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ARTICLE

Short-term, low-dose, non-steroidal anti-inflammatory drug application diminishes premature ovulation in natural-cycle IVF☆

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Dr Satoshi Kawachiya graduated from the medical school of Yamagata University and started his career in obstetrics and gynaecology in 1988. Between 2003 and 2005 he worked at the National Center for Child Health and Development in Tokyo. Later he obtained the official Japanese licence for practising reproductive medicine. In 2005 he moved to Kato Ladies Clinic in Tokyo where he actively participates in the clinical and scientific work related to the centre's programme based on mild assisted reproduction treatments. He regularly gives presentations at domestic and international meetings. His main research interests are minimal ovarian stimulation, natural-cycle IVF, single-embryo transfer and monozygotic twinning.

Abstract A retrospective cohort study was conducted in a private infertility centre to evaluate the use of non-steroidal anti-inflammatory drugs (NSAID) in natural-cycle IVF (nIVF) treatment. A total of 1865 first-rank nIVF cycles performed during 2009–2010 were evaluated. Low-dose, post-trigger NSAID was administered in a non-randomized way in cycles at higher ovulation risk where an imminent LH surge was detected on triggering day. Main outcome measures were premature ovulation rate, embryo transfer rate per scheduled cycle and clinical pregnancy and live birth rates per embryo transfer. NSAID use was associated with a significantly lower risk of premature ovulation (3.6% versus 6.8%, adjusted OR 0.24, 95% CI 0.15–0.39, $P < 0.0001$) and higher embryo transfer rate (46.8% versus 39.5%, adjusted OR 1.38, 95% CI 1.06–1.61, $P = 0.012$) per scheduled cycle. Clinical pregnancy (39.1% versus 35.9%) and live birth rates per embryo transfer (31.3% versus 31.4%) were comparable. In this retrospective series, short-term low-dose NSAID application positively influenced nIVF cycles by diminishing the rate of unwanted premature ovulations and increasing the proportion of cycles reaching embryo transfer.

KEYWORDS: GnRH agonist triggering, IVF, natural-cycle IVF, non-steroidal anti-inflammatory drug, premature ovulation, LH surge

Introduction

In recent years there has been increasing interest in milder approaches to IVF treatment such as minimal ovarian

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stimulation or natural-cycle IVF (nIVF). These approaches decrease the physical burden and psychological distress for patients, increase patient convenience and reduce treatment costs (Devroey et al., 2009; Fauser et al., 2010). nIVF is especially attractive because it is completely safe, requires minimal medication and can be easily repeated over successive cycles. The main drawback of nIVF is its low per cycle efficiency which is partly related to high cancellation rates due to premature LH surge and premature ovulation detected at the time of oocyte retrieval (Pelinck et al., 2002).

Non-steroidal anti-inflammatory drugs (NSAID) may efficiently delay or even prevent follicular rupture by blocking cyclooxygenase-2, which has a key role in the ovulatory process (Russell and Robker, 2007; Takahashi et al., 2010). High-dose NSAID administration in humans during the late follicular phase was recently explored as an effective method for emergency contraception (Hester et al., 2010; Jesam et al., 2010) and a way to control spontaneous ovulations during nIVF (Kadoch et al., 2008; Nargund and Wei, 1996). However, concerns were raised because of potential deleterious effects of high-dose NSAID administration which was shown to cause infertility and adversely affect embryonic implantation in animal studies (Duffy and VandeVoort, 2011; Norman and Wu, 2004).

The objective of this retrospective study is to evaluate the low-dose, short-term use of NSAID in a large series of natural IVF cycles.

Materials and methods

After obtaining informed consent, nIVF was routinely offered to normally cycling (26–35 days) infertile women who ovulated according to their basal body temperature charts (Matsuura et al., 2008). nIVF was usually proposed as a first drug-free and cost-effective treatment option. If no pregnancy was achieved, a series of clomiphene-based minimal stimulation cycles would be offered. Patients were not selected according to their age and because of this the treatment option was offered over a wide age range. After obtaining a normal day-3 baseline, ultrasound scan monitoring was usually started on day 10. Oocyte retrieval was scheduled according to the stage of the spontaneous LH surge (typically when the leading follicle reached 18 mm with a concomitant oestradiol concentration $\geq$250 pg/ml). In the study centre, oocyte retrievals could be scheduled for any day of the week between 8:00 and 17:00 h and the procedure usually only takes 5–6 min. Details of centre's protocol for oocyte retrieval scheduling in nIVF treatment are described in **Figure 1** and **Table 1**.

