Diclofenac Administered After the Initiation of Luteinizing Hormone Surge Increases Live Births in Natural-Cycle IVF-ET: A Cohort Study

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Research

**Keywords:** Diclofenac, ovulation, LH, live birth, IVF-ET

**DOI:** https://doi.org/10.21203/rs.3.rs-33392/v1

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Abstract

**Background**: Diclofenac inhibits follicle rupture and its use in natural-cycle in vitro fertilization and embryo transfer (IVF-ET) has been reported to increase oocyte retrieval chances but has not been reported to improve the therapeutic outcome (live birth). The question is whether the therapeutic utility of diclofenac is demonstrable when administered to a subgroup of women with an imminent LH surge, a higher risk group for premature ovulation.

**Methods**: Infertile women indicated for the natural-cycle IVF-ET between September 2014 and February 2015 (n=183) were recruited in a private infertility clinic and diclofenac use (50 mg suppositories, thrice every 8 h before oocyte retrieval) was offered when their serum LH level was ≥14.0 IU/L on an LH-triggering day (n=137). Of the 137 women, 108 electively used diclofenac and 29 did not. Oocytes were retrieved from both dominant and subordinate nondominant follicles and were fertilized. The resulting blastocysts were frozen, thawed, and transferred one by one in the following spontaneous ovulatory or hormone replacement cycles.

**Results**: Cumulative live birth rate (after the single oocyte retrieval) was calculated from the dominant and nondominant follicles. The live birth rate from dominant follicles was higher in the diclofenac group (21/108, 19%) than in the no diclofenac group (1/29, 3%) (P < .05). Conversely, the live birth rate from nondominant follicles, which had no potential for ovulation, was not different between the diclofenac group (13/108, 12%) and the no diclofenac group (3/29, 10%).

**Conclusion**: Diclofenac improved the live birth rate from dominant follicles when it was administered to women with an imminent LH surge. However, diclofenac did not affect the live birth rate from nondominant follicles which were not at risk of follicle rupture.

Background

Natural-cycle in vitro fertilization and embryo transfer (IVF-ET) without medicine to stimulate follicles is a treatment option for women interested in saving money and facing as few risks as possible and also for women who have failed to conceive by repetitive standard IVF-ET with controlled ovarian hyperstimulation [1]. However, a limitation of natural-cycle IVF-ET is that the success rate is low compared to conventional methods [2]. The reasons are that the number of retrievable oocytes is limited to one and that oocyte retrieval is canceled in as many as 16.6% of patients because of premature ovulation [3].

To overcome this limitation, several modifications have been proposed for natural-cycle IVF-ET. Concomitant oocyte retrieval from subordinate small (nondominant, ≤ 10 mm) follicles, in addition to standard oocyte retrieval from dominant follicles, increases the number of competent oocytes and the live birth rate by roughly two times [4, 5]. In addition, to prevent spontaneous follicle rupture before scheduled oocyte retrieval, gonadotropin-releasing hormone (GnRH) antagonists and nonsteroidal anti-inflammatory drugs (NSAIDs) are used. GnRH antagonists prevent an endogenous luteinizing hormone
(LH) surge, while NSAIDs inhibit follicle rupture that occurs after an LH surge. Therefore, GnRH antagonists and NSAIDs might be used before and after an LH surge, respectively.

NSAIDs inhibit prostaglandin synthetase 2 activity and thereby decrease the production of prostaglandin E2 as the most predominant prostaglandin that plays a paramount role in cumulus–oocyte complex (COC) expansion, COC release, and follicle rupture [6–8]. Therefore, NSAIDs can be used to prevent or delay follicle rupture, allowing scheduled oocyte retrieval without any fear of premature follicle rupture [1].

Studies have shown that NSAIDs in natural-cycle IVF-ET decrease the chances of premature follicle rupture and increase the number of oocytes retrieved but that there is no improvement in the pregnancy rate or live birth rate [9–11]. A possible reason is that NSAIDs increase oocyte retrieval but decrease oocyte quality. Actually, in addition to follicle rupture, intrinsic prostaglandin E2 acts on multiple steps of oocyte maturation to achieve competency, including meiosis resumption, estrogen synthesis, and granulosa cell proliferation [12]. Therefore, the net effect of NSAID-induced prostaglandin E2 decrease on the IVF-ET outcome seems a trade-off between follicle rupture prevention and a detrimental effect on oocyte quality. In this case, the timing of NSAID use with regard to a spontaneous LH surge determines the positive and negative effects of NSAIDs: if NSAIDs administered far before an intrinsic LH surge could cause a detrimental effect as there is no ovulation, while NSAIDs administered after an LH surge decrease the chance of premature ovulation, which might exceed the potential detrimental effect on oocyte quality.

