

Current practice in RECURRENT PREGNANCY LOSS (RPL):

Summarised by Dr.H.S.Palep

It is a variety of infertility, in which couples experience repeated cycles of hope and despair. The fundamental aspiration of the couple to achieve a fulfilling experience of parenthood is shattered. Despite major advances in the field of genetics, biology and immunology RPL still poses a challenge to a treating obstetrician. The cause of RPL is mostly unexplained in many patients and every therapeutic intervention remains controversial.

Couples suffer extreme anxiety, depression, frustration and social isolation. Apart from loss of self esteem and stress, these couples are also subject to big financial setback. The emotional stress is known to affect both cellular and humoral immunity. This in turn releases abortogenic cytokines, thus compounding the problem. Therefore RPL couples approach an obstetrician with great expectations and demand solution to this vexed clinical problem.

Definition:

Three or more consecutive miscarriages up to 28 weeks are defined as RPL.

EPIDEMIOLOGY:

Incidence: 0.6 to 2.3%.

There appears a polygenic mode of inheritance in RPL. Advanced maternal age and partner specificity are important factors. More the numbers of abortions in past poorer is the prognosis. There are three clinical sub groups in RPL>

- Primary : 3 consecutive abortions before 20 weeks
- Secondary : 3 or more pregnancy losses with at least 1 pregnancy ended beyond 20 weeks as live birth or otherwise.
- Tertiary : Mixture of both the groups.

Immunobiology of Pregnancy:

There is now evidence emerging to show that embryo generated Signals modulate maternal immune system.

Sperm penetrates into egg becomes invisible to maternal immune system. It may also promote local immune suppression through release of Prostaglandins (PGs). Cumulus offers first relay system of embryo signalling. At Two cells stage genome is expressed in the embryo, thus making it non self to mother's immune system. Expression of novel genes by it produce unique signals that modulate the

maternal immune system that produce optimum tolerance to embryo without compromising maternal functional capacity to fight the stress or infection.

- Endometrium is primed to make environment more suitable and hospitable for implantation of embryo.

Appropriate interplay of cytokines is the key for a successful pregnancy. Two types of cytokine responses occur in tissues and cells. T-Helper cells 1 (Th₁) are pro-inflammatory in nature and whereas Th₂ type of response is pro pregnancy and anti-inflammatory. Pre implantation embryo protects itself from maternal immune rejection by modulating Th₂ response. Peripheral T lymphocytes release cytokines IL-10 and TGF- β and express progesterone receptors. Decidual NK cells secrete an IFN γ (Th₁ response). NK cell Th₁ action on endometrium improves its vascularity by remodelling spiral arteries to enhance successful implantation. At the same time corpus luteum (CL) releases relaxin. This may also cause Th₁ response. Trophoblast generated hCG supports CL of pregnancy. It secretes progesterone to prime endometrium. T lymphocytes under the influence of Progesterone express progesterone induce blocking factor (PIBF), which is a key regulatory agent for pro pregnancy Th₂ response. HCG supports implantation, but does not reflect the viability of the embryo.

Embryo and trophoblast express immunosuppressive HLA- G, which blocks activated cytotoxic lymphocytes (CTL) and NK cell mediated action. This mediates the facilitation reaction. Thus allogenic reaction of maternal immune system is blocked by non complement fixing antibodies and suppressor cells so as the embryo is tolerated. Thus the dialogue between the embryo and maternal system starts much prior to implantation. A successful pregnancy requires 3 factors.

1. Viable embryo
2. Immune tolerance
3. Receptive Uterus.

Endometrium can be hostile due to immune disruption by dNK cells, altered hormonal priming, infection, deficient integrin expression and adhesion molecule MMPs.

MHC-HLA antigen sharing in couples can adversely affect appropriate signalling by the embryo causing immune rejection.

