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İntrasitoplazmik Sperm Enjeksiyon-Embriyo Transfer Sikluslarında Rutin Luteal Faz Desteğine Gonadotropin Salgılatıcı Hormon Agonisti Eklenmesinin Gebelik Oranları ve Sonuçları Üzerine Etkisi

Effect Of Gonadotropin Releasing Hormon Agonist Addition To Routine Luteal Phase Support In Intracytoplasmic Sperm Injection-Embryo Transfer Cycles On Pregnancy Rates and Outcomes

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ÖZ

Amaç: GnRH-a uzun protokol ve GnRH-anta protokol ICSI-ET siklusla-rında rutin Luteal Faz Desteği (LFD)'ne GnRH-a eklenmesinin gebelik oranlarına ve sonuçlarına etkisini araştırmak

Gereçler ve Yöntem: Bu prospektif randomize çalışmaya ICSI-ET ile tedavi edilen yüz sekiz infertil çift dahil edildi. Hastalar iki farklı ovulasyon indüksiyon protokolüne tabi tutulmak için rastgele olarak iki gruba ayrıldı. Grup I'e GnRH-a uzun protokolü ve grup II'ye GnRH-anta protokolü uygulandı. İki protokol ile tedavi edilecek kadınlar da iki alt gruptan birine rastgele olarak ayrıldı. Ib ve IIb alt gruplarına, rutin LFD'ye [90 mg / gün vajinal progesteron artı 4 mg 17β Estradiol] ek olarak ET'den sonra 5. ve 10. günlerde löprolid asetat (0.5 mg s.c.) enjeksiyonları uygulandı. Diğer iki alt gruba (grup Ia ve IIa) ise sadece rutin LPD verildi.

Bulgular: Toplanan toplam oosit ve MII oosit sayısı, grup Ia'da grup Ib'den anlamlı olarak daha yüksekti. Grup I ve II'de alt gruplar arasında klinik gebelik, yumurtalık hiperstimülasyon sendromu (OHSS), çoğul gebelik, kürtaj, devam eden gebelik ve canlı doğum oranları açısından fark yoktu.

Sonuç: GnRH-a uzun protokol ve GnRH-anta protokol ICSI-ET siklusla-rında GnRH-a'nın rutin LPD'ye eklenmesinin devam eden gebelik, kürtaj, çoğul gebelik, OHSS, klinik gebelik ve canlı doğum oranları üzerinde hiçbir etkisi olmadığı görülmektedir.

Anahtar Kelimeler: gonadotropin, infertilite, luteal faz

ABSTRACT

Aim: To investigate the effect of gonadotrophin releasing hormone agonist (GnRH-a) addition to luteal phase support (LPS) in intracytoplasmic sperm injection-embryo transfer (ICSI-ET) cycles of GnRH-a long protocol and GnRH antagonist (GnRH-anta) protocol, on pregnancy rates and outcomes.

Materials and Method: One hundred and eight infertile couples treated with ICSI-ET were included in this prospective randomized study. Patients were randomly divided into two groups to undergo two different ovarian stimulation protocols. GnRH-a iong protocol was applied to group I and GnRH-anta protocol was applied to group II. Women to be treated by each of the two protocols were also randomly assigned to one of the two subgroups. Subgroups Ib and IIb received leuprolide acetate (0.5 mg s.c.) injections on the 5th and 10th days after ET in addition to routine LPS [90 mg/day of vaginal progesterone plus 4 mg of 17β Estradiol]. Only routine LPS was given to other two subgroups (groups Ia and IIa).

Results: The total number of retrieved oocytes and MII oocytes were significantly higher in group Ia than in group Ib. There were no differences between subgroups in groups I and II regarding clinical pregnancy, ovarian hyperstimulation syndrome (OHSS), multiple pregnancy, abortion, ongoing pregnancy and live birth rates.

Conclusion: Addition of GnRH-a to routine LPS in ICSI-ET cycles of GnRH-a long protocol and GnRH-anta protocol seems to have no effect on ongoing pregnancy, abortion, multiple pregnancy, OHSS, clinical pregnancy and live birth rates.

Keywords: gonadotropin, infertility, luteal phase

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INTRODUCTION

Implantation of the embryo is one of the most important factors affecting the success rate of assisted reproductive technology (ART) (1). Even when high quality embryos are transferred, the pregnancy rate may still be less than expected.

