**Original Article**

**Progesterone Therapy Administered 24 hours Before Embryo Transfer in ICSI Cycle Improves Embryo Implantation and Pregnancy in Women With Luteal Phase Defect.**

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**Summary**

**Background:** Ovulation induction by human menopausal gonadotrophin (HMG) results in temporal luteal phase defect. Luteal support therapies are required to support embryo implantation in stimulated cycle especially in luteal phase defect infertile women.

**Objective:** The objective of the present study was to investigate the clinical significance of progesterone, aspirin and HCG on human embryo implantation in women with luteal phase defect following ICSI and embryo transfer (ET).

**Patients and Methods:** The female patients were divided into six groups depending on the type of the luteal support protocols (LSP). Group 1 (No= 54), received 10 mg oral progesterone (P), group 2 (No= 35) received P plus HCG, group 3 (No= 59) received P plus HCG plus oral aspirin, group 4 (No= 47) received vaginal P administered 24 hours before embryo transfer plus oral aspirin, group 5 (No= 40) received vaginal P administered 12 hours after embryo transfer plus oral aspirin and group 6 (No= 46) received intramuscular P plus oral aspirin. The LSP were continued for at least 12 weeks, when the B-HCG test was positive, (tested two weeks after embryo transfer).

**Results:** Statistical analysis of the clinical data showed no significant differences between the LSP in regard to patient's age, body mass index (B/M2), basal FSH/LH ratio and estradiol concentration at the day of HCG injection. The ICSI rate, percentage s of embryos developed in vitro, and the numbers of the transferable quality embryos were similar in all groups (P>0.05). The pregnancy rate was significantly higher (P < 0.05), in group 4 compared to other groups (38.66% versus 24.51%(G 1), 22.53% (G 2), 28.66% (G 3), 25% (G 4), 21.60% (G 5). The percentages of viable fetal sac development per patient were 31.49 (17/54), in G 1, 42.86 (15/35), in G 2, 49.16 (29/59), in G 3, 59.58 (28/47), in G 4, 32.50 (13/40), in G 5, and 34.79 (16/46), G 6. The percent of viable gestation sac was significantly higher in group 4 compared to other groups (P < 0.05).

**Conclusions:** The administration of 400 mg /day vaginal progesterone 24 hours before ET and 100 mg/day aspirin five days after ET results in significant improvements in pregnancy and embryo implantation rates and development of viable fetuses in luteal phase defect infertile women undergoing ICSI-ET.

**Key Words:** Embryo Implantation, ICSI-ET, Vaginal Progesterone and Aspirin Therapies

**Introduction:**

Progesterone is required for preparation of the uterus for embryo implantation and stabilizes the endometrium during pregnancy. It is well known that any reduction in the concentration of serum progesterone during early stages of pregnancy (specially during the first seven weeks) results in abortion (1-2). Women with luteal phase defect (LPD) are characterized by abnormal corpus luteum function associated with inadequate progesterone secretion. The term LPD is also applied to those women who have a short luteal phase (<11 days period between ovulation and menses) and/or inadequate progesterone action at the level of endometerium, despite its normal production (3). LPD is often due to progesterone production by the corpus luteum and the factors responsible for this dysfunction may be multiple. These factors include reduction in the concentration of FSH in the follicular phase of the menstrual cycle, abnormal secretion of LH and abnormal response of endometerium to progesterone. It has been estimated that 50-60% of infertile women have some sort of LPD (4). Hyperprolactinemia and hyperthyroidism may be associated with LPD in infertile women with abnormal ovulatory cycles and progesterone concentrations (5).

Ovulation induction by exogenous gonadotropin administration in infertile women during in vitro
Materials and Methods

The mean age of the women was 31.8 years with a mean of 8.6 years infertility duration. The age of the husbands was 36.2 years. The mean motility of the sperm was < 50% and mean sperm concentration was > 20 million. The female patients were diagnosed with luteal phase defect. The progesterone concentration on day 21 of the menstrual cycle was < 10 ng/ml. The female patients were grouped into 6 groups depending on the type of luteal support protocols (LSP). Group one (No= 54) received 10 mg of oral progesterone (P) three times per day (Duphston 10 mg tablet, Solvay, U.K.). Group two (No= 35) received P and 1500 international units of human chorionic gonadotropin (HCG) intramuscularly every 10,000 IU HCG (HCG, Profassi, 5000 IU per ampoule, Serano Co., Italy) from cycle day two of menstrual cycle for 10 to 13 days. The dose of HMG was dependent on ovarian response. The basal levels of FSH and LH were assayed on cycle day two. The follicular growth was monitored by serial estradiol concentration assay and transvaginal sonography measurements on cycle day 8, 10, 12 and 13. When the follicular sizes reached to > 16 mm and the estradiol concentration was between 200 - 300 pg/ml/follicle, the patients were injected with 10,000 IU HCG (HCG, Profassi, 5000 IU per ampoule, Serano Co., Italy) to induce the final maturation stage of the dominant follicles. In case of high responders with multiple small follicles and > 3000 pg/ml estradiol concentration, the HCG injection was postponed to avoid the risk of ovarian hyperstimulation syndrome.

