P1L0A1:** 1) 1st pregnancy: Missed abortion 2) 2nd pregnancy: Fresh stillborn at 32 weeks of gestation due to Abruptio Placenta	**LMP**: 16/12/2019 **EDD**: 23/9/2020 **Treatment History:** 1) First visit to author was on 20/02/2020 2) Postive result in ANA test conducted with negative results in other APLA tests 3) LMWH started on 22/2/2020 4) Uneventful pregnancy with FTND on 24//8/2020	Healthy female child of 2.5 kg delivered. Patient pregnant again and due on 25/9/2021
3	20	**G2P0L0A1:** 1) 1st pregnancy: Missed abortion at 4 months; D&E done and reports for the same not available	**LMP:** 17/9/2020 **EDD:** 24/6/2021 **Treatment History:** 1) First visit to author on 21/12/2020 2) Postive result in ANA test conducted 3) LMWH started on 23/12/2020 4) Uneventful pregnancy with delivery on 16/6/2021	Healthy male child of 2.6 kg delivered
4	36	**G5P1A3L1:** 1) 1st pregnancy: FTND 8 yrs ago 2) Three subsequent abortions	**LMP:** 16/8/2020 **EDD:** 23/5/2021 **Treatment History:** 1) First visit to author during 6th week of pregnancy 2) Postive result in ANA test 3) LMWH started in 6th week of pregnancy 4) FTND on 17/5/2021	Healthy female child of 2.6 kg delivered
5	26	**G3P1L0A1:** 1) 1st pregnancy: Missed abortion 2) 2nd pregnancy: LSCS done and no living child	**LMP:** 5/4/2020 **EDD:** 12/1/2021 **Treatment History:** 1) Postive result in ANA test conducted 2) LMWH started from 30/05/2020 3) Patient travelled to maternal home and delivery done on 24/12/2020 through LSCS	Healthy male child delivered

All patients identified with positive ANA results were treated with LMWH and ecosprin .In subsequent patients I did not conduct the other APLA panel due to monetary reasons. Administration of 40 mg SC on alternative days with routine investigation and USGS fructified into an uneventful antenatal period (apart from the first patient who complained of decreased foetal movements), with successful deliveries and uneventful intra-natal and postnatal period. Tab ecosprin was stopped at the onset of ninth month and heparin was stopped one day prior, CBC, PT INR was done and all the patients had normal levels.

Discussion

Immunological responses could have been the cause of many instances of infertility and miscarriages. ANA presence in pregnant women indicates that there may have be an underlying autoimmune process affecting the development of placenta leading to pregnancy loss. Presence of ANA is not uncommon in women with unexplained recurrent miscarriages suggesting the possible role of an autoimmune disorder leading to abortion in at least a subset of patients. A previous study performed to understand the prevalence and significance of ANA in Iranian women with unexplained recurrent miscarriages concluded that the presence of ANA is not uncommon in women with unexplained recurrent miscarriages, suggesting the possible role of an autoimmune disorder leading to abortion in at least of subset of patients.

Gubillos et al. found that 31.8% of patients with an h/o miscarriage have the presence of ANA, and only 5.7% of healthy patients with proven fertility and no pregnancy loss have a presence of ANA. In several studies, high prevalence of low titer ANA has been linked to pregnancy loss. However, the significance of these findings is still not clear.

ANA titers are important in the interpretation of the test but fluctuation in titers has little clinical relevance in autoimmune diseases. A meta-analysis done by Shijuchen, Chixiu Shi concluded that ANA positivity was positively associated with increased RPL risk. ANA positivity is an important risk for RPL and hence needs to be screened. ANA needs to be screened in women diagnosed with RPL and no identified history of autoimmune diseases.

The presence of ANA in RPL might represent a subtle immune abnormality state between mother and conceptus. It has been reported that human IgG ANA could induce pregnancy loss in mice because of an increase in immune complex deposition in placental tissue. However, the exact underlying mechanism as to how ANA leads to pregnancy loss is still unclear. In general, presence of autoantibodies in conjugation with autoimmune induced organ damage could be the probable cause. Placental insufficiency due to autoimmunity related placental damage needs to be further studied. The study speculated that autoimmunity related RPL was most likely an organ specific autoimmune disease in which the specific organ was the placenta which only existed during the pregnancy period.