Ovarian stimulation cycles for ART are associated with supraphysiological steroid hormone levels that are secreted from multiple corpora lutea, which result in low levels of luteinizing hormone (LH) during the luteal phase (2). Subsequently, progesterone levels decrease, endometrial secretory transformation delays and the luteal phase shortens. This is known as a luteal phase deficiency, which is related to reduced embryo implantations, lower pregnancy rates and increased miscarriage rates (3). Therefore, luteal phase support (LPS) is a commonly used practice during ART cycles in order to improve embryo implantation, pregnancy and delivery rates. However, there is a worldwide controversy concerning the best protocol for LPS (4). The type, dose, duration, and starting and stopping time of hormones used for LPS remain controversial.

Human chorionic gonadotrophin (hCG) and progesterone were the first LPS agents with similar effects on pregnancy rates. However, since hCG usage increases the risk of ovarian hyperstimulation syndrome (OHSS), progesterone has been the primary choice for LPS in ART cycles (5). Unfortunately, the optimal protocol for progesterone administration has not yet been defined (6). Some additive agents and alternative medicine methods, such as steroids, oestrogens, ascorbic acid and acupuncture, have also been performed, but none of these have been effective (7-10).

Previous reports have indicated that nasal or subcutaneous administration of GnRH agonists during the luteal phase, in addition to routine LPS, increase pregnancy rates in ART cycles. It is possible that GnRH agonists may support the corpus luteum by inducing LH secretion from the hypophysis and by directly activating local GnRH receptors on the endometrium (11-16). However, there have also been controversial results regarding the beneficial effects of adding a GnRH-a for LPS (17 19). Therefore, in this study, we aimed to investigate pregnancy outcomes following the addition of a luteal phase GnRH-a to routine LPS in ART cycles of a GnRH-a long protocol and a GnRH-anta protocol.

MATERIAL AND METHODS

This randomized study was carried out in the IVF Centre of Zekai Tahir Burak Women's Health Education and Research Hospital between January 2013 and April 2014. Written informed consent was obtained from all enrolled patients, and the study protocol was approved by the Institutional Review Board of the hospital. Patients over 38 years of age, those who had undergone a frozen embryo transfer (ET), those with a chronic disease or drug use because of a systemic disease, those with abnormal thyroid-stimulating hormone (TSH) levels and those in which there was male or female infertility were excluded from the study.

A total of 108 infertile patients treated by intracytoplasmic sperm injection (ICSI)–ET due to unexplained infertility were enrolled in the study. These patients were assigned to one of the two most currently used ovarian stimulation protocols. Specifically, group I (54 patients) underwent the GnRH-a long protocol and group II (54 patients) underwent the GnRH-anta protocol. Patients were randomly assigned to one of the two treatment groups. The aim of this study was to evaluate the effect of the luteal phase GnRH-a administration in each of the two protocols.

In group I, patients underwent the GnRH-a long protocol for pituitary down-regulation using 1 mg/day (s.c.) of leuprolide acetate (Lucrin; Abbott Cedex, Turkey), which was started on the 21st day of the preceding menstrual cycle. When pituitary down-regulation was detected (on the 2nd day of the menstrual cycle; LH < 5 IU/ml, estradiol (E2) < 50 pg/ml and transvaginal ultrasonographic endometrial thickness < 5 mm), the leuprolide acetate dose was reduced to 0.5 mg/day. Also, 150–225 IU/day of human menopausal gonadotropin (Menogon, 75 IU, Ferring Pharmaceuticals) or 150–225 IU/day of recombinant follicle-stimulating hormone (FSH; Gonal F, Sereno, Turkey) was applied intramuscularly from the 2nd day of the menstrual cycle. A GnRH-a (0.5 mg/day) was continued until the hCG injection. In group II, patients underwent a GnRH-anta protocol, and controlled ovarian hyperstimulation (COH) with gonadotrophin began on the 3rd day of the menstrual cycle at a dose ranging from 150–300 IU daily. Once the leading follicle reached a mean diameter of 13–14 mm, a daily GnRH antagonist (Cetrorelix Acetate, Cetrotide, Serono, Turkey) was started at a dose of 0.25 mg and continued until the hCG trigger.

