INTRODUCTION

Controlled ovarian stimulation (COS) using a gonadotropin-releasing hormone (GnRH) antagonist in assisted reproductive technology (ART) has become increasingly favored for the following reasons. For one thing, due to their properties, unlike GnRH agonists, the period required for desensitization is unnecessary. Thus, GnRH antagonists can be used in the late follicular phase, allowing the duration of GnRH antagonist administration to be shorter compared with GnRH agonists. Moreover, the use of GnRH antagonists in ovarian stimulation is associated with smaller doses of gonadotropins administered and thus a decrease in the risk of ovarian hyperstimulation syndrome.
(OHSS) without an appreciable decrease in pregnancy rate. In this regard, the research from our fertility clinic revealed that ART using a GnRH antagonist protocol is associated with a relatively acceptable pregnancy rate across multiple ovarian stimulation protocols.

Currently used GnRH antagonists in COS regimens are injectable preparations. The clinical outcomes of ART using injectable GnRH antagonists are acceptable. However, the pain caused by injections and their relatively high cost are unfavorable for patients. In order to overcome these problems, the development of an orally administrable GnRH receptor antagonist has long been awaited.

Thus far used GnRH antagonists for COS have been structurally related to GnRH (the first generation). Therefore, they inevitably have peptide structures and are difficult to dissolve in water. The property of low aqueous solubility of GnRH antagonists inherent to peptide molecules had hampered the development of oral agents, for which higher water solubility and non-peptide substances are desirable. With this background, a research group at Takeda Chemical Industries scrutinized the in-house chemical libraries and developed oral preparations of a GnRH antagonist for the first time in 1988. After upgrading the candidate drugs for clinical application, they finally achieved the development of relugolix (the second generation) in 2004.

Relugolix is a non-peptide, orally active GnRH antagonist, which binds to GnRH receptors on the anterior pituitary gland with high affinity and thereby suppresses the secretion of gonadotropins from the pituitary gland, resulting in a decrease in blood levels of sex steroid hormones, such as estrogen, progesterone, and testosterone. As such, relugolix has been shown to be effective for sex hormone-dependent diseases, such as uterine fibroids, endometriosis, and prostate cancer.

Relugolix is effective in a relatively short time in men such that serum luteinizing hormone (LH) and testosterone levels decrease within 6 h after a single dose of relugolix. Given the mechanism of action of relugolix, the same would be true for women. It is obvious that the time required to suppress LH secretion is shorter for cetrorelix, an injectable GnRH antagonist. However, as long as GnRH antagonists are used for the purpose of suppressing the LH surge for COS, a slight difference in time until serum LH levels decrease between relugolix and cetrorelix would be clinically negligible. From this perspective, in the present study, we examined whether relugolix could be a substitute for the injectable preparations, thus enhancing convenience for patients undergoing ART.

2 | METHODS

We enrolled women who had natural menstruation and underwent a procedure to retrieve eggs from January 2019 to December 2020 in Women’s Clinic Oizumigakuen, Tokyo, Japan. The main indications for infertility treatment included unexplained infertility, diminished ovarian reserve mainly due to ovarian aging, male infertility, and female infertility, such as endometriosis and tuboperitoneal factors. We excluded women diagnosed with polycystic ovary syndrome (PCOS). The diagnosis of PCOS was based on the diagnostic criteria by Japan Society of Obstetrics and Gynecology. Age was not specified in the entry criteria in this study.

A total of 181 women chose a COS protocol using a GnRH antagonist. We compared two types of antagonists, that is, a subcutaneous injection (cetrorelix acetate; Merck Biopharma) and an oral preparation (relugolix; Takeda Pharmaceutical). Relugolix was launched in Japan in 2019. Cetrorelix acetate has been used during the year 2019, and relugolix has been used during the year 2020. Thus, there were 88 and 93 women who received cetrorelix acetate and relugolix, respectively. Cetrorelix acetate at a dose of 0.125 mg was injected subcutaneously. Relugolix was orally administered at a dose of 20mg. The available relugolix formulation is 40 mg per tablet. It was cut in half with a pill cutter to obtain 20 mg of relugolix. Particularly, we explained to institutional review board (IRB) that we would use half the dose of 40mg relugolix tablet per day and described this to all participants and obtained their consent. Relugolix was taken at least 30 minutes before meal or in a fasting state. Apart from antagonist preparations, the regimen of ovarian stimulation was basically similar.

