The Effects of Depot Leuprorelin on IVF

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Abstract

The trial studied the effects of depot leuprorelin on the IVF cycle and was done on nine couples. A single intramuscular injection of depot leuprorelin was given to the woman a couple days before ovulation. Seven days after ovulation, the serum progesterone level was measured and showed the same normal level as the natural ovulatory cycle. The progesterone levels varied from 12.59 to 96.0 ng/ml. On day three of the menstruation, the hormonal profiles showed a complete pituitary and ovarian suppression. FSH, LH and estrogen levels were less than 4.1 mIU/ml, 2.8 mIU/ml and 9.4 pg/ml respectively. The hMG stimulation took 11 days on average (9-15 days). A hundred and two oocytes were retrieved and among these there were 86 mature oocytes (84.3%). All oocytes were inseminated despite prematurity and resulted in 82.35 per cent fertilization. Normal fertilization occurred in 77.45 per cent (79/102). Good embryos developed in 58.23 per cent (46/79). No more than three embryos were transferred. Four women conceived, among them there was a set of twins. The implantation rate was 44.44 per cent (4/9). One abortion was found in the early first trimester. The take home baby rate was 33.33 per cent (3/9).

Key word : Depot Leuprorelin, IVF

The gonadotropin releasing hormone agonist (GnRH-a) is commonly used in the IVF program at the present time. The benefit inhibits the premature luteinization and leads to a high possibility of reaching the time of eggs retrieval. However, most of the GnRH-a has a short action. The short action was believed to prevent the concerning effects on the luteal phase or in following early pregnancy[1-4] because the administration could be stopped at the appropriate time. But the short acting GnRH-a has to be administrated everyday or every four hours depending on its preparation. This may

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lead to an inconvenience and has an effect on patient compliance.

This study aimed to use the long-acting GnRH-a (depot leuprorelin) in the IVF program as a single muscular injection. The effects of this long-acting GnRH-a on the pituitary suppression, ovarian stimulation, quality and the performance of the oocytes, fertilization and pregnancy outcome were recorded.

MATERIAL AND METHOD

Nine couples were selected randomly from all who were waiting for IVF treatment. All husbands were found to have normal semen analysis. All the women had normal menstruation patterns and had no history of any hormonal treatment at least three months before. Vaginal ultrasonography which was performed a few days before the expectation of ovulation. If the follicular size reached 18 millimeter or greater, depot leuprorelin was administrated as a single intra-muscular injection. Ten days after the injection, serum estrogen and progesterone levels were detected. Then the women were told to return three days after having their period. Serum tests for follicular stimulating hormone (FSH), luteinizing hormone (LH) and the estrogen were done. When the blood tests showed no ovarian activity[5,6], 225 units of hMG (human menopausal gonadotropin) was administrated intramuscularly daily to the women. The ovarian responses were monitored by serum estrogen assays and follicular ultrasonographic scanning.

When at least two follicles had reached 18 millimeters in diameter or more, blood tests for estrogen, LH and progesterone levels were performed. After that the women were given 10,000 units of human hCG (human chorionic gonadotropin) intramuscularly.

Ovum pick up was performed transvaginally 36 to 37 hours after the hCG injection under ultrasonographic guidance. The quantity and quality of oocytes were checked, cultured in human tubal fluid medium (HTF) and incubated in 5 per cent CO_2 atmosphere at 37°C. Four to six hours after incubation, about 100,000 washed sperm per oocyte were inseminated and incubated in the above condition again.

Fertilization was checked twenty hours after incubation. Normal fertilization was defined as an embryo which had two pronuclei (2PN). Embryo formation was also checked the following day.

All the patients were given progestogen (400 mg. vaginal suppository) daily for luteal support until the first pregnancy test.

RESULTS

The studied patients were between 28 and 37 years of age, an average of 34 years old. The main infertility problem was from severe endometriosis (65%). The tubal factor was found to be the second most common (30%) and the other was unknown factor (5%). Average cycle length was 30 ± 4 days. The semen analysis was repeated in all cases and found to be within normal limits[5].

The cycle prior to the stimulated cycle, in all cases, was an ovulatory cycle. The serum progesterone was normal (Table 1). The pituitary function was completely suppressed in all cases within the third day of the following cycle, (Table 1). The

Table 1. Hormonal profile data *prior* to ovarian stimulation.

