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In Vivo Evaluation of the Efficacy and Safety of a Novel Degradable Polymeric Film for the Prevention of Intrauterine Adhesions

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► To cite this version:

Stéphanie Huberlant, Salome Leprince, Lucie Allegre, Sophie Warembourg, Isabelle Leteuff, et al.. In Vivo Evaluation of the Efficacy and Safety of a Novel Degradable Polymeric Film for the Prevention of Intrauterine Adhesions. *Journal of Minimally Invasive Gynecology*, 2021, 28 (7), pp.1384-1390. 10.1016/j.jmig.2020.10.025 . hal-03280567

HAL Id: hal-03280567

<https://hal.umontpellier.fr/hal-03280567>

Submitted on 2 Aug 2023

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1 **Title: In vivo evaluation of the efficacy and safety of a novel degradable polymeric**
2 **film for the prevention of intrauterine adhesions**

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21
22 **Conflict of interest statement**

23 The authors (S.L., X.G., S.H., V.L.) currently have a patent for the polymer and its
24 applications (WO2016020613). Two of the authors (S.H. and X.G.) have developed a
25 start-up company based on the results of the current study, and one of the authors (S.L.)

26 is currently employed in this new company. The rest of the authors do not have any
27 additional conflicts to disclose.

28 **Abstract**

29 *Objective:* To study the safety of DPF (degradable polymeric film) and its efficacy on
30 reducing the risk of intrauterine adhesions (IUA) formation in a rat model.

31 *Design:* A series of Case control studies relying on random allocation where feasible.

32 *Setting:* University and good practice animal laboratories.

33 *Animals:* Animal models comprised female and male OFA (Oncins France Strain A) and
34 female wistar rats.

35 *Intervention(s) and measurements:* OFA rats were used for in vivo evaluation of the
36 impact of DPF on endometrial thickness and in vivo evaluation of the effect on fertility.

37 For in vivo evaluation of the biological response, 40 wistar rats were randomly allocated
38 to intervention and control groups with matched sampling time after surgery. Finally, for
39 the in vivo evaluation of DPF efficacy on IUA prevention, a total of 24 wistar rats were
40 divided into 3 groups that were treated with DPF, hyaluronic acid (HA) gel and a sham
41 group.

42 *Main results:* DPF did not have a significant impact on endometrial thickness and no
43 significant differences in the number of conceived or prematurely terminated pregnancies
44 confirmed its non-inferiority to no treatment. DPF did not induce irritation at 5 and 28
45 days. Finally, DPF significantly reduced the likelihood of complete IUA formation
46 compared to HA gel and sham implanted animals, where only 27% of the animals had
47 their uterine cavity obliterated compared to 80% and 100% respectively.

48 *Conclusion:* DPF is a safe degradable polymeric film that is effective in preventing IUA
49 formation after intrauterine curettage in rats.

50

51 **Keywords:** Adhesions; animal model; Intrauterine; medical device; Synechiae

52 **Introduction**

53 Intrauterine adhesions (IUA) result from an endometrial trauma leading to a partial or
54 complete obliteration of the uterine cavity [1,2]. A Meta-analysis of 10 RCTs involving
55 912 women calculated an IUA prevalence following miscarriage of 19.1% (95% CI 12.8-
56 27.5%) [3]. IUA can also occur after intrauterine surgical interventions for the
57 management of uterine leiomyomas, septa and polyps. Diagnosis can be suspected when
58 pain or abnormal bleeding occur after surgical or obstetrical intrauterine trauma [4].
59 Although the iatrogenic trauma is established as one of the main precipitating factors, the
60 exact underlying pathophysiology of IUA formation is less well known. IUA can lead to
61 primary or secondary subfertility, early or late miscarriage and increase the risk for
62 several obstetric complications including abnormal placentation, postpartum
63 hysterectomy, prematurity and cesarean section [3,5–8]

64
65 Hysteroscopy remains the mainstay for IUA diagnosis and treatment, nevertheless, carry
66 the risk of IUA recurrence [9–11]. Several anti-adhesive strategies including hormonal
67 therapy, intrauterine devices, intrauterine balloons and barrier gels have been evaluated
68 with controversial results [12,13]. Although there is evidence that barrier gels can reduce
69 the incidence and severity of de novo IUA after operative hysteroscopy [13], there is
70 limited evidence relating to their effectiveness on improving pregnancy rates [14]. We
71 previously described the efficacy of a degradable polymeric film (DPF), in a rat model,
72 on post-operative abdominal adhesion [15,16]. The aim of the present study was to assess
73 the safety of DPF, its efficacy on reducing the risk of IUA formation and impact on
74 fertility in a rat model.

