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Effects of acetylsalicylic acid (aspirin) and naproxen sodium (naproxen) on ovulation, prostaglandin, and progesterone production in the rabbit*

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Objective: To determine the effects of acetylsalicylic acid (aspirin) and naproxen sodium (naproxen) on ovulation, ovarian prostaglandins (PG), and P production in the rabbit via in vivo and in vitro studies.

Design: Aspirin and naproxen were administered IV 6.5 and 7 hours, respectively, after hCG administration to New Zealand White adult female rabbits. Laparotomy was performed 24 hours after hCG administration. For in vitro experiments, control animals underwent laparotomy 6.5 (aspirin) and 7 hours (naproxen) after hCG administration. The treated animal received aspirin and naproxen; laparotomy was performed 1 hour later. One ovary was perfused for 6 hours with aspirin or naproxen whereas the contralateral ovary served as a control and was perfused with control medium (M199; GIBCO, Grand Island, New York). Perfusate samples were collected at 1-hour intervals for PG and P determination.

Setting: A conventional laboratory setting.

Interventions: In vivo experiments used IV administration of 100 mg/kg aspirin and 10 and 50 mg/kg naproxen. In vitro perfusion was also carried out with 100 μg/mL aspirin and 10 and 50 μg/mL naproxen added to the perfusate.

Main Outcome Measures: Ovulatory efficiency (no. of ovulations/no mature follicles) and ovarian vein PG and P concentration were determined.

Results: Ovulatory efficiency was 88% for control, 41% for in vivo aspirin-treated, and 40% (10 mg/kg) and 0% (50 mg/kg) for naproxen-treated rabbits. Aspirin and naproxen were associated with decreased ovulatory efficiency when administered in vitro to both in vivo control and in vivo treated ovaries (control-medium = 70%; control-aspirin = 14%; aspirin-medium = 34%; aspirin-aspirin = 0%; control-naproxen = 25%; naproxen-medium = 38%; naproxen = 0% with 10 μg/mL, and control-naproxen = 13%; naproxen-medium = 0%; naproxen = 0% with 50 μg/mL). Prostaglandin F2α was undetectable in the perfusate of those ovaries perfused either with aspirin or naproxen. Ovarian venous concentration of P in the perfusate was similar in all groups.

Conclusions: Aspirin and naproxen significantly reduced ovulatory efficiency and PG production both in vivo and in vitro in hCG-treated rabbits. A critical period of 6.5 and 7 hours after hCG administration was established. Fertil Steril 1996;65:1036–43

Key Words: Aspirin, naproxen, inhibition of ovulation, prostaglandin

The biochemical events involved in mammalian ovulation have been likened to an inflammatory reaction (1). There is evidence that ovarian prostaglandins (PGs) increase significantly just before the time of mammalian ovulation (1–3). One mechanism proposed for the role of PGs in the process of ovulation involves stimulation of collagenase

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through the activation of plasminogen activator (PA) and plasmin, which in turn leads to follicular wall degradation and follicle rupture (4). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, can inhibit ovarian prostaglandin synthesis and ovulation. These compounds appear to suppress ovulation by interfering with the increase in ovarian PGs that normally precedes ovulation (3, 5).

Temporal study of ovarian PG production in the rabbit has demonstrated two phases of PG production: the first, beginning 2 hours after gonadotropin stimulus, and a second increase occurring approximately 5 hours later (3). The optimum time for administration of indomethacin to achieve ovulation inhibition is 7 to 8 hours after the ovulatory process has been initiated by gonadotropin (3, 6). Indomethacin failed to inhibit follicular rupture when administered either 1 or 9 hours after hCG, although PG levels were depressed profoundly. Thus, there appears to be a critical period during which indomethacin must be administered to prevent ovulation (3).

Two NSAIDs, acetylsalicylic acid (aspirin) and naproxen sodium (naproxen) are prescribed widely as analgesic-antipyretic and anti-inflammatory agents. These compounds, like indomethacin, prevent the synthesis of PGs through the inhibition of arachidonate cyclooxygenase, the enzyme that converts arachidonic acid to PG endoperoxides (7). However, disparities exist in the potency of these drugs with respect to their anti-inflammatory activity and their ability to inhibit cyclooxygenase. With regard to anti-inflammatory activity, the potency of aspirin and naproxen is less than indomethacin (7).