Conclusion

In patients with RPL, it is critical to act on a positive ANA result and treat patients for immune related losses despite no other signs of an underlying autoimmune diseases. Many antibodies have been associated with impaired fertility and it is still not completely clear which antibody panel to assess. Hence, patients with RPL and a positive ANA test should be treated with LMWH and Ecosprin, and if an underlying autoimmune disease is the cause then a successful pregnancy can be expected. These patients need to be followed long term for development of autoimmune diseases

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Role of Cytogenetics and Molecular Genenetics in Recurrent Pregnancy Loss (RPL) Genetic workup in patients with Recurrent Pregnancy Loss

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Abstract:

Recurrent pregnancy loss (RPL), a major cause of secondary infertility is a clinical and genetic concern globally. Underlying causes include various factors, biochemical as well as related to chromosomal anomalies. Due to this condition, there have been stratified and extensive studies on such conditions. Extensive research are carried out to understand the relevant problems associated with the aspirant couple and how can they be addressed to get a better chance at pregnancy. Here in particular we are bringing in light two cases that were addressed at our centre with the help of Cytogenetic and Molecular techniques, such as Karyotyping (KT) and Chromosomal Microarray (CMA) available at hands. Also to add, these cases reflected chromosomal anomalies in the investigation. Some extremely small mutations, as low as pointmutations can be missed by both these techniques. So, Whole exome sequencing by Next Gen Sequencing (NGS) can also be performed in addition to investigate the mutation. Such couples undergoing IVF technique can have an added advantage for PGT-M (Preimplantation Genetic Testing for Monogenic disease) and PGT-SR(Pre-implantation Genetic Testing - Structural Rearrangement) if their chromosomal anomalies are known. Simultaneously with these cases we also present a workflow to get better results in the cases of RPL and achieve safe pregnancy.

Keywords: Recurrent Pregnancy Loss (RPL), Karyotyping(KT), Chromosomal Microarray(CMA), Next Gen Sequencing (NGS), Preimplantation Genetic Testing (PGT).

Introduction:

Recurrent Pregnancy Loss (RPL) a serious complication of pregnancies, affecting almost 2%–5% of couples. Among numerous underlying causes, chromosomal anomalies in either of the partners are regarded as important issues, with varying frequencies among different populations (Alibakshi R. et al., 2020; Hyde and Schust, 2015). Recurrent pregnancy loss is the most common complication of pregnancy and occurs in ~ 12-15% of all clinically recognized pregnancies.

Epidemiological studies have revealed that 1-2% of women experience recurrent pregnancy loss. Chromosomal abnormalities have been reported in 50% of aborted fetuses (Viaggi et al., 2013). The chances of chromosomal abnormalities with pregnancy loss depends on many factors, including family history chromosomal anomalies, advanced maternal age and fetal anomalies identified by ultrasound. Identification of chromosomal abnormalities of recurrent pregnancy loss with ultrasound anomalies plays an important role in genetic counseling and future pregnancy.A recent study demonstrates that out of the primary and secondary loss of

pregnancy almost 47% of the cases hadabnormal karyotypes. The overall rate of chromosomal abnormalities in the secondary RPL group was significantly increased compared with the primary RPL group (Nikitina et. al. 2020)

A recent review on recurrent pregnancy loss summarizes research papers across 1980 to 2018 written about recurrent pregnancy loss. From the genetic perspective itprimarily includes pathogenic genetic causes, such as sporadic aneuploidy and translocations. Secondlypotential causes such as smaller CNVs and mutations in genes important in early fetal development also lead to pregnancy failures. In addition, there are likely to be complex genetic contributions, such as multi-factorial inheritance, and changes in methylation (epigenetics) and mitochondrial function, which could be contributing to pregnancy loss (Colley et al. 2019).

Thus it becomes the need of the hour to find a precise line of diagnosis and proper management of couples suffering from Primary and Secondary Pregnancy loss to achieve a full term and healthy pregnancy. Certain cytogenetic and molecular techniques help in guiding us in these terms to achieve our goals. In this paper we have taken two such case examples to understand the better line of diagnostic action.