75

76 **Material and methods**

77 ***Polymer synthesis and shaping***

78 DPF was synthesized at the biopolymer laboratory of Montpellier, France, and previously
79 described by Leprince et al. [16]. For the evaluation of endometrial thickness and fertility
80 impact, DPF was shaped by hot extrusion with a Noztek Powder Filament Extruder
81 (Shoreham, England) to obtain a polymer filament with a diameter of 1.5 mm. For the
82 evaluation of biological response and the evaluation of IUA prevention, DPF was
83 synthesized at Polymer Expert (Pessac, France) and shaped by hot-pressure to obtain a
84 film (10 mg; length 1.3 +/-0.1 cm). The co-polymer was sterilized by irradiation between
85 27.7 and 36.6 kGy by Ionisos (Chaumesnil, France). DPF is now manufactured under the
86 brand name of “Womed Leaf”.

87

88 ***Animal preparation***

89 The evaluation of endometrial thickness and fertility were conducted at the Experimental
90 Department of University of Montpellier (Montpellier, France) on OFA (Oncins France
91 Strain A) female rats (7-8 weeks old) weighing between 250 and 275 grams. OFA male
92 rats weighing between 275 and 300 grams (purchased from Earl Cegav SSC, St Mars
93 d’Egrenne, France) were also required for the fertility experiment. While the evaluation
94 of biological response and the evaluation of IUA prevention were conducted at Phycher
95 Bio Development (Pessac, France), under good laboratory practice, on female wistar rats
96 (SPF Caw; 9-10 weeks old), supplied by Janvier Labs. The animal strain used depended
97 on availability at the time of conducting the experiment. All animal investigations were
98 approved by the Ethics Committee of the French Ministry of Education and Research
99 (contract number 02367.01, task order 1065 and EC N°.76) and carried out in accordance
100 with the EU Directive 2010/63/EU for animal experiments. All efforts were made to
101 minimize animal suffering or distress, and to use the minimum number of animals

102 necessary to produce reliable scientific data. All the animals were in quarantine for one
103 week prior to treatment. They were placed individually in an air-conditioned animal
104 holding facility (22°C +/- 3°C; 30%-70% humidity) with free access to food (SAFE®)
105 and water. They were examined, weighed and their litter changed daily, respecting the
106 guide of good practice and animal welfare.

107

108 OFA rats were anesthetized by intravenous perfusion of ketamine (50mg/kg) and
109 Acepromazine (0.5mg/kg). Anesthesia for wistar rats was induced and maintained by
110 inhalation of an O2-Isoflurane mixture (IsoFlo). Before surgery, OFA and wistar female
111 rats were weighed and injected with buprenorphine (Buprecare at 0.05 mg/kg) and
112 meloxicam (Meloxidolor at 0.5 mg/kg) subcutaneously. After anesthesia, animals were
113 shaved in the abdominal region, disinfected with polyviode iodine and wiped with 70%
114 isopropyl alcohol then a sterile drape placed at the operative site after. One ml
115 subcutaneous Lidocaine 0.1%. was injected prior to the cutaneous incision. An abdominal
116 vertical skin incision was performed followed by dissection with scissors of the
117 subcutaneous and muscle fascia tissues. The peritoneum was opened with scissors,
118 allowing access to the abdominal cavity to rule-out organic disease of the reproductive
119 system (uterus, oviducts, and ovaries). The uterus was exteriorized for the procedure. For
120 both types of animals, Buprenorphine (0.02mg/kg) was systemically administered twice a
121 day for 3 days. At the end of each protocol or in case of reaching the tolerance limit
122 point, animals were euthanized by lethal injection of sodium pentobarbital after
123 anesthesia by ketamine to limit the pain. No animals were reused for other experiments.

124

125 *In vivo evaluation of the impact of DPF on endometrial thickness*

126 The objective was to evaluate the impact of DPF on the rat's uterine horn endometrial
127 thickness as measured histologically. This study was performed on seven OFA female
128 rats. After animal preparation, the right horn's extremity was opened and DPF inserted
129 (Supplementary figure S1A). No DPF was placed in the left horn (control). The horns'
130 extremities were ligated to limit film expulsion (Supplementary figure S1B). Operative
131 and post-operative complications were recorded. Histological analysis was performed
132 after euthanasia and salpingectomy at Day 1 (2 rats), Day 5 (3 rats) and Day 12 (2 rats).
133 The last time point coincides with the expected full degradation of DPF in rats. Horns
134 were split longitudinally, fixed in formalin (formaldehyde 3.5% and phosphate buffered
135 saline) and embedded in paraffin wax. Samples were analyzed after Hematoxylin-Eosin-
136 Safran (HES) staining. The endometrial thickness was measured microscopically in three
137 sections, at the center of the horn and each of the extremities, using NDP view 2,
138 Hamamatsu® software, and the average endometrial thickness calculated for each horn
139 (Supplementary figure S2).

