PRELIMINARY NOTE

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Prostaglandin E2 induces ovulation in prepubertal mice

Prostaglandina E2 induz ovulação em camundongos pré-púberes

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ABSTRACT

The objective of this study was to determine the ability of prostaglandin E_2 (PGE_2) to induce ovulation and expression of PGE_2 receptor (EP2 and EP4) and COX genes (COX-1 and COX-2) in the ovary and pituitary of prepubertal mice. The positive control consisted of the application of 5 µg of gonadotropin-releasing hormone (GnRH, n = 29); the negative control applied 0.5 mL of phosphate buffered saline (PBS, n=31); the treatment tested the application of 250 µg of PGE2 (n = 29), making a total of 89 prepubertal mice (BALB/c). Mice were euthanized 14 to 15 h after treatments to detect ovulation and tissue collection. A Chi-square test was used to compare the proportion of animals ovulating. Gene expressions and number of ovulation were analyzed by one-way ANOVA and Tukey's test was used to compare means among groups. A greater proportion of mice ($P < 0.001$) ovulated after receiving GnRH (89.7%, 26/29) compared to PGE_2 group (58.6%, 17/29). However, the proportion was higher compared to those treated with PBS (0%, 0/31). *Ep2* gene expression in the pituitary was > two-fold higher ($P < 0.05$) in the PGE_2 group compared to the PBS and GnRH groups. Further, PGE_2 stimulated *Cox1* (2.7 fold, $P < 0.05$) while GnRH stimulated *Cox2* expression (6.5 fold, $P < 0.05$) in the pituitary when compared to the PBS group. In conclusion, our results support the hypothesis that PGE_2 can induce ovulation in prepubertal mice with a concomitant increase in *Ep2* and *Cox1* gene expression in the pituitary gland.

Keywords: Ovulation. Prostaglandin. Receptor. Puberty.

RESUMO

O objetivo deste estudo foi determinar a capacidade da prostaglandina E_2 (PGE_2) em induzir a ovulação e expressão do receptor PGE_2 (EP2 e EP4) e genes COX (COX-1 e COX-2) no ovário e na hipófise de camundongos pré-púberes. O controle positivo consistiu na aplicação de 5 µg de hormônio liberador de gonadotrofina (GnRH, n = 29); o controle negativo aplicação 0,5 mL de tampão fosfato-salino (PBS, n=31); o tratamento testado aplicação de 250 µg de PGE2 (n = 29), perfazendo um total de 89 camundongos (BALB/c) pré-púberes. Os camundongos foram sacrificados 14 a 15 h após os tratamentos para detectar ovulações e coleta de tecido. O teste do qui-quadrado foi usado para comparar a proporção de animais ovulando. As expressões gênicas e o número de ovulação foram analisados por ANOVA e o teste de tukey foi usado para comparar as médias entre os grupos. Uma maior proporção de camundongos ($P <0,001$) ovulou após receber GnRH (89,7%, 26/29) em comparação com o grupo PGE_2 (58,6%, 17/29). No entanto, a proporção foi maior em comparação com aqueles tratados com PBS (0%, 0/31). A expressão do gene *Ep2* na hipófise foi duas vezes maior ($P <0,05$) no grupo PGE_2 em comparação com os grupos PBS e GnRH. Além disso, a PGE_2 estimulou a *Cox1* (2,7 vezes, $P <0,05$) enquanto o GnRH estimulou a expressão de *Cox2* (6,5 vezes, $P <0,05$) na pituitária em comparação com o grupo PBS. Em conclusão, nossos resultados suportam a hipótese de que PGE_2 é capaz de induzir ovulação em camundongos pré-púberes com aumento concomitante na expressão dos genes *Ep2* e *Cox1* na glândula pituitária.

Palavras-chave: Ovulação. Prostaglandina. Receptor. Puberdade.

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The identification and study of new molecules to induce ovulation could help introduce important alternatives to increase the use of reproductive biotechniques and, consequently, enhance the productivity in domestic mammals of economic interest. In that regard, before testing alternatives directly in such animals with expensive experiments, it is necessary to study the effect of ovulation inducer in laboratory species. In this context, here we evaluated the effect of PGE_2 on the ovulation of mice. Although the effect of endogenous secretion of PGE_2 in the ovary is well documented (Ben-Ami et al., 2006; Clasadonte et al., 2011; Espey, 1978; Li et al., 2006; Martínez-Boví & Cuervo-Arango, 2016; Murdoch et al., 1993; Niringiyumukiza et al., 2018; Ojeda & Negro-Vilar, 1985; Saksena et al., 1974; Segi et al., 2003; Sirois & Richards, 1992; Sirois et al., 1992), limited information is available regarding the effect of injectable PGE_2 on ovulation in juvenile females. Therefore, the primary objective of this study was to evaluate the effect of PGE_2 administration on ovulation in prepubertal mice. The study of the expression of COX and prostaglandin EP receptor genes in the pituitary and ovary may provide an important step towards the understanding of its mechanism of action and the events that control reproduction. We tested the hypothesis that the administration of PGE_2 will induce ovulation and alter the expression pattern of COX-1, COX-2, EP2, and EP4 genes in the pituitary and the ovary in prepubertal mice.