Human menopausal gonadotropins (hMG) were administered every day from day 2 of the menstrual cycle. Administration of GnRH antagonists was initiated together with gonadotropins when the size of the leading follicle reached 14-16 mm in diameter. Gonadotropins and GnRH antagonists were given until the day of human chorionic gonadotropin (hCG) injection. We injected hCG when the leading follicle reached 17-18 mm in diameter. Eggs were retrieved transvaginally under ultrasound guidance 34 h after the injection of hCG.

We measured the concentrations of follicle-stimulating hormone (FSH), LH, thyroid-stimulating hormone (TSH), prolactin, and estradiol in serum samples taken on day 2-4 of the menstrual cycle before treatment. The serum concentrations of LH, progesterone, and estradiol were further determined on the day of hCG injection. The oocyte maturity was assessed by using eggs of cases assigned to intracytoplasmic sperm injection (ICSI) alone and determining whether they reached the metaphase II or not. In this study, all patients underwent frozen embryo transfer. Oocytes confirmed to be fertilized were cultivated for 5-6 days after the day of collection, and embryos that reached a blastocyst were kept in the liquid nitrogen until transfer. The main outcomes analyzed were the number of oocytes retrieved, fertilization rate, maturation rate of oocytes, the percentage of oocytes that reached the blastocyst stage, and clinical pregnancy rate per embryo transfer.

The data were analyzed by using EZR software (a modified version of R commander). p < 0.05 was considered to be statistically significant. Our institutional review board approved the study protocol and its consent form. We obtained informed consent for this study from all participants.

3 | RESULTS

In the present study, no adverse events related to relugolix were observed. The demographics of study subjects are shown in Table 1. There were no statistical differences between the two groups.
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(cetrorelix versus relugolix) in terms of age, body weight, body mass index (BMI), and causes of infertility. Unexplained infertility accounted for the majority in both groups. Table 2 shows hormonal profiles and clinical data on ovarian stimulation. No significant differences were observed in basal FSH, LH, and estradiol levels. The concentrations of estradiol, progesterone, the number of follicles, and endometrial thickness on the day of hCG injection did not differ between the two groups.

The LH levels on the day of hCG injection in the relugolix group (1.26 ± 0.93 IU/L) were significantly lower than those in the cetrorelix group (2.85 ± 3.02 IU/L). There were no cases in which oocyte retrieval was canceled in both groups. The total doses of gonadotropins (hMG) required were greater in the relugolix group (2802.4 ± 816.9 IU) compared with the cetrorelix group (2440.9 ± 494.1). In addition, the number of days of antagonist administration in the relugolix group (1.71 ± 0.57 days) was significantly longer compared with the cetrorelix group (1.48 ± 0.58 days). There were no cases of OHSS in both groups.

Table 3 shows the data related to ART procedures. The number of oocytes did not differ between the relugolix group (12.2 ± 7.2) versus the cetrorelix group (13.1 ± 7.0). There was no significant difference in the rate of mature oocytes and fertilization rate between the two groups. The blastocyst formation rate was significantly higher in the relugolix group (45.9%) compared with the cetrorelix group (40.5%). Most importantly, pregnancy rate was 47.1% in the relugolix group versus 45.8% in the cetrorelix group with no statistical difference between the two groups.

### DISCUSSION

In this study, we demonstrated that an oral preparation of GnRH antagonist, relugolix 20mg, is effective in preventing premature ovulation when used as an ovarian stimulation method in ART. In addition, the clinical usefulness of relugolix was shown to be comparable with an injectable GnRH antagonist, cetrorelix, in terms of the number of oocytes retrieved, oocyte maturation rate, fertilization rate, blastocyst formation rate, and clinical pregnancy rate. Thus, relugolix, like injectable GnRH antagonists, could be utilized in an ovarian stimulation protocol.

Currently, injectable GnRH antagonist preparations are widely used in COS. There are two injectable GnRH antagonists on the market, cetrorelix acetate, and ganirelix acetate. The methods of antagonist administration vary by fertility center. We employ a fixed dose of antagonists once a day. It has been concluded that the minimum effective dose of cetrorelix to suppress the premature LH surge is 0.125 mg/day. Based on this finding, in the present study, cetrorelix at 0.125mg once a day was administered in COS.
Regarding the dose of relugolix, it was determined as follows. In standard treatment settings for uterine fibroids, relugolix at 40 mg daily is administered, resulting in serum estradiol levels almost equal to postmenopausal levels. The purpose of the administration of a GnRH antagonist in ovarian stimulation for ART is to suppress the LH surge, but not inhibit LH release to such an extent that causes postmenopausal conditions. On the other hand, relugolix at 10 mg produces only a slight decrease in the basal levels of LH so that relugolix at that dose cannot surely prevent premature LH surge. Taking these into consideration, we decided to use 20 mg dose of relugolix per day in this study.