140

141 *In vivo evaluation of the impact of DPF on fertility*

142 Reproductive outcome was evaluated in a prospective randomized study on 20 OFA
143 female rats. Randomization was used to determine which horn to be implanted. The
144 selected horn was incised, DPF was inserted and the horn was sutured while the other
145 horn was used as control. The main endpoint was the number of pregnancies per horn.
146 The purpose of the test was to demonstrate the non-inferiority of the DPF treated horns
147 compared to controls. Female rats were monitored in individual cages for 15 days to
148 allow for abdominal healing and DPF degradation as demonstrated in our previous study
149 [16]. On Day 15, they were mated with 5 OFA males to mitigate potential bias caused by
150 variation in sperm parameters. Fifteen days after mating, manual palpation of the

151 abdomen and abdominal ultrasound were used to confirm pregnancies. Animals were
152 then euthanized and the number of fetuses counted in each horn.

153

154 ***In vivo evaluation of the biological response to DPF***

155 In total, 40 wistar female rats contributed to this experiment. The animals were randomly
156 allocated to one of six groups arranged in 3 pairs: Groups 1 and 2 (5 intervention and 5
157 controls sampled 24 hours after surgery); Groups 3 and 4 (5 intervention and 5 controls
158 sampled 5 days after surgery and Groups 5 and 6 (10 intervention with left horn
159 implantation and 10 controls with left horn incision only, sampled 28 days after surgery).
160 A 4 cm longitudinal midline abdominal incision from the urinary bladder was made. The
161 uterine horn was exteriorized from the abdominal cavity ligated close to the uterine
162 junction with the other uterine horn without cutting off blood circulation. An incision was
163 made and either 3mg DPF introduced (intervention) or a sham incision without DPF
164 implantation (control) depending on the rat group allocation. The incision was then
165 sutured and the uterine horn replaced in the abdominal cavity. Depending on the group
166 allocation, the same procedure was repeated on the second uterine horn if required.

167

168 Based on the *a priori* sampling time, the animal was euthanized and the uterine horns and
169 the draining inguinal lymph nodes were preserved in a formalin - acetic acid – alcohol
170 (AFA) medium, dehydrated then embedded in paraffin wax. Sections of about 4µm were
171 stained with haematoxylin-eosin (H&E) before assessment using the NF-ISO 1993-6
172 grading system (available upon request). The irritation score was calculated based on the
173 mean of the sum of tissue damage (necrosis) and cellular inflammatory parameter scores
174 weighted with a factor of 2 plus the repair phase of inflammation (fibrosis and

175 neovascularization) and fatty infiltrate parameter scores. The irritation index reflecting
176 the intensity of the inflammatory process and the local tissue effects was determined by
177 subtracting the irritation score of control from the score of DPF implanted animals within
178 each of the paired groups. An irritation index of 0 – 2.9, 3.0 – 8.9, 9.0 – 15.0 and >15
179 was considered non-, slightly, moderately, or severely irritant respectively. Distant organs
180 (e.g stomach, intestine, rectum, liver, spleen, inguinal nodes, heart, aorta, pancreas,
181 kidneys and vagina) were macroscopically and microscopically evaluated at 28 days.

182

183 ***In vivo evaluation of DPF efficacy on IUA prevention***

184 In total, there were 24 animals divided into 3 groups of 8 rats each: Group 1 (15 horns
185 abraded and treated with DPF + one control horn); Group 2 (15 horns abraded and treated
186 with hyaluronic acid (HA) gel [0.5 ml, Hyalobarrier, Nordic Pharma, France] + one
187 control horn; Group 3 – sham group - (15 horns abraded but not treated and one control
188 horn). The 3 control horns did not undergo the abrasion procedure and did not receive
189 any treatment and acted as histological references for a healthy horn. From the urinary
190 bladder level a 4 cm longitudinal midline abdominal incision was made. The uterine horn
191 was exteriorized from the abdominal cavity and incised over its entire length. Depending
192 on group allocation, the endometrial lining was abraded by scratching with a scalpel
193 blade to remove the epithelial layer + / - implanted [17]. The uterine horn was then
194 ligated close to the uterine junction with the other horn and 2-3 mm under the abraded
195 area without cutting off blood circulation, to keep the DPF and HA gel in contact with the
196 uterine horn. After 7 days, the animals were euthanized and the uterine horns preserved in
197 AFA, dehydrated then embedded in paraffin wax. Caudal, medial and cervical sections
198 were taken from each horn and stained with H&E. The presence of intrauterine adhesions

199 was evaluated for each uterine horn. The incidence of IUA was defined as the presence of
200 complete adhesion in at least one of the three sections of horn.