Case 1: Karyotype: 46,XY,t(9;20)(p21;p12)

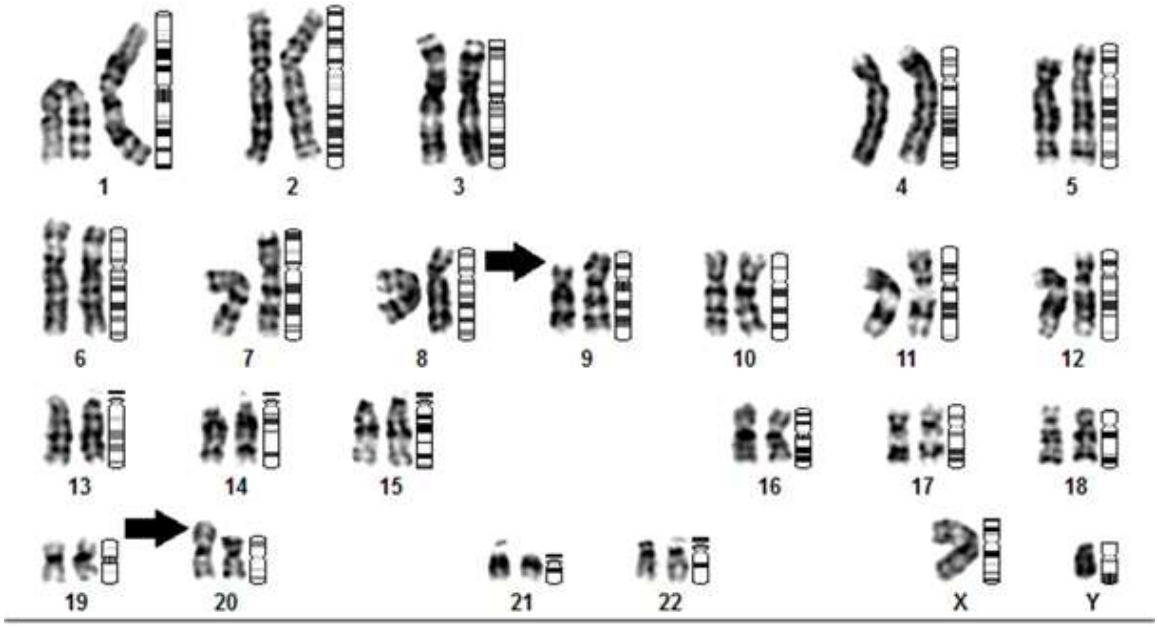

Fig: Abnormal clinical findings of the karyotype, the arrows show the translocation between chromosome 9 and 20

A 33 year old Female with history of first trimester multiple spontaneous abortions were to be investigated. The couple was suggested to undergo Chromosomal analysis from peripheral blood to investigate chromosome anomalies in lieu of the history. On analysis, the female exhibited 46,XY,t(9;20)(p21;p12) chromosomal compliments, suggesting the translocation between chromosome 9 and 20, where p arm of chromosome 9 was translocated on p arm of chromosome 20 at the specific site as mentioned above creating a derivative chromosomes (der9, der20). This balanced translocation leads to the loss/gain of genomic material in the developing foetus which can be the root cause of multiple pregnancy loss. Due to such a translocation, there are increased chances of getting pregnancy loss because of the parental genome.

For study of RPL cases, if classical cytogenetic investigation of POC requires viable tissue but up to 40% of cases undergo culture failure. However a poor chromosome morphology and maternal cell contamination can also give false results (Baxter &Adayapalam, 2013). Therefore an advanced technique is also required to perform exact screening of the genome with higher resolution.

Case 2: ISCN format: arr Xp22.33 (298,292-778,548) X 1

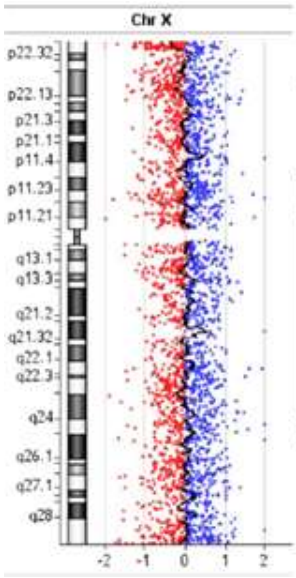

Table Shows Amplification (Amp) / Gain/Loss/Deletion (Del) in the analysed DNA sample.

Amp/Gain/Loss/Del Intervals Table

Sr. No.	Chr	Cytoband	Start-Stop(bp)	Size (kb)	#Probes	Amp/Gain /Loss/Del	P-value	Annotations
1	ChrX	p22.33	298292-2778548	2,480	90	-0.339987	1.08E-10	SHOX, CRLF2, CSF2, IL3RA,SLC25A6, ASMT, ZBED1, CD99, XG, GYG2, PPP2R3B, MIR3690, ASMTLAS, ASMTL, P2RY8, AKAP17A, DHRSX, CD99P1,NCRNA0010, XGPY2

Amplification (Amp) / Gain/Loss/Deletion (Del)
Total Amp/Gain/Loss/Del Intervals: 1

Fig: Deletion X p22.23 as seen in aCGH analysis &table showing the list of functional genes deleted.

