FOLLICULAR PHASE HORMONAL VARIATIONS – A MAJOR RISK FACTOR FOR RECURRENT PREGNANCY LOSS

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Abstract:

Successful pregnancy depends on a multitude of endocrinological events resulting in successful growth and normal development of the fetus. However, certain endocrine anomalies potentially lead to recurrent pregnancy loss (RPL). Luteal phase defect, polycystic ovarian syndrome diabetes mellitus, thyroid disease, and hyperprolactinemia are the most frequently observed endocrinologic disorders implicated in approximately 17% to 20% of recurrent pregnancy loss. However evaluation of hormonal profile during luteal phase has gained much interest than follicular phase. In this study we are analyzing both follicular phase and luteal phase and its correlation with pregnancy loss. A total of 350 non-pregnant women with a history of 2 or more pregnancy losses (mean ± SD: 3.3 ± 1.8, range 3–8) are recruited where their age ranges from 18-35 years (mean ± SD: 26.4 ± 4.5,) Hormonal analysis are carried out for follicle stimulating hormone , luteinizing hormone , prolactin, testosterone, androstenedione, estrogen, progesterone T3,T4 and Thyroid stimulating hormone using automated multiparametric immunoanalyzers. Variations in the hormonal profile were statistically significant ($p < 0.05$) where majority of cases were observed in follicular phase when compared to luteal phase. Therefore it is strongly recommended to analyze both follicular and luteal phase for the evaluation of RPL.

Keywords: Recurrent pregnancy loss, follicular phase, luteal phase.

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Introduction

Recurrent pregnancy loss (RPL) has been defined as 2 or more consecutive pregnancy losses prior to 20 weeks from the last menstrual period; it affects approximately 1% to 2% of women (Ford and Schust, 2009). Approximately 15% of all clinically recognized pregnancies result in pregnancy failure (Chen and Creinin, 2008).). The diagnosis and treatment of RPL is still challenging task due to lack of the exact definition of inconsistency in inclusion and exclusion criteria and also the misconception of over bleeding during menstrual cycle. Proposed etiologies for RPL include parental karyotypic abnormalities, uterine anatomical abnormalities, and hormonal, infectious, immune, and thrombophilic disorders.

Endocrine abnormalities are major risk factor causing substantial proportion of RPL (Porter and Scott, 2005). Following implantation, the progress and maintenance of the pregnancy is associated with sequence of endocrinological events that promotes successful growth and development of the fetus (Arredondo and Noble, 2006). Polycystic ovarian syndrome (PCOS), luteal phase defect (LPD), diabetes mellitus, thyroid disorders, and hyperprolactinemia are among the major endocrinological disorders implicated in approximately 17% to 20% of RPL (Stephenson,1996).

The menstrual cycle has three phases: follicular (before release of the egg), ovulatory (egg release), and luteal phase which is after egg release (Klump *et al.*, 2013). However luteal phase defect has been extensively studied and is strongly correlated with retarded endometrium ,which is a major etiology of RPL (Lee 1987; Bopp and Shoupe,1993; Macklon and Fauser, 2000). Decreased levels of progesterone (Wentz *et al*., 1984;Druckmann and Druckmann, 2004) or decreased length of luteal phase of the cycle (Penton-Voak, 1999; Tavaniotou *et al.*, 2001) has gained much concentration. Further, amenorrhoea (Glueck, 2000), immuno-endocrinological disorders (Lim *et al*., 1996), hyperandrogenism (Carla *et al.*, 2000), obesity (Clifford *et al.*, 1994; Bussen *et al.*,1998;Linné 2004; Homburg,2006) have been diagnosed in a substantial proportion of RPL .

Development and normal function of corpus luteum is initiated during the follicular phase by employing a cohort of growing follicles stimulated by FSH (Montfrans *et al.*,2004). However, endocrine abnormalities during the follicular phase as possible risk factors for recurrent spontaneous abortion has gained minimal focus (Bussen *et al.*,1998). It is necessary to consider and

analyse both the phases of the menstrual cycle, because luteal phase defects are frequently associated with inadequate follicular growth, premature luteinization or basal hormone imbalances during the follicular phase (Bulletti, *et al.*,1996) .Till date no such study have been employed in Mysore district, Karnataka, South India. Therefore in this study we attempted to investigate the frequency of abnormalities of basal hormone concentrations in women with RPL to identify endocrine risk factors during follicular phase as well as luteal phase.