201

202 ***Statistical analysis***

203 Prior to the reproduction study, a power analysis was conducted with a significance level
204 of 5% based on the variance from a statistical two sample t-test resulted in a power of
205 80%. The mean endometrial thickness, calculated from 3 measurements per horn, were
206 compared in the intervention and control arms using the Mann-Whitney U test. The mean
207 number of pregnancies per horn were compared in the intervention and control arms
208 using a paired student's t-test. Fertility non-inferiority was based on the difference in the
209 average number of ongoing and prematurely terminated pregnancies in the DPF and
210 control groups. The *a priori* thresholds for non-inferiority were -1 and +0.5 for the total
211 number of ongoing pregnancies and the number of prematurely terminated pregnancies
212 respectively. The lower and upper bounds of the confidence intervals for the difference in
213 the average number of ongoing and terminated pregnancies respectively were compared
214 to these thresholds to assess non-inferiority. The efficacy of DPF for IUA prevention was
215 assessed using Fisher's exact test because the comparison of proportions of the existence
216 of adhesions was on a small sample of < 30.

217

218 **RESULTS**

219 ***In vivo evaluation of the impact of DPF on endometrial thickness***

220 The endometrial thickness was noted to be thinner on day one in the DPF compared to
221 the control group. However the difference in thickness at all the implantation time
222 periods (one, 5 and 12 days) was not significantly different between groups (Table 1).

223

224 **In vivo evaluation of the impact of DPF on fertility**

225 A total of 144 and 131 pregnancies were clinically observed in the DPF and control
226 groups respectively and this difference was not statistically significant. The average of
227 total pregnancies identified per horn was 7.2 (+/- 2.56) for the DPF group compared to
228 6.5 (+/- 2.81) in the controls, a difference of 0.65, $p=0.38$. While, there were 9
229 prematurely terminated pregnancies in the polymer group and 18 in the control group (p
230 = 0.09). The mean difference in ongoing pregnancy between DPF and control groups was
231 1.1 (95% CI [0.089; + inf]). Whereas the mean difference in prematurely terminated
232 pregnancies was - 0.45 (95%CI [- inf; -0.007]). These results support DPF non-inferiority
233 because the lower bound of the confidence interval for the ongoing pregnancy mean
234 difference in ongoing pregnancy was greater than, and the upper bound of the confidence
235 interval for the mean difference in prematurely terminated pregnancies was lower than
236 the pre-set non inferiority threshold of -1 and + 0.5 respectively.

237

238 ***In vivo evaluation of the biological response to DPF***

239 One animal in the control group was found dead on day 7 and missing small intestines
240 and caecum were the only macroscopic findings recorded at necropsy. Apart from this,
241 we did not observe any unexpected clinical signs in either the DPF or control groups.
242 Macroscopic and microscopic examination of the animals at 28 days did not reveal
243 changes in the main organs of the treated or control groups. There were no macroscopic
244 anomalies seen in the inguinal lymph nodes draining implantation sites. Figure 1
245 illustrates an example of the histopathological examinations of uterine horns at 24 hours,
246 5 days and 28 days after implantation in both the DPF and control groups. Examinations
247 showed that 24 hours after implantation, DPF induced a minimal to mild inflammatory
248 process within the endometrium in 4 out of 5 animals. This was characterized by an

249 infiltration of polymorphonuclear cells and/or macrophages and was associated with
250 flattening of the epithelial cells, focal or localized extensive erosion and a luminal
251 exudate in the DPF group when compared to control uterine horns. At five days, the
252 induced inflammatory process was still observed within the endometrium but at a lower
253 incidence (3/5 animals) and severity when compared to the 24-hour period. At 28 days,
254 the macroscopic examination of the implantation sites showed that the segment of uterine
255 horns between the ligature made during the implantation procedure and the ovaries were
256 hypertrophied and fluid filled in 8/10 and 8/9 females from the DPF and control groups
257 respectively. These changes were bilateral in 7 of the 8 affected animals in each group.
258 Histopathological examinations showed that luminal dilatation, recorded in 5/10 and 8/9
259 females of the DPF and control group, respectively, was the histological correlate of the
260 hypertrophied uterine horns observed at necropsy. Hence, these findings were deemed
261 secondary to the implantation procedure. No mortality attributable to DPF occurred
262 during the study. Table 2 displays the biological response of uterine tissues in DPF-
263 treated and control groups. The DPF was classified as slightly irritant (irritation index
264 3.6) at the end of the 24-hour period and non-irritant at the end of the 5-day period
265 (irritation index 1.6) and 28-day period (irritation index 0) (raw data available on request).