Genetics in spontaneous abortions:

50% to 80% of first trimester abortions are due to abnormal chromosomal or other distinct genetic causes. Thrombo-embolic diseases (FVL Factor V Leiden) and alloimmune pregnancy loss (Shared HLA) could be because of perturbation of gene products. Recurrent aneuploidy exists, usually associated with advanced maternal age. Possibility of live born in future pregnancy could be a Trisomy-21 should be kept in mind. According to CARP et al. finding of abnormal karyotype is 29 % in RPL. Proper counselling is important and pre-implantation genetic diagnosis should always be considered. Selective transfer of euploid embryos is a way to decrease chances of clinical abortions. Therefore karyotyping of abortus is important. If the result shows that embryo is euploid, the cause of RPL is maternal not fetal. But controversy exists with regards to routine testing for fetal genetic testing.

According to Zk Borochowitz, recurrence risk of aneuploidy is 16% (normal is 10 to 15%). Half the structural abnormalities in the fetus are inherited from the parent carrying balanced chromosomal abnormality. Parental chromosomal analysis is now routinely performed in most countries in cases of more than 2 abortions.

RCOG reserves this testing only in case of research trials or in index pregnancy. It is advised to collect the specimen by embryoscopy in utero before it is expelled, where as ACOG does not recommend routine abortus karyotyping.

Pre gestation screening (PGS) is advised in cases of advanced maternal age and also when a strong family history or previous history of anomaly exists.

FISH (Fluorescent in situ hybridization), CGH (Comparative Genome hybridisation) and Iso- thermal genomic amplifications are the methods available. These can help detection of aneuploidy and enable the transfer of only euploid embryos.

Chorion Villous sampling and amniocentesis are advised in high risk cases of known family history of chromosomal abnormalities and advanced maternal age beyond 35 years. It is advised to defer invasive techniques since other non invasive markers are available presently.

Aneuploidy markers in serum:

1. HCG
2. Free beta sub unit of hCG (10weeks)
3. Alpha Fetoprotein
4. E3 (unconjugated estriol)
5. Inhibin A

6. PAPP (Pregnancy associated plasma protein)

USG Markers of Aneuploidy:

1. NT (Nuchal Translucency 11 weeks)
2. NF (Nuchalfold Thickness)
3. Nasal bone
4. TR (Tricuspid regurgitation)
5. Ductus Venosus.

Anomaly scans or genetic sonograms are commonly used to rule out the anomalies now a days.

Maternal system and embryo's response to teratogens:

Many environmental agents and teratogens affect the pregnancy. Chemicals and physical developmental toxicants induce teratogenicity in fetus e.g Diabetes mellitus, ionising radiations, heat shock, chemotherapeutic agents like Cyclophosphamides etc. These induce excess apoptosis, structural malformations and anomalies. Apoptosis is genetically regulated process of cell death. Its function is to eliminate abnormal, misplaced, non functional harmful cells. It controls the over all cell numbers in an organism. Gene P 53 is a tumor suppressor protein, a key regulator of apoptosis in embryo. Both initiator and effector Caspases and TNF- α are pro-apoptotic. Transcription nuclear factor κ B (NF- κ B) is the key molecule which is very active during embryogenesis. During organogenesis the key molecules for preventing cell death are P65 and Bcl 2, which are anti-apoptotic genes.

Any teratogenic insult leads to expression of TNF α and adversely affect the balance of cytokine milieu operating at the fetomaternal interphase. Increase in TNF α leads to dysregulation of transforming growth factor β 2 (TGF β 2) and macrophage colony stimulating factors. TGF β family regulates the cell growth differentiation, migration and extra cellular matrix deposition. Its deficiency can result in pregnancy loss or anomalies. Experimentally TGF β 3 knock out, fetus is born with cleft palate.

Embryoscopic Biopsy offers a better cytogenetic diagnosis, devoid of maternal tissue contamination. It helps in identifying subtle fetal anomalies and uterine malformations.