Institutional Review Board approval was not required for the present study due to its retrospective nature and to the fact that study data was constantly managed in a way which excluded the identification of subjects. The effect of a short-term, low-dose, post-trigger NSAID administration was evaluated in 1865 first-rank nIVF cycles performed between January 2009 and December 2010. Patients with ongoing pregnancies were followed up until their delivery. During the study period, no other drugs (such as a gonadotrophin-releasing hormone (GnRH) antagonist) were used to control the spontaneous LH surge. The NSAID was used in a non-randomized manner based on the physician's decision in some of the cycles where an imminent LH surge was detected (10–30 IU/ml) when reaching follicular maturation. These cycles represented approximately 48% of all first-rank nIVF cycles performed during the study period. The rationale for NSAID application was based on the hypothesis that cycles with imminent LH surge are at higher risk of premature ovulation despite being scheduled with a shorter-time interval (<34 h) between trigger application and oocyte retrieval. The NSAID (Borutaren Sapo; Novartis, Japan) was administered by the patient in two separate 25 mg diclofenac rectal suppositories at 8 and 14 h before oocyte retrieval at a 6-h interval. In patients with a history of NSAID allergy, asthma, peptic ulcer or inflammatory bowel disease, NSAID administration was contraindicated. Transvaginal ultrasound-guided oocyte retrieval was performed without anaesthesia using a very thin 21G needle (Kitazato Medical Co, Tokyo, Japan), which has virtually no dead-space, hence follicular flushing was not considered useful.

After oocyte retrieval any immature (metaphase I or germinal vesicle) oocytes were observed for up to a maximum of 12 h until most of them matured spontaneously. Mature (metaphase II) oocytes were inseminated by conventional IVF or intracytoplasmic sperm injection and normally fertilized 2 pronuclear zygotes were cultured individually in 20 μl of cleavage-stage medium (SAGE; USA) until day 2 or 3. All embryos were cultured at 37°C under a gas phase of 5% O_2, 5% CO_2 and 90% N_2 with full humidity in water jacket, small multigas incubators (Astec; Japan). In a small of proportion cases (7.9%) for different reasons (thin endometrium, endometrial bleeding, unsuspected endometrial polyps detected during oocyte retrieval or patient's personal reasons) cultured cleavage-stage embryos were vitrified (Cryotop; Kitazato) and transferred in a subsequent vitrified–warmed embryo transfer cycle. As an institutional policy, single-embryo transfer was performed in all IVF treatment cycles usually involving a fresh day-2 cleavage-stage embryo. The procedure was performed using transvaginal ultrasound guidance by precisely placing a single embryo

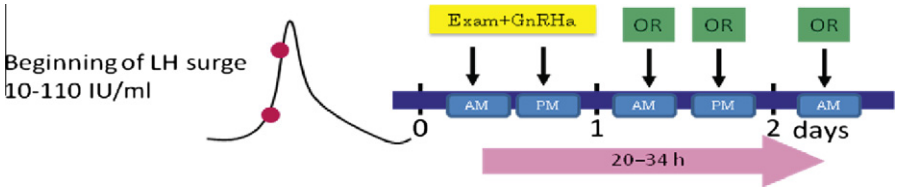

Figure 1 Scheduling of oocyte retrieval in natural-cycle IVF with an imminent LH surge. GnRH = gonadotrophin-releasing hormone; OR = oocyte retrieval.

Table 1 Oocyte retrieval scheduling in nIVF cycles.

Hormonal profile on triggering day	*Triggering*	*NSAID*	*Oocyte retrieval time*
LH <10 IU/ml, progesterone <1.0 ng/ml	At midnight	None	2 days later in the morning
LH 10–30 IU/ml, progesterone <1.0 ng/ml	Immediately	Every 6 h	Next day in the afternoon/2 days later in the morning
LH 30–110 IU/ml, progesterone <1.0 ng/ml	Immediately	Optional	Next day in the morning
LH 110–10 IU/ml, progesterone >1.0 ng/ml	None	None	Same day

NSAID = non-steroidal anti-inflammatory drug.

to the mid-uterine cavity (Bodri et al., 2011). No luteal phase support was administered following embryo transfer.

