Ovarian stimulation with GnRH agonist, but not GnRH antagonist, partially restores the expression of endometrial integrin β3 and leukaemia-inhibitory factor and improves uterine receptivity in mice

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BACKGROUND: The impact of different ovarian stimulation (OS) protocols on endometrial receptivity remains controversial. In this study, the effects of different OS on the expression of endometrial integrin β3 subunit and leukaemia-inhibitory factor (LIF) during the implantation window and the implantation rate in mice were investigated.

METHODS: Three OS protocols were used, involving either pregnant mare’s serum gonadotrophin (PMSG) alone, PMSG plus GnRH agonist or PMSG plus GnRH antagonist. Uterus samples were collected at 48 h after OS or ovulation and were detected with immunohistochemistry, Western blot and RT–PCR analyses. Normal embryos at gestation day 4 were transferred into the uteri of mice in the control and OS groups. RESULTS: All OS groups showed a significant decrease in the expression of both the endometrial integrin β3 subunit and LIF during the implantation window and the implantation rate. Among the three OS groups, GnRH agonist-treated mice showed a higher endometrial integrin β3 subunit and LIF expression and a higher implantation rate. No significant difference was found in the measured indices between the GnRH antagonist and PMSG groups. CONCLUSIONS: OS may inhibit the expression of endometrial integrin β3 subunit and LIF and impair endometrial receptivity in mice. OS with GnRH agonist, but not GnRH antagonist, may partially restore the endometrial physiological secretion and improve uterine receptivity.

Key words: endometrial receptivity/GnRH antagonist/integrin/leukaemia-inhibitory factor/mouse/ovarian stimulation

Introduction

Ovarian stimulation (OS) with gonadotrophins is an important approach in IVF. However, the impact of different OS on endometrium receptivity remains controversial (Al-Inany and Aboulghar, 2002; Fauser and Devroey, 2003; Coccia et al., 2004; Tarlatzis and Bili, 2004). Many studies in human have shown that the periovulatory endometrial characteristics in OS cycles are considerably different compared with the natural cycle (Devroey et al., 2004; Fauser and Devroey, 2003; Saadat et al., 2004; Papanikolaou et al., 2005). This difference could affect luteal phase function and alter endometrial receptivity (Devroey et al., 2004; Kolibianakis et al., 2002), even to the extent of greatly compromising the likelihood of pregnancy (Al-Inany and Aboulghar, 2002; Beckers et al., 2003). An earlier meta-analysis (Al-Inany and Aboulghar, 2002) focusing on five multicentre comparative trials concluded that a small but significant reduction in pregnancy rate was observed per started
cycle using a GnRH antagonist co-treatment protocol compared with the routine GnRH agonist co-treatment OS. Therefore, concerns have been raised regarding the possibility of direct effects of OS on endometrial receptivity and embryonic implantation, especially the possibility that adverse effects may arise from GnRH antagonist co-treatment (Devroey et al., 2004). Concordant evidence of this aspect is accumulating (Mirkin et al., 2004; Horcajadas et al., 2005; Vlahos et al., 2005), whereas the non-concordant results are also appearing (Bahceci et al., 2005; Saadat et al., 2004; Takahashi et al., 2004; Prapas et al., 2005; Simon et al., 2005). This means that a controversial state of this aspect exists and that further clarification is needed.

Regarding the effect of different OS strategies on endometrial receptivity and embryonic implantation in the human, the data shown in previous studies were mainly in the clinical and endometrial histologies (Beckers et al., 2003; Kolibianakis et al., 2002; Mirkin et al., 2004; Papanikolaou et al., 2005; Saadat et al., 2004; Simon et al., 2005; Tavaniotou et al., 2003; Thomas et al., 2002). Little direct data were available in the literature on human endometrial receptivity. Similar situations existed in related studies carried out in mice (Ertzeid and Storeng, 1992, 2001; Van der Auwera and D’Hooghe, 2001).

