

# Suppression of follicular rupture with meloxicam, a cyclooxygenase-2 inhibitor: potential for emergency contraception

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**BACKGROUND:** There is evidence that cyclooxygenase-2 (COX-2) inhibitors can prevent or delay follicular rupture. COX-2 inhibitors, such as meloxicam, may offer advantages over emergency contraception with levonorgestrel, such as extending the therapeutic window for up to 24 h. We assessed the effect of meloxicam administered in the late follicular phase upon ovulation in women.

**MATERIALS AND METHODS:** This was a single center, double blind, crossover study designed to assess the effects in 27 eligible women (18–40 years old, surgically sterilized with regular menstrual cycles) of meloxicam, 15 or 30 mg/day, administered orally for five consecutive days during the late follicular phase, starting when the leading follicle reached 18 mm diameter. Volunteers underwent two treatment cycles separated by one resting cycle, with randomization to dose sequence. Main outcomes were follicular rupture; serum LH, progesterone and estradiol (E<sub>2</sub>) levels; and incidence of adverse events.

**RESULTS:** Twenty-two volunteers completed the study. There were no differences between meloxicam doses in menstrual cycle length. Dysfunctional ovulation was observed in 11/22 (50%) cycles treated with 15 mg/day and 20/22 (90.9%) cycles with 30 mg/day ( $P = 0.0068$ ). All women had normal luteal phase progesterone levels; mean maximal values  $\pm$  SEM were  $42 \pm 4.1$  and  $46.8 \pm 2.6$  nmol/l for 15 and 30 mg/day groups, respectively. There were no serious adverse events, and no changes in LH and E<sub>2</sub> levels or in cycle length.

**CONCLUSIONS:** Meloxicam 30 mg given for five consecutive days in the late follicular phase is safe, effective and may be an alternative form of emergency contraception.

**Key words:** cyclooxygenase-2 inhibitors / meloxicam / emergency contraception / delayed follicular rupture / dysfunctional ovulation

## Introduction

Currently, the synthetic progestogen levonorgestrel (LNG) is the preferred treatment for emergency contraception (EC–LNG) and is widely available; however, women have limited access in some countries because of barriers imposed by conservative groups. Recent evidence suggests that EC–LNG prevents pregnancy when administered prior to the LH surge but has no contraceptive effect if taken once the ovulatory process has been triggered (Croxatto *et al.*, 2004; Novikova *et al.*, 2007). As such, contraceptive failures reported with EC–LNG may be a result of administration too late to disrupt the ovulatory process.

Prevention or delay of follicular rupture with the use of non-steroidal anti-inflammatory drugs (NSAIDs) has been described

previously (Athanasίου *et al.*, 1996; Pall *et al.*, 2001; Bata *et al.*, 2006). The production of prostaglandins, mediated by the activation of the enzyme cyclooxygenase-2 (COX-2) during the ovulatory process, plays an important role in follicular development and rupture (Richards, 2001).

A recent study published by Bata *et al.* (2006) demonstrates that daily administration of meloxicam, a partially selective COX-2 inhibitor, in a dose of 30 mg daily for 5 days at the onset of the LH surge was associated with a significant 5 days delay of follicular rupture as compared with placebo. Another report from our group has demonstrated that the administration of a single dose of meloxicam 15 mg combined with LNG 1.5 mg when the leading follicle has reached 18 mm diameter results in a 2-fold increase in unruptured follicles compared with placebo plus LNG 1.5 mg (Massai *et al.*, 2007).

An advantage of preventing or delaying follicular rupture with a COX-2 inhibitor versus inhibiting the LH surge with LNG is that the former does not suppress the luteal phase. As such, treatment with meloxicam should maintain the normal oscillations of ovarian steroids and preserve normal menstrual cycles. Other benefits are that COX-2 inhibitors are accessible over-the-counter in many countries, are cheaper and may be associated with fewer side effects in women of reproductive age as compared with steroid hormones when given for short periods of time. All this evidence suggests that COX-2 inhibitors can be used for EC, with a wider window of efficacy to prevent or delay follicular rupture compared with LNG.