266

267 *In vivo evaluation of DPF efficacy on IUA prevention*

268 In the sham group, 100% (15/15 horns) had at least one complete IUA (Figure 2A)
269 among the 3 regions of the uterine horn. When comparing the DPF and HA treated
270 groups, 26.7% (4/15) and 80% (12/15) horns had at least one complete adhesion
271 respectively (p=0.009; 66% reduction) (Figure 2).

272

273 **Discussion**

274 ***Summary of findings***

275 Our study showed that, although the endometrium was thinner on day one in the DPF
276 implanted animals, it did not have a significant impact on endometrial thickness at any of
277 the tested time periods or on pregnancy related outcomes compared to the control group.
278 We also demonstrated that DPF was biologically well tolerated with no evidence that it
279 induces any signs of tissue toxicity. Although DPF was classified as slightly irritant 24
280 hours after insertion, this irritation seemed to be short-lived because it did not persist at
281 later assessments. Finally, DPF significantly reduced the likelihood of complete IUA
282 formation compared to HA gel and sham-implanted animals, where 27% only of the
283 animals had at least one complete adhesion compared to 80% and 100% respectively. The
284 presence of IUA in the DPF groups could be due to faster polymer degradation in some
285 rats. Some areas of horns not covered by the DPF due to fragmentation may therefore be
286 more susceptible to adhesion formation. In the case of HA gel, and due to the difference
287 in galenic form (gel vs film), the areas susceptible to adhesion formation would be more
288 numerous, which could explain the higher rate of adhesions in the HA gel group., This
289 observation supports the efficacy of DPF in preventing IUA formation after intrauterine
290 procedures and concurs with previous reports demonstrating its efficacy in preventing
291 intra-peritoneal adhesions [15,16].

292

293 ***Findings in light of what is known***

294 IUA are an important cause of infertility, implantation failure, miscarriage and obstetric
295 complications [3]. The exact mechanism of their formation is unknown and several
296 management modalities have been proposed for their prevention and treatment [14].
297 Although there are several studies evaluating the impact of IUA on different clinical
298 outcomes, there is paucity of information in relation to the efficacy of such interventions

299 on improving fertility [13]. Indeed, although guidelines acknowledge that the use of solid
300 (IUD, balloon) or semi solid (gel) barrier methods reduce postoperative IUA reformation,
301 the use of such barriers is not explicitly recommended, probably due to the lack of
302 fertility data to justify their recommendation [18]. Furthermore, available barrier agents
303 for the prevention of intraperitoneal adhesions cannot be used in the uterine cavity via the
304 vaginal route [13,19]. It is also important to recognize that adhesions can form anywhere
305 within the uterine cavity, hence, it is imperative that a device intended to reduce such
306 adhesions should cover the entire uterine cavity. Indeed, in this study we were able to
307 confirm DPF's efficacy in preventing IUA at multiple uterine sections within the same
308 horn. Furthermore, our study is quite unique in that it also addressed the local and distant
309 tissue response to the polymer film, which is essential information for the development
310 and licensing of such a medical device. Therefore, we believe that the findings of our
311 study demonstrating the efficacy of DPF in preventing IUA, its non-inferiority in relation
312 to fertility outcomes and its minimal biological effect on the endometrium have a
313 significant clinical potential.

314

315 *Strengths, limitations and future implications*

316 We appreciate that our study has some limitations. First, our findings are based on an
317 animal study. Nonetheless, rats are a good model for the evaluation of IUAs and are
318 largely used in similar experimental studies because of the relative ease in managing their
319 reproduction. Furthermore, their bicornuate uterus enables the animal to act as its own
320 control [20]. Therefore, our choice of this model was built on currently available data of
321 similar study designs because demonstrating DPF efficacy in a model with high tendency
322 for IUA formation will likely be effective in a lower risk situation such as post
323 miscarriage curettage [17,20]. Second, in addition to assessing the macroscopic and

324 microscopic effects of DPF on the endometrium and draining lymph nodes, the
325 evaluation of inflammatory markers would have complemented our results [21].
326 However, the ability to test the impact of DPF on endometrial thickness, fertility,
327 biological responses on surrounding tissues and IUA prevention in one study is a major
328 strength to our work. The natural progression now is to validate our results in humans to
329 assess the efficacy of DPF on IUA prevention in women. Some adjustments were
330 performed to adapt the polymeric film to the shape and dimensions of the human uterus
331 and to facilitate its trans-cervical insertion into the uterine cavity while mitigating the risk
332 of any concomitant tissue trauma. This is currently under clinical investigation after
333 hysteroscopic myomectomy to assess DPF efficacy as a preventive barrier against IUA
334 (NCT04381728).

335

336 **Conclusions**

337 In conclusion, DPF has optimal degradation properties, no long-term tissue irritation and
338 no negative impact on fertility outcomes in rats. Moreover, DPF significantly reduced the
339 risk of IUA formation in the tested animals compared to HA gel or no treatment.
340 Therefore, DPF seems to be a potentially promising innovation for the prevention of
341 intrauterine synechiae and their recurrence.

