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Original Article

Use of an oxytocin antagonist in *in vitro* fertilization–embryo transfer for women with repeated implantation failure: A retrospective study☆

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Abstract

Objective: This retrospective study aimed to investigate the use of an oxytocin antagonist in improving the pregnancy outcome of *in vitro* fertilization–embryo transfer (IVF–ET) in patients with repeated implantation failure (RIF).
Materials and Methods: A total of 150 infertile couples with RIF undergoing IVF–ET were divided into three groups. Patients who did not receive atosiban were used as controls (Group 1; $n = 80$). Forty patients received a single bolus dose (6.75 mg, 0.9 mL/vial) of atosiban before ET (Group 2), and 30 patients received a bolus dose of 6.75 mg atosiban followed by infusion at 18 mg/hr for 3 hours immediately after ET (Group 3).
Results: A significantly higher implantation rate (30.21%) was noted in Group 2 compared with Groups 1 and 3 (11.8% and 15.9%, respectively; $p = 0.0006$). The clinical pregnancy rate of Group 2 (37.5%) was significantly higher than that of Groups 1 (12.5%) and 3 (20%) ($p = 0.0057$). The live birth rate was significantly higher in Group 2 (35%) than in Groups 1 and 3 (10% and 16.67%, respectively; $p = 0.0031$).
Conclusion: These results suggest that IVF–ET using lower dosage of atosiban may improve pregnancy outcomes of patients with RIF.

Keywords: Clinical pregnancy rate; Embryo transfer; Implantation rate; Live birth rate; Oxytocin antagonist; Repeated implantation failure

Introduction

Embryo implantation is a complex process, and it is believed that the success of embryo implantation depends on embryo quality and endometrial receptivity. Repeated implantation failure (RIF), despite the transfer of good-quality embryos, is a distressing experience for patients who receive *in vitro* fertilization (IVF) and increases the financial burden on the couple or service provider [1]. RIF is generally defined as failure to conceive after two or more unsuccessful embryo transfer (ET) cycles or the transfer of a total of 10 or more good-quality embryos [2,3]. The cause of RIF can be broadly attributed to embryonic, uterine, genetic, hematological, or immunological causes; however, RIF remains unexplained in most cases [4]. As a result, there is considerable variation in the approach to RIF treatment and management [2].

Arginine vasopressin V1 receptors are present on human myometrial specimens obtained during cesarean section from women at the end of pregnancy (from 32 weeks to term). Oxytocin receptors increase in human parturition and are significantly correlated with the frequency of uterine contractions [5]. Oxytocin and vasopressin receptors may play a role in the regulation of labor, although increased oxytocin release primarily initiates labor contractions. The mixed V1 arginine vasopressin and oxytocin receptor antagonist atosiban is uterus specific, decreases uterine contractions in pregnant women, and is indicated for tocolysis in imminent premature birth [6]. Atosiban was developed specifically to treat preterm labor and is widely used in many countries. A single bolus of atosiban is indicated for acute tocolysis in term labor and arrest of contractions before emergency caesarean section. Use of a bolus dose of 6.75 mg atosiban followed by a maintained dose is an effective management for the treatment of preterm labor. Atosiban has little effect on maternal and fetal heartbeats

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compared with the significant effects of ritodrine but appears to have an immediate and profound effect comparable to that of ritodrine on uterine contractions [7].

Good correlations between oxytocin receptor concentrations and uterine contractility have been observed in both pregnant and nonpregnant states [8]. Therefore, clinical evaluation of atosiban has been proposed for use in dysmenorrhea and infertility cases [8,9]. As such, we have previously reported on a couple experiencing RIF who conceived successfully after an IVF–ET, in which atosiban was administered intravenously during ET, and delivery of the second and third triplets was delayed with the aid of an atosiban infusion [10]. Recently, in a prospective, randomized, placebo-controlled clinical study, treatment with atosiban before ET is effective in priming of the uterus for implantation [11]. Atosiban increases the implantation and clinical pregnancy rates and decreases the miscarriage rate, which may be the result of increased endometrial perfusion and decreased uterine contractility.

The objective of the present study was to determine if the use of atosiban could improve the outcome (implantation rate, clinical pregnancy rate, and live birth rate) of patients with RIF undergoing IVF–ET. In addition, the safety of atosiban use was assessed.

