J Obstet Gynecol India Vol. 57, No. 5 : September/October 2007 Pg 391-397

REVIEW ARTICLE

The Journal of Obstetrics and Gynecology of India

Polycystic ovarian syndrome as a cause of recurrent pregnancy loss

[1] Duru Shah, [2] Sukhpreet Patel

[1] Director and [2] Consultant, Gyanecworld, Mumbai - 400 026.

Introduction

Women with polycystic ovaries (PCOS) frequently present with reproductive dysfunction. However, when these women do finally achieve pregnancy, after intensive treatment, they are faced with a substantially high risk of miscarriage in the first trimester. This review deals with the pathophysiology and management of pregnancy loss in women with PCOS.

Incidence

Recurrent miscarriage, described as the loss of three or more consecutive pregnancies, affects 1% of couples trying to conceive. Amongst the various causes , PCOS is the most commonly identified ultrasound abnormality amongst women with recurrent pregnancy loss [1]. Its incidence ranges from 40 to 56% of women with recurrent pregnancy loss. Conversely, rate of early pregnancy loss is reported to be 30 to 50% in women with PCOS, which is 3 fold higher than the rate of 10 to 15% in normal women [2].

The corpus luteum (CL) of normal early pregnancy

It is clear that a fully functional CL is an important prerequisite for both nidation and the normal progress of early pregnancy. Normal formation and function of the CL and optimal endometrial preparation for implantation of the conceptus depend on a number of factors, a deficiency of any one of which may result in luteal phase inadequacy and thereby predispose to early pregnancy wastage.

The duration of luteal function in the nonfertile menstrual cycle is limited to no more than 10 to 15 days [3]. Theoretically, each menstrual cycle is potentially a fertile one. The mechanism responsible for spontaneous luteolysis, viz., the timely and inevitable demise of the CL when pregnancy fails to occur, remains unknown despite considerable investigations. An important feature of the endocrinology of the early pregnancy is the prolongation or maintenance of luteal function, a process commonly known as CL rescue. Luteal support remains essential until approximately the seventh week of gestation, presumably the time when the trophoblast has acquired sufficient steroidogenic capacity to support the pregnancy by itself. Lutectomy induces abortion in most women if performed prior to 7 weeks of gestation whereas removal of the CL after that time has no effect.

Levels of human chorionic gonadotrophin (hCG) first become measurable in the peripheral circulation by 8 to 10 days following ovulation. hCG appears to be the principal factor responsible for the prolongation of CL function. Luteal phase administration of hCG significantly increases progesterone production and postpones the otherwise inevitable regression of the CL in both women and monkeys. Whether hCG truly acts to rescue the CL by applying a direct tropic stimulus or acts indirectly by inhibiting normal luteolysis is unclear. In any event the CL eventually regresses despite the continued presence of hCG. This refractoriness of the CL to further stimulation apparently results from an uncoupling of the luteinizing hormone LH/hCG receptor with adenylate cycle rather than a down regulation phenomenon since total receptor concentrations remain essentially unchanged during this time.

It is very clear that progesterone is the principal factor responsible for the conversion of a proliferative to a secretory endometrium, a transition that renders the endometrium receptive to the arrival of the conceptus. In in vivo studies it has been shown that progesterone is the only steroid hormone necessary to induce complete decidualization and subsequent decidual prolactin (PRL) synthesis. Under the continued influence of progesterone, the secretory endometrium produces a number of specific proteins that are probably crucial to the implantation process. Progesterone may also inhibit uterine prostaglandin production and has been

Paper received on 25/07/2007; accepted on 25/08/2007

Correspondence :
Dr. Duru Shah
Gynaecworld, Kwality House
Above Chinese Room
Kemps Corner, Mumbai - 400 026.
durushah@hotmail.com

implicated as a factor in conferring immunologic privilege to the embryo, thereby preventing its rejection by the maternal immune system.

The CL in PCOS

Morphologically, PCOs have been shown to have increased number of follicles developing from the primordial stage [4]. Although the reason for this is not known it may be the result of a relative decrease in follicular atresia, with failure of apoptosis [5]. In certain ovarian studies a large number of ovaries were collected from women undergoing total abdominal hysterectomy and bilateral salpingo-oophorectomy for benign gynecological disease. By gross appearance and microscopic dissection a group of ovaries which showed the morphological features of PCO, but which contained a dominant follicle or a CL were identified and collected from women who reported regular cycles [6]. Within these ovaries an apparent increase in the number of degenerating CL and corpora albicantia was noticed compared with normal ovaries. This may be due to a disturbance in the normal process of luteal regression which proceeds, at least in part, by apoptosis. This would provide an additional indicator of a failure of this process in PCO.

