CHRONIC ADMINISTRATION OF NONSTEROIDAL-ANTIINFLAMMATORY DRUGS (NSAIDS): EFFECTS UPON MOUSE REPRODUCTIVE FUNCTIONS.

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SUMMARY:
Although nonsteroidal-antiinflamatory drugs (NSAIDs) are widely employed, reproductive side effects of prostaglandins long-term inhibition remain unknown.
The objective of the present study was to evaluate the effects of chronic low/moderate NSAIDs doses upon mice reproductive functions.
Male or female mice were injected (i.p. for 60 or 35 days respectively) with: ibuprofen doses A, B or C (0.56, 1.12 or 1.68mg/100g/day respectively) or piroxicam doses A, B or C (0.028, 0.056 or 0.084mg/100g/day respectively). Parameters evaluated were: a) in females, spontaneous and induced ovulation, oocyte maturity and spermatozoa migration through genital tract, b) in males, epididymal spermatozoa concentration, motility, viability, resistance to hypoosmotic shock, acrosomal status and membrane maturity and c) in both genders, in vitro and in vivo fertilization, reproductive hormones plasma levels and cyclooxigenase inhibition in reproductive tissues.
In females ibuprofen (dose A) elicited a significant reduction in spontaneous and induced ovulation rates and piroxicam (dose A) diminished the concentration of spermatozoa found in the uterus after mating. Males treated with ibuprofen (dose B) showed a reduction in the in vitro fertilization ability.
Our data reveal that chronic administration of ibuprofen or piroxicam can exert detrimental effects upon reproductive physiology, which depends on the doses and/or the drug employed.

KEY WORDS: Nonsteroidal-antiinflamatory drugs, prostaglandins, reproduction, sperm functional activity, oocyte, ibuprofen, piroxicam.

RESUMEN:
A pesar del frecuente empleo de los antiinflamatorios no esteroideos (AINEs), aún no se conocen los efectos secundarios de la inhibición crónica de las prostaglandinas.
El objetivo del presente estudio fue evaluar los efectos del tratamiento crónico con dosis bajas/moderadas de AINEs sobre la función reproductiva de ratones.
Ratones machos y hembras fueron inyectados (i.p. durante 60 ó 35 días respectivamente) con: dosis A, B o C de ibuprofeno (0.56, 1.12 ó 1.68mg/100g/día respectivamente) or dosis A, B o C de piroxicam (0.028, 0.056 ó 0.084mg/100g/día respectivamente). Los parámetros evaluados fueron: a) en hembras, ovulación espontánea e inducida, maduración ovocitaria y migración de espermatozoides a través del tracto genital, b) en machos y en espermatozoides epididimarios, concentración, motilidad, vitalidad, resistencia al shock hipoosmótico, estadío acrosomal y madurez de membrana y c) en ambos sexos, fertilización in vivo e in vitro, niveles plasmáticos de hormonas reproductivas e inhibición de ciclooxigenasa en tejido reproductivo.
En las hembras, ibuprofeno (dosis A) disminuyó significativamente la ovulación espontánea e inducida y piroxicam (dosis A) disminuyó la concentración de espermatozoides en el tracto genital de la hembra luego de la cópula. Los espermatozoides obtenidos de machos tratados con ibuprofeno (dosis B) mostraron una disminución en su capacidad fertilizante in vitro.
Nuestros datos revelan que la administración crónica de ibuprofeno o piroxicam pueden ejercer efectos deletéreos sobre la fisiología reproductiva, que dependen de la dosis y/o la droga empleada.

KEY WORDS: Antiinflamatorios no esteroideos, prostaglandinas, reproducción, actividad funcional espermática, ovocito, ibuprofeno, piroxicam.

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INTRODUCTION:

Prostaglandins (PGs) have been involved as regulators of several physiological processes related to reproduction (2, 38). Nonsteroidal-antiinflamatory drugs (NSAIDs), substances that interfere with the biosynthesis and/or metabolism of such eicosanoids, may affect male and/or female reproductive processes (5, 8, 15, 17, 32, 39). However, although NSAIDs are widely employed for the treatment of subacute and/or chronic diseases such as muscular pain, headache, lupus erythematosus, rheumatoid arthritis, etc, there is no conclusive knowledge about the possible adverse effects of these agents on reproductive functions. Since some of the mentioned diseases affect men and/or women in their fertile years, more detailed studies about this topic are necessary.

Besides, the majority of the publications on this subject have been performed employing high doses and/or acute administration of these drugs (7, 16, 25, 28), the effects of long-term administration of low or moderate doses of NSAIDs on gamete characteristics and fertility remain to be established.

It was not until 1996, that the first case reports appeared describing transient infertility due to the inhibition of ovulation following treatment with indomethacin, diclofenac, piroxicam and naproxen in female patients with autoimmune diseases and chronic NSAIDs administration (26, 32).