This study was designed to test the efficacy of two doses of meloxicam alone to prevent or delay follicular rupture when it is given very close to or after the LH surge has started.

For this purpose, we compared the effectiveness of meloxicam (15 versus 30 mg given orally) to interfere with the ovulatory process when given for 5 days starting when the leading follicle has reached 18 mm.

## Materials and Methods

### Study design

A total of 27 healthy sterilized women were enrolled between 14th November 2007 and 13th May 2008 in this Phase I randomized, double blind and crossover study at the Instituto Chileno de Medicina Reproductiva. The study protocol was approved by the local Scientific and Ethics Review Committee and by the Eastern Virginia Medical School Institutional Review Board.

Healthy volunteers aged 18–40 years with regular menstrual cycles (24–35 days) were eligible for inclusion if they had been surgically sterilized and were in good health with no contraindications for the use of COX-2 inhibitors.

Each volunteer gave their informed consent and agreed to participate for three cycles: first a drug-treatment cycle, a resting cycle, and then a second drug-treatment cycle. During treatment cycles each participant received a daily oral dose of meloxicam, either 15 or 30 mg for 5 days, starting when the mean diameter of the leading follicle was  $\geq 18$  mm. Enrolled participants were randomized to one of two sequences: 15/30 mg or 30/15 mg. Treatment was administered at the clinic in all cases, under supervision by a nurse/midwife.

Subjects and investigators were blind to which dose was being given until all data collection was completed.

### Follow-up

In both treatment cycles, the following procedures were performed.

#### Ultrasonography

Transvaginal ultrasounds (TVU) were performed to assess the mean diameter of the leading follicle and state of the follicle. Starting on cycle Day  $7 \pm 1$ , TVU was performed at least three times per week until the follicle reached 18 mm. Once the follicle reached 18 mm, treatment was initiated and TVU was performed daily for the next five consecutive days (5-day treatment period) and then, twice a week until follicular rupture or menses occurred. The ultrasound machine used in this study was a Medison SA 6000C or ALOKA prosound SSD-3500SX ultrasound scanner system, with a 7.5-MHz vaginal transducer (Sony Corp, Tokyo, Japan). Two ultrasonographers performed the TVUs; they had been previously trained, and certified by ISUOG 30 June 2005, USA.

#### Blood sampling

A venous blood sample was taken on the same days as TVU, starting on the day when the leading follicle reached 15 mm. The samples were taken immediately before drug administration during the 5-day treatment period.

Levels of LH, estradiol ( $E_2$ ) and progesterone were measured during six consecutive days of treatment and progesterone was measured twice a week from the end of the treatment until the time of menstrual-like bleeding. To avoid missing the LH surge we took additional daily blood samples to measure LH levels, starting when a 14–15 mm follicle was observed.

### Recording chart

During the study, all participants kept a record of occurrence and severity of adverse events, medications and bleeding data in a specially designed diary. This diary was checked at each visit and the data were recorded in the participant chart.

### Serum assays

LH,  $E_2$ , progesterone and hemoglobin were assayed locally using standardized laboratory procedures. For hormone measurements, all the samples from the same subject were run simultaneously. Serum LH was assessed using enzyme immunoassay (EIA, Immunometrics, UK Ltd.). For low- and high-quality control samples, the inter-assay coefficients of variation (CV) were 6.3 and 8.1%, respectively, and the intra-assay CV was 2.6 and 2.3%, respectively.  $E_2$  and progesterone were measured using a radioimmunoassay (Siemens Medical Solutions Diagnostics Products, Los Angeles, CA, USA). For low- and high-quality control samples, the inter-assay CV was 5.5 and 3.8% for  $E_2$  and 7.7 and 4.7% for progesterone, respectively; and intra-assay CV was 2.1 and 2.8% for  $E_2$  and 2.2 and 1.8% for progesterone, respectively.