Endocrinology of pregnancy loss:

Luteal Phase Defect (LPD), Hyper prolactinaemia, polycystic ovary, thyroid dysfunction, decreased ovarian reserve, D.M, and inhibins and activins are chiefly the conditions, where endocrinology play significant role in RPL.

LPD: Corpus luteum supports pregnancy till 9th week. 35% of RPL patients may suffer from luteal phase defect. Diagnosis is done by serum progesterone levels (10Ug / ml on days 22 to 23 days) and/ or by endometrial biopsy (EB) showing a lag of 2 days. EB is more reliable than progesterone level.

Micronised Progesterone supplementation either intravaginal or parenteral route are ideal for treating LPD. LPD and hyperprolactinaemia may be associated. Look for raised prolactin (PRL) level. High PRL interferes with corpus luteal function causing decrease in progesterone support to embryo. Bromocriptin or cabergolin therapy will help.

Thyroid Dysfunction: Hyperthyroidism is associated with poor pregnancy outcome e.g., PTD, IUGR, SB and maternal heart failure etc, but not with RPL or infertility. Hypothyroidism maybe due to Iodine deficiency, auto immunity or post partum thyroiditis. It may also be drug induced. It may cause luteal phase defect leading to RPL. Sub clinical hypothyroidism may result in PTD, reduced IQ in new born and poor fetal outcome. National Academy of Biochemistry recommends upper limit for euthyroid to 2.5 mU/ Lt., when normal range is 4.5 to 5mu/lit. ATA (Anti thyroid antibodies) may be associated with other auto immune disorders, e.g LA, aCI leading to RPL. Therefore when ever ATA are positive always look out for other evidences of auto-immune disorders. ATA with normal thyroid function may not have any impact on pregnancy out come.

Diabetes mellitus: Risk of malformations and pregnancy losses are directly related to poor glycemic control. Hence it is recommended that HbA1c should be maintained at <7.5% level.

POLY CYSTIC OVARIAN SYNDROME (PCOS) : 50% of PCOS end up in early pregnancy loss. Hyperinsulinism, insulin resistance and increased LH with high androgenic milieu, Plasminogen activator inhibitor 1 Gene mutation (4G4G) leading to fibrinolysis causing hypofibrinolysis are possible mechanisms for RPL (early pregnancy loss). This is reversed by Metformin which is pregnancy category B Drug.

Elevated FSH is a sign of poor ovarian reserve with no treatment.

Inhibins are proposed as markers of fetal viability. Their major sites of production are placenta, decidua and fetal membranes.

CD8+ T lymphocyte express PIBF, when exposed to progesterone and produce Th₂ response resulting in selective immune tolerance at maternal fetal inter phase. 33% miscarriages are due to progesterone deficiency. Progesterone supplementation is harmless to fetus or mother, therefore progesterone supplementation can safely be used.

Shazia Mallick of St. Mary's Hospital is against Progesterone supplementation and believes that the tender Loving care (TLC) can give similar results. ACOG guide lines also term Progesterone support in luteal phase is of unproven efficacy. There is a debate on use of even hCG. It is of no benefit in case APS and aneuploidy. It is of particular benefit in women with oligomenorrhea. Trophoblast takes over from corpus luteum at 7 to 8 weeks of gestation, the production of Progesterone. According to Siobahn M Quinby there is no compelling evidence for the hCG supplementation in threatened abortions and in pregnancies following ovulation induction and RPL.

Anti Phospholipid Syndrome (APS): It is an auto immune condition, which affects all organs and systems of body. It is associated with triad of manifestations.

1. Arterial / Venous thrombosis
2. Elevated titers of aPL (aCL, LA) antibodies with mild to moderate thrombocytopenia.
3. RPL / Associated pregnancy complications, e.g., PIH, IUGR, PTD

Etiology:

Environmental factors, genetic susceptibility and infectious agents.