After 35 to 36 hours of HCG injection the patients were prepared for oocyte retrieval and ICSI and ET.

Oocyte Aspiration and ICSI

The oocytes were aspirated by using a vaginal probe transducer (7.0 MHZ, Bruel and Kjaer, Denemark) with a fixed needle guide. A Casmid aspiration needle (Casmid 16g double lumen, Surrey, UK) was used for ovum aspiration. The needle tip was introduced in side the lumen of the dominant follicle and a negative pressure (100-120 mm Hg) was applied by the section pump (Craft suction pump, Craft, London, UK) to aspirate the follicular fluid. All the dominant follicles were aspirated and the follicular fluid was examined under microscope, (Wild M3 high power dissecting microscope, Heerbrugg, Switzerland). The oocytes were cultured in Medicult IVF culture medium (Medicult IVF Co., Denmark) for 4 to 6 hours prior to ICSI.

The oocytes were examined for normality and transferred to hyaluronidase culture medium to remove the cumulus and corona cells. The concentration of hyaluronidase in the culture
of the mean. One way analysis of variance was used for statistical analysis of the data and a P value <0.05 was considered statistically significant (P<0.05). Student t-test, Chi-square and Bonferroni Chi-Square were used to detect the levels of statistical significances (15).

### Results

The clinical data of the female patients are shown in table one. The age of the women, and body mass index were not significantly different between the luteal support protocols (LSP). The basal follicle stimulating hormone / luteinizing hormone (FSH/LH) ratio and the concentration of estradiol at the day of human chorionic gonadotropin (HCG) injection were not significantly different in the LSP (P>0.05).

Table 1 The clinical data of the female patients involved in intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) with different luteal support protocols.*

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<tbody>
<tr>
<td>Number (patient)</td>
<td>54</td>
<td>35</td>
<td>59</td>
<td>47</td>
<td>40</td>
<td>46</td>
</tr>
<tr>
<td>Age (year)</td>
<td>30.8 +/- 4.2</td>
<td>31.1 +/- 2.1</td>
<td>28.1 +/- 3.8</td>
<td>29.8 +/- 2.1</td>
<td>31.2 +/- 2.5</td>
<td>30.4 +/- 3.8</td>
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<tr>
<td>BMI (Kg/m²)</td>
<td>27.7 +/- 2.5</td>
<td>29.5 +/- 2.6</td>
<td>25.6 +/- 1.4</td>
<td>26.9 +/- 2.7</td>
<td>24.3 +/- 3.7</td>
<td>28.7 +/- 3.6</td>
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<tr>
<td>FSH/LH</td>
<td>2.78 +/- 0.3</td>
<td>1.89 +/- 0.2</td>
<td>2.05 +/- 0.1</td>
<td>1.94 +/- 0.2</td>
<td>2.54 +/- 0.5</td>
<td>2.34 +/- 0.1</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1670 +/- 86</td>
<td>1550 +/- 36</td>
<td>1410 +/- 64</td>
<td>1565 +/- 45</td>
<td>1329 +/- 48</td>
<td>1304 +/- 43</td>
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*P>0.05 significantly not different between protocols

BMI: body mass index

FSH/LH: basal FSH/LH ratio

### Statistical Analysis

The data were presented as mean +/- standard error...
The clinical outcome of ICSI-ET is shown in Table 2. The number of the mature oocytes in all luteal support protocols (LSP) was not significantly different ($P>0.05$). The ICSI rate in the protocol three was significantly different from protocol four (83.3 versus 74.5, $P<0.05$). The number of the transferable quality embryos per patient was similar in all LSP groups ($P>0.05$). The pregnancy rate was significantly higher in protocol four compared to other protocols (38.3 versus 24.1, 22.9, 28.8, 25, 21.7, $P<0.01$).

Table 2: ICSI outcome in luteal phase defect infertile women following embryo transfer with different luteal support protocols.

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<tr>
<td>Number (oocytes)</td>
<td>254</td>
<td>185</td>
<td>270</td>
<td>244</td>
<td>210</td>
<td>228</td>
</tr>
<tr>
<td>Number</td>
<td>203/254</td>
<td>167/185</td>
<td>210/270</td>
<td>204/244</td>
<td>186/210</td>
<td>173/228</td>
</tr>
<tr>
<td>Mature (%)</td>
<td>(79.9)</td>
<td>(90.3)</td>
<td>(77.8)</td>
<td>(83.6)</td>
<td>(88.7)</td>
<td>(75.9)</td>
</tr>
<tr>
<td>No oocytes/patient (%)</td>
<td>(4.70)</td>
<td>(5.28)</td>
<td>(4.57)</td>
<td>(5.19)</td>
<td>(4.47)</td>
<td>(4.95)</td>
</tr>
<tr>
<td>ICSI (%)</td>
<td>163/203</td>
<td>127/167</td>
<td>175/210</td>
<td>152/204</td>
<td>142/186</td>
<td>139/172</td>
</tr>
<tr>
<td>ET/patient (%)</td>
<td>151/54</td>
<td>111/55</td>
<td>164/59</td>
<td>136/47</td>
<td>132/40</td>
<td>125/46</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>24.1</td>
<td>22.9</td>
<td>28.8***</td>
<td>38.3**</td>
<td>25</td>
<td>21.7</td>
</tr>
<tr>
<td>Viable fetuses/pregnant (%)</td>
<td>61.54</td>
<td>62.5</td>
<td>70.59</td>
<td>83.3****</td>
<td>60</td>
<td>60</td>
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* $P<0.05$ significantly different from protocol four
** $P<0.01$ significantly different from other protocols
*** $P<0.05$ significantly different from protocol one, two and six
**** $P<0.05$ significantly different from other protocols