Integrin β3 subunit and leukaemia-inhibitory factor (LIF) are the two cellular factors which have been best characterized and largely accepted as the promising candidates of biomarkers of uterine receptivity in both human (Cavagna and Mantese, 2003; Hoozeman et al., 2004; Kondera-Anasz et al., 2004) and mice (Bhatt et al., 1991; Stewart et al., 1992; Illera et al., 2000a). The expression of these two cellular factors in both human and mouse endometrium is in a menstrual cycle-dependent manner, and the maximal expression is observed in the mid-secretory phase of the menstrual cycle, coinciding with the time of implantation (Arici et al., 1995; Lessey et al., 1992, 1994; Cullinan et al., 1996; Damario et al., 2001). It has been found that the expression of both alpha(3)beta(3) integrin and LIF was significantly reduced in endometrium from infertile women (Hambartsoumian, 1998; Lessey et al., 1995; Seli et al., 2005). Although these two cellular factors have not been established as obligatory uterine ones in human implantation (Creus et al., 2002, 2003; Thomas et al., 2003; Devroey et al., 2004), many studies have demonstrated that LIF is one of the obligatory implantation factors in mice (Cavagna and Mantese, 2003; Devroey et al., 2004; Kondera-Anasz et al., 2004).

The purpose of the present study was to investigate the effects of different classical OS protocols (gonadotrophins alone, GnRH agonist co-treatment or GnRH antagonist co-treatment) on endometrial receptivity and embryonic implantation in a human IVF-mimicked mouse model. The expression of integrin β3 subunit and LIF and embryonic implantation were used to evaluate endometrial receptivity and embryonic implantation. Our hypothesis was that different OS protocols would induce different biological and molecular profiles of endometrium that might lead to altered endometrial receptivity and implantation outcome.

Materials and methods

Animals

Animal care and use procedures were in accordance with the institutional guidelines established by the Animal Care and Use Committee (ACUC) of the School of Medicine, Zhejiang University. Virgin 6- to 8-week-old female CD1 mice (Zhejiang University Animal Center, Hangzhou, China) were housed in 12/12 h light/dark cycle (light on from 0:00 to 12:00 hours) at 25 ± 0.5°C and 50–60% humidity. The mice were fed ad libitum with a standard pellet diet and water. Estrus was identified by daily vaginal discharge and smear samples. Only mice that had exhibited estrus for more than two consecutive periods of regular 4-day cycles were used in the present study. Suitable mice [8- to 12-week old and 18–22 g body weight (bw)] were randomly allocated to four groups, with 30 mice in each group. Within each group, 10 mice were either used for OS treatment or allowed to remain in their natural cycle, and the other 20 mice were used as recipients in the embryo-donation model described below.

Ovarian stimulation

The procedures for OS performed in different groups were: (i) GnRH agonist group: GnRH agonist (Decapetyl, Ferring GmbH, Germany) was i.p. injected at 1.5 μg/100 g bw/day from day 3–9 of estrus. At 9.00 a.m. of day 9, the pregnant mare’s serum gonadotrophin (PMSG) (Intervet, USA) was i.p. injected at 40 IU/100 g bw, followed by an injection (i.p.) of hCG (Pregnyl, Organon, The Netherlands) (100 IU/100 g bw) 28 h after the injection of PMSG; (ii) GnRH antagonist group: GnRH antagonist (Cetrotide, Serono, USA) was i.p. injected at 4 μg/100 g bw on day 3 of estrum. PMSG was i.p. injected at 40 IU/100 g bw at 9.00 a.m. of day 9, followed by an injection (i.p.) of hCG (100 IU/100 g bw) 28 h after the injection of PMSG; (iii) PMSG group: The mice were i.p. injected with saline at the same volume from day 3–9 of estrus and then treated with the same procedure as described for the GnRH agonist and GnRH antagonist groups; (iv) Control group: The mice were i.p. injected with saline only at the same volume from day 3 of estrus onwards, following the same injection schedule as described for the three groups above.

Tissue collection and application

Fresh whole uterus samples were collected from OS-treated or naturally ovulated mice at 48 h after OS (treated groups) or ovulation (control group). After quickly being rinsed with cold phosphate-buffered saline, each fresh whole uterus sample was divided into three parts. One part was fixed in 10% formalin and embedded in paraffin for a streptavidin peroxidase (SP)-conjugated immunohistochemical assay. The other two parts of each sample, including both endometrium and myometrium, were stored at –80°C until the extraction of protein and mRNA.