342

343 **Acknowledgements**

344 The authors are grateful to Hubert Taillades, technical manager of at the Experimental
345 Department of University of Montpellier (Montpellier, France) for his expertise in animal
346 trials. The authors would like to thank superviseme ltd - medical writing services
347 (<http://www.superviseme.eu>) for the help with the medical writing and editing of the
348 manuscript.

349

350 **Funding**

351 The reproductive study was conducted with financial support of “Fond de santé de la
352 femme” of the French college of gynecology and obstetrics (CNGOF), and supported by
353 Gedeon Richter France. Salome Leprince’s PhD was supported by SATT AxLR, a
354 technology transfer acceleration company (program “ANTISYN) and the “Languedoc
355 Roussillon Region” (program “Chercheur d’Avenir” attributed to X. Garric). Stephanie
356 Huberlant’s PhD was supported by the University Hospital of Nîmes. This research was
357 supported by the University of Montpellier (BUSR Grant), CHU Caremeau of Nîmes
358 (AOI Grant) and the technology transfer acceleration company SATT AxLR (Program
359 ANTISYN). The in vivo evaluation of biological response and the evaluation of efficacy
360 were performed at Phycher bio développement, a company owned by the Icare group and
361 was funded by Womed SAS.

362

363

364 **Authors’ contribution:**

365 SH: Planning of the project, protocol testing and its implementation, animal surgical
366 preparation and testing, analysis of the data, writing and editing of the article.

367 SL: Planning of the project, test protocols writing, implementation and supervising
368 analysis of the data, writing and editing of the article.

369 LA: Animal surgical preparation and testing

370 SW: Animal surgical preparation and testing , participated in the drafting of the
371 introduction

372 IL: Animal surgical preparation and testing

373 HT: animal surgical preparation and testing

374 XG: planning of the project, analysis of the data, writing and editing of the article

375 RdT: planning of the project, participated of the drafting of the article

376 VL: planning of the project, participated of the drafting of the article

377

378

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453

454 **Captions:**

455 **Tables**

- 456 ● Table 1: Endometrial thickness measurements (μm) in the DPF group and control
457 group, at Day 1, Day 5 and Day 12.
- 458 ● Table 2: Irritation scores of uterine tissues in DPF and control groups at each
459 interval time (24 hours, 5 days and 28 days) and calculated irritation index of
460 DPF.

461

462 **Figures**

- 463 ● Figure 1: Histological examination (HES staining) of the uterine horns: (A,B,C)
464 DPF group at 1 day, 5 days and 28 days respectively (D,E,F) sham group at 1 day,
465 5 days and 28 days respectively.
- 466 ● Figure 2: Prevention of intrauterine adhesion. (A-D) Histology: HES staining of
467 uterine horns sections at 7 days (A) Sham group: the horn is fully closed by
468 complete IUA (B) HA group: the horn is fully closed by complete IUA (C) DPF
469 group: the lumen is visible, no IUA (D) Control group: the lumen is visible, no
470 IUA. (E) Number of uterine horns in percent (%) presenting at least one complete
471 IUA at day 7 following uterine curettage in the sham group, HA group and DPF
472 group.

473

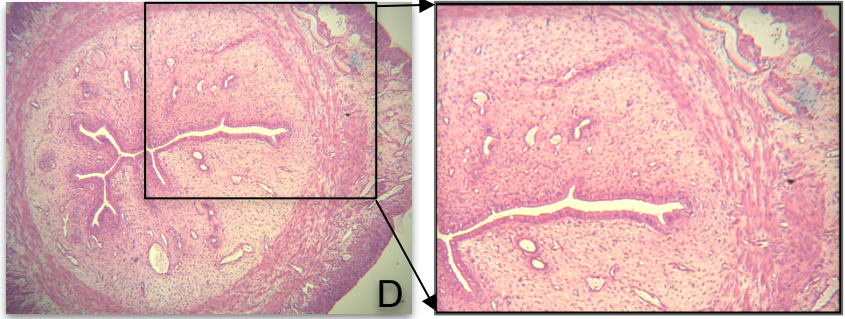
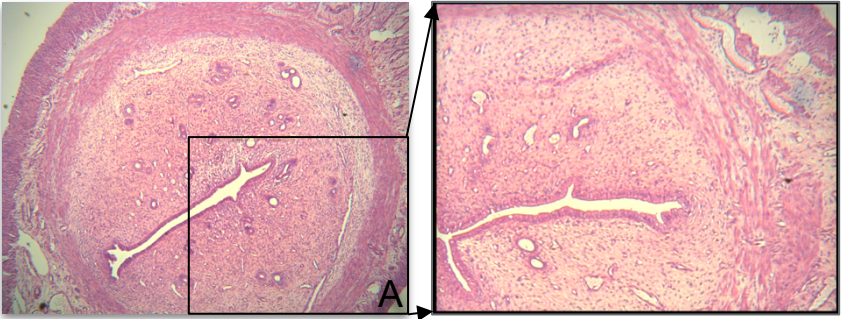
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Fig 1