Recently, we published a paper about the effects of long term administration of aspirin-like drugs upon seminal parameters in humans; we found detrimental effects; reduction in seminal volume, total number of spermatozoa, percentages of motile, viable and morphologically normal cells and diminished seminal fructose levels (21). Nevertheless, it was a retrospective study with all the ethical and methodological limits for human research.

The purposes of this study were to evaluate in adult mice, possible effects of chronic administration of low or moderate doses of ibuprofen or piroxicam upon some parameters that reflect reproductive physiology: in females, ovulation index, oocyte maturity and spermatozoa migration through the genital tract after mating and in males, sperm concentration and motility, sperm membrane maturation, viability, response to hypoosmotic swelling test and spontaneous acrosome reaction. In both genders we also evaluated the in vitro and in vivo fertilization indices, plasma levels of reproductive hormones and cyclooxigenase inhibition in reproductive tissues.

MATERIAL AND METHODS:

Animals

Inbreed sexually mature (70 days) Albino Swiss mice SWR/J(q) were employed. They were maintained on 12:12 h light:dark basis, and at 20 ± 4°C, with mouse pelleted food (Gepsa Feeds, Argentina) and water ad libitum. They were housed in groups not bigger than five animals (all from the same experimental group) in cages of 22x30x9 cm, with wood shavings as bedding material.

Drugs administration

Ibuprofen or piroxicam (Sigma Chemical Co.) were administered in three doses: Ibuprofen A, B or C (0.56, 1.12 or 1.68 mg/100g/day respectively) or Piroxicam A, B or C (0.028, 0.056 or 0.084 mg/100g/day respectively); they were calculated on the basis of commonly human injectable doses.

Ibuprofen was dissolved in propylene glycol (3.4 M) and vehiculized in 0.9% NaCl solution. Piroxicam was dissolved in dimethylsulfoxide (3.5 M) and vehiculized in 0.9% NaCl solution.

Females were injected daily (i.p.) for 35 days (period that covers at least 7 oestrus cycles) with the doses A or C of ibuprofen or piroxicam. Males were injected daily (i.p.) for 60 days (period that covers at least one complete mouse spermatogenic cycle and the epididymal migration) with the doses B or C of ibuprofen or piroxicam. The effects of the doses A of these compounds upon male reproductive function were previously published (34). Control animals were injected only with the solvent and vehicle and for the same period.

An overview of the treatments, parameters and number of animals evaluated is summarized in Table 1.

Gametes

Modified Tyrode´s medium (11) supplemented with 4 mg/ml of fraction V BSA (Sigma Chemical Co.) was employed and gametes were maintained till use in an incubator at 37°C (5% CO_2:95% air) and 100% humidity.

Oocytes were harvested from natural cycling or from superovulated females: 5 IU PMS (i.p.; Sigma Chemical Co.) followed 48 h later by 10 IU hCG (i.p.; Endocorion, ELEA).

The animals were sacrificed (approximately at 9 a.m.) by cervical dislocation or decapitation (in order to obtain plasma for hormone assays) at oestrus morning (detected by male receptivity) or 16-18 h after the hCG injection, and the cumulus-oocyte complexes were collected from the oviducts by puncturing the swollen ampulla and were allocated into center-well dishes with 1 ml Tyrode´s solution.

After male sacrifice (approximately at 9 a.m. by decapitation or cervical dislocation), spermatozoa were obtained by making incisions in the isolated caudal portion of the epididymis and allowing the sperm to extrude into 2 ml of the medium.