To test this hypothesis, this study investigated whether NSAIDs increase the live birth rate in women with increasing serum LH levels who undergo natural-cycle IVF-ET.

**Methods**

**Study design and participants**

We conducted a cohort study on infertile women who underwent natural-cycle IVF-ET in our infertility clinic from September 2014 to February 2015. Natural-cycle IVF-ET with elective use of diclofenac (DCF) was administered as the initial mode of IVF treatment to all patients for whom IVF was the indicated mode of treatment. The inclusion criteria were as follows: (1) no ovary-stimulating hormone used for ovarian stimulation, except nasal buserelin to trigger LH; (2) oocyte retrieval scheduled 32–34 h after an LH trigger; and (3) DCF use when the serum LH level on an LH-triggering day was ≥ 14.0 IU/L was offered by the physician and elected by the patient. We enrolled 183 women who underwent oocyte retrieval in this study. The empirical cut-off to determine DCF use was 14.0 IU/L, corresponding to the 98th percentile of the serum LH level on day 3 and 25th percentile of the serum LH level on an LH-triggering day in our clinic.

**IVF-ET**
Modified natural-cycle IVF-ET with concurrent oocyte retrieval from dominant follicles and nondominant follicles was performed, as described previously [4, 5]. Briefly, each patient's hormonal status was assessed on day 3, and follicular growth was monitored every day or every other day from day 10. When the dominant follicle reached a size of ≥ 16 mm and the serum estradiol level was > 190 pg/mL, 300 µg of nasal buserelin was administered twice at 10:00 p.m. and 11:00 p.m. to trigger an LH surge and oocyte retrieval was scheduled 32–34 h later. Of the 183 patients, 137 had serum LH levels ≥ 14.0 IU/L on an LH-triggering day (highLH group), while the remaining 46 patients had serum LH levels < 14.0 IU/L (lowLH group). Of the 137 patients, 108 agreed to use DCF (DCF + group), while 29 did not (DCF− group).

The DCF + group was administered 50 mg DCF suppositories at 24, 16, and 8 h before the scheduled oocyte retrieval (Fig. 1). In addition, 0.25 mg/day of ganirelix was electively used from day 11 to the morning of the LH-triggering day (n = 49).

Oocytes were aspirated using a 23 G needle (Kitazato Co., Shizuoka, Japan) from a dominant follicle and nondominant follicles. Each oocyte was released from a COC in 3 h, and metaphase II (MII) oocytes were inseminated following the conventional method or by intracytoplasmic sperm injection. Embryos were cultured in 20 µL of Quinn’s Advantage Protein Plus Cleavage Medium (Origio Japan, Kanagawa, Japan) for 2 days and then in Quinn’s Advantage Protein Plus Blastocyst Medium (Origio) for another 3 days. Good-quality blastocysts were frozen, thawed, and transferred one by one in the following spontaneous ovulatory or hormone replacement cycles. Progestin with or without estrogen was administered until week 7 of pregnancy (17α-hydroxyprogesterone caproate, 125 mg intramuscular injection once every 5 days, or dydrogesterone, 30 mg per os thrice a day with or without a daily 0.72 mg estradiol-containing patch).

Outcome variables

The primary outcome was the live birth rate of dominant follicle–derived oocytes (intension-to-treat analysis). Live birth was defined as the delivery of a live baby beyond 22 weeks of pregnancy. The live birth rate of nondominant follicle–derived oocytes was concurrently examined for comparison and was calculated as a cumulative live birth rate of oocytes that had been achieved at the same oocyte retrieval.

Statistical analysis

We used one-way analysis of variance to evaluate statistical differences of continuous data between two categories. To analyze the association between the two variables, we used linear regression and χ² tests for continuous data and categorical data, respectively. If there existed more than one cell that was expected to be < 5, we used Fischer's exact probability test instead of χ² tests. In addition, we used a multiple logistic regression analysis model yielding odds ratios and 95% confidence intervals to identify predictors of live birth outcomes.