Immunohistochemical analysis

Formalin-fixed, paraffin-embedded tissues were sectioned to a thickness of 3 μm. Sections were then deparaffinized in xylene, dehydrated in a series of ethanol solutions and stained using standard immunohistochemistry procedures. Tissue sections were pretreated by boiling in 10 mmol/l citrate buffer (pH 6.0) for 10 min as recommended by the supplier (SP Kit) (Zymed, USA) at dilution of 1 : 75 and 1 : 100 were used, respectively. The secondary antibodies were visualized using the avidin–biotin method recommended by the supplier (SP Kit) (Zymed, USA). The brown colour was developed with diaminobenzidine (DAB Kit) (Zymed).
examiners and was assessed by using the HSCORE. The HSCORE was calculated using the following equation:

$$HSCORE = P(i + 1),$$

where $i$ is the staining intensity and $P$, the percentage of stained epithelial cells at each level of intensity. In order to confirm that the staining was specific, a human endometrium sample from a 29-year-old woman in her mid-secretory phase of menstrual cycle was used as positive control. The negative control was carried out, omitting the primary antibody in the same human endometrial sample.

**Western blot analysis**

Total proteins from the tissues were extracted by RIPA buffer containing 50 mmol/l Tris–HCl (pH 7.4), 150 mmol/l NaCl, 1% NP-40, 0.5% sodium deoxycholic acid, 0.1% SDS, 100 μg/ml PMSF and 100 μg/ml leupeptin. Samples were electrophoresed with sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) using 10% polyacrylamide gels and were transferred to nitrocellulose membranes (Bio-Rad, USA). Membranes were blocked at room temperature for 1 h with 5% fat-free powdered milk in TBS-T (10 mmol/l Tris, 150 mmol/l NaCl, 0.05% Tween-20, pH 8.0). Following three washes with TBS-T, the membranes were incubated overnight with primary antibody in 1% TBS-T at 4°C. For Western blot analysis of integrin β3 and LIF, goat-anti-human polyclonal antibodies (Santa Cruz Biotechnology) at dilution of 1:250 and 1:100 were used, respectively. After incubation, the membranes were washed three times with TBS-T and then incubated with the appropriate secondary antibody at dilution of 1:2000 conjugated to peroxidase (Santa Cruz Biotechnology) at room temperature for 2 h. Following three washes with TBS-T and three washes with distilled water, the bound antibodies were detected by enhanced chemiluminescence (ECL kit) (Santa Cruz Biotechnology). β-Actin was used as an internal control to validate the amount of protein loaded onto the gels.

**RT–PCR analysis**

Poly(A)$^+$ RNA was isolated from the mouse uterus samples using the Micro-Fast kit (Invitrogen, CA, USA), from which single-strand cDNA was synthesized using the cDNA cycle kit (Invitrogen). The purity of the extracted RNA was photometrically tested, and the ratio of the optical density (OD) at 260/280 nm was 1.8–2.0. A total of 20 μl reaction contained 0.5 μg random primer (Sigma-Genosys, Australia), 40 U RNase inhibitor (Promega Corporation, USA), 200 IU M-MLV reverse transcriptase, 2 μl dNTP at 25 pmol/l (Sigma), 4 μl reverse transcriptase buffer (×5) and 2 microgram RNA. Reactions were incubated at 70°C for 5 min, 37°C for 90 min, 95°C for 5 min and then rapidly cooled on ice.

For integrin β3, PCR was performed in a 50 μl volume containing 1 μl RT reaction product, 5 μl PCR buffer (×10), 4 μl MgCl$_2$ at 2.5 mmol/l, 1 μl dNTP at 25 pmol/l, 1 μl integrin β3 sense and antisense primer, 0.5 μl β-actin sense and antisense primer as control and 0.4 μl Taq DNA polymerase (Takara, Japan). LIF was performed as described above except that the dosage of the primers was 1.2 μl for the LIF and 0.4 μl for β-actin.