Reproductive parameters

- *Ovulation index:* was evaluated in natural cycling (at oestrus morning) or in superovulated females; results are expressed as number of oocytes collected from the ampullas/female.
- *Oocyte maturity:* after removal of the cumulus oophorus with hyaluronidase this parameter was determined evaluating, in an inverted microscope at 400x, the percentage of oocytes without a visible germinal vesicle.
- *Spermatozoa migration through the female genital tract*: as previously described (10) around 8:00 a.m. untreated males and treated females were housed together and observed; 110 min after, all mated females were sacrificed and the uterus was flushed into a dish containing 2 ml medium and incubated for 10 min. The oviducts were located into another dish with 1 ml Tyrode, cut into small pieces and taken to the incubator for 10 min. After this period, sperm functional activity was evaluated (sperm concentration, motility, viability and acrosomal status).
- *Sperm concentration and motility:* were measured in a Makler counting chamber (Sefi-Medical Instruments, Israel) under an inverted microscope (Olympus CK2, Japan) at x 200 magnification (19). As previously described, the results were expressed as percentage of motile cells (progressive plus non-progressive spermatozoa). No fewer than 100 gametes were examined (10). In order to evaluate the sperm membrane immaturity, percentages of bending spermatozoa and those with cytoplasmic drop were also determined.
- *Sperm viability:* was evaluated by supravital staining with Hoechst 33258 (H258) (3 mg***/***ml in isotonic solution) (Calbiochem, USA) (37). Using the appropriate ultraviolet fluorescence optics (Axiolab, Zeiss, Germany), spermatozoa showing bright fluorescent nuclei were scored as dead and cells which excluded the H258 were scored as viable. The viability of at least 100 cells was assessed and results were expressed as percentage of viable spermatozoa.
- *Hypoosmotic swelling test (HOST):* as previously described (27), 0.1 ml of sperm suspension was mixed with 1 ml of the hypoosmotic solution (100 mOsm***/***l) for 45 min (37°C). Evaluations were made by phase-contrast microscopy at a magnification of x 400; one hundred or more cells were observed; results are expressed as the percentage of spermatozoa that showed tail swelling.
- *Acrosomal integrity:* as previously described, it was determined by staining with *Pisum sativum* agglutinin labelled with fluorescein isothiocyanate (FITC-PSA) (Sigma Chemical Co., USA) (10). The H258 was dissolved in isotonic solution and added to a final concentration of 1.5 µg***/***ml and then co-incubated with spermatozoa for 10 min. Samples were washed free of unbound stain by centrifugation twice at 400 g for 10 min with 2 ml of isotonic solution; the supernatant was then carefully removed, cells were mounted as smears on glass slides, dried in an incubator and fixed with methanol for 30 sec. The slides were washed with a stream of distilled water for 2 min and after drying, spermatozoa were incubated with 30 µg***/***ml FITC-PSA in isotonic solution for 30 min and washed again with a stream of distilled water for 2 min. Finally, in order to avoid fading of fluorescence, the slides were mounted in mounting medium containing 1 mg***/***ml sodium azide and 100 mg***/***ml 1,4-diazabicyclo (2.2.2) octane (DABCO) in 90% (v/v) glycerol and 10% (v/v) DPBS (pH 9). Acrosomal status and viability were evaluated under an epifluorescence microscope (x 1000). The staining patterns were described as intact or reacted acrosome. At least 100 viable cells were assessed. For the experiments of "spermatozoa migration along the female genital tract after mating", both viable and dead spermatozoa were considered.
- *In vitro fertilization rate:* this procedure was performed according with Yelian and Dukelow (1992). After superovulation induction, the treated females were sacrificed and the oocytes were recovered as described above from the swollen ampulla and placed in 1 ml of Tyrode´s medium and inseminated with ~1 x 10^6/ml epididymal spermatozoa pooled from untreated males.

For the males evaluation, the oocytes of untreated females were pooled, located (n≅20) in a separate center-well dish and inseminated with ~1 x 10^6/ml epididymal spermatozoa of

each treated male.
The culture dishes were kept at incubator for 22 h. After this time, assessment of results was performed employing an inverted microscope and embryos in the pronucleus stage or with two or more blastomeres were considered as fertilized.
- *In vivo fertilization rate and litter size:* males and females were housed together for 4 days (only one oestrus cycle). After twelve days, females were sacrificed and the number of fetuses in the uterus was counted. Each treated male were housed with 2-3 untreated females and each treated female were housed with 2 untreated males.
- *Plasma FSH and testosterone levels:* blood samples were collected immediately after decapitation (at the morning of oestrus in the females), centrifuged for 30 min at 400 g, and plasma was stored at -20°C until assayed. FSH plasma levels were assayed with a rat IRMA kit (Biocode) with a sensibility of 0.2 ng/ml. For testosterone determination a mouse RIA kit (Immunotech) with 0.1 ng/ml of sensibility was employed. The final determinations were performed in an automatic gamma counter (ANSR – Abbott).
- *Cyclooxigenase inhibition:* as previously described (3), the ability of reproductive tissues to radio-convert C^{14} arachidonic acid into PGs was determined employing a liquid chromatography technique. Uterus and seminal vesicles were obtained 20 h after the last drug injection, in order to investigate the possible chronic cyclooxigenase inhibition exerted by the NSAIDs chronic treatment upon reproductive tissues. The PGs used as controls were 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, PGE_2 and TXB_2 (Sigma Chemical Co.). The quantifications were performed in a beta-counter (Beckman LS 7000) and the results were expressed as proportion of labelled arachidonic acid converted into PGs.

Statistical analysis

Results were processes with the software program Infostat (Infostat 1.1, version 2000, grupo Infostat, National University of Cordoba, Argentine). Values are expressed as Mean±SEM or percentage. As adequate, Student's "t"- test, ANOVA or Chi-square test were applied. When necessary, percentages were converted to a Gaussian distribution by arcsin transformation. All *p* values ≤ 0.05 were considered statistically significant.