For each group, the starting dose of gonadotropins was arranged according to their body mass index, age and the anticipated ovarian response. The gonadotropin dose was then adjusted based on follicular development as determined by transvaginal ultrasonography and serum E2 levels. Following the detection of three or more follicles with an 18-mm mean diameter, a single dose of 10 000 IU (Pregnyl, Organon, Netherlands) was used to induce oocyte maturation. Oocyte retrieval was performed transvaginally with ultrasound guidance 36 hours later. Fertilization was then done with an ICSI in all couples and a uterine ET was performed 3 or 5 days after ICSI. For standard luteal phase supplementation, all women received 90 mg/day of vaginal progesterone (Crinone vaginal gel; 8%; Merck-Serono, Germany) and 4 mg of 17b E2 (Estrofem; Novo Nordisk, Bagsvaerd, Denmark) starting on the oocyte retrieval day. E2 was continued until the pregnancy test was performed after the ET, while progesterone was continued until the 12th week of gestation by women with a positive pregnancy test.

Patients to be treated by each of the two protocols were randomly assigned to one of the two subgroups. Randomization was done with the use of a computer-generated randomization list. Women randomized to subgroups Ib and IIb received leuprolide acetate (Lucrin; 0.5 mg s.c.) injections on the 5th and 10th days after ET in addition to the routine LPS mentioned above. The other two subgroups (groups Ia and IIa) received only the routine LPS.

Serum beta hCG levels were measured 12 days after ET to confirm the presence of a pregnancy. A clinical pregnancy was defined as the presence of at least one gestational sac in which the foetal heartbeat was positive with transvaginal ultrasonography. When a pregnancy proceeded beyond 20 weeks of gestation, it was defined as an ongoing pregnancy.

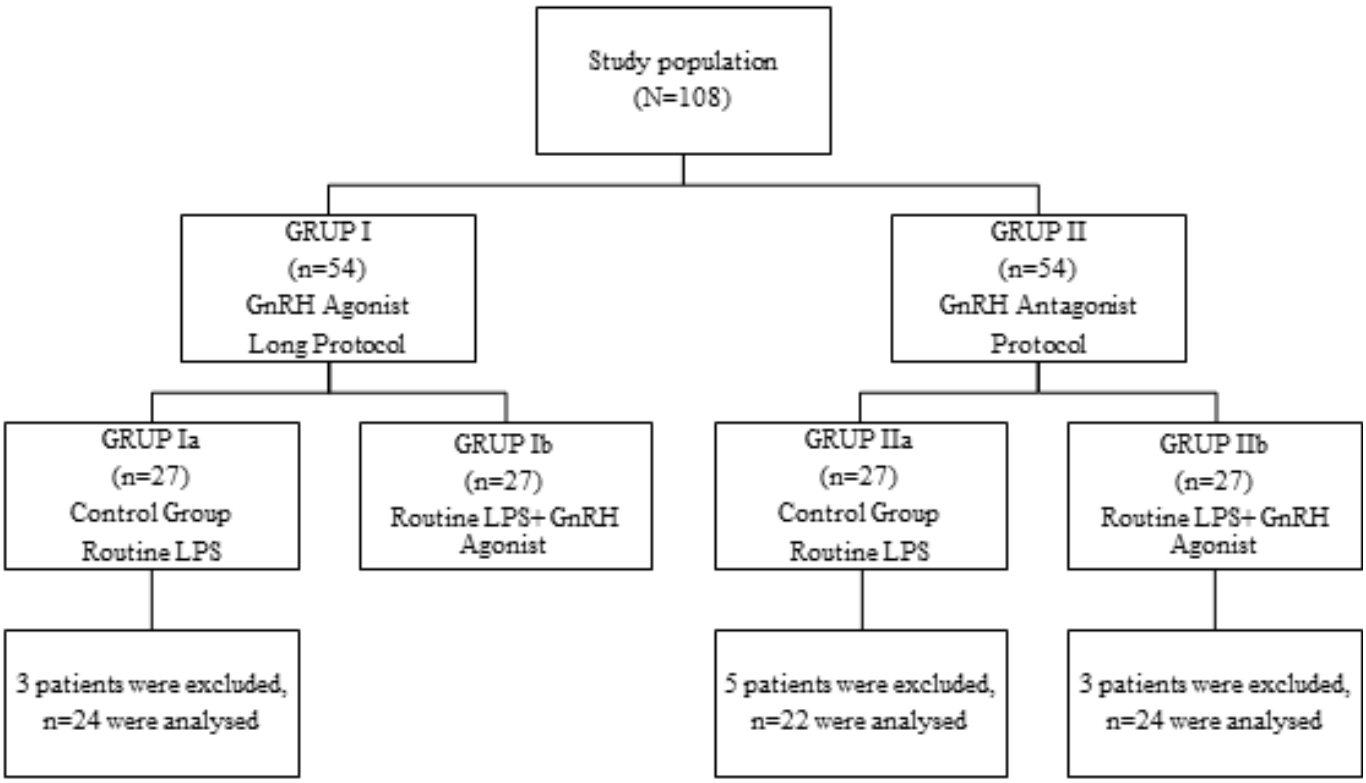
The Statistical Package for the Social Sciences (SPSS) 15.0 for Windows was used for statistical analyses. The Kolmogorov-Smirnov test was used to determine the distribution of data normality. The data are presented as mean ± standard deviation (SD) or median (minimum maximum) for continuous variables, and as a number (percentage) for categorical variables. The variables with normal distributions were compared between groups by independent samples tests. The Mann-Whitney U test was used to analyse non-normally distributed data. The chi-square test was used to compare categorical variables. A multivariable adjusted analysis of covariance (ANCOVA) including statistically significant variables was used to investigate potential relationships between LPS and the GnRH agonist and clinical or ongoing pregnancies. A p value < 0.05 was considered to be statistically significant.