The Committee for Ethics in Animal Experimentation from the Oswaldo Cruz Foundation in Rondônia (Fiocruz-Rondônia) approved all procedures performed in the experiment described in this manuscript (Protocol 2014/12). Prepubertal female BALB/c (n = 89), between 18 and 22 days of age and weighing 20-25g, had access to food and water *ad libitum*.

To induce follicle growth, prepubertal mice were treated with an intraperitoneal (i.p.) dose of 0.2 UI eCG per g body weight (equine chorionic gonadotrophin (Novormon®, Zoetis-Pfizer, Brazil); total dose of 5 IU per mice) at the end of the light cycle. After 48 to 50 h, mice were randomly assigned to one of the 3 experimental groups, and given a single i.p dose of 1) 20 µL PBS per g body weight (Dulbecco-modified phosphate-buffered saline; Biodux®, Brazil; total dose of 0.5 mL per mice; n = 31, placebo control, PBS group); 2) 0.2 µg GnRH per g body weight (gonadotrophin-releasing hormone, Gonaxal®, Biogenesis-Bagó, Argentina; total dose of 5 µg per mice, n = 29; positive control, GnRH group), or 3) 10 µg PGE_2 per g body weight (Sigma-Aldrich, USA; total dose of 250 µg per animal, n = 29; PGE group). Mice were euthanized by cervical dislocation 14 to 15 h after the administration of treatments according to Bogle et al. (2011). The oviducts, ovaries, and pituitary were collected. The ovaries and pituitary were then placed in a 1.5 mL cryotube filled with 1 mL of RNALater (Sigma-Aldrich, USA). The cumulus-oocyte complexes (COCs) were detected in the oviduct ampullae under a stereomicroscope.

Total RNA was extracted from the pituitary and ovaries of a subset of mice (n = 4 each group) selected at random before euthanasia. After complete removal of the RNALater from the samples, the tissues were macerated mechanically under liquid nitrogen and 600 µL of 1% β-mercaptoethanol (Sigma-Aldrich, USA) was added before total RNA was extracted according to the PureLink® RNA Mini kit (Carlsbad, CA, USA) protocol. cDNA synthesis and quantitative real-time-PCR (qRT-PCR) were performed according to Pfeifer et al. (2018). Primers used to evaluate the qRT- PCR is described in Table 1.

Statistical analyses were performed using SAS 9.0 statistical program (Statistical Analysis System Institute, Inc., Cary, NC, USA, 2004). The proportion of female mice that ovulated was analyzed using the Chi-square test. The mean number of COC collected per animal and the gene expression was analyzed by one-way ANOVA and Tukey's test was used to compare the means among groups. A P-value equal to or less than 0.05 was considered statistically significant.

No mice ovulated in the control group, while a greater proportion of mice ovulated in the PGE_2 group ($P < 0.001$; Table 2). Furthermore, the number of ovulations per mouse was higher in PGE_2 treated mice than the PBS treated ones ($P < 0.001$). The results of this study supported the primary hypothesis that treatment with PGE_2 induces ovulation in prepubertal mice. Although previous studies have demonstrated the involvement of PGE_2 in the ovulatory events of mammals (Li et al., 2006; Martínez-Boví & Cuervo-

Table 1 – Primer designed and used in the quantitative real-time polymerase chain reaction (qRT-PCR) amplification of mRNA

Gene	Primer direction	Primer sequence (5′ - 3′)
Gapdh	forward	ACACTGAGGACCAGGTTG
	reverse	TGGTCGTTGAGGGCAATG
βactin	forward	AGGCATCCTGACCCTCAAGTA
	reverse	GCTCGTTGTAGAAGGTGTGGT
Cox1	forward	TGCATGTGGCTGTGGATGTCATCAA
	reverse	CACTAAGACAGACCCGTCATCTCCA
Cox2	forward	GAGTGGGGTGATGAGCAACTATTCC
	reverse	CTGTAGGGTTAATGTCATCTAGTCT
Ep2	forward	GCTCCTTGCCTTTCACAATCTT
	reverse	CAGGACCGGTGGCCTAAGTA
Ep4	forward	GCACTGCGTGGGAAGAGACT
	reverse	ATGGTACCTGTAGGGTGGGG