Infections:

H.Pylori, HIV, Skin infections, Hepatitis C, Varicella Zoster (Chicken Pox) etc.

These infections are now believed to stimulate the expression of antibodies that recognise epitopes (molecular mimicry), e.g., β 2GPI. These antibodies bind to membrane phospholipids resulting in auto immunity, creating a state of hypercoagulability.

Anti phospholipid antibodies (aPL) inhibit arachidonic acid (AA), which is the source of PGI₂ tilting the balance in favour of TXA₂(throxane A₂). TXA₂ causes vasoconstriction and procoagulant platelet activation. aPL levels are the

prognostic markers. When aPL level is just >40 GPL units the risk is considered medium to high. Drug therapy may be beneficial. Very High levels of aPL > 80 GPL units indicate a very high risk, ending in Fetal demise.

Heparin and Low dose Aspirin are used to combat this hypercoagulable state. UH (unfractionated Heparin), more commonly LMWH (Low Molecular Weight Heparin) are used now a days.

RPL before 10 weeks with no history of prior A/V or small vessel Thrombosis, LMWH in dose of 1mg/ kg /day as prophylaxis started soon after the USG evidence of cardiac activity is established and continued till a day before delivery. Risks of heparin therapy are haemorrhage, osteopenia, thrombocytopenia and epidural haematoma.

If the RPL has been occurring after 10 weeks and there exist the evidence previous thromboembolic episodes, a full dose Heparin therapy, 1mg/kg every 12hr should be started. If the patient is on Warfarin, it should be withdrawn in view of its teratogenicity. Low dose aspirin can also be given additionally, which should be stopped by 34 weeks. Monitoring LFT, RFT, Platelet count and PTT, regular and USG Doppler study is very important if the heparin is used. It is prudent to be on alert for PIH and other pregnancy related complications. There is absolutely no role for Glucocorticoids in aPL. Hydroxy chloroquin is preferred in pregnancy with SLE. Advocated dose is <6.5mg/kg for anti platelet action. Intra venous immunoglobulin (IVIg) therapy is indicated only as a second line in cases of failure with heparin therapy. If the fetal or maternal compromise is suspected and early delivery should be planned.

Hereditary thrombophilia causing coagulation defects are more common in II and III trimester RPL and IUGR, PIH, SB and Abruption placenta.

F xiii and Fv L (Factor V Leiden), Homocystein, fibrinogen, cytokines (IL-6, TnF α), Fii (prothrombin generation) and TG (Thrombin generation), M.P (Microparticles) are thrombotic.

Protein C, Protein S, anti thrombin, TFPI (Tissue factor pathway inhibitor) and fibrinolytic system are anticoagulant.

Imbalance of these factors either due to deficiency or excess can be a cause of RPL.

Heparin increases protection against TNF α and improves trophoblastic function.

Normal serum fibrinogen level is 60mg/100ml.

Two weekly infusions of 250 mg/bag in fibrinogen deficiency is the protocol to bring the fibrinogen level to normal. Complication that should be anticipated is a catastrophic thrombosis. Low molecular weight heparin (LMWH) can be used as prophylaxis.

Uterine anomalies:

Incidence of RPL with uterine anomalies is 0.2% to 10%. Anomaly incidence in general population is 1%.

Conditions, e.g., sub septate, arcuate, bicornuate uterii, intra uterine adhesions, polyps and fibroids associated with RPL.

Most common cause amongst them is subseptate uterus. Poor placentation due to avascular septum and distorted uterine cavity and in co-ordinated uterine action could be the causes. Surgical hysteroscopic resection of the septum is now preferred mode of treatment. In bicornuate uterus metroplasty can be done. Arcuate and T shaped uterii are commonly associated with II trimester loss. Prophylactic cerclage is ideal intervention, when the cervical length is < 25mm in II trimester and funnelling greater than 25% of the length of the cervix. HSG, USG, Doppler, IVP, KUB, Sonohysterogram, hysteroscopy are all the investigations useful in diagnosis.