ET: the number of transferable quality embryos transferred per woman

Discussion:
The age, BMI, basal FSH/LH ratio and estradiol concentration on the day of HCG injection were similar in the luteal support protocols (LSP). This indicates that these variables did not have a significant interaction on ICSI outcome. Similar observations were reported by other investigators (16). The ICSI rate in the protocol three was significantly higher compared to protocol four, but was not significantly different from other protocols. This may be due to the increased number of the mature oocytes available for ICSI in protocol three (17). The number of the transferable quality embryos transferred per patient was not significantly different between the LSP, which indicates that they have no significant effects on either pregnancy or embryo implantation rates (18).

Protocol one, two, five and six had significantly lower pregnancy and embryo implantation rates compared to protocol three and four. The decrease in pregnancy and implantation rates in these protocols may be due to limited intestinal absorption of oral progesterone and the lower half-life of progesterone (two hours) (19-21). The result was relatively improved in protocol three following the addition of HCG and aspirin to oral progesterone compared to other protocols except protocol four.

The intermittent low-dose HCG was used to avoid the risk of ovarian hyperstimulation syndrome and also to avoid down regulation of LH receptors. The administration of low dose aspirin orally inhibits the function of cyclo-oxygenase enzyme, which results in the inhibition of thromboxane-A2 production, (a powerful platelet activator causing platelet aggregation and vasoconstriction) (22). Inhibition of the action of cyclo-oxygenase results in the inhibition of the production of prostaglandin F2-alpha and this improves corpus luteum function (23). It is well known that aspirin significantly reduces gonadotropin-induced ovarian prostaglandin production (24).

The reason for the administration of aspirin on day five after embryo transfer was because aspirin also has anti-inflammatory action. Prostaglandin stimulates inflammatory cells (such as monocytes, lymphocytes, neutrophils and macrophages) and the release of interleukin-1-beta (which has a powerful inflammatory effect) and nitric oxide. These factors are necessary for the invasion of trophoblast in the endometrium during the opening of the implantation window. Therefore the early administration of aspirin may have adverse effect on the invasion step of embryo implantation (25).

The pregnancy and embryo implantation rates were highly significantly ($P<0.01$) increased in the protocol four compared to other protocols including the protocol three. The increase in uterine contractility at the time of embryo transfer in women undergoing IVF-ET was found to associate
with lower clinical pregnancies. This may be the reason of lower pregnancy and embryo implantation rates in the protocol three compared to the protocol four, since in the protocol three the vaginal progesterone was administered to the female patients 12 hours after embryo transfer (26). Similar results were reported by other investigators, which confirm the data of the present work (27). The delay in progesterone supplementation initiation in women undergoing IVF-ET can lead to decrease in embryo implantation and pregnancy rates (28). Uterine tissue levels of progesterone have been shown to be much higher after vaginal progesterone supplementation compared with intramuscular progesterone (IM) supplementation, although serum progesterone levels are higher in IM progesterone group (29).

It was observed that physiological synchronized endometrium transformation took place under vaginal progesterone but not oral or IM progesterone supplementation (30). The significant reduction in pregnancy and embryo implantation in the protocol six compared to the protocol three and four may be due to an inability of IM progesterone to reach the uterine tissue directly passing through the liver and lousing its bioactivity. Vaginal progesterone reaches the uterine tissue directly without passing through the liver (31-32). Complete diffusion in uterine myometrium occurred within six hours of the vaginal administration of progesterone and in addition to the direct diffusion, an active mechanism may be involved in the progesterone transport as demonstrated by means of hysterosalpingography of uterus and uterine tubes (33-34).

The best ICSI outcome was observed in the protocol four which may be due to the fact that the early vaginal progesterone supplementation before embryo transfer results in decreased uterine contractions during the time of ET and also vaginal progesterone causes normal physiological synchronized endometrium transformation in addition to the supportive action of aspirin on embryo implantation (23, 25, 32).

In conclusion, supplementation of vaginal progesterone (400 mg) 24 hours before embryo transfer with low dose of oral aspirin (100 mg aspirin) 5 days after embryo transfer results in significant improvements in pregnancy rate and viable fetal development in the luteal phase defect infertile women undergoing ICSI-ET.

21. Levine, H., and Watson, N. "Comparison of the pharmacokinetics of Crinone 8% administered vaginally versus