Materials and methods

Patient selection

This study was approved by the Institutional Review Board of National Cheng Kung University Hospital. A total number of 150 infertile couples, who attended the Assisted Reproductive Technology Center at National Cheng Kung University Hospital, with a history of two or more previous IVF failures after the transfer of good-quality embryos, were collected retrospectively. The study was performed between October 2006 and December 2009. After starting ovarian stimulation and oocyte retrieval, patients received different doses of atosiban (Tractocile; Ferring AB, Limhamn, Sweden) infusion or no atosiban during the procedure of ET, in addition to the medications that are used routinely in our IVF program.

The control group (Group 1; $n = 80$) included patients with RIF who did not receive atosiban during ET. A placebo was not administered because the variables that were chosen as primary and secondary endpoints were objective and, thus, not likely to be influenced by patients' will. Forty patients received a single bolus dose (6.75 mg, 0.9 mL/vial) of atosiban intravenously before ET (Group 2), with an infusion time of more than 1 minute. Thirty patients received a bolus dose of 6.75 mg atosiban intravenously followed by continuous infusion at 18 mg/hr for 3 hours (Group 3).

Ovarian stimulation

All of the women in this study underwent a controlled ovulation hyperstimulation protocol as determined by their primary physician depending on patient age, antral follicle count, and serum follicle-stimulating hormone levels. Protocols included a long luteal-phase gonadotropin-releasing hormone (GnRH) agonist protocol, a short GnRH agonist protocol, or the GnRH antagonist protocol.

Briefly, patients who underwent the long GnRH agonist protocol started leuprolide acetate (Lupron; Farma Laigle for Takeda, France) in the mid-luteal phase of the preceding cycle, and patients who received the short GnRH agonist protocol started leuprolide acetate from Day 2 of their menses. Transvaginal ultrasound and serum laboratory endocrine level assessment were then performed on Day 2 of their menses to confirm pituitary suppression. Controlled ovarian stimulation was initiated until the day of oocyte maturation trigger. Superovulation was obtained through the use of gonadotropins. Patients who received the GnRH antagonist protocol were evaluated on Day 2 of their menses, and gonadotropins were administered. Cetrorelix (Cetrotide; Baxter Oncology, GmbH, Germany) was initiated daily once the leading follicle was 14 mm and was continued until the day of trigger. All patients received recombinant gonadotropin Follitropin α (Gonal-F; Merck Serono, SpA, Rome, Italy) at appropriate daily doses (75–225 IU) for ovarian stimulation.

When the leading follicles reached 18–20 mm in diameter, final oocyte maturation was induced through a subcutaneous injection of 250-μg recombinant human chorionic gonadotropin (hCG; Ovitrelle, Merck Serono, SpA), and transvaginal oocyte retrieval was performed 34–36 hours later. Oocyte insemination, intracytoplasmic sperm injection, and *in vitro* embryo culture were performed according to standard protocol. Fertilization was assessed the next day (Day 1) and embryo cleavage 24 hours later. Embryo morphology was studied on Day 3 based on the number of cells and fragmentation using the standard morphological criteria. A good Day 3 embryo was defined as one with the number of blastomeres greater than or equal to eight, fragmentation of less than 20%, and without multinucleated blastomeres. All ET procedure was carried out on Day 3 with good-quality embryos. The same equipment and technique was used for all ETs. Only easy ETs have been accepted for the study. No complication of ET procedure was noted in this study.

The luteal phase was supported by transvaginal progesterone gel (Crinone 8%; Fleet Laboratories Ltd., Hertfordshire, UK) in all patients. Serum hCG was assayed 14 days after ET; in the case of positive hCG (serum hCG level > 30 mIU/mL), pregnancy was confirmed by transvaginal ultrasound to detect the presence of one or more gestational sacs 1 week later. Clinical pregnancy was defined as ultrasound detection of an intrauterine gestational sac with a fetal pole and cardiac activity between 6 weeks' and 7 weeks' gestational age.

Endpoint and statistical analysis

The primary endpoint of the study was implantation rate. Secondary endpoints were clinical pregnancy rate, live birth rate, incidence of adverse effects, and complications. Data were expressed as percentages and mean standard deviations.

Table 1
Patient characteristics

Group and parameter	Group 1 ($n = 80$) (control group)	Group 2 ($n = 40$) (single bolus dose of Tractocile)	Group 3 ($n = 30$) (infusion dose of Tractocile)	p
Age (mean)	34.8 ± 3.76	34.63 ± 4.21	35.3 ± 3.59	0.76
Number of previous failed cycles	3.08 ± 0.93	2.6 ± 0.54	3.2 ± 0.79	0.11
Length of infertility (yr)	4.45 ± 2.36	4.98 ± 3.06	4.67 ± 2.81	0.59
Body mass index	22.03 ± 3.24	21.63 ± 3.06	21.14 ± 2.23	0.39
Etiology of infertility				
Tubal factor	31.25 (25/80)	32.5 (13/40)	36.67 (11/30)	0.68
Male factor	36.25 (29/80)	32.5 (13/40)	26.67 (8/30)	0.61
Anovulation	13.75 (11/80)	17.5 (7/40)	10 (3/30)	0.47
Unexplained infertility	18.75 (15/80)	17.5 (7/40)	26.67 (8/30)	0.59

Values are expressed as mean ± standard deviation, and percentages, and also the number of positive finding/the total number in the group.