The objectives of the present study were to determine the effects of aspirin and naproxen on ovulation and ovarian PG and P production in the rabbit and to correlate observations with dose and time of administration of these agents. Both in vivo and in vitro experiments were performed. The direct effects of both compounds on the ovary were evaluated by means of an in vitro ovarian perfusion system.

MATERIALS AND METHODS

Animals

Sexually mature New Zealand White rabbits (mean weight: 3.5 kg) were used. The rabbits were caged individually for a minimum period of 3 weeks under controlled light and temperature and given Purina Rabbit Chow animal food (Ralston Purina Co., St. Louis, MO) and water ad libitum. All studies were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

In Vivo Administration of Aspirin and Naproxen

The effects of time and dose of different NSAIDs, such as indomethacin, on ovulation and follicular PGs in the rabbit have been studied extensively (3). Frazer and Ritter (8) showed that, in rabbits, aspirin, at the dose of 10 mg/kg, inhibits prostanoid production by different tissues and plasma, although the ovarian tissue has not been studied we used 10 mg/kg as starting dose. The in vivo experiments of the present study were designed to determine the dose and time course effects of aspirin and naproxen on ovulation and ovarian PGs production.

The first group of experimental rabbits received 100 IU hCG and aspirin (Warner Chilcott Laboratories, Morris Plains, NJ; 10 mg/kg, n = 8 animals; 20 mg/kg, n = 4 animals; 50 mg/kg, n = 2 animals, and 100 mg/kg, n = 2 animals) 8 hours after hCG administration, via marginal ear vein. Another group of seven rabbits received 100 mg/kg aspirin at 6.5 to 7 hours and an additional three rabbits received 100 mg/kg aspirin at 7.5 to 8 hours after hCG administration. Aspirin was dissolved immediately before use in 1 M Tris buffer (pH 8.4) at room temperature and diluted with saline solution (sodium chloride 0.9%) to 20 mg/mL. Control rabbits (n = 7 animals for both dose and time studies) received the same dose of hCG and vehicle.

The second group of experimental rabbits received 100 IU hCG and naproxen (Syntex Laboratories, Inc., Palo Alto, CA; 10 mg/kg, n = 3 animals; 50 mg/kg, n = 7 animals) via marginal ear vein, at 6.5, 7, and 8 hours after hCG administration. Naproxen was administered as a 0.3% solution in 5% sodium bicarbonate. Control rabbits (no. 7 animals) received the same dose of hCG and vehicle.

Treated and control animals were anesthetized with 32 mg/kg IV sodium pentobarbital, heparinized with 120 U/kg heparin sulfate, and subjected to laparotomy 24 hours after hCG administration. The numbers of mature follicles (>1.5 mm in diameter) and of stigma observed on the ovarian surface were recorded and ovulatory efficiency (the percentage of mature follicles that had ruptured) was determined for each ovary (9). The presence of hemorrhagic stigma was used to confirm ovulation. The ovarian vessels were isolated, the ovarian vein was ligated and cannulated, and ovarian vein blood samples were collected from each ovary (13). Peripheral blood samples also were collected. The samples were frozen and maintained at −20°C for future assay of P and PGs.

In Vivo and In Vitro Administration of Aspirin and Naproxen

The objective of this component of the study was to evaluate the effects of aspirin and naproxen on

ovulation, steroidogenesis, and PG production in vitro by means of an in vitro perfusion system. Rabbits were divided into "in vivo treated" and "in vivo control" groups. Animals (n = 8) belonging to the in vivo treated group received 100 mg/kg IV aspirin 6.5 hours after hCG administration. Control rabbits (n = 6) received the vehicle alone with the same time schedule. For the naproxen experiments, in vivo treated animals received an IV injection of naproxen (10 mg/kg, n = 4 animals; 50 mg/kg, n = 4 animals) 7 hours after hCG administration. Control rabbits (n = 8) received the vehicle alone with the same time schedule.

Animals were anesthetized with 32 mg/kg IV sodium pentobarbital, heparinized with heparin sulfate 120 U/kg, and subjected to laparotomy at specific times after hCG administration. The perfusion technique and surgical procedure have been used extensively in our laboratory and the details have been described previously (9, 10). Briefly, each ovarian artery and vein was cannulated in situ, ovarian blood samples were collected, and the ovary with its ovarian artery and vein and the supporting adipose tissue was removed and placed in the perfusion chamber. Peripheral blood samples also were collected to determine prostaglandin F (PGF) metabolites concentration.