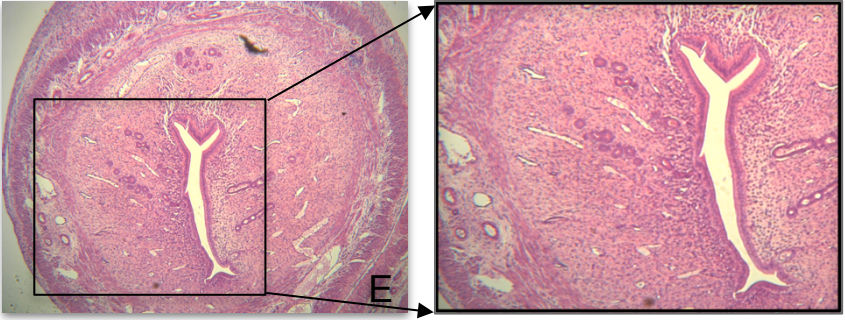
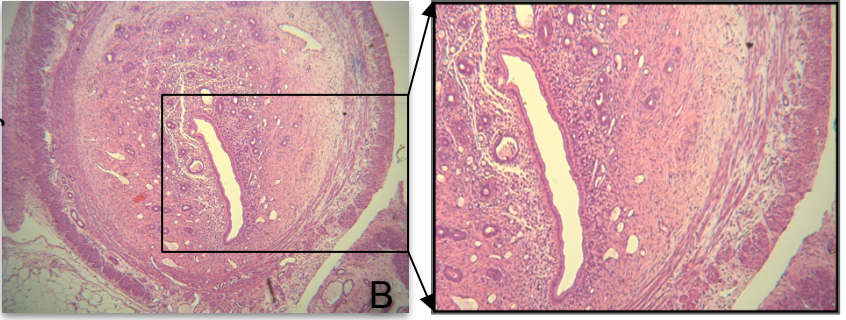
DPF group