The primers used were as follows: (i) integrin β3: sense: 5’-GCTTCTGAGGACACTGAT-3’; antisense: 5’-GGACAACTGC TGGCAGTCTTT-3’, which yield a 337 bp PCR product. (ii) LIF: sense: 5’-CCTACTGCTGCTGTCTCTG-3’; antisense: 5’-GCTCCA CCAAATGCTGCT-3’, which yield a 288 bp PCR product. (iii) β-actin: sense: 5’-TTCCAGCTTCTTCTG-3’; antisense: 5’- TTGCGCTCAGGAGCAAT-3’, which yield a 224 bp PCR product. All primers were obtained from Bio Basic (Shanghai, China). PCR was performed using the following conditions: for integrin β3, an initial denaturation at 94°C for 5 min, followed by 28 cycles of 30 s at 94°C, 30 s at 60°C and 30 min at 72°C; for LIF, an initial denaturation at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 65°C and 30 min at 72°C. All PCR was terminated with a final extension at 72°C for 10 min. The PCR products were subjected to electrophoresis on 2% agarose gels and visualized by ethidium bromide staining and then verified with β-actin as a control.

**Embryo collection and transfer**

Successfully mated female mice were identified with vaginal plugs. The day the vaginal plug presented was designated gestation day 1. Pregnant donor mice were killed by cervical dislocation on gestation day 2. The oviducts were excised and flushed with human tubal fluid (HTF) medium (Irvine Science, USA). Morphologically normal day 2 embryos (2- to 4-cell) were pooled after collection. The quality of embryos was evaluated based on cell numbers, size of blastomeres and cytoplasmic fragments. The embryos were cultured in HTF medium, supplemented with 10% synthetic serum substitute under oil at 37°C in a humidified atmosphere with 5% CO$_2$. On day 4, normal embryos that reached the morula/blastocyst stage were randomly divided and transferred into the mice in control and OS groups. There were 20 mice in each group. Six embryos were transferred into the right uterine horn of each mouse in the estrus of natural cycle (control group) or at 48 h later after being given HCG (three OS groups) in a way of embryo-donation model. Mice were placed in a separate cage supplied with ad libitum food and water. The recipients were killed on day 10 of gestation, and the conceptuses were removed from the uteri.

The number of implantation sites, moles (resorbing conceptuses) and live fetuses in the right uterine horn were recorded. The implantation rate was calculated as: total number of implantation sites, moles and live fetuses/number of embryos transferred.

**Statistical analysis**

Mann–Whitney U-test was used to compare the expression of endometrial integrin β3 and LIF in different OS groups and the control group. The effect of OS with different treatment on embryonic implantation rate was analysed by Chi-square test. All P-values are two-sided, and $P < 0.05$ was considered statistically significant. Data were analysed using the commercially available software package SPSS 11.0 (SPSS, Chicago, IL, USA).

**Results**

**Immunohistochemical localization of endometrial integrin β3 subunit and LIF during the implantation window of mice**

Immunohistochemical detection of integrin β3 subunit and LIF was observed mainly in glandular epithelial cells and luminal epithelial cells of murine endometrium during the implantation window (Figures 1A and 2A). Compared with the control group (HSCORE: 2.44 ± 0.09), OS groups showed lower staining intensities of integrin β3 subunit (HSCORE: 2.31 ± 0.09 in GnRH agonist group; 2.09 ± 0.08 in GnRH antagonist group and 2.06 ± 0.10 in PMSG group) during the implantation window of the treated mice ($P$-values of 0.009, <0.001 and <0.001, respectively; Figure 1B). OS groups also showed lower staining intensities of LIF (HSCORE: 1.67 ± 0.10 in GnRH agonist group; 1.44 ± 0.05 in GnRH antagonist group and 1.41 ± 0.07 in PMSG group) than that of the control group (HSCORE: 1.84 ± 0.08) during the implantation window ($P$-values of 0.121,
<0.001 and <0.001, respectively) (Figure 2B). Among OS groups, the staining intensities of integrin β3 subunit and LIF during the implantation window in the GnRH agonist group were significantly higher than that in GnRH antagonist group (P-values of <0.001 in integrin and <0.001 in LIF, respectively) or PMSG group (P-values of <0.001 in integrin and <0.001 in LIF, respectively) (Figures 1B and 2B).