### Meloxicam

Meloxicam (Chemopharma S.A., Chile) was provided as 15 mg tablets for oral use. Silesia Laboratories S.A. (Santiago, Chile) manufactured placebo pills. Meloxicam (2 pills, 15 mg each) or meloxicam (1 pill, 15 mg) plus placebo (1 pill) were dispensed. The capsules for meloxicam and placebo were identical in appearance in order to mask the dose. Drug was stored in a temperature controlled room at 17–25°C until dispensing. The same supplier distributed all pills in vials numbered to match an *ad hoc* randomization list. Daily dosing was considered to be adequate as the terminal elimination half-life is reported to be between 13 and 20 h and the apparent clearance (CL/F) is between 0.4 and 0.6 l/h (Gates *et al.*, 2005).

### Data analysis

The effect of treatment on the occurrence and timing of follicular rupture was focused on the 6-day period starting on the day of treatment. Spermatozoa do not retain the ability to fertilize the oocyte for longer than 5 days after coitus; therefore, if follicle rupture does not occur within this period, the probability of conception has been shown to be zero (Wilcox *et al.*, 1995). The following results are centered in the 6 days period of observation beginning when the leading follicle reached 18 mm of diameter, which was the day when the first dose of meloxicam was given.

Cycles were classified according to the occurrence of follicular rupture within the 6-day period and hormone levels. The proportion of cycles presenting no follicular rupture, ovulatory dysfunction (discussed below) or follicular rupture within the 6-day period was compared between meloxicam 15 mg and meloxicam 30 mg groups.

The following end-points and definitions were pre-established for data analysis:

- (i) Length of the cycle: number of days from the first day of menses until the day before the next menstrual-like bleeding, both inclusive. The first day of the luteal phase was the day in which the follicular echo-image disappeared and the last day was the day before the next menstrual-like bleeding.
- (ii) Follicular rupture: abrupt disappearance or a reduction in size of at least 50% of the echo-image of a leading follicle that had attained at least 15 mm in diameter.
- (iii) Ovulation: follicular rupture preceded 24–48 h by a LH peak of at least 21 IU/l and followed by a serum progesterone concentration > 12 nmol/l.
- (iv) Ovulatory dysfunction: follicular rupture not preceded 24–48 h by an LH peak or preceded by a blunted LH peak (<21 IU/l), or not followed by elevation of serum progesterone level > 12 nmol/l

When the LH serum level was > 15 IU/l on the same day as the onset of treatment, it was considered that treatment was given after the ovulatory process had been triggered by the gonadotrophin surge, and the cycle was classified as 'treatment after the onset of the LH surge'. Differences in the number of cycles presenting no follicular rupture and ovulatory dysfunction or follicular rupture within the 6-day period was analyzed by Fisher's exact test. Analysis of variance was used to analyze differences in the length of the cycles and LH and E<sub>2</sub> values. Differences in the length of luteal phase, follicular diameter and maximal values of progesterone were analyzed using Student's t-test. Statistical tests used are indicated in the text and tables, as appropriate. Statistical analyses were performed using Graph Pad Prism software, version 3.02. Data are presented as mean ± SEM unless otherwise stated. A value of  $P < 0.05$  was considered significant.

**Table I** Baseline data for 22 volunteer women enrolled in a study of the effects of 5 days treatment with oral meloxicam, a cyclooxygenase-2 inhibitor, on follicle rupture

	Mean	SEM	Range
Age (years)	35.7	0.78	31–40
Parity	3.07	0.13	2–5
Weight (kg)	59.7	1.58	46.5–72.2
Height (m)	1.56	0.01	1.47–1.62
BMI	24.7	0.63	20.4–29.4
Hemoglobin (g/dl) at screen	13.7	0.13	11.0–14.9
Hemoglobin (g/dl) end study	13.6	0.16	10.07–15.3

## Results

A total of 27 women were enrolled in the study after giving informed consent. Five women were discontinued for the following reasons: thyroid disorder (1), anemia (1), decided not to participate (2) and ovulation with a follicle < 18 mm (1). Therefore, 22 women completed the study. Baseline data for the 22 participants are shown in Table I.

Each participant was exposed to both doses of meloxicam, the 15 mg/day (low dose) and the 30 mg/day (high dose), giving a total of 44 treated cycles. Data from 44 treated cycles were analyzed.