Materials and method

The study was conducted after obtaining Institutional ethical committee clearance (IHEC-UOM No.52/Ph.D/2011-12). This case-control study was carried out at Molecular Reproductive and Human Genetics laboratory, University of Mysore, Mysore. A total of 350 non-pregnant women with the previous history of pregnancy loss, who were attending different hospitals and clinics in Mysorecity, South Karnataka were recruited for the present study .The age ranged from 18-35 years (mean ± SD: 26.4 ± 4.5,) with a history of 2 or more (mean ± SD: 3.3 ± 1.8, range 3–8) previous pregnancy losses (table 1). Exclusion factors included chromosome abnormality, uterine malformations, polycystic ovaries, chlamydia infection, HIV/ HBsAg positive, bacterial vaginosis, antiphospholipid antibodies, diabetes, obesity, anti-cardiolipin and clinically proven endocrinological complications. Therefore considering the above mentioned factors 50 individuals were excluded from the study.

Control subjects (n = 300) were nulligravida women of similar age (mean ± SD: 33.3 ± 4.7 years, range 24–41) with no previously recognized miscarriage, and no clinical evidence of endocrine abnormality.

All the subjects were recruited after informed written consent was obtained. All the study subjects including the control group were not pregnant at the time of the study, nor were under the medication likely to affect the results. Subjects were ascertained through gynecologist of different hospitals and clinics in and around Mysore. A detailed family, occupational, reproductive and clinical histories were recorded through pre designed genetic registry with more than two rounds of interaction with the study subjects. The body mass index (BMI) of each study and control subject was calculated using the formula weight (kg)/height (m^2) (study group mean ± SD: 21.5 ± 6.4 kg/m^2, range 3.3 17–28 kg/m^2; control group mean ± SD: 21.8 ± 1.29 kg/m^2, range 21–25 kg/m^2).

Women with a BMI ≥25 kg/m^2 were regarded as obese and were excluded from the study. Study group was categorized into four groups according to the age range from 18years to 40 years. The pregnancy loses experienced by the study group ranged from 2-8 losses.

Endocrine evaluations

Peripheral blood was collected from both the group during the early follicular phase and leutal phase which was later subjected for centrifugation to collect the serum. Hormonal analyses was performed for follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, estrogen progesterone T3,T4 and Thyroid stimulating hormone (TSH) using commercially available kit by BioMerieux France through automated multiparametric immunoanalyzers (VIDAS, BioMerieux, France). The manufacturer's instructions. were referred for the normal ranges .

Statistics

The results of the study and control groups were analysed by Student's *t*-test using SPSS version 4 software. All reported values are given as mean ± SD and 95% confidence interval (95% CI). $P < 0.05$ was regarded as significant

Results

The age group and the frequency of pregnancy losses are depicted in table 1. Table 2 represents the frequency of abnormal endocrine test results in both groups. The total percentage of increased or decreased levels of hormones in follicular phase as well as luteal phase is summarized in figure 1. Significant variations from the normal range of hormonal levels were observed in both the follicular and luteal phase when compared to control group as depicted in table 3. Out of 300 subjects 217 cases had abnormal hormonal levels in follicular phase and 83 cases in luteal phase (table 3). Analysis carried during follicular phase depicted significant variation from normal values were in Estrogen , Prolactin, T4, TSH and progesterone. Statistical analysis for Student's *t*-test at 95% confidence interval (95% CI) resulted in significant values.

Discussion:

Successful progression of the pregnancy after the implantation is dependent on a sequence of endocrinological process resulting in the successful growth and development of the fetus (Arredondo and Noble, 2006). Endocrine-related pregnancy failures are likely to occur early in

gestation because the successful attachment of the embryo and early implantation are exquisitely controlled by the local hormonal milieu (Smith and Schust, 2011). The abnormalities in the hormonal profile observed in our study are summarized as follows.

Follicle Stimulating Hormone is produced by the brain via the pituitary gland to stimulate follicles in the ovary to grow (Styne-Gross *et al*., 2004). Reduced ovarian reserve and poor quality oocytes is strongly associated with elevated basal FSH level resulting in RPL (El-Toukhy *et al*. 2002) . In our study 87 (29%) cases were diagnosed with elevated levels FSH which correlates with the previous studies (Abdalla and Thum, 2004, Styne-Gross *et al*., 2004) . Of which 39 (13%) cases were observed with elevated levels during follicular phase. Though number of cases were less the statistical analysis depicted significant variation ($p > 0.5$) when compared to the control group during follicular phase. FSH is associated with diminished ovarian reserve which contributes to RPL and therefore should be strongly considered as a part of regular work-up for RPL (Trout and Seifer ,2000).