Expression of endometrial integrin β3 subunit and LIF mRNA during the implantation window

Both integrin β3 subunit and LIF mRNA were expressed in endometrium of mice during the implantation window in the natural control group and all the OS groups (Figures 3A and 4A). Compared with the control group, the integrin β3 subunit mRNA expression levels, normalized by β-actin expression levels, in all three OS groups were significantly decreased (P-values of 0.049, 0.016 and 0.009, respectively; Figure 3B). LIF mRNA expression levels were also reduced in three OS groups (P-values of 0.023 in GnRH agonist versus control group, 0.009 in GnRH antagonist versus control group and <0.001 in PMSG versus control group, respectively) (Figure 4B). Among the three OS groups, integrin β3 subunit and LIF mRNA expression levels in GnRH agonist group were significantly higher than those in the other two OS groups (integrin β3 subunit: P-values of 0.037 in GnRH agonist versus GnRH antagonist group and 0.017 in GnRH agonist versus PMSG group, respectively; LIF: P-values of 0.049 in GnRH agonist versus GnRH antagonist group and 0.018 in GnRH agonist versus PMSG group, respectively) (Figures 3B and 4B).

Expression of endometrial integrin β3 and LIF proteins during the implantation window

The protein expression of endometrial integrin β3 subunit and LIF during the implantation window of the mice was confirmed in all groups by Western blotting (Figures 5A and 6A). Consistent with the results of mRNA expression, the protein levels of both integrin β3 subunit and LIF, normalized with β-actin expression level, in OS groups were lower than those in the control group (integrin β3 subunit: P-values of 0.028 in
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GnRH agonist versus control group, 0.009 in GnRH agonist versus control group and <0.001 in PMSG versus control group, respectively; LIF: P-values of 0.016 in GnRH agonist versus control group, <0.001 in GnRH antagonist versus control group, and <0.001 in PMSG versus control group, respectively; Figures 5B and 6B). Among the three OS groups, both integrin β3 subunit and LIF expression levels in GnRH agonist group were significantly higher than those in the other two OS groups (integrin β3 subunit: P-values of 0.028 in GnRH agonist versus GnRH antagonist group and 0.019 in GnRH agonist versus PMSG group, respectively; LIF: P-values of 0.013 in GnRH agonist versus GnRH antagonist group, and <0.001 in GnRH agonist versus PMSG group, respectively; Figures 5B and 6B).

**Embryonic implantation rate**

In each group of the present study, 120 normal mouse embryos that reached the morula/blastocyst stage at day 4 were transferred to the corresponding mice. The number of implantation sites, moles (resorbing conceptuses) and live fetuses at gestation day 10 in each group was 58 in the control group, 44 in GnRH agonist group, 28 in GnRH antagonist group and 20 in PMSG group. The embryonic implantation rate in the control group (48.4%) was significantly higher than that in GnRH antagonist group (23.4%, P < 0.001) and PMSG group (16.7%, P < 0.001). Among the three OS groups, the embryonic implantation rate in GnRH agonist group (36.7%) was also significantly higher than those in the other two OS groups (P-values of 0.018 in GnRH agonist versus GnRH antagonist group and <0.001 in GnRH agonist versus PMSG group, respectively).
Discussion

The present study is a pilot, prospective, randomized and controlled comparison study on the effects of different OS strategies on the endometrial receptivity and embryonic implantation in the human IVF-mimicked mouse models. Using immunohistochemical, Western blot and RT–PCR analyses, we demonstrated that integrin β3 subunit and LIF were expressed in the endometrium of mature female mice during the implantation windows. There were significant differences in the expression of endometrial integrin β3 subunit and LIF between OS groups and the control group during the implantation window of mice. By using an embryo-donation model, we also demonstrated that the embryonic implantation rates in all three OS groups were lower than that in the control group. Immunohistochemical analysis showed that the integrin β3 subunit and LIF were mainly located in endometrial glandular epithelial and luminal epithelial cells. The immunohistochemical localization of both integrin β3 subunit and LIF in the mouse uterus at the time of implantation window was similar to that in the previous studies in mice (Bhatt et al., 1991; Illera et al., 2000b). Our results are not only consistent with the existing viewpoint that endometrial expression of integrin β3 subunit and LIF positively correlates with endometrial receptivity and embryonic implantation (Cavagna and Mantese, 2003; Hoozemans et al., 2004; Kondera-Anasz et al., 2004), but also infer that OS may impair endometrial receptivity at least in part by inhibiting the expression of endometrial integrin β3 subunit and LIF.