One-way analysis of variance was used for analysis of continuous variables. All p values were two sided, and values less than 0.05 were considered statistically significant.

Results

Group characteristics are summarized in Table 1. There were no significant differences among the three groups in terms of age, number of previous cycles, length of infertility, etiology of infertility, or body mass index. Table 2 shows the reproductive outcomes of all groups. There were no significant differences among the three groups in terms of the mean number of good-quality embryos transferred.

Patients in Group 2 experienced a significantly higher implantation rate (30.21%) compared with those of Groups 1 and 3 (11.8% and 15.9%, respectively; $p = 0.0006$). The clinical pregnancy rate was significantly increased to 37.5% in Group 2 versus 12.5% in Group 1 and 20% in Group 3 ($p = 0.0057$). In addition, a significant increase in the live birth rate was observed in Group 2 (35%) compared with those in Groups 1 and 3 (10% and 16.67%, respectively; $p = 0.0031$). Although the multiple pregnancy rate seemed higher and the miscarriage rate seemed lower in Group 2, there were no significant differences in the multiple pregnancy rate or the miscarriage rate between the groups (Table 2).

No relevant adverse effects were observed in Groups 2 and 3. One patient experienced mild nausea and another experienced mild abdominal discomfort, but these symptoms were probably not associated with the use of atosiban.

Discussion

The causes of RIF in assisted reproduction are complex and numerous, which include embryonic defects, abnormalities of the uterine cavity, reduced endometrial receptivity, or multifactorial causes (e.g. immune system, hydrosalpinx, and genetic). Good quality of embryos and optimal intrauterine environment are the basic determinants of the whole IVF–ET procedure. When RIF is associated with good-quality embryos, a uterine or endometrial factor is more likely to be present [12]. Implantation and pregnancy rates are inversely correlated with the frequency of uterine contractions. Increased uterine contractile activity may expel embryos from the uterus [13]. As psychological stressors are associated with preterm labor [14], elevated uterine contractile activity (a component of uterine receptivity) may also be related to the increase of stress or anxiety during ET. In RIF, psychological factors should also be evaluated. One study reports that more than 40% of infertile women who were seen for a new course of assisted reproductive technology treatment had a psychiatric disorder (anxiety and/or depression) [15]. The negative impact of the psychological stress may contribute to implantation failure, which in turn reduces IVF success [16]. Psychological stress may be associated with uterine contractility and poor IVF outcomes. The intervention of psychological support and drug prescription

Table 2
Clinical outcome of the study

Group and parameter	Group 1 ($n = 80$) (control group)	Group 2 ($n = 40$) (single bolus dose of Tractocile)	Group 3 ($n = 30$) (infusion dose of Tractocile)	p
Number of embryos transferred (mean)	2.23 ± 1.10	2.35 ± 0.99	2.37 ± 1.14	0.85
Implantation rate[a]	11.8 (21/178)	30.21 (29/96)	15.9 (11/69)	0.0006*
Clinical pregnancy rate	12.5 (10/80)	37.5 (15/40)	20 (6/30)	0.0057*
Miscarriage rate	20 (2/10)	6.67 (1/15)	16.6 (1/6)	0.62
Live birth rate	10 (8/80)	35 (14/40)	16.67 (5/30)	0.0031*
Multiple pregnancy rate	10(1/10)	40 (6/15)	16.67 (1/6)	0.22

Values are expressed as mean ± standard deviation, percentages, and also the number of positive finding/the total number in the group.
*p Values less than 0.05 were considered statistically significant.
[a] Number of gestational sacs detected by ultrasound/number of transferred embryos.

may help to reduce this burden, and the use of atosiban may improve the IVF outcome in anxious or depressed patients with RIF.