RESULTS:

Effects of NSAIDs upon female reproductive function

The administration of the dose A of ibuprofen for 35 days to adult female mice induced a significant reduction in spontaneous as well as in induced ovulation index ($p< 0.05$) (Figure 1). Nevertheless, the higher doses of ibuprofen here employed (dose C) did not significantly modify this parameter. Piroxicam, doses A or C, did not affect this variable either (Induced ovulation –oocytes/female-: control 19.7 ± 1.5, n=21, piroxicam dose A 17.9 ± 2.4, n=22; control 21.6 ± 4.1, n=10, piroxicam dose C 16.4 ± 4.3, n=10. Spontaneous ovulation –oocytes/female-: control 10.0 ± 0.6, n= 10, piroxicam dose A 9.6 ± 0.7, n=9).

In order to evaluate if an "acute administration" of ibuprofen (dose C) exerts a comparable effect upon ovulation rates vs chronic ones (35 days), females were injected for only two days (those of PMS and hCG administration) with this drug. Ovulation indices from their respective controls, but were significantly higher than those obtained after chronic administration (36.1 ± 4.3, n=8; 22.8 ± 3.2, n=18 respectively; $p< 0.05$).

Oocyte maturity was not modified by NSAIDs administration (ibuprofen or piroxicam) in any of the doses here assayed (results not shown). In vitro or in vivo fertilization indices remained unaltered also (Table 2).

On oestrus day, no changes were detected in plasma FSH levels (females treated for 35 days: control 17.7 ± 1.8 ng/ml, n=13; ibuprofen dose A 21.8 ± 3.2 ng/ml, n=8; control 23.7 ± 2.4 ng/ml, n=10; ibuprofen dose C 22.4 ± 3.4 ng/ml, n=11).

When sperm migration along the female genital tract was evaluated, we found that piroxicam administration (dose A) elicited a significant decrease in the concentration of spermatozoa found in the uterus (control: 6.9 ± 1.5 $x10^6$/ml, n=8; piroxicam: 3.6 ± 1.2 x 10^6/ml, n=9; $p< 0.05$). Ibuprofen (dose A) did not significantly modify this parameter. However, it must be remarked, that in all groups (control or NSAIDs treated animals) we detected a physiological reduction in oviductal sperm concentration vs uterine one ($p< 0.05$). When comparing sperm functional activity of samples obtained from the oviduct with those obtained from the uterus, we detected in the last ones, a reduction in motility and in viability and an increase in the percentage of acrosome reacted cells. These effects were obtained in control as well as in NSAIDs treated animals (Table 3).

We did not detect any effect of NSAIDs upon the conversion of □abeled arachidonic acid to PGs in the uterus from females treated

with ibuprofen dose C (Table 4) or with piroxicam (results not shown).

Effects of NSAIDs upon male reproductive function

As can be seen in Table 5, the treatment of adult male mice for 60 days with the dose B of ibuprofen or piroxicam did not exert any effect in the functional activity of epididymal spermatozoa. Similar results were obtained with the highest dose of ibuprofen or piroxicam (results not shown).

In vitro fertilization index of spermatozoa obtained from males treated with ibuprofen (dose B) was significantly lower than that of control ones (Table 6). No significant changes in this parameter were evoked by dose C of ibuprofen or piroxicam. Neither the proportion of pregnant females mated with treated males nor the litter size were significantly modified by any of the NSAIDs treatments here assayed.

Plasma concentration of FSH and testosterone were 46.6 ± 3.9 ng/ml (n=10) and 5.8 ± 1.3 ng/ml (n=24) respectively in control animals and they were not modified by ibuprofen (doses C or B) or piroxicam (dose C) treatment.

No alterations in the proportion of □abeled arachidonic acid converted into PGs were detected in the seminal vesicles of animals treated with the highest doses of ibuprofen (Table 4) or piroxicam (results not shown).

DISCUSSION:

In the present paper we evaluated the effects of chronic administration of low or moderate doses of ibuprofen or piroxicam upon adult mice reproductive function; both NSAIDs frequently used in human therapy. Although these drugs have been employed for decades, we did not find studies evaluating the effects of low doses applied in long term protocols upon reproductive function.

Effects of NSAIDs upon female reproductive function

The participation of PGs in mammalian ovulatory process is well documented (38). On this basis, the significant decrease in the ovulation rate elicited by ibuprofen (dose A), can be attributed to eicosanoid synthesis inhibition. However, we can not discard some effects on the phenomena previously occurring, i.e. during oogenesis.

Piroxicam did not exert any significant effect on ovulation. In this context, it must be considered that the potency of a NSAID for inhibiting ovulation is correlated with their anti-inflammatory ability (7, 8, 25); consequently could not be the same for ibuprofen or piroxicam. On the other hand, the highest dose of ibuprofen did not significantly alter ovulation indices. It has been reported that some NSAIDs do not exert a dose-dependent action on ovulation (39) and that, in prolonged NSAIDs treatment, the eicosanoid synthesis reduction is not the same for the different PGs series and also, that the recuperation in the synthesis is uneven (18).