RESULTS

In this study, 108 patients were included and patients in each of the two ovarian stimulation protocols were initially at a ratio of 1:1 (group I:group II). Groups (groups I and II) were randomly divided into two subgroups (groups

Ia/Ib and groups IIa/IIb, respectively) at a ratio of 1:1. Eleven patients (3 in Group Ia, 5 in Group IIa and 3 in Group IIb) were excluded because of failed fertilizations; this left 24 patients in group Ia, 27 patients in group Ib, 22 patients in group IIa and 24 patients in group IIb eligible for the final analysis (Figure 1).

Figure 1: Study population and randomization



The groups I and II had statistically similar subgroups in terms of age, body mass index, infertility duration, and basal FSH and LH levels ($p > 0.05$ for all). Basal E2 levels were significantly greater in group Ib than in group Ia ($p = 0.035$), but this significant difference was not present for group II ($p = 0.104$) (Table1).

Table 1. Demographic and basal characteristics of the groups

	Group I (n=51)		p	Group II (n=46)		p
	Grup Ia (n=24)	Grup Ib (n=27)		Grup IIa (n=22)	Grup IIb (n=24)	
Woman's age (years)	28.0 (23.0-35.0)	30.5 (24.0-37.0)	0.920	28.5 (23.0-38.0)	30.0 (26.0-38.0)	0.333
BMI(kg/m^2)	26.2 ±4.3	26.0 ±4.5	0.872	23.9 ±3.6	24.4 ±3.8	0.650
Infertility duration (years)	5.2 (1.5-14.0)	4.7 (1.5-18.0)	0.754	3.0 (1.0-9.0)	4.0 (1.0-15.0)	0.380
Basal FSH (mIU/ml)	6.6 (3.8-11.6)	6.7 (4.0-13.0)	0.890	6.1 (4.0-11.6)	7.1 (4.5-19.3)	0.080
Basal LH (mIU/ml)	5.0 (2.0-21.0)	5.1 (2.4-22.0)	0.905	4.8 (2.4-24.4)	4.6 (1.0-13.5)	0.691
Basal E_2 (pg/ml)	38.0 (11.8-63.0)	47.5 (21.8-62.3)	**0.035**	40 (28.0-78.9)	34.0 (36.0-57.0)	0.104

Data presented as mean±standard deviation, median (minimum-maximum values) or n (%).
BMI: Body mass index; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E_2: Estradiol
$p<0.05$ was considered as statistically significant.

ART cycle characteristics of the groups are shown in Table 2. The number of total retrieved oocytes and MII oocytes were significantly higher in group Ia than in group Ib ($p = 0.001$ and $p = 0.004$, respectively). However, the other variables listed in Table 2 did not reveal any significant differences between the groups.