This is an atypical case of deletion that would be easily missed if the diagnostic tool used was just Karyotyping. Standard GTG banded chromosomal karyotype at 450 to 600 band resolution can detect only whole chromosome aneuploidy and balance translocation >5Mb deletion and duplication (Lomax et al., 2000). Chromosomal Microarray(CMA) increase the finding capacity of genetic alteration which is responsible for fetal death by providing coverage of the entire genome at higher resolution by detection limits as small as 50 to 100 kb deletions and duplications (Strassberg et al., 2011). Molecular cytogenetic technique like CMA,is a high-resolution genome analysis technique which does not require live cells; it can be used on DNA from tissue samples. The deletion of 2Mbp would have been very easily missed out with the help of Karyotyping. As a result since the karyotype was normal, CMA was suggested to the patient looking at the clinical history of four recurrent pregnancy losses. This deletion was thus affirmed with the help of aCGH leading to the clinical finding of the deletion on X chromosome which is primarily responsible for a lot of functions, especially if the foetus is male, since it is in hemizygous condition. Thus this technique played a pivotal role in understanding the developmental problem that might have happened with every pregnancy. Also in addition when certain cases if not solvable by Karyotyping as well as aCGH, can be further referred for Next Gen Sequencing (NGS) based tests. NGS can narrow the problem down to even Single Nuclear

Polymorphsims (SNPs) or mutations if any, enabling us to understand the clinical problem in hand more diversely.