Moreover, PG effects are related with the individual concentration of each PG as well as with their relative concentration (18). These aspects explain results here obtained with the both doses of ibuprofen here assayed. Therefore, it can not be discarded that the higher doses of NSAIDs could provoke compensatory mechanisms not evoked by the smaller ones, which could include up-regulation of receptors, increase in the cyclooxigenase synthesis (24), decrease in the concentration and/or activity of PGs catabolic enzymes (24, 25) and/or increase in the synthesis of leucotrienes, substances also involved in the ovulatory process (38, 39).

When ibuprofen was daily injected for 35 days there were no chronic inhibition of cyclooxigenase activity in reproductive tissues. In order to evaluate if an acute administration of the same agent exerted a comparable effect on ovulation, two single doses of ibuprofen were injected simultaneously with the PMS and hCG. In chronic treated animals a significant lower ovulation index than in acute treated ones was detected. This finding suggests that chronic administration of ibuprofen exerts more severe alterations and/or some other effects beyond the cyclooxigenase inhibition.

Although it is known that the cumulus-oocytes complexes synthesize PGs that participate in the fertilization process (6, 12), in our study we did not find any effect of NSAIDs upon in vitro fertilization indices, which reached similar values to those previously obtained in our laboratory (10, 20).

In vivo fertilization rates or the litter size remained unchanged in treated females. Since ibuprofen (dose A) diminished the ovulatory index, these results seem to be contradictory. Nevertheless, these findings are explained by the fact that in our experimental design, in order to avoid implantation inhibition and/or abortions previously reported (29, 33), drugs injection were interrupted the day before male and female joining.

When we evaluated the effects of female piroxicam administration (dose A) upon sperm migration through the genital tract, we found a reduction in the concentration of uterine spermatozoa. An involvement of PGs in sperm migration was previously suggested by several authors (5, 38). When functional

activity of spermatozoa obtained from the oviduct was compared with those of gametes collected from the uterus, results obtained were similar to those reported in previous papers (10, 35). Briefly, the reduction of sperm concentration, motility and viability and the increase in the percentage of acrosome reacted sperm, are explained by the occurrence of physiological processes such as sperm capacitation or acrosome reaction induced by female micro-environmental signals and not by the NSAIDs injected.

Finally, NSAIDs treatment did not modify the plasma levels of FSH; this finding is in accordance with those of Matsumoto et al (2001) and Sato et al (1974).

Effects of NSAIDs upon male reproductive function

When analyzing our results, it must be taken into account that the spermatozoa have been obtained from the caudal portion of the epididymis, and consequently they have not been in contact with the seminal PGs. Nevertheless, PGs are also synthesized in another portions of the reproductive tract (including testis and epididymis) (4, 9). Moreover, it is known that epididymal, as well as ejaculated spermatozoa, synthesize PGs (14, 30). It is also relevant that in contrast with the bibliography, in our experimental model, NSAIDs were injected for 60 days, period that covers spermatogenesis and epididymal transit.

Functional activity of epididymal spermatozoa was not modified by NSAIDs treatments. In a previous paper employing the dose A of ibuprofen or piroxicam, we obtained similar results (34). Despite the species-specific differences that must be considered, in humans, we found that the chronic administration of low or moderate doses of NSAIDs (mainly aspirin) exerted detrimental effects. Seminal volume, total number of spermatozoa, motility, viability, percentage of morphologically normal cells and seminal fructose levels diminished significantly (21). These discrepancies can be explained by functional membrane changes evoked by seminal plasma components on ejaculated spermatozoa (36).

The detrimental effect upon the in vitro fertilization index when ibuprofen (dose B) was administered, is in accordance with previous studies by Aitken and Kelly (1985), who reported that the PGs synthesized by the spermatozoa, particularly from the E family, are involved in the fertilization process. The increase induced by PGs on the fertilization rate has been related to the ability of these eicosanoids to enhance sperm Ca^{++} inflow (31). Furthermore, the addition of three NSAIDs in a mouse in vitro fertilization assay decreased the fertilization index (13, 15). Since our results were evoked only by the smaller dose of ibuprofen and not by the higher one, the possible compensatory mechanisms above suggested (for ovulation response) would be operating.

Although it is well known that seminal PGs are involved in the migration of the spermatozoa through the female genital tract (2), we did not find any effect of male chronic NSAIDs treatment upon the percentage of pregnant females or in the litter size. The low pregnancy rates found in all the groups studied here (control or treated males) can be explained by the stressful stimulus of the daily puncture and/or injection. In other experiments we detected alterations in the erection and/or ejaculation processes without affecting the sperm quality or the plasmatic levels of FSH or testosterone (results not shown)**.**

In conclusion, our data suggest that chronic administration of low or moderate doses of NSAIDs exerts several detrimental effects upon male and female reproductive physiology, which depends, among other factors, from the dose and/or the drug employed. Although possible species-specific effects would be operating, when NSAIDs are administered to patients in their fertile years, these results must be taken into account.

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Table 1: Summary of the treatments applied and parameters and animal number evaluated.