The fate of a pregnancy relies on adequate maternal production of estrogens until the placenta is able to replace the corpus luteum as the main source of steroids (Nepomnaschy ,2006). Elevated levels of estrogen during the follicular phase, causes a decline in FSH (Kolibianakis *et al.*, 2004), and has significant correlation with diameter of the gestational sac and is associated with variability in the rate of embryo growth during the first 12 gestational weeks (Ohtsuka *et al*., 1995). Estrogen play an important role as a modulator of uterine vascularity (Goswamy *et al.*,1988,). First-trimester endocrinology is determined on estrogen biosynthesis and secretion and deficiency of estrogen leads to RPL (Schindler, 2004). In our study we observed decreased level of estrogen in 136 cases (45.3%) of which majority of variation were observed in follicular phase 97 (32.3%) and rest 39 cases (1.3%) belonged to luteal phase. This study correlates with previous studies (Trout and Seifer,2000; Albrecht *et al.*,2000) which is considers decreased level of estrogen as a risk factor for RPL with statistically significant difference (p value < 0.05) when compared to the control group.

Luteinizing hormone and FSH, which are produced by the pituitary gland, promote ovulation and stimulate the ovaries to produce estrogen and progesterone (Westergaard *et al*.,2000). Earlier to the process of ovulation the LH surge begins about 34 to 36 hours prior. Higher levels of LH, even though still within normal range, are associated with an increased risk of

miscarriage (Balasch *et al*. 2001). Increased serum concentrations of LH in the follicular phase, has been associated with decreased reproductive function (Ayabe *et al*.,1994). In this study we observed 218 cases with increased LH which accounts for 42 % and decreased levels were observed in 15 cases which accounts for 5%. LH variation during follicular phase was observed in 142(47.3%) and in 76 (25.3%) case showed abnormalities in luteal phase. It has been stated that high basal LH concentrations, leading to premature luteinization, may be found in up to 33% of women with recurrent spontaneous abortion (Clifford *et al.*, 1994; Chuang *et al*., 2003;Gürbüz *et al.*, 2004).

Normal development of the fetus and its sustenance is also based on the normal thyroid hormones. Hypothyroidism and hyperthyroidism are considered to have negative effects on normal development of the fetus. Hypothyroidism is often correlated with infertility and it has a higher risk of RPL (Glinoer ,2000). The fetus's thyroid gland is not fully functional until after 12 weeks of pregnancy. If the mother does not have sufficient thyroid hormones, she may be at increased risk of miscarriage (Roberts and Murphy, 2000).In this study we observed 47 cases with hypothyroidism which accounts for 15.6%. Hyperthyroidism was observed in 9 cases (3%) which is not a significant risk factor. Negro *et al* in 2010 demonstrated that the increased rate of pregnancy loss in women with TSH levels between 2.5 and 5.0 mIU/liter adds strong support to re-defining the normal range of TSH during pregnancy, especially in the first trimester which accords with our study result (Roberto *et al.,* 2010). Significant association was observed in our study where 129 cases (42%) were recorded with increased TSH which correlates with the previous studies (Abalovich *et al., 2*002; Benhadi *et al., 2*009). Among which 92 cases were observed in follicular phase (30.6%) and 37 cases in luteal phase (12.3%).Based on this evidence, it appears that hypothyroidism is strongly associated with a increased risk for RPL.

The increased serum concentrations of prolactin shows few women with a history of recurrent spontaneous miscarriage in the follicular phase (Renzo *et al*.,2005; Rai and Reagan 2006). Appropriate levels of circulating prolactin play an important role in maintaining early pregnancy, Prolactin decreases the life and activity of the corpus luteum, which is required to sustain pregnancy during the first 7 weeks (Hirahara *et al*.,1998). Prolactin levels are often correlated with ovulatory dysfunction. Hyperprolactinemia may be associated with recurrent pregnancy loss because of the variation in alterations in the hypothalamic-pituitary-ovarian axis, which causes impaired folliculogenesis and abnormality in oocyte maturation, and/or a short luteal phase (Orhan and

Aydin, 2004). Increased frequencies of Hyperprolactinemia (Ando *et al.,* 1994) and of hypersecretion of LH (Balen *et al.,* 1993) have been reported in women with RPL. In this study we observed an higher frequency of increased prolactin which sustain the higher risk of RPL. We observed 215cases (71.7%) with hyperprolactinaemia of which 172 (57.3%) cases were observed in follicular phase which is a significant data observed in our study. This study shows women with a history of RPL have raised serum concentrations of prolactin in follicular phase.