The perfusion system consists of a chamber containing the ovary, an oxygenator, a reservoir, and a pulsatile roller pump that maintains perfusate flow at 1.5 mL/min, the approximate blood flow to the estrus rabbit ovary (Ahren K, Janson PO, Selstam G, abstract). The oxygenator was gassed with 95% O_2-5% CO_2. Ovaries were perfused for 6 hours at 37°C in 150 mL medium M199 supplemented with 200 U/L heparin sulfate, 20 U/L insulin, 50 mg/L streptomycin sulfate, 75 mg/L penicillin G, and 3% bovine serum albumin at pH 7.4 (perfusion medium). The in vitro treated ovaries were perfused with the perfusion medium supplemented with either 100 μg/mL aspirin or 10 or 50 μg/mL naproxen. Ovaries were excluded from the study if they contained only immature follicles (<1.5 mm).

In the in vivo treated animals group, perfusion was begun 1 hour after in vivo aspirin or naproxen administration. One ovary was perfused in vitro either with 100 μg/mL aspirin or 10 and 50 μg/mL naproxen and the contralateral ovary was perfused with perfusion medium and vehicle.

In the in vivo controls group, perfusion of ovaries removed from in vivo control rabbits was initiated either 6.5 or 7 hours after in vivo administration of hCG and one ovary was perfused either with 100 μg/mL aspirin or 10 or 50 μg/mL naproxen and the contralateral was perfused with perfusion medium and vehicle. The concentration of aspirin in the perfusate was based on the data obtained in the rabbit by Buchanan et al. (11). The concentration of naproxen was based on the effective plasma levels detected in human after oral administration of the compound at a similar dose used in our experiments (7, 8).

Ovaries were observed periodically for evidence of follicle growth and rupture. A follicle was considered to be ruptured when the cumulus containing an oocyte was observed protruding from the ovarian surface. Ovarian effluent perfusate samples were collected at 0, 0.5, 1, 2, 4, and 6 hours and replaced with an equal volume of fresh medium. Samples were stored at −20°C until assayed for P and $PGF_{2\alpha}$ concentration. Ovulatory efficiency was calculated for each group.

Progesterone and Prostaglandin Assays

Progesterone standards and samples were measured using a commercial RIA kit (Diagnostic Products Corp., Los Angeles, CA) in which the P antibodies bound covalently to the inner surface of polypropylene assay tubes. All samples and 100 μL P standards were assayed in duplicate. For each experiment all samples were assayed in a single assay to avoid error due to interassay variation. Ovarian vein and perfusate samples were diluted before assay because of their high concentration of P. The intra-assay variation was 6.6%.

Plasma PGF metabolites were assayed by RIA as described previously (12). The intra-assay variation was <8%. Perfusate levels of $PGF_{2\alpha}$ were measured using by $PGF_{2\alpha}$ antibodies that cross-react <2% with other PGs tested. For each prostanoid measured, the intra-assay coefficient of variation was <8%. Samples from each experiment were assayed simultaneously to avoid error due to interassay variation.

Statistical Analysis

Data for P, PGF metabolites, and $PGF_{2\alpha}$ levels at each time period and ovulation time were evaluated by analysis of variance with $P < 0.005$ considered significant for major effects (i.e., effects due to treatment). Data from ovaries of the same rabbit were treated as repeated measures. Two group comparisons were made by Student's t-test. Comparison of ovulatory efficiency was performed using χ^2 analysis.

RESULTS

The Effect of In Vivo Administration of Aspirin

Aspirin displayed a significant ($P < 0.05$) reduction of ovulatory efficiency as compared with controls when administered 8 hours after hCG administra-

Table 1 Follicle Characteristics of In Vivo Control and Aspirin-Treated (+8 Hours) Ovaries

	Control	10 mg/kg	20 mg/kg	50 mg/kg	100 mg/kg
Total no. of ovaries	13	15	8	4	4
Unruptured follicles	20	59	32	13	17
Ovulated follicles	74	71	41	19	20
Total no. of follicles	94	130	74	32	37
Follicles per ovary*	6.8 ± 0.5	7.9 ± 0.8	8.5 ± 0.7	7.5 ± 0.6	8 ± 0.4
Ovulation (%)	79	55†	56†	59†	54†

* Values are means ± SEM.
† $P < 0.05$.

tion. A dose-related effect was not observed (Table 1). The effects of the of 100 mg/kg aspirin at different times after hCG administration are recorded in Table 2, between 6.5 and 7 hours aspirin displayed the greatest effect (41%).