Gender	Parameters	IBUPROFEN						PIROXICAM					
		A		B		C		A		B		C	
		C	NSAIDs	C	NSAIDs	C	NSAIDs	C	NSAIDs	C	NSAIDs	C	NSAIDs
Females	Ovulation Index (induced)	22	26			17	19	21	22			10	10
	Ovulation Index (spontaneous)	13	11			12	12	10	9			-	-
	Oocyte maturity	58 cells	50 cells			47 cells	26 cells	44 cells	38 cells			29 cells	31 cells
	Spermatozoa migration*	12	9			-	-	8	9			-	-
	In vitro fertilization rate	199 cells	219 cells			237 cells	137 cells	226 cells	274 cells			132 cells	91 cells
	In vivo fertilization rate	13	12			5	10	10	10			10	10
	Litter size	10	11			4	6	8	7			6	5
	Plasma FSH levels	13	8			10	11	-	-			-	-
	Cyclooxigenase inhibition	-	-			5	5	-	-			5	5
Males	Sperm functional activity*			37	30	7	7			20	18	9	7
	In vitro fertilization rate			196 cells	171 cells	121 cells	117 cells			89 cells	126 cells	94 cells	124 cells
	In vivo fertilization rate			23	16	14	16			18	14	14	12
	Litter size			3	7	3	6			6	3	2	2
	Plasma FSH levels			3	6	7	6			-	-	-	-
	Plasma testosterone levels			8	8	8	8			-	-	8	8
	Cyclooxigenase inhibition			-	-	5	5			-	-	5	5

Ibuprofen: A, B, C: 0.56, 1.12, 1.68 mg/100 g/day respectively. Piroxicam: A, B, C: 0.028, 0.056, 0.084 respectively. C: Control animals; NSAIDs: treated animals. Values indicate the number of animals except when indicated (cells = oocytes). *: differences in the final number of animals consigned in results, can be explained by missing values or statistically excluded ones.

Table 2: Effects of nonsteroidal-antiinflammatory drugs (NSAIDs) (ibuprofen or piroxicam) administration to adult female mice upon in vitro or in vivo fertilization indices.

Variables	IBUPROFEN				PIROXICAM			
	doses A		doses C		doses A		doses C	
	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs
% of in vitro fertilized oocytes	81, 9% (199)	82.6% (218)	90.7% (237)	89.0% (137)	85.4% (226)	81.7% (274)	80.3% (132)	83.5% (91)
In vivo % of pregnant mice	76.9% (13)	91.7% (12)	80.0% (5)	60.0% (10)	80.0% (10)	70.0% (10)	70.0% (10)	65.0% (10)
Number of fetuses	8.4 ± 0.4 (10)	9.5 ± 0.7 (11)	9.2 ± 0.5 (4)	8.5 ± 0.2 (6)	9.6 ± 0.7 (8)	8.1 ± 1.2 (7)	9.2 ± 1.1 (6)	9.0 ± 1.0 (5)

Ibuprofen (doses A: 0.56 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.) or piroxicam (doses A: 0.028 mg/100g/day or doses C: 0.084 mg/100g/day; i.p.) were injected daily to adult female mice for 35 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. In vitro fertilization indices, are expressed as percentages of fertilized oocytes that were obtained by ampullar puncture after induced superovulation. In vivo fertilization indices are expressed as percentages of pregnant mice after housing for 4 days (one oestral cycle) with untreated males; 12 days after, females were sacrificed and the number of fetuses was determined. Results are expressed as percentages or Mean ± SEM. In parentheses: number of oocytes or female mice evaluated.

Table 3: Functional activity of spermatozoa obtained from the reproductive tract from female mice (treated with ibuprofen or piroxicam) after mating with untreated males.

SPERM VARIABLE	IBUPROFEN				PIROXICAM			
	CONTROL UTERUS	CONTROL OVIDUCT	UTERUS	OVIDUCT	CONTROL UTERUS	CONTROL OVIDUCT	UTERUS	OVIDUCT
Progressive (%)	45.7 ± 6.1 [a] (12)	20.0 ± 11.5 [a] (3)	46.2 ± 5.7 [b] (9)	7.0 ± 7.0 [b] (2)	43.6 ± 6.5 (8)	25.0 ± 14.4 (3)	22.9 ± 8.3 (9)	---
Non-progressive (%)	10.5 ± 2.9 (12)	13.3 ± 6.7 (3)	12.8 ± 2.7 (9)	13.5 ± 13.5 (2)	12.9 ± 2.1 (8)	11.0 ± 11.0 (3)	15.8 ± 4.8 (9)	—
Non-motile (%)	43.7 ± 5.1 [c] (12)	66.7 ± 17.6 [c] (3)	41.1 ± 5.7 [d] (9)	79.5 ± 6.5 [d] (2)	43.6 ± 5.1 (8)	64.0 ± 7.4 (3)	61.2 ± 8.9 (9)	—
Viable (%)	47.7 ± 6.3 [e] (12)	24.3 ± 5.0 [e] (10)	49.6 ± 6.6 [f] (10)	27.1 ± 6.9 [f] (7)	56.9 ± 6.2 [g] (8)	33.1 ± 5.6 [g] (7)	40.0 ± 7.0 [h] (9)	18.4 ± 3.4 [h] (5)
Acrosome reacted (%)	51.4 ± 10.2 (11)	70.3 ± 13.0 (6)	54.1 ± 12.2 (9)	74.1 ± 7.5 (8)	33.3 ± 2.7 [i] (7)	55.5 ± 4.7 [i] (8)	32.4 ± 6.0 [j] (7)	60.3 ± 8.3 [j] (5)