Women with an elevated testosterone concentration have reported a similar relationship between free testosterone concentrations and future pregnancy outcome (Liddell *et al.*, 1997). It is unable to detect raised concentrations of serum testosterone in women with RPL, as testosterone is the androgen with the highest sensitivity for the disorder (Nardo *et al*., 2002). However, high serum concentrations of testosterone, free testosterone and dehydroepiandrostenedione are associated with an increased RPL rate (Tulppala *et al*., 1993). In this study we observed elevated testosterone in 63 cases which account for 21 percent of which 48(16%) cases were observed in follicular phase. Though the percentage seems to be insignificant the statistical analyses proved significant variation in testosterone (p value is >0.05). Therefore we conclude that an elevated testosterone index can be used a prognostic factor for a subsequent miscarriage in women with RPL and is a more significant predictor than an advanced maternal age ≥40 years.

Normal developmental activity during the pre-implantation period is stimulated by progesterone and estrogen (Renzo *et al.,* 2005). For the successful implantation and maintaince of pregnancy Progesterone is very essential (Arredondo and Noble, 2006). Therefore, disorders related to inadequate progesterone secretion by the corpus luteum are likely to affect the outcome of the pregnancy. Earlier studies indicate that decreased levels of progesterone results due to impaired folliculogenesis which play a major role in RPL (Pluchino *et al.,* 2014) Progesterone also has a immunosupressent effect which is essential to retain and maintain pregnancy to avoid fetal rejection(Choi *et al.,* 2000). It also inhibits prostaglandins and suppress the premature uterine contraction . Our results accords with the earlier studies (Renzo Di *et al.*,2005;Hussain *et al.*,2012) with 165 (55%) cases exhibiting low levels of progesterone where 121(40.3%) cases fall into follicular phase where low levels of progesterone are often associated with early RPL .