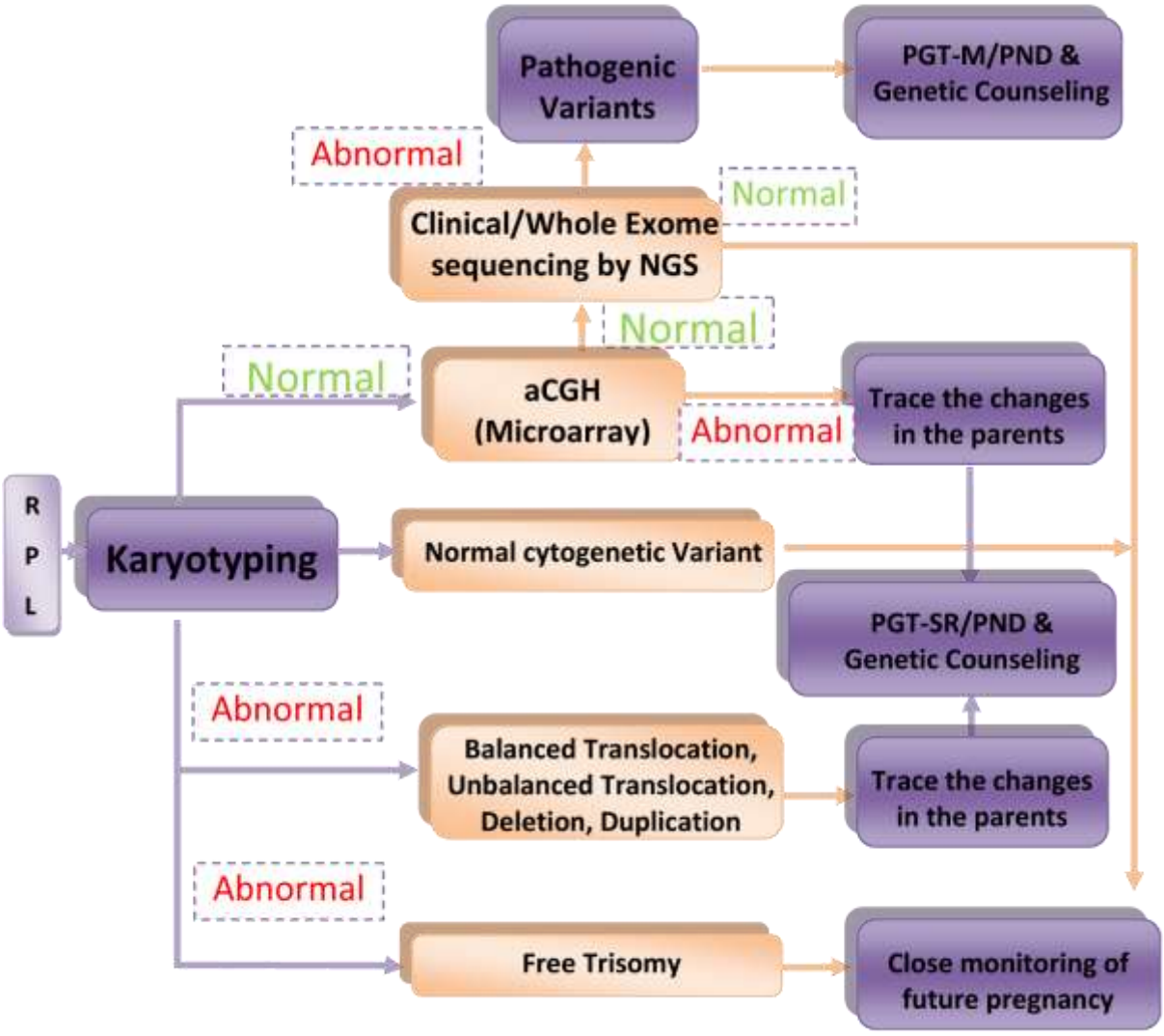

Fig: Cytogenetic workflow of a couple having Recurrent Pregnancy Loss (RPL)

Discussion:

Recurrent pregnancy loss (RPL) is a global concern these days. Early pregnancy loss, also referred to as miscarriage or spontaneous abortion, is defined as the loss of a clinical pregnancy before 20 completed weeks of gestational age (18 weeks after fertilization) or, if gestational age is unknown, the loss of an embryo/fetus of <400 g(Zegers-Hochschild F. et. al., 2009; Ford & Schust 2009). Therefore we have created a workflow at our centre trying to achieve the best diagnosis with the available instrumentation at hand. The first and cheapest available investigation that can be done in case of RPL is Karyotyping (KT). Karyotyping is gold standard to find chromosomal anomalies, if any in any of the parents. If the Karyotype is normal, there are chances that there is a minor change (<10Mbp) in the genetic makeup which is not detectable by chromosomal analysis.In such cases Chromosomal Microarray (CMA) is the next tool for reliable analysis (Pauta et. al., 2018). CMA particularly screens the genome for roughly 500Kbps of microdeletion or duplication. This technique empowers us to diagnose a very small change that could be missed by Karyotyping. This article focuses on 2 cases of Recurrent Pregnancy Loss (RPL) where genetic workup was performed to understand the etiology of the recurrence.

Case I in particular had multiple miscarriages, in particular first trimester abortions. The couple was advised karyotyping before further pregnancies. When both were investigated one of the partners was found to bear a balanced translocation between 9 and 20 creating derivative chromosomes of 9 and 20. This was easily identified with the help of Karyotyping in strong contrast to case II. In case II the karyotyping had appeared normal, still not able to justify the query of multiple abortions. As a result Chromosomal Microarray (CMA) was suggested to the couple. Further analysis exhibited a deletion in chromosome X in one of the partner showing the cause of RPL. In advancement of both the cases diagnosis by PGT-SR (Pre-implantation Genetic Testing for Structural Rearrangements) was suggested to avoid further pregnancy loss. As a result of which the first couple was successful to achieve a full term pregnancy with PGT of 8 embryos out of which 2 viable ones were transferred and the second couple with deletion on chromosome ‘X’ is still in follow up. If both of these techniques fail to find any anomaly then the couple would be suggested to get screened for monogenic diseases dealing with point mutations. This can be done successfully by performing Clinical/Whole Exome Sequencing by NGS(Next Genome Sequencing). If any mutations are registered then couple would be further suggested for PGT-M (Pre-implantation Genetic Testing for Monogenic Diseases). Both techniques will help us pick up the right embryos for implantation, ultimately leading to higher chances of sustainable pregnancy.

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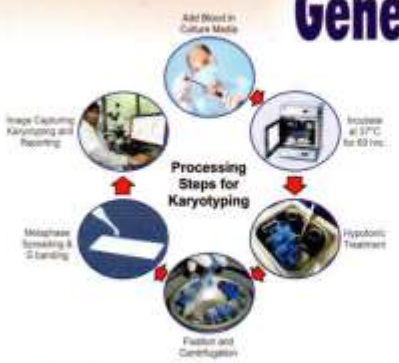