Ibuprofen (doses A: 0.56 mg/100g/day; i.p.) or piroxicam (doses A: 0.028 mg/100g/day; i.p.) were administered to adult female mice during 35 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. The spermatozoa were evaluated 110 min after the female accepted the untreated male for mating. Values indicate Mean ± SEM. In parentheses: number of animals evaluated. In each row, identical letters indicate significant differences ($p < 0.05$).

Table 4: Effects of ibuprofen administration to adult female or male mice upon percentages of radioconversion of arachidonic acid to prostaglandins in uterus or seminal vesicles.

ARACHIDONIC ACID METABOLITES	% OF RADIOCONVERSION			
	UTERUS		SEMINAL VESICLES	
	CONTROL (5)	IBUPROFEN (5)	CONTROL (5)	IBUPROFEN (5)
6 – ceto - $PGF_{1\alpha}$	4.74 ± 0.64	5.36 ± 3.27	7.44 ± 1.57	7.36 ± 1.53
$PGF_{2\alpha}$	3.84 ± 0.33	3.76 ± 0.18	6.37 ± 1.16	6.72 ± 1.49
PGE_2	3.82 ± 0.19	4.18 ± 0.29	11.21 ± 2.32	11.96 ± 2.45
TXB_2	2.37 ± 0.24	2.62 ± 0.15	4.50 ± 0.78	5.60 ± 1.55

Ibuprofen (doses C: 1.68 mg/100g/day; i.p.) was administered to adult female mice for 35 days and to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol) in the same volume and period. PG: prostaglandin; TX: tromboxan. Values indicate Mean ± SEM. In parentheses: number of animals evaluated.

Table 5: Effects of nonsteroidal-antiinflammatory drugs (NSAIDs) (ibuprofen or piroxicam) administration to adult male mice upon functional activity of epididymal spermatozoa.

SPERM VARIABLE	IBUPROFEN		PIROXICAM	
	CONTROL	NSAIDs	CONTROL	NSAIDs
Body weight (g)	27.6 ± 0.5 (24)	27.5 ± 0.5 (19)	26.7 ± 1.1 (10)	26.5 ± 0.5 (13)
Concentration (x 10^6/ml)	11.6 ± 0.9 (37)	11.1 ± 1.0 (30)	15.3 ± 1.9 (20)	14.6 ± 2.1 (18)
Motile (progressive + non-progressive) (%)	63.7 ± 2.2 (30)	62.8 ± 2.8 (29)	74.2 ± 2.0 (17)	67.2 ± 3.1 (18)
Viable (%)	71.3 ± 2.1 (16)	71.7 ± 2.2 (16)	73.2 ± 1.3 (17)	75.6 ± 2.4 (18)
Swollen (HOST) (%)	60.3 ± 3.1 (17)	57.5 ± 3.6 (15)	69.9 ± 1.4 (17)	69.4 ± 2.2 (17)
Acrosome intact (%)	78.9 ± 1.1 (8)	77.4 ± 3.5 (8)	88.4 ± 1.4 (9)	88.9 ± 1.2 (10)
Bending and/or with cytoplasmatic drop (%)	17.0 ± 1.9 (16)	17.4 ± 2.0 (19)	14.4 ± 1.7 (24)	17.4 ± 2.3 (22)

Ibuprofen (doses B: 1.12 mg/100g/day; i.p.) or piroxicam (doses B: 0.056 mg/100g/day; i.p.) were administered to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. The spermatozoa were obtained from the caudal epididymis. Values indicate Mean ± SEM. In parentheses: number of animals. HOST= hypoosmotic swelling test.

Table 6: Effects of nonsteroidal-antiinflammatory drugs (NSAIDs) (ibuprofen or piroxicam) administration to adult male mice upon in vitro or in vivo fertilization indices.