### Follicular outcome

#### Ovulation within the 6-day period of observation

The outcome of the leading follicles within the 6-day period following treatment is shown in Table II. Ovulation occurred in half of the cycles treated with 15 mg/day; however, women treated with 30 mg/day ovulated in only 2/22 cycles (9%). This difference reached statistical significance ( $P = 0.0068$ , Fisher's exact probability test).

Follicular diameters observed in both treatment groups when normal ovulation occurred were smaller than diameters observed in dysfunctional cycles (Table III).

#### Ovulatory dysfunction within 6-day period

The proportion of cycles showing ovulatory dysfunction is shown in Table II. Ovulatory dysfunction was observed in 10/22 (45.5%) cycles with 30 mg/day and only in 6/22 (27%) of cycles treated with 15 mg/day. This difference did not reach statistical significance.

Ovulatory dysfunction was characterized by a blunted LH peak in only four cycles and by follicular rupture taking place more than 48 h after the LH peak in the remaining 12 cycles. Unruptured follicles were observed in 10/22 (45.5%) of the cycles following high dose and 5/22 (23.0%) of the cycles following low dose meloxicam within the 6-day observation period. However, follicular rupture had occurred in all of the treatment cycles by the time of the next menstrual-like period.

With the lower meloxicam dose, follicles reached a larger diameter when ovulation was dysfunctional ( $P < 0.0001$ , Student's *t*-test). This difference was also observed with 30 mg/day but since only two normal ovulations occurred, statistical analysis was not applied (Table III).

**Table II** Proportion of cycles presenting ovulation, dysfunctional ovulation or an unruptured follicle within the 6-day period of observation after the administration of meloxicam

	Meloxicam 15 mg/day	Meloxicam 30 mg/day	All
Ovulation (follicular rupture within 48 h of LH peak)	11/22(50%)**	2/22 (9%)	13/44 (29.5%)
Dysfunctional ovulation (follicular rupture 48 h–6 days after LH peak or blunted LH peak)	6/22 (27%)	10/22 (45.5%)*	16/44 (36.4%)
Unruptured (follicular rupture more than 6 days after LH peak)	5/22 (23%)	10/22 (45.5%)*	15/44 (34.1%)

\*\* $P = 0.0068$  (15 versus 30 mg/day group).

\* $P = 0.015$  (30 mg/day group; dysfunctional ovulation or unruptured versus ovulation). Fisher's exact probability test.

**Table III** Maximum follicle diameters during treatment cycles (mm, mean  $\pm$  SEM)

	N	Meloxicam 15 mg/day (range)	N	Meloxicam 30 mg/day (range)
Ovulation	11	21.0 $\pm$ 0.6 (18.3–24.6)	2	20.2–19
Dysfunctional ovulation	11	31.5 $\pm$ 1.7* (25.6–44.9)	20	30.9 $\pm$ 1.10 (18–39.2)

\* $P < 0.0001$  Student *t*-test (15 mg/day group; ovulation versus dysfunctional ovulation).

**Table IV** Different parameters of treatment cycles after meloxicam

	N	Max LH (IU/l)	Max estradiol (pmol/l)	Max progesterone (nmol/l)	Luteal phase length (days)	Cycle length (days)
15 mg Meloxicam (mean $\pm$ SEM)						
Ovulation	11	43.7 $\pm$ 5.0	691 $\pm$ 69	50 $\pm$ 3.9	13.7 $\pm$ 0.4	28.9 $\pm$ 1.0
Dysfunctional ovulation and unruptured follicles	11	63.4 $\pm$ 10	704 $\pm$ 43	42 $\pm$ 4.1	11.2 $\pm$ 0.7* ( $n = 6$ )	28.7 $\pm$ 0.7
30 mg Meloxicam (mean $\pm$ SEM)						
Ovulation**	2	47–56.5	526–546	44.5–25.3	13–13	26–26
Dysfunctional ovulation and unruptured follicles	20	44.7 $\pm$ 4.4	752 $\pm$ 61	46.8 $\pm$ 2.6	12.3 $\pm$ 1.3 ( $n = 10$ )	29.6 $\pm$ 1.1

\* $P = 0.003$  versus Ovulation (Student *t*-test).