Infections and RPL:

It is not clear whether association of TORCH infections in RPL is pathogenic or opportunistic. RCOG guideline no.17, 2003 recommends that in RPL there is no need for TORCH screening. Chlamydia, Bacterial Vaginosis are associated with increased incidence of PTD. Gram negative and bacterial culture respond to Metronidazole.

Mycoplasma, Ureaplasma etc are sensitive to erythromycin or tetracyclin.

Infections by H.Pylori and others are now linked to aPL through molecular mimicry of β_2 GPL.

How should we investigate a case of RPL?

There is a lot of controversy as to how a patient of RPL should be investigated.

ACOG protocol recommends investigations after 2 consecutive pregnancy losses. RCOG and ESHRE protocols demand investigations only after 3 or more losses.

Biochemical Pregnancy Loss:

1. USG: no evidence of gestational sac
2. β hCG in the range of 10 – 1000 IU/l and two readings showing rising levels.
3. Menstruation delayed by at least 1 week.

As per ACOG guidelines routine parental karyotype is advised and if one of the parent is a carrier of balanced translocation prenatal diagnosis is mandatory.

Fetal karyotyping is not recommended on routine basis.

RCOG guidelines advise only in index pregnancy or in a research trial.

For ruling out uterine anomalies 3D USG and hydrosoneography are advised.

APS testing is recommended in all II trimester abortions. Confirm auto-immune pathology only if LA and aCL tested at least twice 6 weeks apart. Assessment of β 2GPI is more relevant than LA and aCL is the current opinion. Full panel aPL include LA, aCL, aPE, aPS, APC, aPG, aPL, aPA, a β 2GPI. In early pregnancy loss aPS and aPE testing is more fruitful.

RCOG guidelines assert that there is insignificant evidence to investigate for LPD.

ACOG guidelines do not recommend ATA, TORCH panel, allo immune testing.

Bloodsugar, Thyroid function tests are mandatory.

For clinical trial framework, karyotyping of fetus, NK cells, luteal phase endometrial biopsy and homocystein levels are recommended.

Prognostic Markers in RPL

1. More the number of previous pregnancy losses, poorer prognosis.
2. Secondary aborters have better prognosis.
3. Previous aneuploid abortion – a better prognosis, If aneuploidy repeats advise PGD
4. Concurrent infertility : poor prognosis
5. Advanced maternal age is again a sign of poor prognosis.
6. Presence of APCA (anti paternal complement dependent anti bodies) – successful pregnancy outcome.
7. NK cells increase in peripheral blood – poor prognosis
8. Late pregnancy loss – Worse prognosis.

Perusing through this summary of the current available knowledge and experience on this vexed problem of RPL One can conclude that the management of RPL is complex and controversial. Since RPL is multi factorial, it requires multiple types of interventions. Addressing and achieving the homeostasis of immune system operating at the fetomaternal interphase is the main focus of any therapy. There is currently sufficient experimental evidence to show that non specific immune stimulation with CFA (complete Freund's adjuvant) improved reproductive performance in animals (Todder V et al, Am.Journal . Rep. Immunology 1990, 24; 63-6).

The current understanding immunobiology clearly established the fact that Th₂ immune response in mother protects the pregnancy. Any therapeutic intervention that can achieve this object can deliver results in RPL. As can be seen clearly from the following scientific research based information both Cap. SUJAT and Cap. TORCHNIL are powerful immuno-modulators, anti-oxidants and anti-inflammatory agents.

Cap. SUJAT and Cap. TORCHNIL as therapeutic interventions have been used by large number of Obstetricians all over the country for now more than a decade with great success and satisfaction.

TORCHNIL consist of 11 and SUJAT 9 highly acclaimed Rasayana Herbs.