Results
Clinical characteristics

Of 13 clinical characteristics studied, 3 were statistically different between DCF + and DCF− groups: serum LH levels on an LH-triggering day and two infertility causes (male factor and unexplained infertility) (Table I).

|                      | (A) DCF+ (n = 108) | (B) DCF− (n = 29) | P (A) vs. (B) | (C) LowLH (n = 46) |
|----------------------|--------------------|-------------------|---------------|--------------------|
| Age (year)           | 37.1 ± 0.4         | 37.3 ± 0.7        | n. s.         | 37.7 ± 0.6         |
| Estradiol on day 3 (pg/ml) | 34 ± 2            | 30 ± 4            | n. s.         | 38 ± 3             |
| LH on day 3 (IU/L)  | 5.9 ± 0.3          | 5.8 ± 0.5         | n. s.         | 4.6 ± 0.4          |
| AMH on day 3 (ng/ml) | 3.6 ± 0.3          | 4.1 ± 0.6         | n. s.         | 3.7 ± 0.5          |
| Use of ganirelix    | 19 (18%)           | 9 (31%)           | n. s.         | 21 (46%)           |
| Estradiol on triggering (IU/L) | 299 ± 7       | 280 ± 20          | n. s.         | 281 ± 11           |
| Day of triggering   | 14.3 ± 0.3         | 14.5 ± 0.6        | n. s.         | 13.1 ± 0.5         |
| LH on triggering (IU/L) | 28.4 ± 1.2       | 30.7 ± 3.1        | <0.001        | 9.0 ± 1.7          |
| Causes of infertility* |                   |                   |               |                    |
| Tubar factor        | 9                  | 1                 | n. s.         | 2                  |
| Male factor         | 29                 | 14                | <0.05         | 15                 |
| Unknown             | 69                 | 13                | <0.05         | 29                 |
| Others              | 3                  | 1                 | n. s.         | 0                  |

* Multiple causes included.

DCF, diclofenac; LH, luteinizing hormone; AMH, anti-Müllerian hormone; n.s., not significant.

The lowLH group, who did not use DCF, were using ganirelix more frequently and buserelin earlier compared to DCF + and DCF− groups.
**Live birth outcomes**

The live birth rate from dominant follicle-derived oocytes was high in the DCF+ (19%) compared to the DCF− (3%) group ($P < .05$) and similar to that in the lowLH group (17%). In contrast, the live birth rate from nondominant follicle-derived oocytes was similar in all three groups: DCF+ (12%), DCF− (10%), and lowLH (17%) (Table II).

**Table II**  Outcomes of IVF-ET
|                                      | (A) DCF+  |          | (B) DCF− |        |
|--------------------------------------|-----------|----------|----------|--------|
|                                      | \( n = 108 \) |          | \( n = 29 \) |        |
| **Dominant follicle**                |           |          |          |        |
| Number of patients with intact      | 100       | 93%      | 19       | 65%    |
| dominant follicles for puncture     | \( \% \) patients |          |          |        |
| Number of total oocytes retrieved   | 84        | 84%      | 11       | 58%    |
| \( \% \) puncture                   |           |          |          |        |
| Number of MII oocytes               | 82        | 98%      | 9        | 82%    |
| \( \% \) total oocyte               |           |          |          |        |
| Number of blastocysts               | 34        | 41%      | 3        | 33%    |
| \( \% \) MII oocyte                 |           |          |          |        |
| Number of live births               | 21        | 62%      | 1        | 33%    |
| \( \% \) blastocyst                 |           |          |          |        |
| Number of live births               | 21        | 19%      | 1        | 3%     |
| \( \% \) total patient              |           |          |          |        |
| **Nondominant follicles**            |           |          |          |        |
| Number of patients from whom more   | 103       | 95%      | 26       | 90%    |
| than one oocyte was retrieved        | \( \% \) patient |          |          |        |
| Mean ± SEM (total) of retrieved     | 7.1 ± 0.5 | (769)    | 6.3 ± 1.0 | (183) |
| oocytes                             |           |          |          |        |
| Number of MII oocytes               | 143       | 19%      | 25       | 14%    |
| \( \% \) total oocyte               |           |          |          |        |
| Number of blastocysts               | 35        | 24%      | 13       | 52%    |
|                                      |           |          |          |        |
| Developmental stages affected by DCF |
|-------------------------------------|