Vascular endothelial growth factor (VEGF), one of the major factors associated with angiogenesis and change in vascular permeability, plays an important role of in the primate CL [7]. In women with PCOS the levels of VEGF are high, and the ovaries show increased vascularity [8]. Morales et al [9], have introduced the concept of interaction between the occurrence of blood in the luteal cavity with the nature of the final demise of the CL suggesting that only CL in which a blood clot and a large luteal cavity are present progress to formation of true corpora albicantia. In the absence of such a blood clot, they believe that the CL does not undergo extensive hyalinization resulting in the formation of a corpus albicans but, instead, involutes to form small clusters or scars of pigment-filled granulosalutein cells. If the enhanced vascularity in the PCO is associated with increased vascular permeability, then this may account for the finding that the occurrence of hemorrhage in and around the CL of PCO is greater than that observed in luteal tissue from morphologically normal ovaries. Additionally, it might explain the increased number of corpora albicantia found in PCO .

A recent study aimed to study any obvious morphological differences in CL structure between normal and ovulatory PCO. However, it was unable to demonstrate so. However, it remains to be determined whether there are differences in the occurrence of apoptosis and regression or changes in the vasculature of the ovulatory PCO's CL that could result in the persistence of regressing luteal structures or affect luteal steroidogenesis.

Pathophysiology of recurrent pregnancy loss in PCOS

The suggested mechanisms for PCOS related pregnancy loss are various. Previous studies have reported that women who are either hyperandrogenic or who hypersecrete LH are at an increased risk for pregnancy loss following either spontaneous or assisted conception. However, a recent study has revealed no significant difference in the prospective live-birth rate between those women with either an elevated serum follicular phase LH concentration (>10 IU/L) or an elevated testosterone level (>3nmol/L) compared to those with normal levels [10] . Besides, Clifford et al [11], in a prospective randomized placebo controlled study revealed that the prepregnancy pituitary suppression of high endogenous LH does not improve the live birth rate of women with recurrent miscarriage and PCO.

Hyperinsulinemia seems to be the consistent factor in PCO related pregnancy loss. It is an independent risk factor for early pregnancy loss and is found to decrease levels of glycodelin and insulin growth factor protein-1 (IGFBP-1), two major endometrial proteins. Jakubowicz et al [12] compared the serum glycodelin and serum IGFBP-1 levels in women with PCOS who experienced early pregnancy loss with those who did not. Their study revealed lower serum glycodelin levels during week 3 to 8, and lower serum IGFBP-1 during week 9 to 11 in women with early pregnancy loss with PCOS. This finding could implicate endometrial epithelial and stromal dysfunction during preimplantation and early pregnancy as a possible mechanism for early pregnancy loss in PCOS. These decreases are likely to be secondary to hyperinsulinemia and reduced sensitivity.

Management of recurrent pregnancy loss in PCOS

After a brief explanation of the pathogenesis of recurrent pregnancy loss in PCOS, we can conclude that its management involves two main areas. Firstly, the reduction of insulin, and secondly, providing progesterone support.

Insulin lowering drugs

Since hyperinsulinemia is an independent risk factor for early pregnancy loss in PCOS, its correction should reduce the miscarriage rates in these patients.

Administration of metformin throughout pregnancy to women with PCOS reduces the rate of pregnancy loss dramatically from 41.9% to 8.8% [13]. Thus, the rate of early pregnancy loss of 8.8% in women treated with metformin is similar to the rate of 10 to 15% reported for clinically recognized pregnancies in normal women, suggesting that insulin resistance is an independent risk factor for PCO related pregnancy loss.

Besides, insulin reduction with metformin increases follicular and luteal phase serum glycodelin and IGFBP-1 concentrations and enhances luteal phase uterine vascularity and blood flow in women with PCOS [14]. These changes may reflect an improved endometrial milieu for the establishment and maintenance of pregnancy with metformin therapy.

To minimize side effects such as nausea, many clinicians now recommend that metformin be started at a dose of 500mg once daily after dinner for one week and then gradually increased to 500mg twice daily for a week, and finally to 500mg three times a day. Continuation of metformin throughout pregnancy is however controversial. Metformin is a pregnancy Category B drug, and although there have been no reports of fetal abnormalities, earlier reports suggested stopping the drug as soon as pregnancy is diagnosed [15].