One study reports that atosiban was used in pregnancies with extremely preterm premature rupture of membranes (at 18 weeks) and assisted in prolonging a pregnancy successfully [17]. Similarly, atosiban may increase the success rates of infertility treatment through a possible reduction in the uterine contractile activity, which may help increase uterine receptivity during ET, especially in women with RIF. Pierzynski et al [9] first reported that atosiban reduced intense spontaneous uterine contractility as visualized by transvaginal sonography, leading to improved uterine receptivity and resulting in successful embryo implantation during ET after endometrial synchronization with the donated oocyte recipient. Subsequently, we presented a woman who was first treated with atosiban for RIF and successfully conceived [10]. The delivery of her second and third triplets during preterm labor was then delayed through the use of an atosiban infusion. Thus, atosiban has been used to inhibit uterine contractility in both nonpregnant and pregnant women. Recently, Moraloglu et al [11] demonstrate that treatment with atosiban before ET affects the endometrial environment, including a decrease in uterine contractile activity, an increase in endometrial perfusion, and the inhibition of endometrial prostaglandin production, which improves pregnancy outcomes of IVF–ET.

A significant decrease in uterine contractility activity visualized by transvaginal sonography has been noted after 1 hour of intravenous atosiban infusion [9]. Although we did not use ultrasonography to study uterine contractility after intravenous infusion of atosiban in this study, patients in Group 2 had a significantly higher implantation rate (30.21%) compared with those of the control group (Group 1: 11.8%). Similarly, patients in Group 3 had a slightly higher implantation rate (15.9%) than those in the control group. A plausible explanation for the benefits of atosiban during ET may be a decrease in uterine contractile activity, which results in a significantly higher embryo implantation rate. In this study, we noted that the multiple pregnancy rate of Group 2 was coincidentally higher than those of Groups 1 and 3. Because the patients in our study had experienced two or more previous IVF failures, elective single ET seemed unsuitable for them. However, the subject of reducing the likelihood of multiple pregnancies in women with RIF requires more investigation.

Examination between the dosage of atosiban and our endpoints revealed that the dosage of atosiban was inversely correlated with implantation rate, clinical pregnancy rate, and live birth rate. Moraloglu et al [11] have demonstrated that the total administered dose of atosiban treatment in IVF–ET is 37.5 mg, with 46.7% clinical pregnancy rate and 20.4% implantation rate. This atosiban dosage is between the administered dosages of Groups 2 and 3 in our study, but it still shows high pregnancy outcomes. The inverse correlation between atosiban dosage and implantation rate seems contradictory. The reason for this contradiction is unknown.

Genetic expression of oxytocin and its receptor is detected in human cumulus cells surrounding the oocytes [18]. Oxytocin may enhance blastocyte formation and play a physiological role in embryogenesis of fertilized mouse oocytes. Messenger RNA expression of the oxytocin receptor has been demonstrated in mouse oocytes and embryos up to the blastocyst stage [19]. Its expression increases immediately after fertilization, which is compatible with a possible role of oxytocin in this process, and then, oxytocin receptor messenger RNA gradually decreases after the four-cell stage of pre-embryonic development [19]. Besides, oxytocin advanced the leutinizing hormone surge, and oxytocin is a component of an autocrine/paracrine intraovarian regulatory system responsible for the episodic release of progesterone from the bovine corpus luteum during the early luteal phase. Conversely, atosiban significantly reduces the number of progesterone peaks, and it affects the endocrinology of the ovulation cycle in nonpregnant women [20,21]. The pharmacokinetics of atosiban in nonpregnant patients is similar to that in pregnant women [22]. Preimplantation rabbit embryo development is not affected by atosiban in concentrations 50-fold higher than the mean plasma concentration reached during regular therapy [23]. In this study, no significant maternal complications or fetal anomalies were noted in the atosiban-treated group compared with those in the control group. However, the suitable dosage of atosiban used in humans during ET still needs to be clarified.

Results of this study clearly showed a significant improvement in implantation rate, pregnancy rate, and live birth rate in the atosiban-treated group compared with those in the control group. To our knowledge, this is the first case-series study evaluating the efficacy of using different dosages of atosiban in patients with RIF undergoing IVF–ET. Although the small sample sizes of our groups limited the statistical power of our observation, this study demonstrated that patients with RIF may benefit from atosiban treatment. In summary, we suggest that the use of atosiban may be safe and effective for IVF–ET of women with RIF. The use of atosiban likely inhibits uterine contractility, which may be a good method to help minimize the psychological burden after consultation in women undergoing IVF–ET, especially women with RIF. Atosiban may improve pregnancy outcomes of IVF–ET in women with RIF. However, further well-designed, prospective randomized placebo-controlled trials with large numbers of patients are needed to compare variant dosages of atosiban infusion during ET in women with RIF.

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