The primary outcome was the premature ovulation rate and total (fresh and vitrified) embryo transfer rate per scheduled cycle. Secondary outcome measures were clinical pregnancy and live birth rate per embryo transfer. Clinical pregnancy was defined as a positive fetal heartbeat detected at week 6–7 of pregnancy by transvaginal ultrasound. Miscarriage was defined as pregnancy loss occurring before week 22. Metric variables were analysed by the

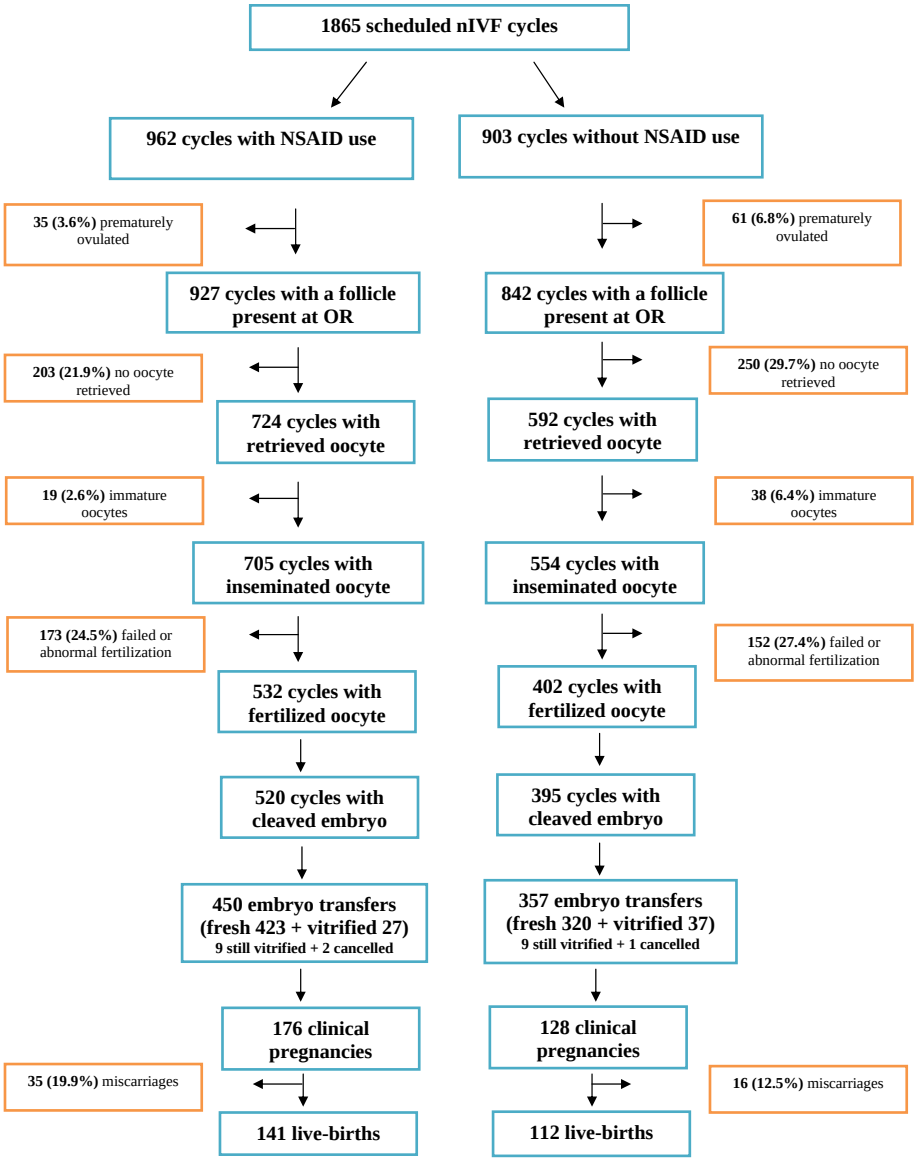

Figure 2 Flow chart of patient events in natural-cycle IVF (nIVF) with and without non-steroidal anti-inflammatory drug (NSAID). OR = oocyte retrieval.

independent t-test and nominal variables were analysed by the chi-squared test. $P < 0.05$ was considered statistically significant. The association between NSAID use and premature ovulation rate and the rate of embryo transfer per scheduled cycle was evaluated by a multiple logistic regression analysis (adjusted for patient age, LH, oestradiol concentration and follicular size on triggering day, and time interval between triggering and oocyte retrieval time).

