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, percentages 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 5), 21.60% (G 6). 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

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fertilization and embryo transfer (IVF-ET) treatment usually results in a temporary LPD. This causes suppression in progesterone secretion by the corpus luteum, which affects human embryo implantation. The corpus luteum requires continual stimulation by LH to produce adequate secretion of progesterone, which is necessary for the induction of secretary transformation of the endometerium so that normal embryo implantation can be maintained successfully in the pregnant women (6). Gonadotropin releasing hormone analogue (GnRH-a) has been used for the induction of ovulation and prevention of premature LH surge in infertile women undergoing IVF-ET. This GnRH-a treatment is associated with persistent blockage of LH output for at least 10 days after discontinuation of GnRH-a administration causing LPD (7).

The increase in the estradiol/progesterone ratio in the stimulated cycles has an inhibitory action on embryo implantation in human and animal studies (8-10).

These observations clearly indicate that the application of luteal support therapies in the form of progesterone and/or human chorionic gonadotropin (HCG) have a beneficial effect on embryo implantation and maintenance of pregnancy in IVF stimulated cycles in infertile women with LPD or without LPD.

The objective of the study was to evaluate the clinical significance of vaginal progesterone (administered before embryo transfer), Aspirin and human chorionic gonadotropin (HCG) on human embryo implantation and pregnancy rate in luteal phase defect infertile women undergoing intracytoplasmic sperm injection and embryo transfer (ICSI-ET) treatment.

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 72 hours (Pregnyl 1500 IU, N. V. Organon, Oss Holand). Group 3 (No= 59) received P plus HCG and 100 mg oral aspirin. The aspirin was given 5 days after embryo transfer. Group four (No= 47) received 400 mg per day vaginal progesterone (Cyclogest 400 mg, Hoechst-Roussal, Uxibridge, UK), and was administered 24 hours before embryo transfer plus aspirin. Group five (No= 40) received vaginal P 12 hours after embryo transfer plus aspirin. Group six received 125mg intramuscular injection of progesterone (Primolute Depot 125 mg progesterone, Schering Co., Germany) twice weekly after embryo transfer. The LSP started after embryo transfer (except in group 4) and continued for 12 weeks when the women diagnosed to have a positive B-HCG test.

Ovulation Induction

The female patients received 2 to 3 ampoules of human menopausal gonadotropin (HMG, Pergonal 75 IU FSH and 75 IU LH per ampoule, Serono 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/mI 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

medium was 80 IU. The oocytes were pipetted several times by using denuding micropipette (Denuding micropipette, billdalsvagen, Sweden) and washed by culture medium prior to insemination. The immature oocytes with or without germinal vesicle were cultured for 24-48 hours until they reached the maturation stage with first polar body (11-12). The spermatozoon was immobilized by rubbing the tail with micropipette against the bottom of the culture dish. The mature oocyte was held in position so that the polar body was in 6 or 12 o'clock position by using the holding micropipette (ICSI micropipette, CCD, France) to avoid damage to the spindle of the polar body during the insemination procedure. The injection needle was introduced in side the cytoplasm of the oocytes and the spermatozoon was released with minimum amount of medium. The inseminated oocytes were cultured in IVF medium and examined 17 to 19 hours after insemination for the presence of two pronuclei and extrusion of the second polar body. The embryonic development of the fertilized oocytes was checked next day and when the embryos reached to 4-cell or 8-cell stage, they were prepared for embryo transfer (13).

Embryo Transfer

The bladder was empted before embryo transfer. The embryos were transferred two or three days after insemination. The cervix was cleaned with culture medium and the external embryo transfer catheter was used for canalization of the cervical canal (Casmid double lumen embryo transfer catheter, Casmid Co., UK). The internal catheter was washed with IVF culture medium and the embryos were withdrawn from the embryo culture dish. The embryos were located in side the internal catheter and kept between two layers of air bubbles and culture medium. This pattern of location of the embryos between two layers of air bubbles was used to protect the embryos from surface tension changes during the passage of the internal catheter through the cervical canal. Two to four embryos were transferred per patient. The external and the internal catheters were washed and examined under a microscope to check any retained embryos. In case of retained embryos, the embryo transfer procedure was repeated. The woman remained in the supine position for 20 minutes and kept in bed for two hours before discharge. The female patients received luteal support therapies depending on the type of luteal support groups as mentioned above. The pregnancy was tested after two weeks of embryo transfer by the B-HCG test and the embryo implantation and viable fetal sac development were examined after five weeks of embryo transfer by ultrasound examination (14).

Statistical Analysis

The data were presented as mean +/- standard error 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 signifcances (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.*

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

Groups	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5	Proptocol 6
Number (patient)	54	35	59	47	40	46
Age (year)	30.8 +/- 4.2	31.1 +/- 2.1	28.1 +/- 3.8	29.8 +/- 2.1	31.2 +/- 2.5	30.4 +/- 3.8
BMI (Kg/m^2)	27.7 +/- 2.5	29.5 +/- 2.6	25.6 +/- 1.4	26.9 +/-2.7	24.5 +/- 3.7	28.7 +/- 3.6
FSH/LH ratio	2.78 +/- 0.3	1.89 +/- 0.2	2.05 +/- 0.1	1.94 +/- 0.2	2.54 +/- 0.5	2.34 +/- 0.1
Estradiol level at HCG day	1670 +/- 86	1550 +/- 36	1410 +/- 64	1565 +/- 45	1329 +/- 48	1304 +/- 43

P >0.05 significantly not different between protocols
BMI: body mass index
FSH/LH: basal FSH/LH ratio

The clinical outcome of ICSI-ET is shown in table two. The number of the mature oocytes in the 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 the 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). The pregnancy rate in the protocol three was significantly higher than protocol l, 2, and three (28.8 versus, 24.1, 22.9, 21.7, respectively, P<0.05). The percentage of the viable fetuses was significantly higher in the protocol four compared to other protocols (83.3 versus 61.5, 62.5, 70.6, 60, 60, P<0.05).

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

Groups	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5	Protocol 6
Number (oocytes)	254	185	270	244	210	228
Number Mature (%)	203/254 (79.9)	167/185 (90.3)	210/270 (77.8)	204/244 (83.6)	186/210 (88.7)	173/228 (75.9)
No oocytes/ patient (%)	254/54 (4.70)	185/35 (5.28)	270/59 (4.57)	244/47 (5.19)	210/40 (4.47)	228/46 (4.95)
ICSI No (%)	163/203 (80.3)	127/167 (76.1)	175/210 (83.3)*	152/204 (74.5)	142/186 (76.3)	139/173 (80.4)
ET/patient (%)	151/54 (2.79)	111/35 (3.17)	164/59 (2.78)	136/47 (2.89)	132/40 (3.30)	125/46 (2.72)
Pregnancy rate (%)	24.1	22.9	28.8***	38.3**	25	21.7
Viable fetuses/pregnant Women (%)	61.54	62.5	70.59	83.3****	60	60

*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) (19-2 1). The result was relatively improved in protocol three following the addition of HCG and aspirin to the 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-l-beta (which has a powerful inflammatory effect) and nitric oxide. These factors are necessary for the invasion of trophoblast in the endometerium 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 endometerium 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 reachs the uterine tissue directly without passing through the liver (31-32). Complete diffusion in uterine myometerium 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 endometerium 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.

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