Then, based on this study, one may ask whether the optimal dose of relugolix for ovarian stimulation is really 20 mg daily. It is to be noted that the LH levels at the time of hCG administration were significantly lower in relugolix (20 mg) group relative to the cetrorelix (0.125 mg) group. On the other hand, the total doses of gonadotropins were greater in relugolix group than those in the cetrorelix group. Based on these observations, it appears that suppression of gonadotropin secretion is more potent with relugolix compared with cetrorelix as long as 20 mg of relugolix and 0.125 mg of cetrorelix are compared. On the other hand, in the setting of COS, the intensity of ovarian stimulation is the sum of endogenous gonadotropins and exogenous gonadotropins. If the doses of exogenous gonadotropins are kept constant and the secretion of endogenous gonadotropin is reduced, the stimulation of the ovaries is lessened, resulting in the prolongation of follicular development. This may explain observed longer duration of the antagonist administration in the relugolix group compared with the cetrorelix group. The half-lives of relugolix and cetrorelix are 67–79 h and 5–10 h, respectively, with relugolix being much longer. The observed difference in LH levels between the two groups may be in part explained by the difference in half-life. Given a longer half-life of relugolix, extending the dosing interval of relugolix from 24 h to 36 h is worth considering.

On balance, we reasoned that the daily dose of relugolix could have been reduced a little more. When it comes to the optimal dose of relugolix, it would be less than 20 mg and more than 10 mg. A dose of 15 mg may be worth considering as an appropriate dose. However, the dose of relugolix on the market is only 40 mg. It is difficult to reduce it to less than 20 mg in the actual medical practice. But from a different perspective, the outcomes of ART, including the number of oocytes collected, oocyte maturation, fertilization rate, and clinical pregnancy rate, were essentially the same between the relugolix group and the cetrorelix group. Therefore, for the time being, it appears that relugolix 20 mg daily is considered justifiable.

The blastocyst formation rate was significantly higher in the relugolix group compared with the cetrorelix group. However, the difference seems to be marginal. In addition, when looking at the data in more detail, mature oocyte rate, fertilization rate, and pregnancy rate were all higher in the relugolix group, although not significant. At present, it is difficult to find a likely mechanism through which relugolix directly might have an impact on ART outcomes. Rather, it is conceivable that the average age in the relugolix group being slightly younger, though not significantly different from the cetrorelix group, could be a plausible explanation for the observed differences in ART outcomes.

In the present study, no apparent adverse events were observed with relugolix. Relugolix, a non-peptide small molecule compound (an N-phenylurea derivative), possesses high affinity for GnRH receptors on pituitary gonadotropin cells and exhibits antagonistic activity. It is, however, structurally quite distinct from peptide GnRH analogues. Although, when used as an ovarian stimulation regimen, the period of usage is limited to a few days, and the possible influence on developing eggs has not been fully investigated at this time. Therefore, careful follow-up of children born from mothers who conceived by use of relugolix is warranted.

This paper retrospectively analyzed clinical data at a fertility clinic and thus entailed some limitations. First, this study is not a randomized cohort study and warrants large-scale clinical trials in the future. Secondly, regarding the dose of relugolix, a dose of 20 mg was shown to be as effective as the subcutaneous preparation. However, the study on the optimal dose of relugolix for COS is currently insufficient, and further refined studies are required.

To summarize, relugolix, a novel orally active GnRH antagonist, when used in COS for ART, seems to be safe and well-tolerated and offers clinical outcomes comparable to an injectable GnRH antagonist, cetrorelix. In particular, it is beneficial for patients to avoid two injections on the day they receive GnRH antagonists. Moreover, it might be preferable for both patients and physicians to have an alternative to an injectable GnRH antagonist.

**CONFLICT OF INTEREST**

All the authors state explicitly that there are no conflicts of interest in connection with this article.

**HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT**

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation.
(institute and national) and with the Helsinki Declaration of 1964 and its later amendment. Our IRB approved the study protocol and its consent form, and we obtained informed consent for this study from all participants.

**ORCID**

Michiko Hamada [https://orcid.org/0000-0002-5942-9800](https://orcid.org/0000-0002-5942-9800)

Rena Ishii [https://orcid.org/0000-0002-4067-1066](https://orcid.org/0000-0002-4067-1066)

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