The Effect of In Vivo and In Vitro Administration of Aspirin

Ovulatory efficiency of control ovaries removed from in vivo control rabbits and perfused in vitro with perfusion medium and vehicle was 70%. Contralateral ovaries perfused in vitro with 100 μg/mL aspirin displayed a significant ($P < 0.001$) reduction of ovulatory efficiency (14%). Ovulatory efficiency of ovaries removed from in vivo treated (100 mg/kg) animals and perfused in vitro with perfusion medium and vehicle was 34%. Aspirin, administered in vitro to contralateral ovaries, totally inhibited follicular rupture (Fig. 1).

The Effect of In Vivo Administration of Naproxen

Naproxen at a dose of 10 mg/kg did not reduce ovulatory efficiency when administered 6 hours after hCG injection (no. of ovaries: 4; unruptured follicles: 8; ovulated follicles: 12; ovulatory efficiency: 60%). In contrast a significant ($P < 0.05$) reduction of ovulatory efficiency (Table 3) was observed when naproxen was administered at a dose of 50 mg/kg. The

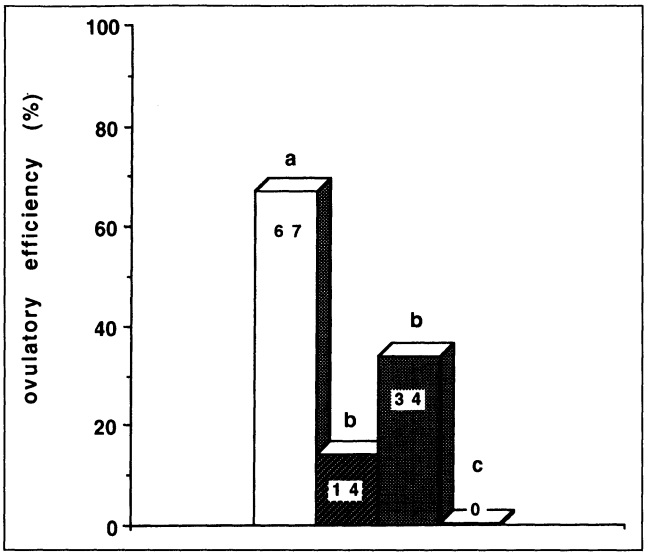

Figure 1 In vitro ovulatory efficiency (percentage of mature follicles) of control and aspirin perfused ovaries. One of the ovaries removed from in vivo control (control-aspirin) and in vivo treated (aspirin-aspirin) was perfused with aspirin (100 μg/mL). The other one with perfusion medium (control-medium; aspirin-medium). Bars with different superscripts represent significantly different values ($P < 0.01$). □, control-medium; ▨, control-aspirin; ▩, aspirin-medium; ■, aspirin-aspirin.

lower dose significantly ($P < 0.05$) reduced ovulatory efficiency (40%) and the higher dose totally inhibited follicular rupture in animals treated with naproxen 7 hours after hCG administration. No inhibitory effect on ovulatory efficiency was observed when naproxen was administered 8 hours after hCG.

The Effect of In Vivo and In Vitro Administration of Naproxen

Ovulatory efficiency of control ovaries removed from in vivo control rabbits and perfused in vitro with perfusion medium and vehicle was 70% in the first group of experiments. Contralateral ovaries perfused in vitro with 10 μg/mL naproxen displayed a significant ($P < 0.001$) reduction of ovulatory effi-

Table 2 Follicle Characteristics of In Vivo Control and Aspirin-Treated (100 mg/kg) Ovaries at Different Times

	Control	+6.5 to 7 hours	+7.5 to 8 hours
Total no. of ovaries	15	14	6
Unruptured follicles	22	69	27
Ovulated follicles	88	48	30
Total no. of follicles	100	117	57
Follicles per ovary*	6.9 ± 0.6	8.7 ± 0.5	8.3 ± 0.9
Ovulation (%)	88	41†	53†

* Values are mean ± SEM.
† $P < 0.05$.