Sham group

24 hours



5 days



28 days

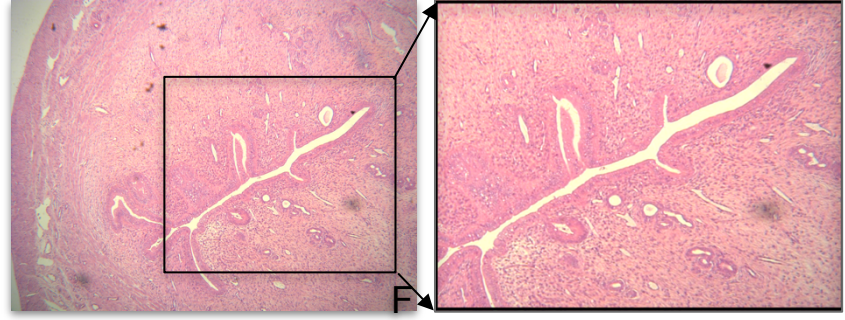
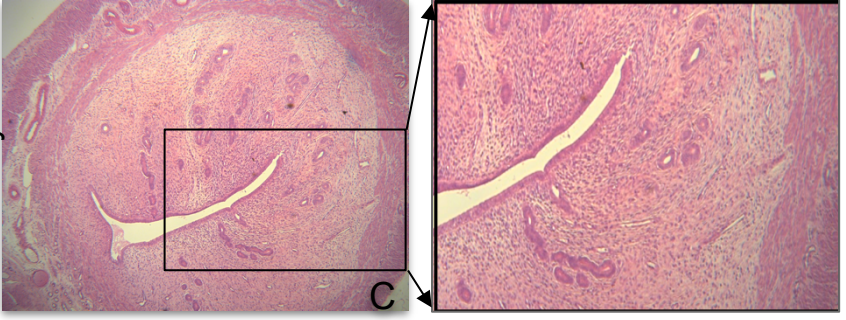
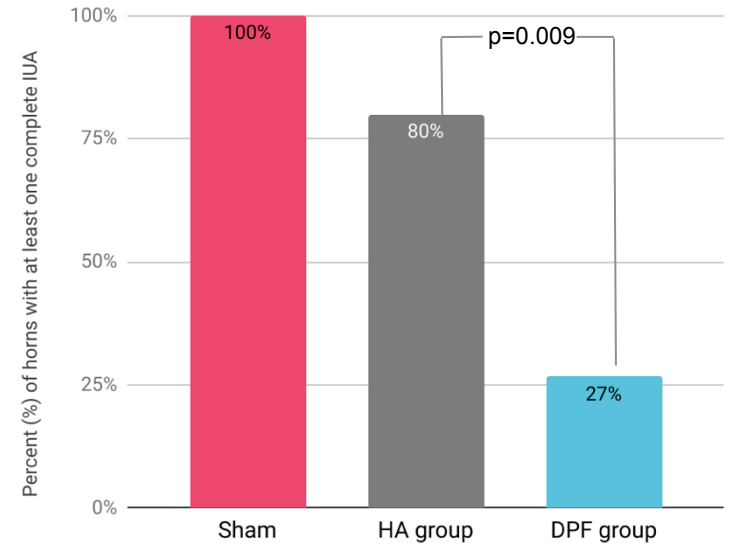
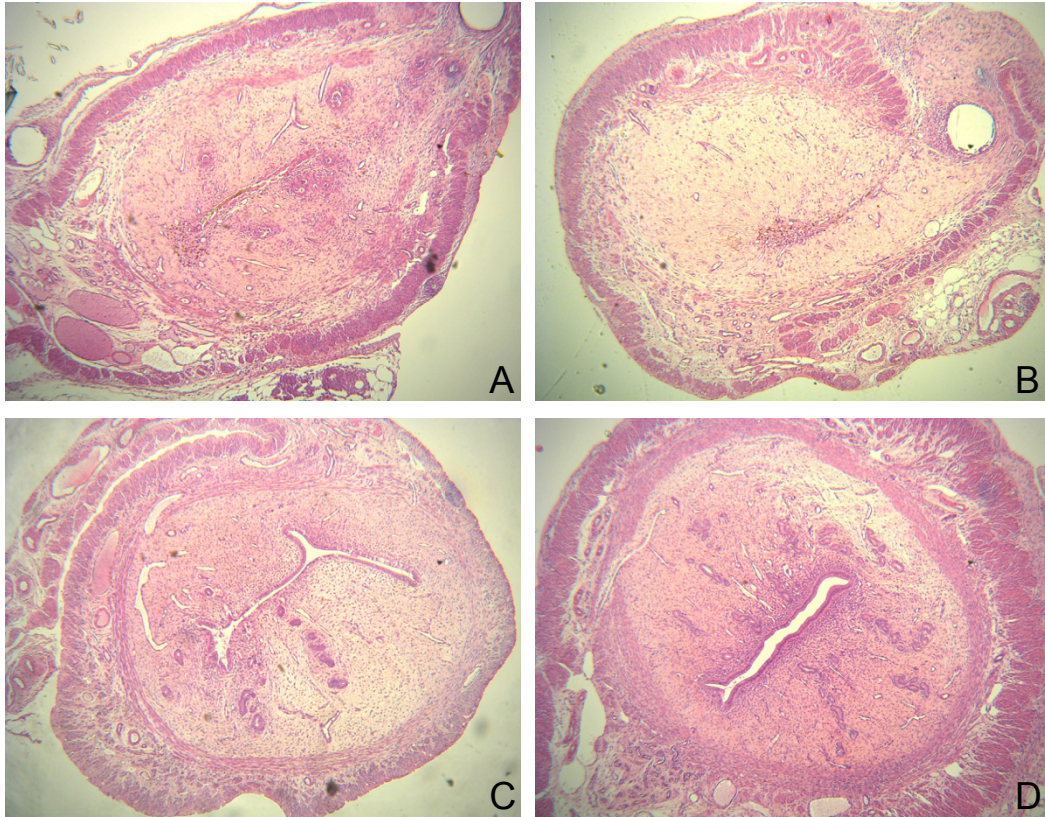


Fig 2



	DPF group (n=7)	Control group (n=7)	p
Day 1	337.3 +/-118.9	693 +/- 371.8	0.09
Day 5	897+/-441	927.8 +/- 226	1
Day 12	647.3+/- 147.9	777 +/- 406.5	0.2

Table 1: Endometrial thickness measurements (μm) in the DPF group and control group, at Day 1, Day 5 and Day 12

Table 2: Mean irritation scores of uterine tissues in DPF and control groups at each interval time (24 hours, 5 days and 28 days) and calculated irritation index of DPF.

Time-period	Group	Mean Irritation Score	DPF Irritation Index
24-hour	Treated with DPF	3.6 +/- 2.95	3.6 (Slightly irritant)
	Sham group	0.0 +/- 0.0	
5-day	Treated with DPF	1.6 +/-1.57	1.6 (Non irritant)
	Sham group	0.0 +/- 0.0	
28-day	Treated with DPF	0.0 +/- 0.0	0.0 (Non irritant)
	Sham group	0.0 +/- 0.0	