Progesterone support

Since progesterone is the essential hormone during the first trimester of pregnancy, early pregnancy support in the form of progesterone supplementation is a logical treatment modality.

Progesterone supplementation should be started in the luteal phase and continued until 7 to 8 weeks gestation, when the placenta assumes the role of progesterone production and an ultrasound can document fetal normality. Initiation of progesterone therapy after missing menses is not adequate, because the nidation site has not been properly prepared. Table 1 depicts progestogen used for supporting pregnancy.

Table 2. Progestogens used for early pregnancy support.

1.	Natural Progesterone /Micronized progesterone
	oral
	vaginal
	rectal
	intramuscular
2.	Other Progestogens
	dydrogesterone
	17 alpha hydroxyprogesterone caproate
	allylestrenol

Natural progesterone

Micronized progesterone

The clinical usefulness of progesterone has been limited by its poor absorption after oral administration and its susceptibility to rapid first pass liver metabolism. A number of synthetic oral progestogens have been developed to overcome the low oral bioavailability of progesterone. However, the use of synthetic progestational agents in pregnancy has been condemned. Compared to the natural hormone, synthetic progestins may have a luteolytic effect on the CL and can produce glandular stromal disparity, thereby worsening rather than improving the situation. Furthermore, because 19 nor progestins can masculinise a female fetus and possibly result in cardiovascular and limb reduction defects, their use in early pregnancy is contraindicated.

Several oral micronized progesterone formulations have been developed to overcome the problems associated with the absorption of previously available oral progesterone preparations and the adverse metabolic effects of the synthetic progestogens. Micronization in combination with lipophilic vehicles enhances absorption. A natural progesterone formulation with improved bioavailability would prove a distinct advantage over other available therapies [16].

Progesterone is lipophilic in nature and diffuses freely into cells, where it binds to the progesterone receptors and exerts progestational activity. The steroid-receptor complex binds to DNA in the nucleus, thereby inducing the synthesis of specific proteins.

Micronised progesterone may be administered orally or parenterally. However, orally delivered progesterone has low bioavailabity [12]. Furthermore, metabolites of orally administered progesterone may induce significant hypnotic effects. It may also be administered by vaginal or rectal suppositories. Vaginal suppositories are associated with an unpleasant discharge and serum level after their administration is influenced by the vehicle in which the steroid is given [17].

Intramuscular natural progesterone

The intramuscular route remains the most reliable method of achieving predictable serum progesterone levels. However, it is uncomfortable as it requires daily injections to maintain appropriate serum concentrations. This may be of concern for patients who are in need of a long-term treatment for pregnancy support. Furthermore, intramuscular progesterone administration may lead to marked inflammation at the injection site, resulting in redness, pain and even sterile abscess formation.

Pharmacokinetics of natural progesterone

Progesterone is well absorbed when administered rectally or vaginally. Rectal or vaginal administration of 100-400mg produces concentrations in the luteal range, which are maximal within 1 to 8 hours and then decline over 24 hours.

Devroey et al [18] and Cornet et al [4] have demonstrated increased bioavailablity and reduced variability of absorption when an

oral micronized progesterone preparation was administered vaginally. By using the vaginal route of administration, these investigators suggested that there is less intra- and interindividual variability than after oral administration and that the higher bioavailability and more sustained serum levels provide a more physiologic endometrial response, especially when full secretary transformation is required.

Progesterone is almost completely absorbed after administration by oral route when its preparation is micronized, but due to the important metabolic inactivation during the first hepatic pass, bioavailability of oral progesterone is notably poor reaching values lesser than 10% [19].

Intramuscular administration of progesterone is followed by rapid absorption. High plasma concentrations are reached within 8 hours [20]. Serum progesterone levels are more sustained after intramuscular than vaginal or oral use, suggesting that the intramuscular site of injection may function as a depot.

Vaginal or rectal micronized progesterone can be used in a range of 600 to 800µg per day in two to three divided doses. It should preferably be started immediately after ovulation and continued till 7 to 8 weeks of pregnancy.

Natural progesterone is also available as oil based injection. The recommended dosage is 50 to 100mg deep intramuscularly daily.

Vaginal or intramuscular progesterone : the first uterine pass effect

The ability of natural vaginal progesterone to induce secretory transformation of the endometrium has been repeatedly demonstrated in literature.