Conclusion

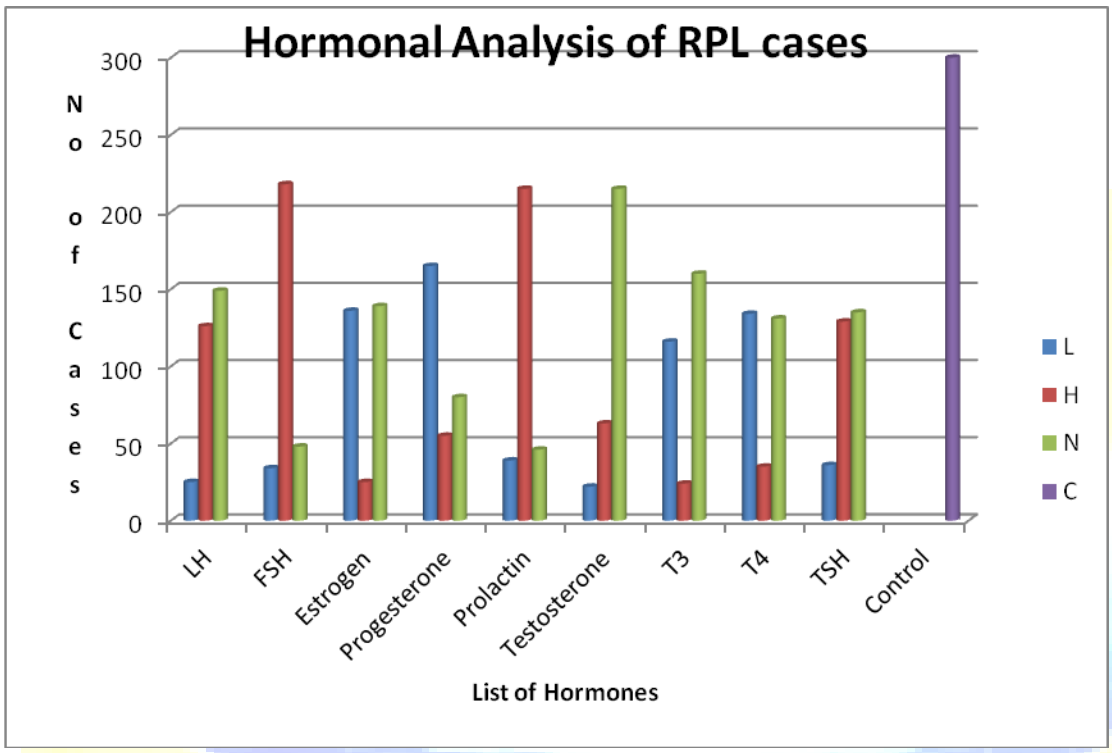
Endocrine etiologies including thyroid disorders, luteal phase defect, and PCOS, may be found in as many as 1 in 5 women with RPL. Based on the available data, we recommend measuring TSH, antithyroglobulin, and antithyroperoxidase antibody levels in the evaluation of patients with RPL. Hypothyroidism and hyperthyroidism can have negative effects on fetal development. Though the association of TSH levels and RPL is minimal it is strongly recommended to screen TSH because both hypothyroidism and hyperthyroidism are easy to correct medically which can facilitate successful pregnancies in future. Endocrine therapeutic strategic approach is recommended to suppress the 'endocrine-mediated' pregnancy loss. In summary, endocrinological abnormalities obsereved in our study shows listed below .Decreased levels of progesterone with 162 cases exhibiting low levels of (54%), decreased level of estrogen in the follicular phase in 136 cases (45.3%). In this study we observed hyperprolactinaemia in 172 which accords for 71.7% which predicts a strong association of Prolactin and RPL. In our study though we observed very few cases which are deviated from the normal range in the follicular phase, the statistical analyses proved significant correlation when compared to the control group. Increased endocrine abnormality was observed in follicular phase than in luteal phase. Therefore it is strongly recommended to analyse both follicular and luteal phase for the evaluation of RPL.

Acknowledgement :

Authors acknowledge DOS in Zoology,University of Mysore,entire team of Molecular reproductive and human genetics laboratory . Our thanks to all the doctors and staff different hospitals for helping us with sample procurement in Myore district and Mediwave institute of medical science. We thank Rajiv Gandhi National Fellowship for the funding.

Conflict of interest: None

Figure1: Summary of variation in hormonal levels observed in both follicular and Luteal phase.



FSH: Follicular stimulating hormone;LH: luteinizing hormone; TSH: Thyroid stimulation hormone.

Table 1: Frequency of pregnancy loss in correlation with different age group.

Age	No of Cases	No of Pl	2 PL	3PL	4PL	5PL	6PL	7PL	8PL
18-20 years	74	2-4.	45	21	11	0	0	0	0
21-25 years	99	2-8.	22	25	29	13	18	4	5
25-30 years	92	2-6.	25	32	41	8	9	0	0
31-35 years	16	2-4.	7	13	6	0	0	0	0
36-40 years	19	2-4.	8	6	5	0	0	0	0

PL: pregnancy loss.

Table2: The frequency of abnormal endocrine test results in both follicular and luteal phase

Hormones	Follicular Phase			Luteal Phase			Control	
	Mean	St Dev	Sig. (2-tailed)	Mean	St Dev	Sig. (2-tailed)	Mean	St Dev
LH	4.94	1.88	0.000	3.78	2.17	0.036	5.22	1.45
FSH	7.25	2.38	0.000	4.26	1.67	0.001	7.91	2.43
Estrogen	7.25	38.43	0.000	1.43	32.58	0.001	94.60	33.60
Testosterone	0.49	0.25	0.000	1.37	28.26	0.0278	0.51	0.25
Prolactin	28.45	12.6	0.000	32.32	27.43	0.000	21.4	9.63
T3	1.27	0.43	0.009	1.15	0.39	0. 362	1.24	0.43
T4	76.62	19.56	0.000	74.27	3.29	0.000	84.77	18.59
TSH	3.29	1.74	0.000	2.94	1.72	0.651	3.01	1.67
Progesterone	0.56	0.21	0.000	0.89	0.31	0.000	1.67	0.24

FSH: Follicular stimulating hormone;LH: luteinizing hormone; TSH: Thyroid stimulation hormone.