Evaluation of each developmental stage by the ratio of the embryo that had proceeded from the previous developmental stage (Table II) showed that as expected, the unruptured dominant follicle rate increased from 65% in the DCF– to 93% in the DCF + group \((P<.05)\), which was similar to that in the lowLH group (91%). The oocyte recovery rate (number of oocytes retrieved per follicle puncture) from unruptured dominant follicles was also high in the DCF+ (84%) compared to the DCF– (58%) group \((P<.05)\), and the difference in MII oocytes rate (the number of MII oocytes per retrieved oocyte) was marginal (41% vs. 33%; \(P<.07\)). However, this difference was not significant in the latter two developmental stages (blastocysts vs. MII oocytes and the live birth rate vs. blastocysts). In contrast, the measures in each developmental stage were not different in nondominant follicle–derived oocytes, except blastocysts versus MII oocytes.

| Multiple regression analysis |
|-----------------------------|

Multivariate logistic regression analysis of factors affecting dominant follicle–derived live birth rate in the highLH group identified, 3 characteristics (young age, high serum LH level on an LH-triggering day, and the use of DCF) out of 13 (Table I) as independent factors (Table III). Other background factors different between DCF+ and DCF– groups, that is, antagonist use, two infertility causes (male factor, unexplained infertility), and interaction between the serum LH level on the LH-triggering day and DCF use, did not affect the dominant follicle–derived live birth rate. In contrast, regression analysis did not identify any factor as a significant valuable for the nondominant follicle–derived live birth rate.

| Table III  Logistic regression analysis of factors affecting the live birth rate in women with serum LH level \(\geq 14.0\) IU/L on the LH-triggering day |
| Dominant follicle–derived live birth rate | Non-dominant follicle–derived live birth rate |
|----------------------------------------|--------------------------------------------|
| \( P \) | OR | 95\%CI | \( P \) | OR | 95\%CI |
| Age (year) | <0.00 | 0.763 | [ | 0.646 | - | 0.879 | ] | n. s. |
| LH level on triggering (IU/L) | <0.02 | 1.067 | [ | 1.024 | - | 1.115 | ] | n. s. |
| Diclofenac use (yes or no) | <0.01 | 9.866 | [ | 1.691 | - | 191.3 | 41 | ] | n. s. |

DCF, diclofenac; LH, luteinizing hormone; OR, odds ratio; CI, confidence interval; n.s., not significant.

**Discussion**

DCF use increases the live birth rate in a modified natural-cycle IVF-ET setting in women with high serum LH levels on an LH-triggering day, which means that DCF is beneficial when administered after commencement of the intrinsic LH surge. In addition, the increase in the live birth rate is because of not only inhibition of premature follicle rupture but also restoration of some developing potentials of oocytes.

**DCF improves the live birth rate**

Studies have shown no improvement in the live birth rate as an outcome of natural-cycle IVF-ET, despite some improvement in the oocyte recovery rate [9–11], which is in contrast to our result of a significant increase in the live birth rate after DCF use. There are several reasons for the detection of a significant effect. First is the study design; we selected a subgroup of women with high serum LH levels (≥ 14.0 IU/L) on an LH-triggering day and compared the outcomes between DCF users and non-users in the subgroup. Consequently, our observation simply meant that DCF use benefits women with an imminent or ongoing LH surge compared to DCF non-users with an imminent LH surge. In this context, Kawachiya et al. conducted a similar study using a different control group: DCF use was evaluated in women with an imminent or ongoing LH surge (10–110 IU/L), and the result was compared to DCF non-users without an LH surge (serum LH level < 10 IU/L) instead of DCF non-users with an LH surge [10]. The authors detected no increase in the live birth rate. The comparison between the two user groups was, however, biased in terms of the LH surge, which corresponds to a comparison between DCF + and lowLH groups in our study (Table II): we, too, did not observe a difference in the live birth rate.
The second possible reason is the difference in the drug types (DCF vs. indomethacin), dose (25 vs. 50 mg), administration route (per rectal vs. per os), duration (16–48 h prior to oocyte retrieval), and administration interval (6–8 h) of NSAIDs. However, there is little information available to discuss these issues.

The third possibility is that the oocyte deteriorates long after the LH surge and that DCF protects oocytes against this time-dependent deterioration. Healthy normal follicles ovulate in 34–36 h of the LH surge, whereas follicles that are not healthy do not rupture beyond the time and have low competent oocytes. Thus, follicles that exist until oocyte retrieval in the DCF – groups may be more likely to be unhealthy and thus have low competent oocytes. Prostaglandin E2 accelerates oocyte maturation process irreversibly, and its inhibition may delay the process and extend the oocyte longevity. However, no information is available on the competency of oocytes that exist in the dominant follicle long after the LH surge.