Case Serial No.	Prior $\triangle P_4$ ng/ml	Day 3 hormone profiles			
		$\triangle P_4$ng/ml	E_2pg/ml	FSH mIU/ml	LH mIU/ml
1	24.50	0.1	5.3	1.1	1.3
2	14.59	1.2	7.7	1.0	1.2
3	31.10	0.5	5.0	4.1	2.8
4	96.00	1.0	3.0	1.0	1.0
5	36.20	1.1	7.0	1.0	1.0
6	29.30	0.0	9.4	1.1	2.3
7	12.59	0.2	5.0	2.8	1.2
8	14.79	0.0	2.6	1.1	2.6
9	13.68	0.2	0.0	1.5	0.8

Table 2. Number of hMG injections and hormonal profiles before ovulation induction.

	No. of injection (days)	E_2 pg /ml	LH mIU/ml	$\triangle P_4$ng /ml	Endometrial thickness (mm.)
Average	11	1697.99	1.03	0.29	11.40
Range	9 - 15	877- 3200	0.5-1.3	0.0-0.5	10-12.4

Table 3. Oocyte quantity and quality.

	No. oocytes	Mature (%)
Total	102	86 (84.3%)
No. of oocytes /cycle (by average)	11.3	9.5
No. of oocytes/person (min.-max.)	5-15	4-13

Table 5. Data of the embryo classification.

Embryo quality	Number	%
Good	46	58.23
Fair	22	27.85
Poor	11	13.92
Total	79	100

Table 4. Fertilization and its abnormalities.

	Number	%
Type of fertilization	18	17.65
1PN	2	1.96
2PN	79	77.45
3PN	3	2.94

Table 6. Pregnancy outcome.

Pregnancy	Number	%
Beta-hCG > 100 mIU/ml	4	44.44
Abortion	1	11.11
Twins	1	11.11
Delivery	3	33.33

ultrasonography of both ovaries showed no evidence of cyst formation and the uterine cavity was empty.

The average number of hMG injections was 11 injections and varied from 9 injections to 15 injections. At the day of ovulation induction the hormonal profiles showed good response of the ovaries with no spontaneous premature leutenization and appropriate estrogen level, Table 2.

Oocyte collection was done thirty seven hours after ovulation induction. The number of oocytes varied from 5 oocytes per woman to 15 oocytes per woman. Oocyte quality and quantity data is shown in Table 3 . There were 102 oocytes retrieved and among these there were 86 mature oocytes (84.3%)

All oocytes were inseminated four to six hours after collection. All specimens were incubated in an IVF incubator as already described. Around 16 to 20 hours after the insemination, fertilization was checked and the data is shown in Table 4. About 82.35 per cent of the oocytes were fertilized and 77.45 per cent were normal fertilization (2PN). Abnormal fertilization (1PN and 3PN) was found to be 4.9 per cent as shown in Table 4.

All specimens were incubated again but abnormal fertilized oocytes and non- fertilized ones were cultured separately. Twenty four hours later, normal fertilized oocytes were developed into cleavage stage embryos. The embryos were classified into good, fair and poor quality as described before[7,8]. There were 58.23 per cent good embryos and only 13.92 per cent were of poor quality. The data is shown in Table 5.

Embryo transfer was done on the second day after oocyte collection. The number of embryos transfered was at most three embryos, so as to prevent a multiple pregnancy. In this study the pregnancy outcome was calculated from only one cycle (the stimulated cycle), so the other embryos were frozen and stored in liquid nitrogen. Four cases showed evidence of pregnancy. Unfortunately

one of the four pregnancies was aborted two weeks later. There was a set of twins, Table 6. All of the three pregnancies progressed well and normal healthy children were delivered.

DISCUSSION

At the present time, assisted reproductive technology has yielded a very high success of pregnancy outcome. So many techniques have been developed for these aspects and among these, *in vitro* fertilization is common. This technique requires ovarian hyper-stimulation to harvest a large amount of eggs. In the past, ovarian stimulation was very simple but it yielded only a small amount of eggs. One of the problems was prior ovulation due to premature luteinization[9]. This led to low quality of the eggs and finally a low pregnancy rate. Nowadays, gonadotropin releasing hormone agonist (GnRH-a) is used in the ovarian hyperstimulation cycle to prevent premature luteinization. Depot leuprorelin acetate is a long acting GnRH-a. It is used to treat pelvic endometriosis by monthly injection. For infertile couples who suffer from pelvic endometriosis, long acting GnRH-a has been used both for the treatment of the disease and the following IVF program. Successful pregnancies have been reported from this technique.