Table 3 Follicle Characteristics of In Vivo Control and Naproxen-Treated (50 mg/kg) Ovaries at Different Times

	Control	+6 hours	+7 hours	+8 hours
Total no. of ovaries	15	4	6	4
Unruptured follicles	22	10	42	3
Ovulated follicles	88	6	0	29
Total no. of follicles	100	16	42	32
Follicles per ovary*	6.9 ± 0.6	4 ± 2	7 ± 0.9	8 ± 1.9
Ovulation (%)	88	37†	0	91

* Values are mean ± SEM.
† $P < 0.05$.

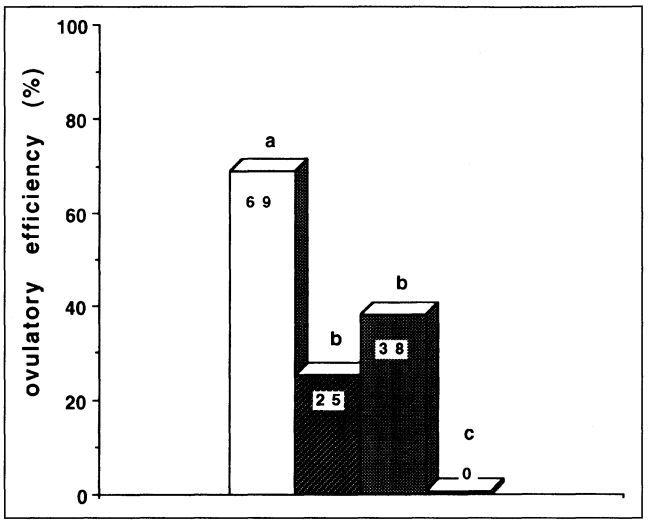

Figure 2 In vitro ovulatory efficiency (percentage of mature follicles) of control and naproxen perfused ovaries. One of the ovaries removed from in vivo control (control-naproxen) and in vivo treated (naproxen-naproxen) was perfused with 10 μg/mL naproxen. The other one with perfusion medium (control-medium; naproxen-medium). Bars with different superscripts represent significantly different values ($P < 0.01$). □, control-medium; ▨, control-naproxen; ▩, naproxen-medium; ■, naproxin naproxin.

ciency (25%). Ovulatory efficiency of ovaries removed from in vivo treated animals (10 mg/kg) and perfused in vitro with perfusion medium and vehicle was 38%. Naproxen administered in vitro to contralateral ovaries completely inhibited follicular rupture (Fig. 2).

In the second group of experiments, ovulatory efficiency of control ovaries removed from in vivo control rabbits and perfused in vitro with perfusion medium and vehicle was 77%. Contralateral ovaries perfused in vitro with 50 μg/mL naproxen displayed a significant ($P < 0.001$) reduction of ovulatory efficiency (13%). Ovulatory efficiency of ovaries removed from in vivo treated (50 mg/kg) animals and perfused in vitro with perfusion medium and vehicle was 0%. Naproxen administered in vitro to contralateral ovaries also completely inhibited follicular rupture (Fig. 3).

Effect of Aspirin and Naproxen on P and Prostaglandin Production

To verify the effect of aspirin and naproxen on ovarian PG production, ovarian venous concentration of PG was determined from ovarian venous blood obtained at the time of ovarian removal. Prostaglandin F metabolites were significantly ($P < 0.05$) reduced by aspirin and naproxen treatments: controls = 422 ± 56 pg/mL, aspirin = 169 ± 31 pg/mL, naproxen = 228 ± 51 pg/mL (conversion factor to SI unit, 2.820).

Concentration of $PGF_{2\alpha}$ in the perfusate was suppressed completely in both groups perfused with aspirin or naproxen (Fig. 4). In contrast, $PGF_{2\alpha}$ levels increased progressively, during perfusion, in the effluent of ovaries perfused with control-medium. The increase was more significant ($P < 0.01$) in the control ovaries removed from in vivo control rabbits than in the ovaries removed from in vivo aspirin-treated animals. This increase did not differ in naproxen perfusion experiments.

Perfusate P levels were similar in all group of perfused ovaries: control-medium = 5.9 ± 0.3 μg/h per ovary, control-aspirin = 7.5 ± 0.2 μg/h per ovary, aspirin-medium = 7.6 ± 0.3 μg/h per ovary, aspirin-aspirin = 5.5 ± 0.3 μg/h per ovary; control-medium = 8.0 ± 0.5 μg/h per ovary, control-naproxen = 9.7 ± 0.5 μg/h per ovary, naproxen control = 8.1 ± 0.4 μg/h per ovary, aspirin-aspirin = 9.0 ± 0.2 μg/h per ovary (conversion factor to SI unit, 3.810).