Table 2: ART cycles characteristics of the groups

	Group I (n=51)		p	Group II (n=46)		p
	Grup Ia (n=24)	Grup Ib (n=27)		Grup IIa (n=22)	Grup IIb (n=24)	
Total gonadotrophin dose used (IU)	1950.0 (900.0-3675.0)	1800 (900.0-4050.0)	0.758	1750.0 (750.0-4050.0)	2100.0 (400.0-4650.0)	0.386
E_2 levels on hCG day (pg/ml)	2570.0 ±993.0	2361.0 ±693.0	0.383	2298.0 ±1085.0	1782.0 ±729.0	0.067
Endometrial thickness on hCG day (mm)	10.2 (7.9-21.0)	9,7 (5-16)	0.265	10.1 (7.0-13.0)	9.4 (7.0-13.0)	0.117
Total oocytes retrieved	17 (6-22)	10 (3-26)	**0.001**	10 (3-28)	11 (3-18)	0.665
MII oocyte number	11 (5-18)	8 (1-22)	**0.004**	8 (2-24)	7 (3-13)	0.740
No. of transferred embryos One Two	 21(87.5) 3(12.5)	 27(96.4) 1(3.6)	0.233	 16(72.7) 6(27.3)	 19(82.6) 4(17.4)	0.431
Embryo grading (quality) 1 (high quality) 2 (low quality)	 20(83.3) 4(16.7)	 27(96.4) 1(3.6)	0.169	 21(95.4) 1(4.6)	 23(100.0) 0 (0.0)	0.489

Data presented as mean±standard deviation, median (minimum-maximum values) or n (%).
E_2: Estradiol; hCG: Human chorionic gonadotropin
p<0.05 was considered as statistically significant.

There were no significant differences between the subgroups with respect to clinical pregnancy, OHSS, multiple pregnancy, abortion, ongoing pregnancy and live birth rates in groups I and II (p > 0.05 for all) (Table 3). In this study, all ongoing pregnancies resulted in a live birth.

Table 3: Clinical outcomes of the groups

	Group I (n=51)		p	Group II (n=46)		p
	Group Ia (n=24)	Group Ib (n=27)		Group IIa (n=22)	Group IIb (n=24)	
Clinical pregnancy rate	8 (33.3)	6 (22.2)	0.375	5 (22.7)	9 (37.5)	0.235
OHSS rate	1 (4.2)	0 (0.0)	0.658	2 (9.1)	1(4.2)	0.608
Multiple pregnancy rate	1 (4.2)	0 (0.0)	0.471	1 (4.5)	0 (0.0)	0.489
Abortion rate	3 (12.5)	1 (3.7)	0.902	1 (4.5)	3 (12.5)	0.608
Ongoing pregnancy rate	5 (20.8)	5 (18.5)	0.989	4 (18.2)	6 (25.0)	0.722
Live birth rate	5 (20.8)	5 (18.5)	0.989	4 (18.2)	6 (25.0)	0.722

Data presented as n (%).
OHSS: Ovarian hyperstimulation syndrome
p<0.05 was considered as statistically significant.

We performed a multivariable-adjusted ANCOVA including the total number of retrieved oocytes, MII oocytes and basal E2 levels, which were statistically significant variables between the groups. We also excluded the effect of these parameters on pregnancy to investigate the effect of LPS with the GnRH-a on clinical pregnancy, ongoing pregnancy and live birth. The results of this analysis suggest there was no difference between subgroups with respect to clinical pregnancy, ongoing pregnancy rates or live birth rates (p > 0.05 for all) (Table 4).

Table 4: The results of univariate regression analysis

	Clinical pregnancy Adjusted p	Ongoing pregnancy rate Adjusted p
Total oocytes retrieved	0.528	0.910
MII oocyte number	0.567	0.915
Basal E_2 level	0.086	0.089
E2: Estradiol p<0.05 was considered as statistically significant.		

DISCUSSION

LPS during ART cycles with COH is a routine practice in infertility treatments. Because stimulated ART cycles are generally resulted with luteal phase deficiency that decreases the treatment success. However, the best LPS protocol remains controversial. Progesterone use has been the standard option for LPS. However, several other options have also been investigated including hCG, recombinant LH and E2. In recent years, a novel approach for LPS with a GnRH agonist during ART cycles has emerged.