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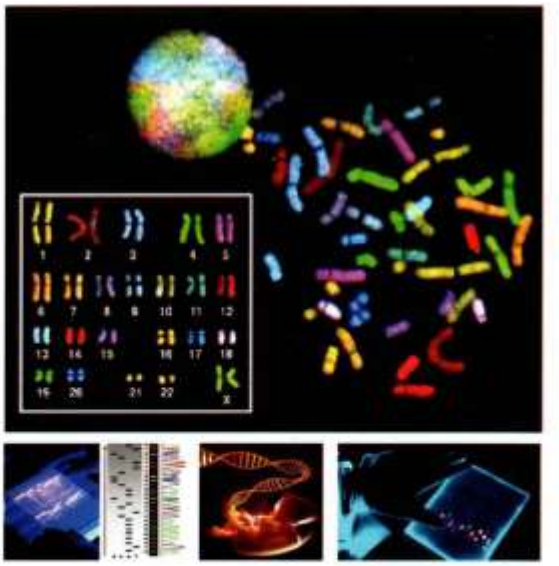

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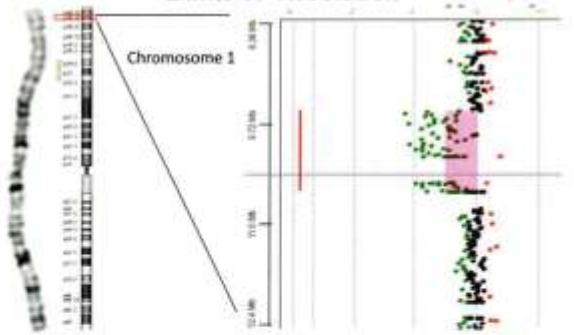

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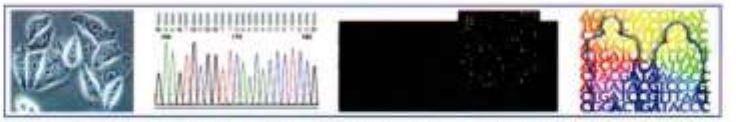

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Efficacy Of Cervical Cerclage In High Risk Twin Pregnancy

A CASE REPORT

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AUTHORS CONTRIBUTION

- Dr. Sonia R. Chandnani conducted the procedure and suggested for case to be published.

- Dr.Chaitali G. Viras wrote the manuscript and Dr.Kajal Tejani provided data for the same.

- All authors reviewed the final manuscript.

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ABSTRACT

Preterm birth (PTB) remains the foremost global cause of perinatal morbidity and mortality, hence the prevention of spontaneous PTB is of critical importance. In an attempt to prevent PTB in multiple pregnancies, cervical cerclage, in combination with other treatments, has been strongly advocated. This is because, cervical cerclage is an intervention that is recommended in women with an incompetent cervix at high risk of preterm birth but, despite this, many women still deliver prematurely, as the biological mechanism is still incompletely understood. Cervical insufficiency is a risk factor for spontaneous midtrimester abortion or early preterm birth. The use of prophylactic antibiotics is predicated largely on the basis that they reduce the rate of bacterial vaginosis and asymptomatic bacteriuria.

Keywords: Preterm delivery, Incompetent Cervix, Cervical cerclage, midtrimester abortion

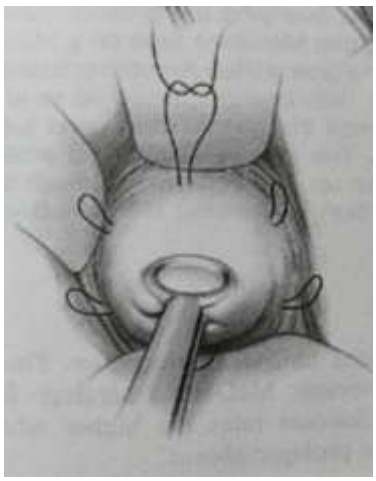
INTRODUCTION

Incompetent cervix is characterized by painless cervical dilatation in the mid trimester or perhaps early in the third trimester, with prolapsed and ballooning of the membranes into the vagina, followed by the rupture of membranes and expulsion of an immature fetus. Unless effectively treated, this sequence tends to repeat in each pregnancy. Although the cause of cervical incompetence is obscure, previous trauma to the cervix (dilatation and curettage, conization, cauterization or amputation) appears to be the factor in many cases. In other instances, abnormal cervical development, including that following exposure to diethylstilbestrol in utero, plays a role. The diagnosis of incompetent cervix is largely made based on a history of one or more prior mid-trimester losses. The treatment of cervical incompetence is surgical, consisting of reinforcement of the weak cervix by some type of purse-string suture. The pathophysiology of preterm birth is multifactorial, very complex and not clearly understood. The cervical cerclage is an intervention that is usually recommended in women with an incompetent cervix at high risk of preterm birth, as the biological mechanism is still incompletely understood. Cervical insufficiency stands as one of the risk factor for spontaneous recurrent mid-trimester losses or early preterm birth. In our case we managed cervical insufficiency with the administration of perioperative antibiotic treatment along with emergency cervical cerclage.