Variables	IBUPROFEN				PIROXICAM			
	doses B		doses C		doses B		doses C	
	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs	Control	NSAIDs
% of in vitro fertilized oocytes	73.5%[a] (196)	59.1%[a] (171)	66.1% (121)	62.4% (117)	80.9% (89)	73.8% (126)	83.0% (94)	85.5% (124)
% of pregnant mice	13.0% (23)	43.7% (16)	21.4% (14)	37.5% (16)	33.3% (18)	21.4% (14)	14.3% (14)	16.7% (12)
litter size	7.0 ± 1.5 (3)	7.9 ± 1.0 (7)	8.7 ± 0.3 (3)	7.8 ± 1.2 (6)	6.8 ± 1.6 (6)	3.3 ± 0.9 (3)	7.5 ± 2.5 (2)	8.5 ± 0.5 (2)

Ibuprofen (doses B: 1.12 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.) or piroxicam (doses B: 0.056 mg/100g/day or doses C: 0.084 mg/100g/day; i.p.) were injected to adult male mice for 60 days. Control animals were injected with the solvent (propylene glycol or dimethylsulfoxide respectively) in the same volume and period. In vitro fertilization indices, are expressed as percentages of fertilized oocytes that were obtained by ampullar puncture after induced superovulation. In vivo fertilization indices are expressed as percentages of untreated pregnant mice after housing for 4 days (one estral cycle) with treated males; 12 days after, females were sacrificed and the number of fetuses was determined. Results are expressed as percentages or Mean ± SEM. In parentheses: number of oocytes or female mice evaluated. **a**= p< 0.05.

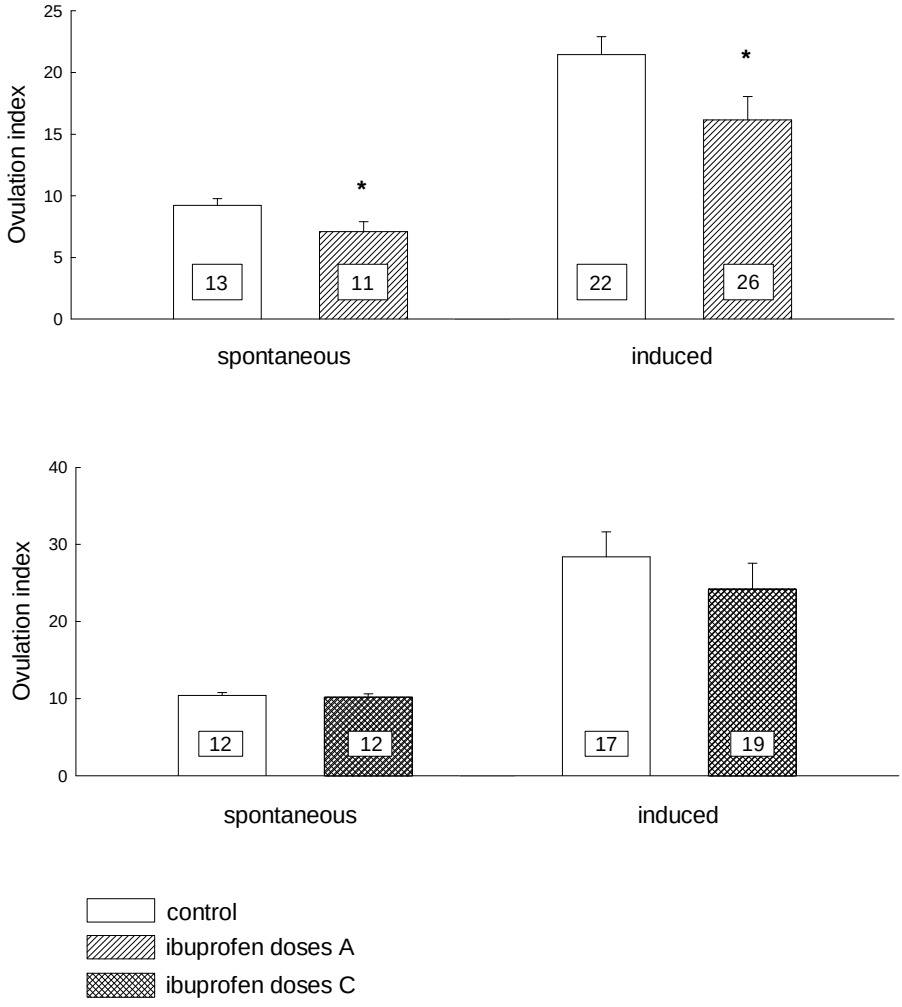

Figure 1: Ovulation indices (number of oocytes/female) of adult female mice injected for 35 days with ibuprofen (doses A: 0.56 mg/100g/day or doses C: 1.68 mg/100g/day; i.p.). Control animals were injected with the solvent (propylene glycol) in the same volume and period. The oocytes were obtained by ampullar puncture after induced superovulation (PMS – hCG; induced) or after oestrus determination (spontaneous). Results are expressed as Mean ± SEM. In the bottom of the bars: number of female mice evaluated. *= significant differences vs control (p< 0.05).

HEAD PAGE: Chronic NSAIDs treatment and mouse reproductive functions