Table 3: Represents the percentage of abnormal hormonal levels observed in both follicular and luteal phase.

Hormones	Follicular Phase(%)		Luteal Phase(%)	
	Low	High	Low	High
FSH	5.6	29	2.6	13
LH	4.3	47.3	7	25.3
Estrogen	32.3	4	1.3	4.3
Progesterone	40.3	7.6	14.6	10.6
Prolactin	6	57.3	7	14.3
Testosterone	4.3	16	3	5
T3	29	4.6	9.6	3.3
T4	32	6.3	12.6	5.3
TSH	7.3	30.6	4.6	12.3

FSH: Follicular stimulating hormone;LH: luteinizing hormone; TSH: Thyroid stimulation hormone.

References:

- Abalovich M., Gutierrez S., Alcaraz G., Maccallini G., Garcia A., and Levalle O., 2002.Overt and Subclinical Hypothyroidism Complicating Pregnancy.Thyroid,12(1),pp 63-68.
- Abdalla H., Thum M.Y, 2004. An elevated basal FSH reflects a quantitative rather than qualitative decline of the ovarian reserve. Hum Reprod.,19 ,pp 893–898.
- Albrecht E.D, Aberdeen G.W, Pepe G.J,2000.The role of estrogen in the maintenance of primate pregnancy ,182(2) ,pp 432–438.
- Ando. N., Gorai. I., Hirabuki. T., *et al.*, 1994 Prolactin disorders in patients with habitual abortion. Nippon Sanka Fujinka Gakkai Zasshi, 44 ,pp 650–656.
- Arredondo. F, Noble L.S,2006. Endocrinology of recurrent pregnancy loss**.** Semin Reprod Med. ,24(1) ,pp 33-9.
- Ayabe. T., Tsutsumi. O., Momoeda. M., Yano. T., Mitsuhashi. N., Taketani. Y.,1994.Impaired follicular growth and abnormal luteinizing hormone surge in luteal phase defect. Fertil Steril. ,61(4) ,pp 652-6.
- Balasch .J., Vidal. E., Peñarrubia .J., Casamitjana.R., Carmona. F., *et al.*,2001 .Suppression of LH during ovarian stimulation: analysing threshold values and effects on ovarian response and the outcome of assisted reproduction in down-regulated women stimulated with recombinant FSH. Hum. Reprod,16 (8), ,pp 1636-1643.
- Balen A.H., Tan S.L., and Jacobs H.S.,1993 Hypersecretion of luteinizing hormone: a significant cause of infertility and miscarriage. *Br. J. Obstet. Gynecol.*,100**,** pp 1082–1089.
- Benhadi. N., Wiersinga W.M., Reitsma J.B., Vrijkotte T.G.M., and Bonsel G.J.,2009. Higher maternal TSH levels in pregnancy are associated with increased risk for miscarriage, fetal or neonatal death. Eur J Endocrinol ,160**,**pp 985-991.
- Bopp. B,, Shoupe. D., (1993). Luteal phase defects. The Journal of Reproductive Medicine, 38(5), ,pp 348-356.
- Bulletti, C., Flamigni, C., and Giacomucci, E.,1996 Reproductive failure due to spontaneous abortion and recurrent miscarriage. Hum. Reprod. Update, 2**,**pp 118–136.

- Bussen. S., Sütterlin. M., and Steck. T., (1998).Endocrine abnormalities during the follicular phase in women with recurrent spontaneous. Human Reproduction,14(1) ,pp 18-20 .
- Carla P.R., Ana A.M., 2000. Endocrinopathies Associated with Recurrent Pregnancy Loss. Semin Reprod Med,18(4) ,pp 357-362.
- Chen B.A., Creinin M.D., 2008. Medical management of early pregnancy failure:efficacy.Semin Reprod Med,26(5) ,pp 411-422
- Choi B.C., Polgar K., Xiao L., and Hill J.A .,2000. Progesterone inhibits in-vitro embryotoxic Th1 cytokine production to trophoblast in women with recurrent pregnancy loss. Hum. Reprod, 15 (1) ,pp 46-59.
- Chuang C.C., Chen C.D., Chao K.H., *et al.*,2003. Age is a better predictor of pregnancy potential than basal follicle-stimulating hormone levels in women undergoing in vitro fertilization. Fertil Steril ,79,pp 63– 68.
- Clifford. K., Rai. R., Watson. H., *et al.*,1994. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. Hum Reprod ,9,pp 1328–1332.
- Druckmann.R. , Druckmann Marc.-A.,2005.Progesterone and the immunology of pregnancy . The Journal of Steroid Biochemistry and Molecular Biology, 97(5) ,pp 389–396
- El-Toukhy. T., Khalaf. Y., Hart. R.., Taylor. A., and Braude. P.,2002. Young age does not protect against the adverse effects of reduced ovarian reserveÐan eight year study. Hum. Reprod., 17, 1519-1524. Fertility and Sterility, 74(2) ,pp 394–397.
- Ford H.B., and Schust D.J.,2009.Recurrent pregnancy loss: etiology, diagnosis, and therapy. Rev Obstet Gynecol,2(2) ,pp 76-83.
- Glinoer. D.,2000. Thyroid immunity, thyroid dysfunction, and the risk of miscarriage: a` propos article by Vaquero et al. Mild thyroid abnormalities and recurrent spontaneeous abortion: diagnostic and therapeutical approach. Am J Reprod Immunol,43(4) ,pp 202–203