The objective of this research was to find out the roles of depot leuprorelin in the IVF program as reported by many centers. Depot leuprorelin was given to the women a few days before ovulation of the prior cycle and it was able to cause ovulation. Serum progesterone was detected ten days after the administration and the hormonal level varied from 12.59 to 96 ng /ml (Table 1). The progesterone level was the same as that found in the natural ovulatory cycle. Three days after menstruation, the pituitary and ovarian hormonal profiles were tested. The hormonal profiles showed a complete pituitary suppression and complete ovarian suppression in all cases (Table 1). Human menopausal gonadotropin injection was given to the woman until the follicles reached their maturity. The number of injections varied from 9 to 15 days, which seems to be a bit longer than in conventional IVF (Table 2). At the time of ovulation induction, luteinizing hormone and progesterone level were detected and showed no possibility of spontaneous luteinization. The endometrial thickness varied from 10 to 12.4 mm an average of 10.4 mm. The endometrial thickness was comparable to conventional ovarian hyperstimulation and acceptable for embryo implantation (Table 2). This showed that depot leuprorelin was able to suppress the LH surge effectively in all cases but there was no effect on the intra uterine endometrium. Ovum pick up was done thirty six hours following the hCG. Injection. A hundred and two oocytes were recovered, an average of 11.3 oocytes per cycle. The oocytes were classified as mature and pre-mature. Eighty six oocytes out of a hundred and two were found to be mature oocytes (Table 3). This was also no different from the conventional IVF cycle.

Twenty hours after insemination, fertilization was examined. Normal fertilization was found to be 77.45 per cent and abnormal fertilization was 4.9 per cent. 17.65 per cent showed no fertilization and was comparable to conventional IVF (Table 4). All normal fertilized oocytes had developed to be embryos. The quality of the embryo was classified as already described. Forty six good quality embryos developed (58.23%). Fair and poor embryos were 27.85 per cent and 13.92 per cent respectively (Table 5). Not more than three embryos were transferred back to the women. The other embryos were frozen and stored in liquid nitrogen for transfer in the next cycle. The result of the successful pregnancy in this study was calculated from the stimulated cycle only. The data from the thawing cycle was not included in this study. Four pregnancies occurred with one set of twins. The pregnancy rate was 44.44 per cent. Unfortunately there was one abortion early in the first trimester. The other pregnancies progressed well and all reached the full term course. They all gave birth of healthy children without any anomalies. The take home baby rate was 33.33 per cent (Table 6). This seems to be no different from conventional IVF[12].

SUMMARY

According to the results above, we concluded that depot leuprorelin is useful and quite safe in ovarian hyperstimulation. Single intramusculalr administration depot leuprorelin is convenient and enough to completely suppress both pituitary and ovarian function. Furthermore, it prevented premature luteinization in all the studied cases. The quantity and quality of both eggs and embryos developed were comparable to conventional IVF. The pregnancy rate and take home baby rate were also not different.

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ผลของดีโป ลิวโปรเรลิน เมื่อใช้ในขบวนการของเด็กหลอดแก้ว

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ได้ทำการศึกษาผลของ depot leuprorelin เมื่อนำมาใช้ในการรักษาร่วมในขบวนการของเด็กหลอดแก้ว ทั้งนี้เพื่อประสิทธิภาพในการป้องกันการตกไข่ก่อนกำหนด ได้ทำการศึกษากับคู่สมรสที่มีบุตรยากและมีข้อบ่งชี้ที่ได้รับการรักษาโดยวิธีการของเด็กหลอดแก้ว โดยฉีด depot leuprorelin สองวันก่อนจะมีการตกไข่ในรอบเดือนก่อนที่จะทำการรักษาด้วยวิธีเด็กหลอดแก้ว พบว่า depot leuprorelin สามารถกระตุ้นให้มีการตกไข่ในรอบเดือนก่อนที่จะมีการกระตุ้นไข่ได้ร้อยละ 100 สามารถกดการทำงานของต่อมใต้สมองและป้องกันการตกไข่ก่อนกำหนดในรอบเดือนที่กระตุ้นไข่ได้ร้อยละ 100 จำนวนวันในการใช้ยาฉีดเพื่อการกระตุ้นไข่โดยเฉลี่ย 11 วัน (9–15 วัน) ไข่ที่เก็บได้มีความสมบูรณ์เต็มที่ร้อยละ 84.3 การปฏิสนธิเกิดขึ้น ร้อยละ 82.5 และพัฒนาเป็นตัวอ่อนได้ร้อยละ 77.45 เป็นตัวอ่อนที่มีคุณภาพดีร้อยละ 58.23 ตัวอ่อนจำนวนไม่เกินสามตัวได้ถูกนำกลับสู่โพรงมดลูกของมารดา พบว่ามีการตั้งครรภ์ได้ร้อยละ 44.44 ทั้งนี้เป็นการแท้งบุตรหนึ่งราย เป็นการตั้งครรภ์แฝดสองหนึ่งราย อัตราการคลอดบุตรปรกติร้อยละ 33.33

คำสำคัญ : ดีโป ลิวโปรเรลิน, ขบวนการเด็กหลอดแก้ว

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