Table 2 – Proportion of prepubertal mice that ovulated and mean ± S.E.M. of the number of ovulations per mice treated intraperitoneally with 0.5 ml PBS, 5 µg GnRH and 250 µg PGE_2 (Porto Velho, 2021)

Ovarian response	Treatments			
	0.5 mL PBS	5 µg GnRH	250 µg PGE_2	P-Value
Proportion of mice that ovulated (%)	0/31 (0.0%)^{A}	26/29 (89.7%)^{B}	17/29 (58.6%)^{C}	<0.001
Number of ovulations per ovulating mice	0.0 ± 0.0^{A}	7.1 ± 0.9^{B}	3.3 ± 0.7^{C}	<0.001

PBS, phosphate-buffered saline; GnRH, gonadotropin-releasing hormone; PGE_2, prostaglandin E_2. ^{ABC} Within rows, values with no common superscript are different ($P < 0.05$).

Arango, 2016; Murdoch et al., 1993; Saksena et al., 1974; Tsafriri et al., 1972), to the best of our knowledge, this is the first report to determine that PGE_2 per se can induce ovulation in prepubertal mice. A greater proportion of mice ovulated after treatment with GnRH compared to those treated with PBS or PGE_2 ($P < 0.001$). The number of ovulations per mouse was also higher in the GnRH group compared to PGE_2 and PBS groups ($P < 0.001$; Table 2). The relative abundance of *Cox1*, *Cox2*, *Ep2,* and *Ep4* mRNA is depicted in Figure 1. There was no difference ($P > 0.05$) in ct values of βactin and *Gapdh* among groups for the ovarian tissue and pituitary gland.

The relative expression of *Cox1* in the ovary was higher ($P < 0.001$) for GnRH treated mice compared to PBS and PGE_2. *Cox2* expression was higher ($P < 0.05$) in mice treated with GnRH compared to PBS, while PGE_2 group expression was intermediate. The GnRH and PGE_2 treatments did not affect the expression of *Ep2* and *Ep4* in the ovary ($P > 0.05$). Prostaglandin E_2 treatment did not alter *Ep2*, *Ep4*, *Cox1,* or *Cox2* mRNA levels in the ovaries. However, whether the PGE_2-induced ovulations result from the local ovarian effect, direct effect on the pituitary, through modulation of GnRH at the hypothalamic levels, or some combination of these mechanisms remains to be explored further.

Although the main objective of this study was to evaluate the effect of PGE_2 on ovulation in prepubertal mice, we also attempted to elucidate the pattern of mRNA transcripts for PGE receptors and COX enzymes involved in cell receptivity and synthesis, respectively, in tissues where prostaglandins may act, such as the ovary (Espey, 1978; Evans et al., 1983; Fortune et al., 2009; Martínez-Boví & Cuervo-Arango, 2016) and the pituitary (Clasadonte et al., 2011; Myren et al., 2012; Naor et al., 2007; Randel et al., 1996). In the pituitary gland, the *Cox1* and *Ep2* expression was 2.7 (range: 1.5 to 3.9) and 2.0-fold (range: 1.5 to 2.7) higher ($P < 0.05$; respectively) for mice treated with PGE_2 compared to PBS. In addition, mice treated with PGE_2 had a higher relative expression of *Ep2* than the GnRH group ($P < 0.05$). The relative expression of *Cox2* was higher ($P < 0.05$) in mice treated with GnRH compared to PBS, while the PGE_2 group was not different from either GnRH or PBS. The GnRH and PGE_2 treatments did not change the expression of *Ep4* receptors in the pituitary ($P > 0.05$).

In the present study, PGE_2 induced ovulation in 58% of the prepubertal eCG-stimulated mice within 14 to 15 h compared to almost 90% mice after GnRH treatment. Furthermore, the number of ovulations per mouse was less than half in comparison to GnRH (3.3 versus 7.1). The higher relative amounts of mRNA transcripts for *Ep2* and *Cox1* detected in the pituitary of PGE_2 treated mice corroborates this statement. Compared to the control and GnRH treatment, PGE_2 treatment upregulated the *Ep2* gene expression in