DISCUSSION

The role of PGs in the process of ovulation has been reviewed in various animal models (1–4, 13). In the rabbit, both the rise in PGs and subsequent ovulation can be prevented by the IV or intrafollicular administration of either indomethacin or anti-$PGF_{2\alpha}$ antiserum (13–15). In the rabbit, two phases of PG production have been established during the process of ovulation (3). The second phase, which begins approximately 3 hours before follicle rupture, may be related to an increase in follicular proteolytic

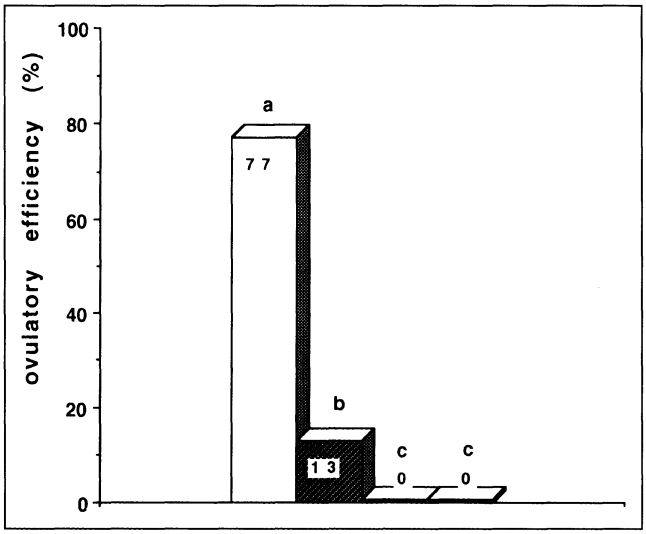

Figure 3 In vitro ovulatory efficiency (percentage of mature follicles) of control and naproxen perfused ovaries. One of the ovaries removed from in vivo control (control-naproxen) and in vivo treated (naproxen-naproxen) was perfused with naproxen (50 μg/mL). The other one with perfusion medium (control-medium; naproxen-medium). Bars with different superscripts represent significantly different values ($P < 0.01$). □, control-medium; ▨, control-naproxen; ▩, naproxen-medium; ■, naproxen-naproxen.