CASE REPORT

A 33-years female, short stature, G_3 A_2 L_0having twin pregnancy with gestational diabetes mellitus along with the history of hypothyroidism and obesity , BMI> 35 (Body mass Index); referred to us by a Radiologist with lower abdominal pain, bleeding per vagina at 14 weeks of amenorrhea. Ultrasonography was suggestive of twin live uterine pregnancies with funneling of cervical canal and cervix measuring 1.6 cms. Her pre-operative blood profile and an emergency cervical cerclage was done with informed consent. In addition to cerclage, vaginal culture and peri-operative antibiotics were administered. Emergency cervical cerclage was done using McDonald procedure with prolene number 1 suture and the procedure was uneventful, and the patient was on intra-venous antibiotic treatment for five days. The C-Reactive Protein (CRP) value remained high till 35th week of her pregnancy. She delivered twins of 2.8 kgs and 2.3 kgs respectively. One of the fetuses had cyst in the liver and another one had single developed lung. The continuous monitoring on ultrasound showed that the funneling of the cervical canal till 35th week. In view of medical disorders , physician and endocrinologist were in treatment loop constantly. Despite of multiple health issues, the patient responded well to the emergency medical management and continuous surveillance with successful outcome.

Image 1:



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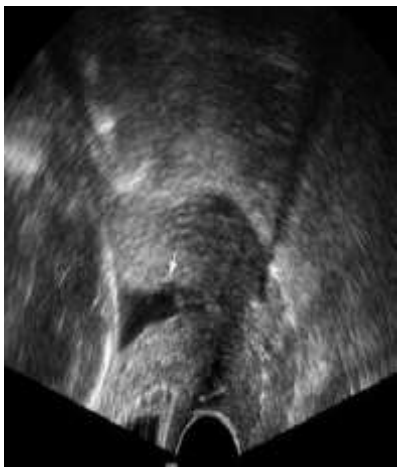

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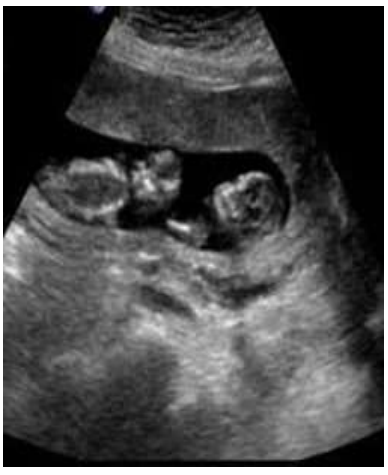

DISCUSSION

Preterm labor has multifactorial etiology, of which the anatomical causes account for 9%. Cervical Factors:- most common anatomical factor ; Pre-term labor has multifactorial Cervical length and strength together with the quality of cervical mucus contribute in retaining the pregnancy in the uterus and in preventing the entry of potential pathogens ascending from the vagina. Uterine Factors: - Uterine malformations , Congenital :Septate uterus Bicornuate, Unicornuate, Acquired Intrauterine adhesions Fibroids (> 6 cm size, protruding into uterine cavity) , Short cervix- congenital/developmental (DES exposure) Cervical surgery-conisation/ablation/excisional procedure Obstetric injuries-difficult instrumental vaginal deliveries, cesarean section after full dilatation. If the likely cause of recurrent pregnancy loss can be determined treatment is to be directed accordingly.

The treatment of cervical incompetence is surgical, consisting of reinforcement of the weak cervix by some type of purse-string suture. Bleeding , uterine contractions, or ruptured membranes are usually contraindications to surgery. The more advanced the pregnancy, the more likely surgical intervention will stimulate preterm labor or membrane rupture.cerclage procedures done in the late midtrimester after cervical dilatation and effacement have already occurred and are called “emergency”or “rescue” procedures. Bulging membranes are associated with significantly increased failure rates and infection is always a threat. Amino reduction at the time of emergency cerclage may improve pregnancy prolongation.weekly ultrasonic surveillance of the lower uterine segment between 14 and 27 weeks may prove useful in some women. Unfortunately, rapid effacement and dilatation develop even with such precautions. Success rates approaching 85-90 % are achieved with both Mc Donald and Shirodkar cervical cerclage techniques, but here we used McDonald technique aiming to reserve the complicated Shirodkar technique for cerclage failure or structural cervical abnormality. All these were ruled out by proper pre-operative profile and peri-operative care with the assistance of the intensivist and intra-operative ultrasound guided suturing was done for accuracy with proper operative position and Foley’s catheter in-situ. The complications, especially infection, have been identified to be much less frequent when cerclage was performed by 18 weeks. Risk factors associated to emergency cervical cerclage also need precised and timely monitoring for the successful outcome of the pregnancy.