Several studies have reported beneficial effects of GnRH agonists in LPS. In 2004, Tesarik et al. (13) added 0.1 mg of triptorelin in LPS 6 days after ICSI in 276 oocyte donation cycles and found that implantation and birth rates were higher with triptorelin compared to a placebo. In 2006, Pirard et al. (12) randomized 23 patients into 5 groups to determine whether the administration of intranasal buserelin could support the luteal phase and improve ART outcomes. They pointed out that implantation and live birth rates increased with advancing buserelin doses when compared to the control group. They also concluded that buserelin may affect the follicular maturation trigger and provide LPS in ART cycles. In another randomized study, Tesarik et al. (11) evaluated the effect of a GnRH-a (0.1 mg triptorelin on day 6 after ICSI), in addition to routine LPS with progesterone, E2 and hCG on ICSI outcomes in both GnRH-a and -anta treated ovarian stimulation cycles. The authors reported that LPS with the GnRH-a administration improved implantation and live birth rates significantly in both agonist and antagonist cycles. Furthermore, these authors suggested that LPS with a GnRH-a may enhance ICSI outcomes by a combination effect on the embryo and the corpus luteum in both agonist and antagonist cycles. In 2009, Isık et al. (16) also reported increased implantation, clinical pregnancy and live birth rates by adding a single subcutaneous dose (0.5 mg) of leuprolide acetate 6 days after ICSI to routine LPS in antagonist cycles of 164 patients.

In contrast, there are several studies showing that the administration of GnRH agonists in LPS do not have a beneficial effect on pregnancy rates. In a large double-blind study, Ata et al. (19) randomized 570 ICSI-ET patients to receive either 0.1 mg of triptorelin injections or a placebo 6 days after ICSI in addition to routine LPS with progesterone. They found no significant differences between the groups regarding implantation, clinical pregnancy and multiple pregnancy rates. In a recent study by Inamdar et al. (17), 426 patients treated with a long agonist protocol were randomized to receive three 1 mg doses of leuprolide acetate or placebo injections 6 days after ovum pick-up (OPU) in addition to routine LPS with progesterone. Moreover, similar implantation, clinical pregnancy and multiple pregnancy rates were reported between the GnRH-a and placebo groups.

In the present study, we also found no benefits of adding GnRH agonists to routine LPS. There were also no increases in OHSS and multiple pregnancy rates in patients receiving GnRH agonists in the luteal phase. However, caution is recommended until more detailed information is available concerning the effect of GnRH agonists administered in the luteal phase over the different aspects of the reproductive system.

The flare-up effect of GnRH agonists continues for 3 days and is then suppressed (20). Therefore, a single dose may be insufficient. One of the reasons for the administration of GnRH agonists twice during the luteal phase in this study was to prolong the flare-up effect of the GnRH-a. In COH protocols, after a down regulation of GnRH receptors in the endometrium, there may be a resistance to a single dose of GnRH-a in the luteal phase. Therefore, this was one of the reasons GnRH agonists were administered twice during the luteal phase in this study.

The exact mechanism of beneficial effects of GnRH-a administration with LPS is not well defined. GnRH agonists may stimulate the corpus luteum by inducing LH secretion from the hypophysis or by directly activating local GnRH receptors on the endometrium with certain doses (11). Endometrial GnRH receptors positively affect implantation of the embryo by modulating several paracrine factors, such as transforming growth factor, fibronectin, matrix metalloproteinase and L-selectin, which are important in the implantation process (21). It has also been argued that GnRH agonists have a direct impact on the early embryo (13). In addition, GnRH agonists stimulate in vivo and in vitro placental hCG production (22,23). In support of these claims, a GnRH antibody was detected in serum samples of women who had an abortus history with low levels of hCG (22), a finding that may confirm the importance of the GnRH and hCG relationship on normal early pregnancy physiology (13). One of the reasons for the administration of a second GnRH-a dose during the luteal phase in this study was to investigate the effect of the GnRH-a on abortion prevention. There was no significant difference between the subgroups with respect to abortion rates.

As mentioned above, it remains controversial whether adding GnRH agonists with LPS has beneficial effects on ART cycles. Another controversy concerns the optimal dose of the GnRH-a. We did not compare different GnRH-a doses in our present study and this may be a limitation.

DISCUSSION

Our findings suggest that a subcutaneously administered GnRH-a (0.5 mg) on the 5th and 10th days after ET with routine LPS in ICSI-ET cycles of GnRH-a long and GnRH-anta protocols did not improve ART cycle outcomes. However, the relatively small sample size seems to be a limitation in establishing the exact role of GnRH-a administration in the luteal phase. Therefore, we believe that further studies with larger sample sizes and different doses of GnRH agonists are still required.

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