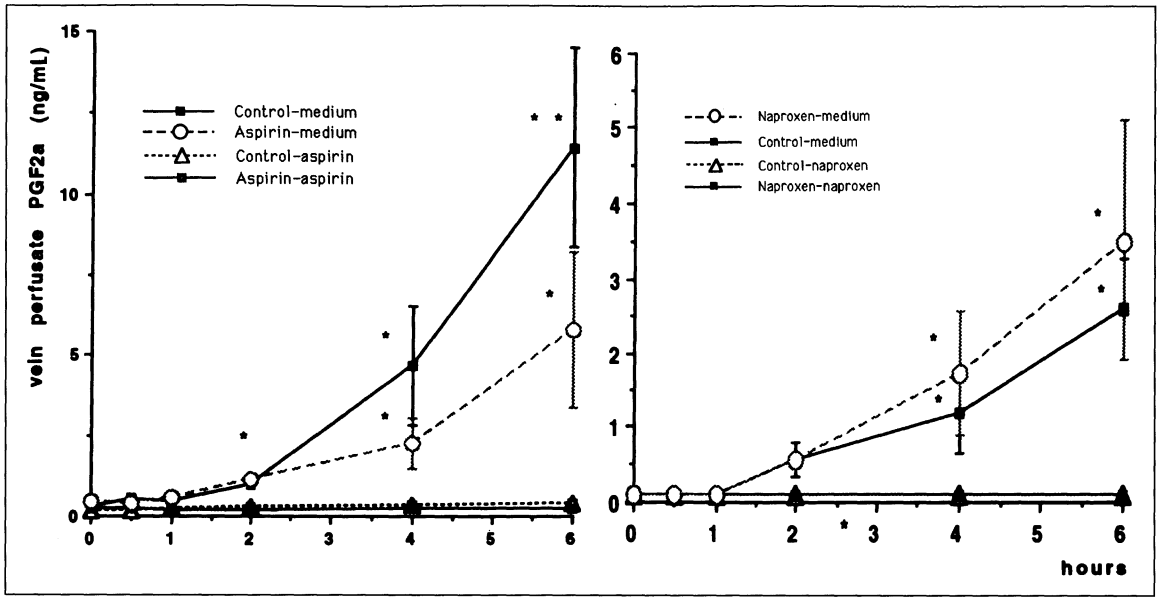

Figure 4 $PGF_{2\alpha}$ concentration in the vein perfusate of control, aspirin, and naproxen perfused ovaries. One of the ovaries removed from in vivo control (control-aspirin; control-naproxen) and in vivo treated (aspirin-aspirin; naproxen-naproxen) was perfused with 100 μg/mL aspirin or 50 μg/mL naproxen. The other one with perfusion medium (control-medium; aspirin-medium; naproxen-medium). *Significant difference ($P < 0.01$) versus aspirin-perfused or naproxen-perfused ovaries. **Significant difference ($P < 0.01$) versus in vivo treated ovaries perfused with medium.

activity near the time of ovulation (4). Indomethacin prevents ovulation only when administered 8 hours after hCG administration, although PG levels are depressed profoundly after drug administration at any time (3). Thus, there appears to be a critical interval in which indomethacin must be administered for blockade of ovulation. Moreover, as recent data in the rat suggest, indomethacin specifically may block cyclooxygenase activity and inhibit ovulation by interfering with the formation of lipoxygenase products (16).

The in vivo results of the present study indicate that aspirin and naproxen must each be administered at a critical time for the reduction in ovulatory efficiency after aspirin administration and for complete naproxen-induced inhibition of ovulation. The critical time ranges between 6.5 and 7 hours after hCG administration and is related temporally to the second, more significant, rise in PG production. This interval begins 5 hours after hCG injection and reaches its peak 8 hours after the administration of the ovulatory stimulus, 1 hour before the anticipated time of ovulation (3).

In the gonadotropin-primed immature rat model, there appears to be a correlation between potency of NSAID preparations and their inhibitory effect on ovulation and on ovarian PG synthesis (17). In this model, indomethacin was more effective as an inhibitor of ovarian PG synthesis and ovulation than the other compounds. The order of potency of the different agents is similar to their anti-inflammatory efficacy, with acetylsalicylic acid displaying the least activity (17). In concordance with this observation, in the present study, aspirin, administered in vivo, reduced ovulatory efficiency, but did not inhibit completely ovulation even at the highest used dose, whereas naproxen displayed 100% inhibition when administered at a dose of 50 mg/kg.

One explanation for the low efficacy of aspirin in vivo may be based upon the action of aspirin as an inhibitor of cyclooxygenase. Inhibition of this enzyme may result in the diversion of arachidonic acid to the lipoxygenase pathway. Arachidonic acid serves as the common substrate for synthesis of PGs and leukotrienes (LTs) through the cyclooxygenase and lipoxygenase pathways, respectively (18). In the rat, both nonpeptidic and peptidic LTs, compounds that are involved in hypersensitivity and inflammation (18–20), increase in the ovary during ovulation (16, 18). These eicosanoids ultimately might contribute to the degradative processes that lead to follicle rupture. Various experimental models of inflammation suggest that indomethacin-induced inhibition of PG synthesis tends to increase the synthesis of LTs (20, 21). In indomethacin-induced inhibition of rat ovulation, the lipoxygenase pathway eventually is suppressed with very high doses (18). Aspirin does not inhibit lipoxygenase, even at a very high dose; therefore, the increase in LTs is likely to occur, leading to ovulation and as a result only partial inhibi-

tion of ovulation as observed in aspirin-treated animals. Moreover, the in vivo experiments in the present study are consistent with the lower anti-inflammatory effect of aspirin in comparison with that of compounds that inhibit both lipoxygenase and cyclooxygenase.

A dose-related effect was not observed in rabbits treated with aspirin. A possible explanation for this phenomenon may be based on the notion that a moderate amount of PGE, still present during exposure to low doses of aspirin, can exert some degree of feedback inhibition of arachidonic acid release from phospholipid (20). Such inhibition may prevent arachidonic acid accumulation sufficiently to affect the lipoxygenase pathway until the dose of aspirin was high enough to virtually block the synthesis of PGs. Thus, inhibitory effect of PGs on arachidonic acid formation would disappear, making this substrate available for the lipoxygenase pathway (18). Therefore, the increase in LT synthesis and their active involvement in the process of ovulation would mask the dose-related inhibitory effect of high doses of aspirin on PGs production.