Experiments with vaginal progesterone administration have shown endometrial tissue concentrations of progesterone relative to low serum concentration. These findings led to the postulate of a first uterine pass effect for drugs administered vaginally.

The comparisons between vaginal and intramuscular progesterone have provided contradictory results. Various studies have advocated the use of the vaginal route due to higher implantation and lower miscarriage rates [21] or due to better histological findings [22]. Because of better steady progesterone concentrations. Some have supported the intramuscular route [23].

Miles et al [24] using a human ex-vivo uterine perfusion model demonstrated the first uterine pass effect. Tritiated progesterone was directly applied over the rim of vaginal tissue remaining attached to the cervix and removed at time of hysterectomy. Progesterone had diffused to the entire uterus and reached a steady state at the end of 4-5 hours. Concentrations of 185±155mg per 100mg of myometrial tissue were attained 4 hours after application of progesterone. The authors concluded that a 'first uterine pass effect' occurs when drugs are delivered vaginally, thereby providing an explanation for the high uterine concentrations relative to the low serum concentration observed after vaginal administration.

There is increasing evidence in literature that serum progesterone concentrations after vaginal progesterone application is not indicative of the effect that vaginally administered progesterone has on the endometrium [19].

Significantly higher progesterone concentrations were detected in the uterine artery than in the radial artery in postmenopausal women undergoing hysterectomy who received micronized progesterone in an oil based solution before the surgery, providing further evidence of the preferential drug distribution to the uterus after vaginal application.

Mechanisms of uterine tropism of vaginal progesterone

Theoretically, at least four different mechanisms can explain this phenomenon:

1. Passive diffusion through tissues: The direct vagina to uterus diffusion through tissues has been demonstrated in the study by Miles et al [24].

2. Passage through the cervical lumen: Passage through the cervical canal has been proposed as a possible mechanism explaining the direct diffusion from the vagina to the uterus. Recent work has provided evidence of intraluminal transport activated by uterine peristalsis [25].

3. Absorption through venous or lymphatic systems: Hormones placed in the vagina are absorbed as through other mucosas. Lymph plays an equally important role in absorption of hormones as venous blood, as it has been recognized as an important carrier of steroid hormones.

4. Countercurrent transport: Countercurrent transport is a physiological exchange mechanism known to take place between fluids flowing in opposite directions. Support for the hypothesis of local transfer between vessels resulting in countercurrent vagina to uterus transport was provided by Einer-Jensen [8]. These authors found higher progesterone conccentrations in the uterine arterial

blood of pigs as compared with other arteries after vaginal administration of progesterone.

The comparison between intramuscular and vaginal progesterone has led to controversial results as regards the superiority of one or the other in inducing secretory endometrial transformation. However, there is increasing evidence in literature to favor the use of vaginal progesterone.

Safety of natural progesterone

There is no reliable evidence that indicates natural progesterone to be teratogenic. If used in physiological doses there is no reason to expect any fetal malformation.

Addition of estradiol to natural progesterone

Since luteal phase endometrium also requires estradiol support, various studies have been attempted to investigate whether hormone substitution with progesterone and estradiol would be more successful in improving pregnancy rates. Although Fateni et al [26] demonstrate that the addition of estradiol to progesterone in the luteal phase after stimulation does not enhance the probability of pregnancy, Gorekemli H et al [27] others show an increase in implantation and pregnancy rates.

Gleicher et al [28] have further demonstrated that combined estradiol and progesterone substitution of the luteal phase of ovulation induction cycles increases the overall pregnancy rate, especially in women below the age of 38 years and in nulliparous females.

Human chorionic gonadotrophin (hCG)

Human chorionic gonadotropin (hCG), a polypeptide hormone produced by the human placenta, is composed of an alpha and a beta subunit. The alpha subunit is essentially identical to the alpha subunits of the human pituitary gonadotropins, LH and FSH, as well as to the alpha subunit of human TSH. The beta subunits of these hormones differ in amino acid sequence.

Commercially hCG is available as a water soluble glycoprotein derived from human pregnancy urine. The sterile lyophilized powder is stable. When reconstituted the solution should be refrigerated and used within 30 days.