Previous studies on early mouse pregnancy have shown that both integrin β3 subunit and LIF appeared in the endometrium coinciding with the implantation window and might be used as markers of endometrial receptivity (Bhatt et al., 1991; Illera et al., 2000b). Targeted disruption of the LIF gene (Stewart et al., 1992) or blockade of the alpha(v)beta(3) integrin could adversely affect implantation in mice (Illera et al., 2000a). This likely explains why the lower expression of either endometrial integrin β3 subunit or LIF in OS groups, especially in the GnRH antagonist or PMSG alone group, is associated with a lower embryonic implantation rate.

The adverse effects of superovulation with gonadotrophins on implantation in mice have been documented in previous studies (Fossom et al., 1989; Ertzeid and Storeng, 2001; Van der Auwera and D’Hooghe, 2001). These studies showed that the implantation rate was significantly less in the superovulated mouse recipients by using a mouse embryo-donation model similar to the one used in the present study. A decreased implantation rate was supposed to be due to the changes in the uterine milieu, especially due to the change in endometrial receptivity. Unfortunately, all of these studies did not provide detailed information on the change in endometrial receptivity after OS treatment. The present study, for the first time, provides

![Figure 5](https://example.com/figure5.png)

**Figure 5.** (A) Expression of endometrial integrin β3 subunit protein during the implantation window of mice. The band of β-actin was used as an internal loading control in each lane. (B) Normalized expression intensity of endometrial integrin β3 subunit protein and its comparison among natural control and three ovarian stimulation (OS) groups. Data are expressed as mean ± SE of the integrin β3 subunit/β-actin protein ratio. a, P < 0.05 compared with control; b, P < 0.05 compared with GnRH antagonist (GnRHant) group and pregnant mare’s serum gonadotrophin (PMSG) group.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** (A) Expression of endometrial leukaemia-inhibitory factor (LIF) protein during the implantation window of mice. The band of β-actin was used as an internal loading control in each lane. (B) Normalized expression intensity of endometrial LIF protein and its comparison among natural control and three ovarian stimulation (OS) groups. Data are expressed as mean ± SE of the LIF/β-actin protein ratio. a, P < 0.02 compared with control; b, P < 0.02 compared with GnRH antagonist (GnRHant) group and pregnant mare’s serum gonadotrophin (PMSG) group.
evidence that the endometrial expression of integrin β3 subunit and/or LIF is downregulated following OS and that this down-regulation negatively impacts endometrial receptivity.

Up to now, the effects of different OS strategies, including OS with GnRH agonist or GnRH antagonist co-treatment, in human and other mammals on both endometrial receptivity and embryonic implantation are still unclear (Coccia et al., 2004; Ertzeid and Storeng, 2001; Devroey et al., 2004). In the present comparison study, we demonstrated for the first time that, among three OS groups in mice (GnRH agonist, GnRH antagonist and PMSG alone), differences arose both in the expression of endometrial integrin β3 subunit and LIF during the implantation window and in the embryonic implantation rate. Compared with the PMSG alone protocol, OS with GnRH agonist co-treatment showed elevated expression levels of endometrial integrin β3 subunit and LIF and a higher embryonic implantation rate, suggesting that OS with GnRH agonist co-treatment, but not with GnRH antagonist co-treatment, may potentially improve uterine receptivity, and this improvement of uterine receptivity in mice might be partially through the restoration of physiologically endometrial secretion changed in the OS cycle.