\*\*Individual values of two cycles.

All cycles with ovulatory dysfunction had  $E_2$  levels that were comparable with ovulatory cycles. All women had normal progesterone levels during luteal phase. Maximal values of progesterone during the luteal phase, showed no difference between doses of meloxicam. These data are shown in Table IV.

When cycles with unruptured follicles or ovulatory dysfunction were added together, the overall percentage of disrupted ovulatory processes among cycles treated with 30 mg/day was significantly increased versus the low dose ( $P = 0.0068$ , Fisher's exact probability test).

### Follicular outcome in relationship to the LH peak

Follicular outcome was analyzed in relation to the LH surge-treatment interval. Volunteers were grouped according to the time that they received the treatment before or after the onset of LH peak. This is shown in the Table V. Dysfunctional ovulation was observed when meloxicam was administered before the onset of the LH peak in 5/11 (46%) cycles and in 14/14 (100%) cycles with 15 and 30 mg/day dose, respectively ( $P = 0.0026$ , Fisher's exact probability test). The association between dose of meloxicam and outcome of the follicle when treatment was administered after the LH peak was not statistically significant. When the higher dose of meloxicam was administered on the day of the LH peak or later, a trend towards dysfunctional ovulation was observed but this did not reach statistical significance owing to the small number of cycles in this group.

**Table V** Proportion of cycles presenting ovulation or dysfunctional ovulation within the 6-day period of observation following the administration of meloxicam in relation with the LH peak

	15 mg/day	30 mg/day
Treatment start before LH peak		
Ovulation	6/11 (54%)	0/14 (0%)
Dysfunctional ovulation	5/11 (46%)	14/14 (100%)*
Treatment start after LH peak		
Ovulation	5/11 (46%)	2/8 (25%)
Dysfunctional ovulation	6/11 (54%)	6/8 (75%)

\*Two-tailed  $P = 0.0026$ .

Fisher's exact probability test (15 versus 30 mg/day group).

### Bleeding pattern

Cycle length did not differ between doses and between outcomes of the leading follicles. The mean  $\pm$  SEM length of the menstrual cycle was 28.9  $\pm$  1.0 days in ovulatory cycles and 28.7  $\pm$  0.7 days in dysfunctional/unruptured follicles for the lower dose, and 26 days in ovulatory cycles and 29.6  $\pm$  1.1 days in dysfunctional/unruptured follicles for the higher dose (Table IV).

### Adverse events

Adverse events were observed in 6/22 (27.3%) of cycles treated with 15 mg/day and in 2/22 (9.1%) with 30 mg/day during the 5-day

treatment period. These events included low abdominal pain in 3/22 versus 1/22 cycles, bloating in 1/22 versus 2/22 and hot flushes in 2/22 versus 0/22, for low and high dose meloxicam, respectively. There were no severe adverse events observed in this study.

## Discussion

The results of this pilot study confirm that the administration of meloxicam during the late follicular phase interferes with the ovulatory process, either preventing follicular rupture or causing dysfunctional ovulation. Differences between doses in the outcome of the leading follicle were observed, but we found no differences in other biological variables, such as cycle length or endocrine profiles of the cycles. Unruptured follicles were observed in a higher proportion of the high dose than the low dose cycles (45.5 versus 23%, respectively), however, all of the unruptured follicles within the 6-day period of observation had ruptured by the time of the next menses. Our observation of a normal cycle profile with meloxicam is consistent with results obtained by other authors with different meloxicam doses or different NSAIDs (Pall et al., 2001; Bata et al., 2006). The final destiny of the oocytes has yet to be determined. Are the oocytes trapped in the follicle or are they released, and if so, can they be fertilized? These are questions that need to be answered to confirm a local effect of meloxicam on the final event of follicular development, follicular rupture and release of a mature oocyte rather than an effect on hormone secretion or action. Recent studies in monkeys have explored the destiny of the oocytes and the results showed that even though follicular rupture occurs with treatment of monkeys with meloxicam during the periovulatory period, oocytes may remain trapped in the follicle and thus may be unavailable for fertilization (Hester et al., 2009).