Results

A total of 962 (51.6%) cycles with NSAID use were compared with 903 (48.4%) cycles without. A chart representing the flow of patients is shown in **Figure 2**. Baseline characteristics and outcome of nIVF cycles according to NSAID use are summarized in **Table 2** and **Figure 3**. NSAID use is associated with a significantly lower risk of premature ovulation detected at oocyte retrieval (3.6% versus 6.8% per scheduled cycle, $P = 0.0038$) and lower risk of not retrieving any oocyte (empty follicle) (21.9% versus 29.7% per oocyte retrieval, $P = 0.0039$). The proportion of mature eggs (including spontaneously matured oocytes) (97.4% versus 93.6%) and fertilization rates (75.5% versus 72.6%) were not statistically significantly different. In total, more scheduled cycles reached embryo transfer in the NSAID group (46.8% versus 39.5%, $P = 0.047$). Clinical pregnancy (39.1% versus 35.9%), live birth rates per embryo transfer (31.3% versus 31.4%), miscarriage rates (19.9% versus 12.5%) and live birth rate per scheduled cycle (14.7% versus 12.4%) were comparable between treatment groups. After adjusting for confounding factors related to baseline nIVF cycle characteristics (based on patient age, oestradiol, LH concentration and follicular size on triggering day and the time interval between triggering and oocyte retrieval) in a multivariate regression analysis, NSAID use was associated with a lower rate of premature ovulation (adjusted OR 0.24, 95% CI 0.15–0.39, $P < 0.0001$) and a higher probability of reaching embryo

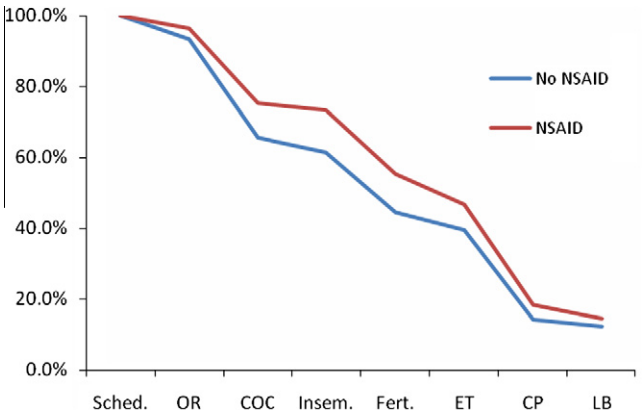

Figure 3 Natural-cycle IVF outcome per scheduled cycle according to non-steroidal anti-inflammatory drug (NSAID) use. Sched = scheduled cycle; OR = oocyte retrieval; COC = cumulus–oocyte–complex; Insem = inseminated cycle; Fert = fertilized; ET = embryo transfer; CP = clinical pregnancy; LB = live birth.

Table 2 Basal characteristics and outcome of nIVF cycles according to non-steroidal anti-inflammatory drug use.

Study groups	*NSAID group*	*No NSAID group*	*P-value*
Scheduled cycles	962 (51.6)	903 (48.4)	–
Patient age (years) (range 24–49)	36.2 ± 4	36.5 ± 4.1	NS[d]
On triggering day			
LH (IU/ml) (range 10–30)	17.3 ± 5.4	18.9 ± 5.9	<0.0001[d]
Oestradiol (IU/ml)	304 ± 69	283 ± 96	<0.0001[d]
Leading follicle size (mm)	18.1 ± 1.7	18.5 ± 2.1	<0.0001[d]
Time between triggering and OR (h) (range 20–34)	29.2 ± 2.5	28.2 ± 2.7	<0.0001[d]
Premature ovulation[a]	35 (3.6)	61 (6.8)	0.0038[e]
Retrieved oocytes[a]	724 (75.3)	592 (65.6)	NS[e]
Inseminated oocytes[a]	705 (73.3)	554 (61.4)	0.015[e]
Fertilized (2PN) oocytes[a]	532 (55.3)	402 (44.5)	0.007[e]
Embryo transfers[a,b]	450 (46.8)	357 (39.5)	0.047[e]
Clinical pregnancies[c]	176 (39.1)	128 (35.9)	NS[e]
Live births[a]	141 (14.7)	112 (12.4)	NS[e]
Live births[c]	141 (31.3)	112 (31.4)	NS[e]

Values are means ± SD or *n* (%).
NS = not statistically significant; OR = oocyte retrieval.
[a]Percentage of scheduled cycles.
[b]Summing fresh and vitrified/warmed embryo transfers.
[c]Percentage of embryo transfers.
[d]Independent t-test.
[e]Chi-squared test.

transfer (adjusted OR 1.38, 95% CI 1.06—1.61, $P = 0.012$). In addition no differences were observed in gestational age at delivery (38.7 ± 1.7 versus 38.9 ± 2.3 weeks) and birthweight of singleton newborns (3023 ± 417 versus 3020 ± 510) between treatment groups.