Administration of hCG stimulates the CL to produce progesterone. It is only in situations where the LH receptor population is inadequate that it may not be effective. In certain circumstances direct progesterone supplementation is more effective than hCG administration to improve the luteal function. Progesterone production is improved by the administration of hCG only in a normally functioning CL whereas its effect is minimal on a malfunctioning CL. If the CL is hyporesponsive to hCG during the transitional period of early pregnancy, treatment with exogenous hCG is not likely to be helpful. But if there is a specific defect in postovulatory LH secretion or in trophoblastic hCG production, then exogenous administration of hCG would be useful. hCG has a longer half-life than LH which is an advantage in treating inadequate luteal function. But hCG therapy is more expensive and cannot be self-administered.

Dose: The recommended dose and frequency of hCG administration are empiric and vary among the same investigators. In order to achieve complete luteinisation of the preovulatory follicle, 10,000 IU of hCG should be administered at the approximate time of ovulation followed by a dose of 1500-5000 IU every 3-4 days. Treatment is stopped after the 12th postovulatory day to avoid a high incidence of pseudopregnancy and with the assumption that if pregnancy is achieved, exogenous hCG should no longer be necessary. Because of its long half-life (about 12 hours), pregnancy testing is rendered invalid for 7 days after the last hCG injection.

The steroidogenic response of the deficient CL depends on the timing of hCG administration. When hCG treatment is begun immediately after the LH surge, there is no increase in estradiol and progesterone concentrations. Yet the same treatment regimen begun on luteal day 5 produces an immediate and marked steroidogenic response that remains sustained throughout the luteal phase. When hCG treatment is begun in the midluteal phase, circulating levels of progesterone closely approximate the pattern normally observed in early pregnancy; concentrations transiently increase but then decline despite continued treatment whereas estradiol levels remain elevated. When hCG is first administered in the late luteal phase, the progesterone response is much more brief and attenuated with levels falling steadily during treatment. This uncertainty of response, along with mode of administration and the problem of detecting an early pregnancy with hCG use makes this treatment unattractive as a first choice. But if there is non-availability of natural progesterone, then hCG would be a better option as compared to a progestational agent. Momoeda et al [29] studied whether luteal phase defect is in part, causally related to insufficient gonadotrophin stimulation. and found that hCG readily stimulated progesterone production in nonfunctioning CL whereas its stimulating effect is minimal in functioning corpus luteum. Hence they suggest that luteal phase deficiency is not caused by inadequate GnRH stimulation and does not benefit from hCG administration.

Safety

hCG has been included in the pregnancy category C of the FDA classification. Animal reproduction studies have not been conducted with hCG. It is also not known whether hCG can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. hCG should be given to a pregnant woman only if clearly needed.

Other recommended Therapies

The efficacy and safety of combined enoxiparin – metformin was prospectively assessed in women with PCOS with one or more previous spontaneous abortion, thrombophilia, and / or hypofibrinolysis. This combination was found to reduce pregnancy loss in these women with PCOS.

Although earlier studies [14,15] have reported the reversal of the apparently deleterious effects of high LH, by LH suppression with GnRH analogues [12], more recent studies have failed to do so [11].

Conclusion

In conclusion, polycystic ovaries are found more often amongst women with recurrent pregnancy loss. Hyperinsulinemia seems to be the key factor responsible for this pregnancy wastage. The use of insulin lowering drugs is therefore, the mainstay of therapy for women with PCOS and recurrent pregnancy loss. Adjuvant therapy with support in the form of progesterone and hCG has also been recommended.