Unfortunately, rapid effacement and dilatation develop even with such precautions. Success rates approaching 85-90 % are achieved with both Mc Donald and Shirodkar cervical cerclage techniques. The complications, especially infection, have been identified to be much less frequent when cerclage was performed by 18 weeks.

Other associated risk factors could be:

- Exposure of the fetal membranes to vaginal bacteria may increase the risk of chorioamnionitis, intraamniotic infection, hematosepsis of mother, or even maternal death because of severe infection.
- Vaginal bleeding
- A tear in the cervix (cervical laceration)
- Preterm premature rupture of the membranes — when the fluid-filled membrane that surrounds and cushions the baby during pregnancy (amniotic sac) leaks or breaks before week 37 of pregnancy
- Suture displacement
- Cerclage to be avoided if there is purulent discharge from the cervix, ruptured membranes, severe vaginitis, HSV infection, vaginal bleeding, coagulopathy or a dead fetus.

CONCLUSION

Compared with no treatment, cervical cerclage reduces the incidence of preterm birth in women at risk of recurrent preterm birth without statistically significant reduction in perinatal mortality or neonatal morbidity and uncertain long-term impact on the baby. Ceasarean section is more likely in women who had cervical suture inserted during pregnancy.The decision on how best to minimise the risk of recurrent preterm birth in women at risk, either because of poor history of a short or dilated cervix, should be 'personalised', based on the clinical circumstances, the skill and expertise of the clinical team and, most importantly, woman's informed choice.

DECLARATION OF PATIENT CONSENT

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient has given her consent for her clinical information and images to be reported in the journal the patient understands that her name and initials will not be published and due efforts will be made to conceal her identity, but anonymity cannot be guaranteed.

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CONFLICTS OF INTEREST: THERE ARE NO CONFLICTS OF INTEREST.

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MCQ on RPL

1) What is cut off cervical length for cervical cerclage?
   a) 20 mm
   b) 25 mm
   c) 30 mm
   d) 22 mm
2) What is the most common cause of pregnancy loss in 1st trimester?
   a) Genetic
   b) Idiopathic
   c) APLA
   d) Endocrine
3) Parental karyotype analysis is strongly indicated if karyotype of abortus is:
   a) Normal
   b) Aneuploidy
   c) Unbalanced translocation
   d) None of above
4) Initial choice for imaging in RPL is:
   a) USG
   b) MRI
   c) HSG
   d) Hysteroscopy
5) Which test is not a part of APLA testing?
   a) Anticrdiolipin antibody
   b) Lupus anticoagulant
   c) Antinuclear antibody
   d) Beta 2 glycoprotein 1 antibody
6) Chronology of funnelling of internal OS is:
   a) TVU
   b) TUV
   c) UVT
   d) VTU
7) Contraindications for emergency cerclage include:
   a) Ruptured membranes
   b) Uterine contractions
   c) None
   d) Both a and b
8) Progesterone supplementation in RPL, if required, should be continued up to:
   (a) 8 weeks
   (b) 20 weeks
   (c) 12 weeks
   (d) 16 weeks
9) Method to improve live pregnancy rate in RPL as a result of sperm abnormality:
   (a) ICSI
   (b) PICSI
   (c) IMSI
   (d) All of above
10) Lateral Metroplasty is indicated in:
   (a) T shaped uterus
   (b) Bicornuate uterus
   (c) Septate uterus
   (d) All of above

11) Sperm factors responsible for RPL:
    (a) High DNA fragmentation index
    (b) Y- microdeletions
    (c) Sperm aneuploidy
    (d) All of above
12) Even after thorough evaluation, cause of RPL is unidentified in how many cases?
    (a) 25 %
    (b) 35 %
    (c) 50 %
    (d) 40 %

Answers:
(1) b (2) a (3) c (4) a (5) c (6) a (7) d (8) b (9) d
(10) a (11) d (12) c

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