**DCF affects multiple steps more than follicle rupture**

In addition to inhibiting premature follicle rupture, DCF improves developmental measures of oocytes in multiple steps. All measures that are relevant to dominant follicle–derived oocyte quality, i.e., oocyte maturation, blastocyst formation (number of blastocysts per MII oocyte), and live birth rate (number of live births per blastocyst), are low in DCF non-users and high in both DCF users and the lowLH group. This comparison might indicate that dominant follicle–derived oocyte quality decreases with a long lag time after an LH surge and DCF antagonizes this. Because the spontaneous LH surge begins before extrinsic LH triggering in both DCF users and non-users, i.e., the HighLH group, their oocytes are exposed to increased serum LH levels for > 34 h (average lag time between LH triggering and follicle puncture), which might hurt matured oocytes. The gonadotropin-release lasting time of the GnRH agonist–induced LH surge is shorter (20 h) compared to the spontaneous LH surge (48 h); nevertheless, a GnRH agonist–induced LH surge effectively causes oocyte maturation [13]. Thus, a long-lasting spontaneous LH surge may be necessary not only for oocyte maturation per se but also for stimulating corpus luteum function.

**DCF action on nondominant follicle–derived oocytes**

DCF specifically affects dominant follicle–derived but not nondominant follicle–derived oocytes. With regard to nondominant follicle–derived oocytes, there is no difference in developmental measures between DCF users and non-users, except for the blastocyst formation rate. We believe that this is an alpha error, because the difference is not detected in the live birth rate as the end outcome. Another possibility is oocyte maturity underestimation, which makes the number of MII oocytes small, so the blastocyst formation rate calculated by division of the underestimated number of MII oocytes increases.

Nondominant follicle–derived oocytes appear insensitive to elevated serum LH levels in terms of IVF-ET outcomes. Low LH receptor expression in nondominant follicles might explain the less sensitivity of nondominant follicles to increased LH [14]. This less insensitivity might favor consistent IVF-ET outcomes using nondominant follicle–derived oocytes.

**Limitations**
This study had a few limitations. First, it was an observational study in which DCF use was electively determined. The number of DCF non-users was less than one-third of DCF users, which led to a low statistical power to detect differences in each developmental stage. Background characteristics were not the same between DCF + and DCF− groups and therefore needed to be adjusted by logistic regression analysis. In addition, the cut-off (14.0 IU/L) of serum LH levels was empirically set in order to eliminate any chance of premature rupture of dominant follicles; however, a cut-off to maximize the outcome might be higher than it.

Conclusions

DCF use prior to follicle puncture in natural-cycle IVF-ET might improve the live birth rate in women with an imminent LH surge. A more suitable cut-off should be determined in a larger and preferentially randomized study.

Abbreviations

COC; cumulus–oocyte complex; DCF: diclofenac; GnRH: gonadotropin-releasing hormone; IVF-ET: vitro fertilization and embryo transfer; LH: luteinizing hormone; MII: metaphase II; NSAIDs: nonsteroidal anti-inflammatory drugs.

Declarations

Ethics approval and consent to participate

The application of DCF for IVF-ET as well as the study was approved by the institutional review board (2010-2, 2014-04 and 2019-04). Only patients who accepted the treatment and gave informed consent were enrolled.

Consent for publication

Not applicable.

Availability of data

All data analyzed during this study are included in this article as tables and a figure.

Competing interests

All authors have declared that they have no competing interest.

Funding

This study was supported by Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology, Japan (19K09748).
Authors’ contributions

ST conceived and designed the study, performed oocyte retrieval, and collected, analysed, and interpreted the data. HO revised the paper critically for important intellectual content. All authors approved the final version of the report. TO, TU, FA and collected, cleaned and analyzed the data. SM analysed and interpreted the data, searched the literature, and wrote the report.

Acknowledgements

The authors would like to thank Ms. K. Nakazato and Enago (www.enago.jp) for the English language review.

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Figures

Study design. Infertile women who underwent modified natural-cycle IVF-ET for 6 months since September 2014 were enrolled in the study. The use of DCF was electively decided when the serum LH level was ≥14.0 IU/L. Patients who had a serum LH level < 14.0 IU/L but used DCF (n = 6) were excluded. DCF, diclofenac; LH, luteinizing hormone.