Discussion

The short-term, low-dose, post-trigger NSAID application decreased the rate of premature ovulations and was associated with a higher proportion of cycles that reached embryo transfer. Prostaglandin, which is an important mediator of the ovulation process in humans, is produced within the ovulatory follicle by cyclooxygenase-2 (Russell and Robker, 2007; Takahashi et al., 2009). The inhibition of cyclooxygenase-2 by NSAID limits prostaglandin production, prevents follicular rupture and prevents oocyte release (Duffy and Stouffer, 2002).

Although the first successful IVF treatment was performed in an unstimulated cycle (Steptoe and Edwards, 1978), it was quickly abandoned in favour of ovarian stimulation, which has became the standard of care. In recent years however, a renewed interest has emerged in patient-friendly, low-risk, low-cost IVF treatments and nIVF was 'rediscovered' (Rongieres-Bertrand et al., 1999). A systematic review on the efficacy of nIVF (Pelinck et al., 2002) was performed in 2002 summarizing the findings of 20 studies comprising a total of 1800 initiated nIVF cycles. It has concluded that only 46.6% of initiated cycles reached embryo transfer resulting in a 7.2% ongoing pregnancy rate per initiated cycle which may explain the low clinical acceptance of this treatment option. Pelinck et al. (2002) have already highlighted that the main reasons for high cycle cancellation rates are difficulties in controlling the spontaneous LH surge and unwanted premature ovulations detected at oocyte retrieval. Different groups have tried to develop modified nIVF protocols where the LH surge is modified by the use of GnRH antagonist (Pelinck et al., 2005; Phillips et al., 2007; Rongieres-Bertrand et al., 1999) and premature ovulation is controlled by NSAID application (Kadoch et al., 2008).

In the context of nIVF, NSAID were successfully applied by Kadoch et al. (2008) to diminish the rate of unwanted premature ovulations. In their retrospective, observational study, an NSAID was non-randomly used in one-third of 255 nIVF cycles and it was associated with a significantly diminished rate of premature ovulation (6% versus 16%) and a non-significantly higher clinical pregnancy rate per initiated cycle (13% versus 6%). Although their findings are completely in line with the present study, their modified nIVF protocol was slight different: NSAID were used in a high-dose regimen (150 mg indomethacin/day) over a course of several days concomitantly with a daily GnRH antagonist to control the LH surge (Kadoch et al., 2008).

In the context of conventional IVF treatment, NSAID have been used to obtain analgesia after oocyte retrieval (Akande et al., 2006; Kailasam et al., 2008) or before embryo transfer (Bernabeu et al., 2006; Dal Prato and Borini, 2009; Moon et al., 2004). Similarly to the present study, pregnancy rates were not adversely affected (and in some studies implantation rates were increased) and miscarriage rates were comparable supporting the safety of NSAID administration in the peri-implantation period. This is also supported by the present series where there were no significant differences in basic perinatal outcome between treatment groups. This might be related to the fact that a 25 mg NSAID dose used twice with a 6-h interval is completely eliminated by the time of oocyte retrieval and does not have any potential negative effect afterwards.

The main limitation of this study is that NSAID were administered in a non-randomized manner; hence differences in outcome might be related to uncontrolled confounding factors. Nonetheless, a multivariate regression analysis was used to control for important baseline cycle characteristics such as patient age, hormonal concentrations and follicular size on triggering day and the time interval between triggering and oocyte retrieval. A large placebo-controlled randomized clinical trial is still warranted to evaluate more precisely the effect of low-dose NSAID administration in nIVF cycles. An evaluation of any potential benefit of NSAID application in those nIVF cycles where LH surge has not yet started might also be undertaken.

In conclusion, in this large retrospective series, NSAID application in nIVF cycles with an imminent LH surge on triggering day was associated with a reduced rate of premature ovulation and a higher probability of reaching embryo transfer.

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