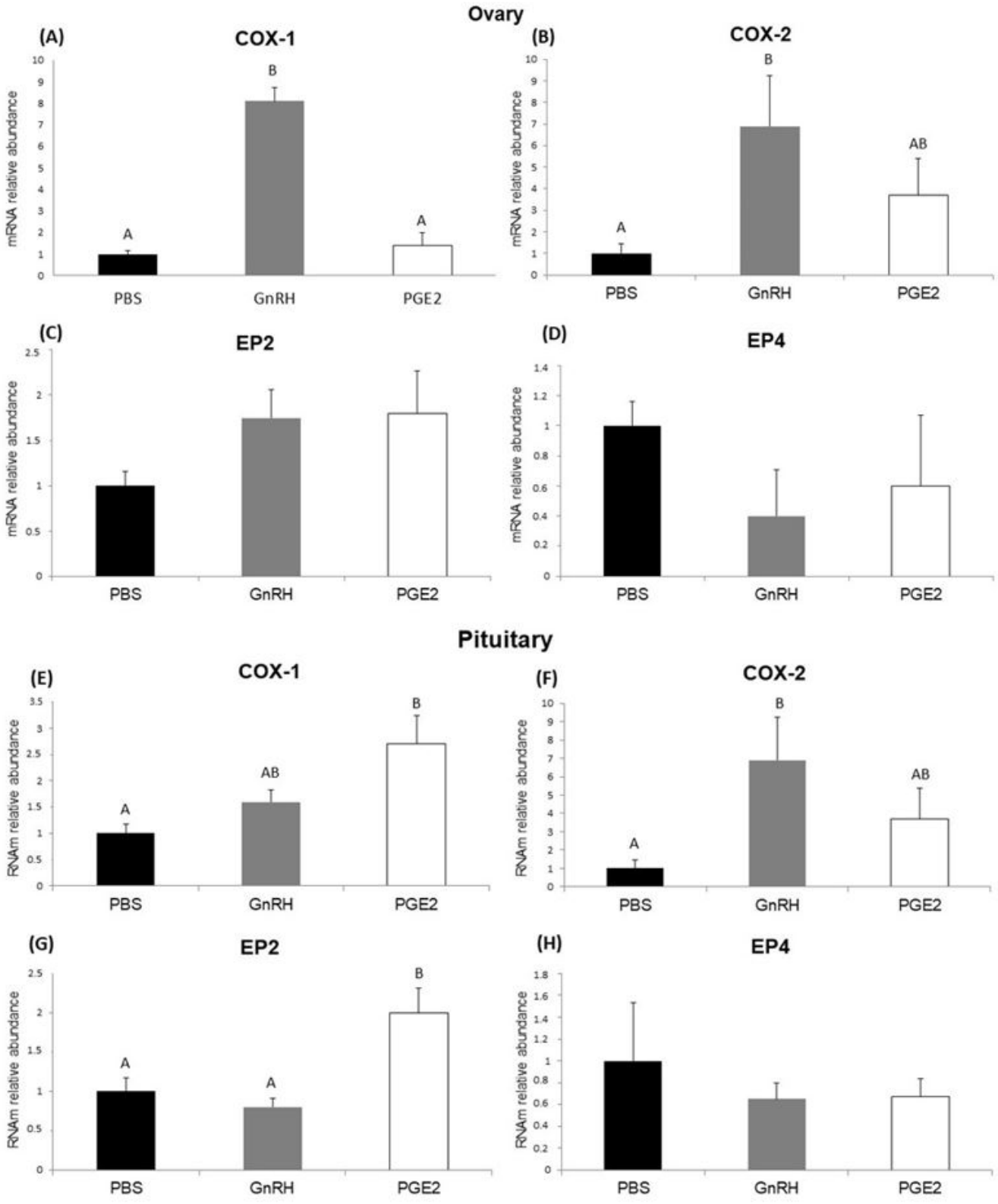

Figure 1 – Relative mRNA transcript abundance in the ovary (A-D) and the pituitary (E-H) in prepubertal female mice (n = 4 per group) induced to ovulate with PBS, GnRH, or PGE2. Different letters indicate differences among groups ($P < 0.05$). Relative abundance was normalized based on the βactin and Gapdh expression.

the pituitary. PGE_2 differentially stimulated the *Cox1* while GnRH enhanced *Cox2* expression in the pituitary.

In conclusion, our results support the hypothesis that PGE_2 can induce ovulation in prepubertal mice with a concomitant increase in *Ep2* and *Cox1* gene expression in the pituitary gland at 14-15 h after treatment. Further studies are needed to elucidate the local versus the central role of prostaglandins in follicle rupture and to verify the proposed mechanisms of action.

Conflict of Interest

The authors declare no conflicts of interest.

Ethics Statement

The Committee for Ethics in Animal Experimentation from the Oswaldo Cruz Foundation in Rondônia (Fiocruz-Rondônia) approved all procedures performed in this experiment (Protocol 2014/12).

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