Glueck C.J. , Awadalla S.G. , Phillips.H., Cameron. D., Wang. P., Robert N.F.,2000. Polycystic ovary syndrome, infertility, familial thrombophilia, familial hypofibrinolysis, recurrent loss of in vitro fertilized embryos, and miscarriage Fertil Steril.,74(2) ,pp 394-397.

- Goswamy R.K., Williams.G., and Steptoe P.C.,1988. Decreased uterine perfusion – a cause of infertility. Hum. Reprod ,3 ,pp 955–959.
- Gürbüz B., Yalti S., Ozden S., Ficicioglu.C.,2004. High basal estradiol level and FSH/LH ratio in unexplained recurrent pregnancy loss. Arch Gynecol Obstet.,270(1) ,pp 37-39.
- Hirahara.F., Andoh. N., Sawai. K., Hirabuki. T., Uemura. T., Minaguchi. H., 1998.Hypecprolacrinemic recurrent miscarriage and results of randomized bromocriptine treatment trials. Fertil Steril, 70 ,pp 246-252
- Homburg. R.,2006.Pregnancy complications in PCOS Clinical Endocrinology & Metabolism ,20(2), ,pp 281–292.
- Hussain. M., El-Hakim. , and Cahill D.J.,2012.Progesterone supplementation in women with otherwise unexplained recurrent miscarriages. J Hum Reprod Sci, 5(3) ,pp 248–251.
- Klump K.L., Keel P.K., Racine S.E., Burt S.A., Burt A.S., *et al*., 2013. The interactive effects of estrogen and progesterone on changes in emotional eating across the menstrual cycle.J Abnorm Psychol ,122 (1) ,pp 131–7.
- Kolibianakis E.M., Albano. C., Camus. M., Tournaye. H., Van Steirteghem A.C., and Devroey. P.,2003. Initiation of gonadotropin-releasing hormone antagonist on day 1 as compared to day 6 of stimulation: effect on hormonal levels and follicular development in in vitro fertilization cycles. J Clin Endocrinol Metab ,88,pp 5632–5637.
- Lee.,Chong. S.,1987. Luteal Phase Defects.Obstetrical & Gynecological Survey.42(5) pp 267-274
- Liddell H.S., Sowden. K., and Farquhar C.M.,1997. Recurrent miscarriage: screening for polycystic ovaries and subsequent pregnancy outcome. Aust. N.Z.J. Obstet. Gynaecol., 37,pp 402–406.
- Lim K.J.H., Odukoya O.A., Li T.C., and Cooke I.D.,1996. Cytokines and immuno-endocrine factors in recurrent miscarriage. Hum. Reprod. Update,2,pp 469–481.