The findings of the present study support the conclusion that meloxicam, a COX-2 inhibitor, can prevent or delay follicular rupture, even when it is given after the onset of the LH surge, without disrupting the endocrine profile of the cycle.

When meloxicam was given before the LH surge, the surge was not suppressed but dysfunctional ovulation was observed in all cycles treated with the high-dose. This effect was less pronounced with the low-dose, where 50% of cycles had follicular rupture during the first 48 h after the onset of the LH peak, corresponding with apparently normal ovulation. When treatment was given after the ovulatory process had been triggered by the LH surge, follicular rupture was delayed by more than 48 h (classified as dysfunctional ovulation) in 54 and 75% of cycles with the low- and high-dose, respectively. The difference between doses did not reach statistical significance probably because of the low number of cycles being compared, and to prove this hypothesis it is necessary to have a larger group of women treated at the time of LH surge. We conclude from these results that, independent of the time when treatment was given in relation to the LH peak, treatment was more efficient for delaying follicular rupture and causing dysfunctional ovulation with the dose of 30 mg/day: this is the principal difference between the mechanism of action of meloxicam and that shown for LNG. When LNG is administered early during the follicular phase it impairs the ovulatory process and luteal function (Durand et al., 2001; Hapangama et al., 2001; Marions et al., 2002, 2004; Croxatto et al., 2004; Okewole et al., 2007). A possible effect of LNG on the endometrium has been

studied in several publications and it seems that LNG has no effect on the expression of implantation molecules *in vivo* or *in vitro* (Durand et al., 2001; Marions et al., 2002; do Nascimento et al., 2007; Lalitkumar et al., 2007; Meng et al., 2008). However, there are still no studies on the effect of meloxicam on the endometrium. There is only one publication on the effect of meloxicam in gravid myometrium *in vitro* and this showed a modest inhibitory effect on contraction peaks (Sawdy et al., 2003). One of the limitations of our study was that we did not assess the pharmacokinetics of meloxicam, so we cannot calculate this information in our volunteers. It is of interest to compare our results with those obtained when LNG 1.5 mg was administered when the follicle had attained  $\geq 18$  mm (Croxatto et al., 2004). The proportion of cycles without follicular rupture was 25/57 (44%) and those with ovulatory dysfunction 20/57 (35%) with LNG; in a subsequent study (Massai et al., 2007) in which LNG was given together with meloxicam 15 mg when the leading follicle reached 18 mm, the proportion of cycles without follicular rupture was 12/31 (39%) and ovulatory dysfunction increased to 14/31 (45%); therefore the proportion of cycles having ovulatory dysfunction or unruptured follicles is similar with LNG alone, LNG combined with meloxicam or meloxicam alone (45.5%, present study). The contraceptive efficacy of these three treatments may be similar, but a potential advantage of meloxicam is that it interferes with the ovulatory process when given after the onset of the LH surge, whereas LNG has to be given before the LH surge. Although the efficacy to disrupt the ovulatory process is similar, the window of effectiveness is 24 h wider for meloxicam than for LNG. Moreover, a COX-2 inhibitor is cheaper and more accessible than steroidal drugs in many countries, does not alter the endocrine profile of the cycle, and causes no menstrual disturbance. The main disadvantage of meloxicam is the need to take it for several days.

The results of this study suggest that the use of meloxicam in a dose of 30 mg/day for five consecutive days may be a good alternative to EC-LNG in places where access to dedicated EC products is restricted.

## Acknowledgements

The views expressed by the authors do not necessarily reflect the views of Eastern Virginia Medical School. We are grateful to all the volunteers who participated in this study. The statistical analysis was carried out with the help of Ms H. María Luisa Forcelledo. The authors thank Laboratorios Silesia for producing the placebo capsules.

## Funding

Support for this subproject CIG-07-117 was provided by CICCR, a program of CONRAD, Eastern Virginia Medical School.

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Submitted on July 31, 2009; resubmitted on October 10, 2009; accepted on October 14, 2009