SUJAT (Ingredients)

SHATAVARI	ASPARAGUS RACEMOSUS
VIDARI	IPOMEA DIGITATA
SHRINGATAK	TRAPA BISPINOSA
AMLAKI	EMBELICA OFFICIANALE
BALA	SIDA CORDIFOLIA
ASHWAGANDHA	WITHANIA SOMNIFERA
YASHTIMADHU	GLYCERRHIZA GLABRA
SARIVA	HEMIDESMUS INDICUS
GOKSHURU	TRIBULIS TERRESTRIS

TORCHNIL (ingredients)

YASHTIMADHU	GLYCERRHIZA GLABRA
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GUDUCHI	TINOSPORA CORDIFOLIA
LAGHU KANTAKARI	SOLANUM XANTHOCARPUM
BRIHAT KANTAKARI	SOLANUM INDICUM
PIPPALI	PIPER LONGUM
GOKSHURU	TRIBULIS TERRESTRIS
BHARANG MOOL	CLERODENDRUM SERRIATUM
DADIM PATRA	PUNICA GRANATUM
USHEER	ANDRPOGAN MURICATUM
RASNA	VANDA ROXBURGHII
MANJISHTA	RUBIA CORDIFOLIA

We published our first report in June 1997, a study on 26 patients. We were able to show almost 95% live births and superior outcome in terms of better birth weights, when compared with controls, which were given modern medical treatment. Herb extracts constituting of Sujat and TORCHNIL were studied and researched at prestigious institutions to establish their mechanism of action.

At BARC aqueous extracts of SUJAT were tested for anti oxidant action on rat liver cell mitochondria by Dr.T.P.A Devsagayam et al. At 0.1 % concentration SUJAT showed comparable anti-oxidant protection effect to Trolox and superior to Glutathione. (Research Communications in Pharmacology and Toxicology: Emerging Drugs vol II).

At UICT Dr.Sadhana Sathye et al, have reconfirmed the anti –oxidant effect of Sujat and established the anti-oxidant action of Torchnil. Immuno-modulatory action was studied at UICT which showed that both C. Sujat and C. Torchnil are very good immune-modulators. It was concluded that TORCHNIL produced significant immune-modulation, both non specific and specific humoral immunity. It also produced significant cell mediated immunity. In conclusion, our opinion is Torchnil has very good immuno modulating potential.

TORCHNIL herbs were studied individually in-vitro culture of HIV for their action of inhibition on RT (reverse Transcriptase) and P24 antigen. Tribulis terrestris, Vetivera zizanoid and Solanum xanthocarpum showed highest inhibition of

P24 compared to Lamivudin. Solanum xanthocarpum & Punica granatum have showed very good inhibition power against RT enzyme superior to AZT.

Number of clinical trials confirmed the effectiveness of these agents in preventing PIH, Improving birth weights and overall fetal outcome of pregnancy both in normal as well as in RPL. Number of specialists involved in ART have found the benefit of these formulations in implantation failures.

Mechanism of action of Sujat and Torchnil is based on their immunomodulatory, antioxidant and anti-inflammatory actions. Immunology plays a great role in successful pregnancy and especially so in RPL.

Pregnancy is a condition of high energy demand producing great oxidative stress. Powerful anti oxidant ability of Sujat and Torchnil are of great value in RPL, where oxidative stress is very high.

Our post market research shows very great acceptance of these formulations by both the patients and Obstetricians with no known side effects what so ever.

With this background evidence Cap Sujat and Cap Torchnil combination is the choice of most obstetricians to the vexed problem of RPL. Many obstetricians have integrated these drugs along with their modern interventions synergistically. They have also been used independently proving safety, efficacy and cost effectiveness. Over more than a decade of their use no undesired side effects have been reported in post marketing survey comprising more than 250 obstetricians.

Recommended dose: Cap. Torchnil 1 BD 3- 6 months before conception. After conception Cap. Sujat 1 TDS along with TORCHNIL till the term.

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