- Linné Y.,2004. Effects of obesity on women's reproduction and complications during pregnancy.Obesity reviews,5(3),pp137–143.
- Macklon N.S., and Fauser 2000. Impact of ovarian hyperstimulation on the luteal phase. J. Reprod. Fertil,55,pp 101–108.
- Nardo L.G., Christodoulou.D., 2007. Anti-Müllerian hormone levels and antral follicle count in women enrolled in in vitro fertilization cycles: relationship to lifestyle factors, chronological age and reproductive history. Gynecol Endocrino,23,pp 486–493.
- Nepomnaschy P.A., Welch K.B., McConnell D.S., Low B.S., Strassmann B.I., England B.G.,2006. Cortisol levels and very early pregnancy loss in humans. Proc Natl Acad Sci U S A,103(10) ,pp 3938-42.
- Ohtsuka. T., Taga. M., Suzuki. T., Minaguchi. H., 1995. Relation between gestational sac diameter, crown-rump length, and maternal serum estradiol, progesterone, and prolactin levels in early pregnancy. Arch Gynecol Obstet,256(1) ,pp 5-8.
- Orhan. B., Aydin. A., 2004. Luteal phase defect: myth or reality. Obstetrics and Gynecology Clinics of North America,31(4) ,pp 727–744
- Penton-Voak I.S. , Perrett D.I., Castles D. L., Kobayashi. T., Burt D. M.,1999. Menstrual cycle alters face preference. Nature, 399,pp 741-742 .
- Pluchino. N., Drakopoulos. P., Wenger J.M., Petignat. P., Streuli. I., *et al* ., 2014. Hormonal causes of recurrent pregnancy loss (RPL).HORMONES, 13(3) ,pp 314-322
- Porter T.F., Scott J.R., 2005.Evidence-based care of recurrent miscarriage. Best Pract Res Clin Obstet Gynaecol,19,pp 85-101.
- Rai. R., Regan. L.,2006. Recurrent miscarriage. Lancet,368,pp 601–611.
- Renzo. Di., Carlo. G., Alberto. M., Miroslava.G., Gerli, S.G.2005. Progesterone and pregnancy.Current Opinion in Obstetrics & Gynecology,17(6) ,pp 598–600.
- Roberto. N., Alan. S., Riccardo. G., Andrea. T., Tiziana M., and Alex. Stagnaro-G.,2010. Increased Pregnancy Loss Rate in Thyroid Antibody Negative Women with TSH Levels between 2.5 and 5.0 in the First Trimester of Pregnancy. J Clin Endocrinol Metab, September,95(9) ,pp 44 –48.

- Roberts C.P., and Murphy A.A.,2000. Endocrinopathies associated with recurrent pregnancy loss. Seminars in Reproductive Medicine,18,pp 357 – 362.
- Schindler A.E.,2004.First trimester endocrinology: consequences for diagnosis and treatment of pregnancy failure. Gynecological Endocrinology,18(1) ,pp 51-57 .
- Smith M.L., Schust D.J.,2011. Endocrinology and recurrent early pregnancy loss.Semin Reprod Med,29(6) ,pp 482-90
- Stephenson M.D.,1996. Frequency of factors associated with habitual abortion in 197 couples. Fertil Steril,66,pp 24–29.
- Styne-Gross. A., Elkind-Hirsch. K., and Scott R.T.,2004.Value of basal FSH concentrations: prognostic implications for pregnancy outcome. Human Reproduction,19(10),pp2426-2427.
- Styne-Gross. A., Elkind-Hirsch. K., Scott. R .,2005. Obesity does not impact implantation rates or pregnancy outcome in women attempting conception through oocyte donation. Fertil Steril,83,pp 1629 –1634.
- Tavaniotou. A., Albano. C., Smitz. J., and Devroey.P.,2001.Comparison of LH concentrations in the early and mid-luteal phase in IVF cycles after treatment with HMG alone or in association with the GnRH antagonist Cetrorelix. Human Reproduction,16(4),pp 663-667.
- Trout S.W., Seifer D.B.,2000. Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values? Fertility & sterility,74,pp 335-337.
- Tulppala. M., Palosuo. T., Ramsay. T., *et al.*,1993 A prospective study of 63 couples with a history of recurrent abortion: contributing factors and outcome of subsequent pregnancies. *Hum. Reprod.*, 8, pp764–770
- van Montfrans J.M., van Hooff M.H.A., Tanahatoe S.J., Sadrezadeh. S., Martens. F., *et al.*, 2004.Basal FSH concentrations as a marker of ovarian ageing are not related to pregnancy outcome in a general population of women over 30 years. Human Reproduction,19(2),pp 430-434.

- Wentz A.C., Herbert C.M., Maxson W.S., Garner C.H.,1984. Outcome of progesterone treatment of luteal phase inadequacy.Fertility and Sterility ,41(6),pp 856-862.
- Westergaard, L.G., Laursen S.B., and Andersen C.Y., 2000. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum. Reprod,15,pp 1003–1008.