In contrast, naproxen displayed a dose-related effect and completely blocked ovulation in vivo at a dose of 50 mg/kg. This compound is approximately 20 times more potent than aspirin as an anti-inflammatory agent and similarly to indomethacin it might suppress the lipoxygenase pathway at a high dose (7, 18), blocking the contribution of LTs to follicle wall rupture.

In this study, the in vivo and in vitro results of experiments with aspirin indicate that the compound inhibits ovulation more efficiently when administered in vitro. Aspirin completely inhibited ovulation in ovaries perfused in vitro (100 μg/mL) after removal from in vivo treated animals (100 mg/kg). An explanation for the discrepancy between in vivo and in vivo and in vitro results might be that aspirin administered in vitro in this system is not metabolized rapidly to salicylate and therefore may display a high level of potency. In vivo, the half-life for aspirin is approximately 15 minutes; the compound is hydrolyzed rapidly, mainly in the liver, to salicylate, which is eliminated within 2 to 3 hours (7). Therefore, aspirin mainly acts as a precursor for salicylate and, in its anti-inflammatory effect, salicylate is the active agent (22). There is a great disparity between the potency of aspirin and salicylate, with aspirin approximately 20 times more potent than salicylate in inhibiting cyclooxygenase (22).

The in vivo and in vitro results with naproxen demonstrate that at the low dose the effect of naproxen on ovulatory efficiency was additive when administered both in vivo and in vitro. The compound completely inhibited ovulation in ovaries perfused in vitro 10 μg/mL after removal from in vivo treated animals (10 mg/kg). This result may be related to the longer time of ovarian exposure to this agent during the periovulatory period, to more homogeneous tissue distribution, and to its low rate of metabolism in vitro.

Our data indicate that ovarian P production was unaffected by aspirin and naproxen administration in vivo and in vitro. This observation is in agreement with the report that luteinized unruptured follicles can be produced by the administration of indomethacin in combination with hCG (23). These results confirm that follicle luteinization can occur in the absence of follicular rupture. Thus, the process of ovulation and the subsequent transformation of granulosa cells into functional luteal cells seem to be independent phenomena.

Killick and Elstein (24) demonstrated that NSAIDs can induce luteinized unruptured follicles in women if administered at a high dose during the periovulatory period, a phenomenon that appears to be associated with a significant decrease in the synthesis of ovarian eicosanoids (25). A therapeutic means of delaying follicle wall rupture has obvious potential as a nonhormonal form of oral contraception, but the high doses of NSAIDs required might not be without hazard. On the other hand, more concern can arise from the likely negative effect of long-term NSAIDs treatment on the efficiency of the ovulatory process. Chronic autoimmune inflammatory diseases, which necessitate long-term administration of NSAIDs, occur frequently in women and often are diagnosed in young fertile women. The usual single dose of aspirin is 300 mg to 1 g, to be repeated every 4 to 8 hours, of naproxen is 250 to 375 mg, given twice daily (7). The doses used in this study, adjusted to the weight, correspond, in humans, to an administration of 500 mg to 3 g (10 to 60 mg/kg) for aspirin and of 500 mg to 2 g (10 to 40 mg/kg) for naproxen. Long-term treatment of high doses of NSAIDs, similar to those used in our study, involve administration of these drugs during the periovulatory interval of menstrual cycles. Such long-term administration may cause an increase of the frequency of luteinized unruptured follicles among fertile women and consequently a higher incidence of infertility in this specific population.

In conclusion, both aspirin and naproxen significantly reduced ovulatory efficiency in vivo and in vitro in hCG-treated rabbits. The potency of the compounds in blocking ovulation parallels their anti-inflammatory action. Moreover, aspirin and naproxen induced a significant decrease in ovarian PG production, corroborating a role for these metabolites in the process of ovulation. A critical period has been observed after the preovulatory gonadotropin stimu-

lus during which aspirin or naproxen must be administered to display their maximum effect. This critical time in the rabbit correlates with the second, larger PG increase that precedes follicle wall rupture. Progesterone production did not change during aspirin and naproxen administration despite the presence of unruptured follicles, confirming that ovulation and follicle luteinization represent two distinct and independent events.

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