References

1. Gallardo-Lozano E, Ayon P, Neuspiller F. A study of two different routes of administration of micronized progesterone in assisted reproduction.Ginecol Obstet Mex 2004;72:407-10.
2. Bulletti, C, deZiegler, D, Flamigni,C,et al,(1997) Targeted drug delivery in gynecology; the first uterine pass effect. Hum Reprod1997; 12:1073-9
3. Glueck CJ, Wang P, Goldenberg N et al. Pregnancy loss, polycystic ovary syndrome, thrombophilia, hypofibrinolysis, enoxaparin, metformin. Clin Appl Thromb Hemost 2004;10:323-4.
4. Cornet D. Alverez S. Antoine JM et al. Pregnancies following ovum donation in gonadal dysgenesis. Hum Reprod. 1990:5:291-3.
5. Dallenbach, Hellnig G; The endometrium in natural and artificial luteal phase. Human Reprod 1988;;3:165-8.
6. Devroey P, Palermo G, Bourgam C et al. (1989). Progesterone administration in patients with absent ovaries. Int J Fertil 1989; 34:188-93.
7. Devroey P. Palermo G. Bourgain C et al. Progesterone administration in patients with absent ovaries. In J Fertil 1990;34:188:93.
8. Einer-Jensen N. Countercurrent transfer between blood vessels in the ovarian adnexa. Acta Obstet. Gynecol Scand1992;71;566-7.
9. Morales, C, Garcia-Pardo L, Reymundo, C et al. Different patterns of structural luteolysis in the human corpus luteum of menstruation. Hum Reprod 2000;15:2119–28.
10. Rai R, Backos M, Rushworth F et al. Polycystic ovaries and recurrent miscarriage- a reappraisal. Hum Reprod. 2000;15:612-5.
11. Clifford K, Rai R, Watson H et al. Does Suppresing luteinising hormone secretion reduce the miscarriage rate ? Results of a randomised controlled trial. BMJ 1996;312:1508-11.
12. Jakubowicz D, Iuorno M, Jakubowicz S et al. Effects of metformin on early pregnancy loss in the Polycystic Ovary Syndrome. J Clin Endocrinol Metab.2002;87:524-9.
13. Hargrove JT. Maxson WA. Wentz AC. Absorption of oral progesterone is influenced by vehicle and particle size. Am J Obstet Gynecol.1989;161:948-51.
14. Homburg R, Levy T, Berkowitz D et al. Gonadotropin releasing hormone agonist reduces the miscarriage rate for pregnancies achieved in women with polycystic ovary syndrome. 1993 Fertil Steril 1993;59:527-31.
15. Homburg, R, Amsterdam A. Polycystic ovary syndrome – loss of the apoptotic mechanism in the ovarian follicle? J. Gynaecol. Invest 1998;21:552–7.
16. Hughesdon PE. Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called 'hyperthecosis'. Obstet. Gynecol. Surv.1988;37:59–77.
17. Jakubowicz D, Seppala M, Jakubowicz S et al. Insulin reduction with metformin increases luteal phase serum glycoden and insulin-like growth factor-binding protein 1 concentrations and enhances uterine vascularity and blood flow in the polycystic ovary syndrome. J Clin Endocrinol Metab 2001;86:1126-33.
18. Devroey P, Smitz J, Camus M et al. Progesterone administration in patients with absent ovaries. Int J Fertil 1989;34:188-93.
19. Magness RP, Ford SP. Estrone, estradiol-17 beta and progesterone concentrations in uterine lymph and systemic blood throughout the porcine estrous cycle. J. Anim Sci 1983;57:449-55.
20. Mason HD, Willis DS et al. Estradiol production by granulosa cells of normal and polycystic ovaries: relationship to menstrual cycle history and concentrations of gonadotropins and sex steroids in follicular fluid. J. Clin. Endocrinol. Metab 1994;79;1355–60.
21. Lunn SF, Fraser HM, Mason HD. Structure of the corpus luteum in the ovulatory polycystic ovary. Hum Reprod 2002 J;17:111-7
22. Salat-Baroux, J, Cornet, D, Alvarex S et al. (1988). Pregnancies after replacement of frozen-thawed embryos in a donation program. Fertil Steril 1988;49:817-21
23. Simon J, Robinson D, Andrews, M et al. The absorption of oral micronized progesterone: the effect of food, dose proportionally and comparison with intramuscular progesterone. Fertil Steril 1993; 60.26-33.
24. Miles. R, Paulson. R, Lobo. R et al (1994) Pharmacokinetics and endometrial tissue levels of progesterone after administration by intramuscular and vaginal routes a comparative study. Fertil Steril 1994;62:485-90.
25. Kunz G, Herbertz M, Noel M et al. (1998). Sonographic evidence for the involvement of the utero-ovarian countercurrent system in the ovarian control of directed uterine sperm transport. Hum Reprod Update,1998;4:667-72.

26. Fatemi HM, Kolibianakis EM, Camus M. Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: a randomized controlled trial. Hum Reprod.2006;21:2628-32.

27. Gorkemli H, Ak D, Akyurek C et al. Comparison of pregnancy outcomes of progesterone or progesterone + estradiol for luteal phase support in ICSI-ET cycles. Gynecol Obstet Invest 2004;58:140-4.

28. Gleicher N, Brown T, Dudkiewicz A et al. Estradiol/progesterone substitution in the luteal phase improves pregnancy rates in stimulated cycles—but only in younger women. Early Pregnancy. 2000 ;4:64-73.

29. Momoeda M, Tsutsumi O, Morita et al. Differential effect of exogeneous hCG on progesterone production from normal or malfunctioning corpus luteum. Hum Reprod 1998;13:1907-11.