The mechanisms underlying the observed difference in endometrial receptivity among the different OS protocols, especially the modest endometrial receptivity in GnRH antagonist co-treatment OS, are unclear. In the human, a significantly lower serum estradiol concentration was observed on the day of HCG administration in the OS with GnRH antagonist co-treatment (Albano et al., 2000). This observation is in agreement with the results shown in the previous mouse studies, which reported that both integrin β3 subunit and LIF expression in the endometrium were under maternal control and were regulated by circulating estrogen levels (Bhatt et al., 1991; Yang et al., 1996; Illera et al., 2000b). A recent study reported that the profile of endometrial gene expression during the window of implantation was significantly different when comparing OS cycles using a GnRH agonist versus a GnRH antagonist (Mirkin et al., 2004). These pharmacological or pharmacodynamic properties in GnRH antagonist co-treatment cycles may be associated with the differences in aforementioned endometrial receptivity indices. However, definite conclusions about this issue cannot be drawn now (Ortmann et al., 2001; Coccia et al., 2004; Griesinger et al., 2004; Tarlatzis and Bili, 2004). Further studies are required to establish a clear causative relation.

In the human IVF clinic, either the commonly used OS with GnRH agonist co-treatment or the uncommonly used OS with GnRH antagonist co-treatment is intended to reduce cycle-cancellation rate and to improve the overall IVF outcome (Coccia et al., 2004; Hughes et al., 1992; Shapiro and Mitchell-Leef, 2003). By relying on competitive receptor blockade rather than receptor desensitization, OS with GnRH antagonist co-treatment is considered more advantageous in IVF over the OS with GnRH agonist co-treatment (Al-Inany and Aboulghar, 2002; Shapiro and Mitchell-Leef, 2003; Weiss et al., 2003; Coccia et al., 2004; Tarlatzis and Bili, 2004). However, an earlier meta-analysis concluded that a small but significant reduction in pregnancy rate was observed per started cycle using a GnRH antagonist co-treatment protocol compared with the routine GnRH agonist co-treatment OS (Al-Inany and Aboulghar, 2002). Furthermore, although some recent studies showed that no significant difference in either endometrial maturation (Saadat et al., 2004) or pregnancy outcomes (Takahashi et al., 2004; Bahceci et al., 2005; Prapas et al., 2005) was found between different OS protocols, the concurrent relevant studies (Horcajadas et al., 2005; Mirkin et al., 2004; Simon et al., 2005; Vlahos et al., 2005) still infer the possibility of adverse effects of OS on endometrial receptivity and embryonic implantation, including the possibility that arose specifically from GnRH antagonist co-treatment (Devroey et al., 2004). In the present study, the observed alteration in endometrial receptivity, as shown by significantly lower expression of both endometrial integrin β3 subunit and LIF and a significantly decreased embryonic implantation rate in GnRH antagonist co-treatment OS, supports the inference mentioned above and may provide some extra insights into this issue. Respecting the effect of GnRH antagonist co-treatment OS on endometrial receptivity, the results obtained in the present study were contrary to those from a recently reported study, in which it was shown that the endometrial development after GnRH antagonist co-treatment mimics the natural endometrium more closely than after GnRH agonist co-treatment (Simon et al., 2005). The conflict might be due to the differences between human and mice and/or the different indices measured between the two studies. Further studies are needed to establish their true effects on human IVF outcome and to optimize each of these two OS protocols (Felberbaum and Diedrich, 2003; Weiss et al., 2003; Prapas et al., 2005).

The present study used a human IVF-mimicked mouse model to investigate the effects of different classical OS protocols on the endometrial receptivity and embryonic implantation. Although there are considerable similarities amongst mammals in the early stage of development, it is difficult to extrapolate the information obtained from this mouse model directly to the human IVF clinic. Indeed, numerous differences exist in this aspect between the human and the mouse (Dey et al., 2004; Wimsatt, 1975; Lee and DeMayo, 2004). Hence, the extrapolation mentioned above should be done with caution. Moreover, because of a relatively small size of each group and the uncertain roles of both integrin β3 and LIF in endometrial receptivity and embryonic implantation in either humans or mice, further studies are needed to clarify the reality of all inferences and extrapolations on the basis of the present results.

In conclusion, our results indicate that routine OS protocol may have a negative influence on endometrial receptivity and embryonic implantation in mice. Conversely, OS with GnRH agonist, but not GnRH antagonist, appears to reverse the expression of endometrial integrin β3 and LIF and improves the uterine receptivity in mice. In the human IVF clinic, the low implantation rate observed with OS protocol in combination with GnRH antagonist might partly result from the impaired endometrial receptivity. Further studies in this aspect are needed to provide more definitive answers.

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