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ROLE OF PROSTAGLANDINS IN FOLLICULAR RESPONSES TO LUTEINIZING HORMONE

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SUMMARY

Levels of prostaglandins of the F series have been found to increase several-fold during the pre-ovulatory period in follicles of rabbits, rats, and swine, reaching maximal levels shortly before ovulation. In rabbits and rats, the increase can be induced by exogenous luteinizing hormone (LH). The increase in rats is preceded by increases in ovarian levels of cyclic AMP and progesterone. Systemic administration of indomethacin prevented the increase of ovarian prostaglandin F and blocked ovulation in rabbits and rats. Intrafollicular injection of indomethacin, in rabbits, at dosages which were ineffective when given via systemic injection, blocked the elevation of follicular prostaglandins and ovulation. Prostaglandin $F_{2\alpha}$ antiserum also blocked LH-induced ovulation when injected intrafollicularly. Intrafollicular injection of prostaglandin $F_{2\alpha}$, in rabbits in the absence of LH resulted in release of ova from 40 p. 100 of injected follicles, and in resumption of meiotic division of oöcytes in 90 p. 100 of injected follicles. Prostaglandin E_2 was much less effective. These results provide evidence in support of a physiologic role of follicular prostaglandin $F_{2\alpha}$ in the process of ovulation and oöcyte maturation in response to LH.

INTRODUCTION

Prostaglandins have been implicated, on the basis of *in vitro* studies, as playing an intermediate role in the mechanism by which luteinizing hormone (LH) activates ovarian adenylate cyclase, thereby stimulating steroid biosynthesis (KUEHL *et al.*, 1970). Subsequently, a considerable body of evidence has been accumulated, suggesting roles of prostaglandins in three additional ovarian responses to LH, *viz.* ovulation (ARMSTRONG and GRINWICH, 1972 ; ORCZYK and BEHRMAN, 1972 ; GRINWICH, KENNEDY and ARMSTRONG, 1972), oocyte maturation (TSAFRIRI *et al.*, 1972 ; ARMS-

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TRONG, MOON and ZAMECNIK, 1974), and luteinization (KOLENA and CHANNING, 1972 ; ELLSWORTH and ARMSTRONG, 1974). This paper reviews recent *in vivo* evidence bearing on the possible involvement of prostaglandins in each of these follicular responses to LH.

EXPERIMENTAL

I. — *Pre-ovulatory changes of ovarian prostaglandin F levels Correlation with other ovarian responses to LH*

A) *Rats.*

Ovarian prostaglandin F (PGF) levels have been measured by radioimmunoassay, at various times in relation to ovulation in prepubertal rats in which first estrus has been induced prematurely by injection of pregnant mare serum gonadotropin (PMS) (ARMSTRONG and ZAMECNIK, 1975). As summarized in figure 1, ovarian PGF

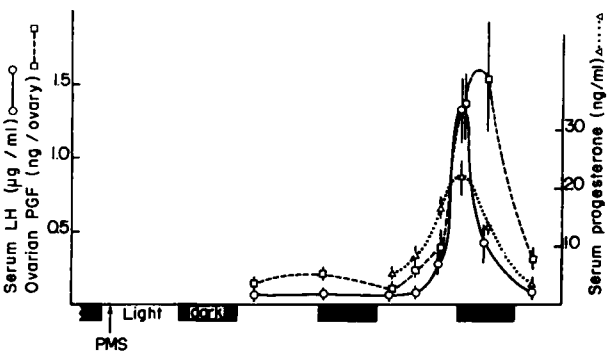

FIG. 1. — *Proestrous elevation of serum LH, serum progesterone and ovarian prostaglandins F in prepubertal rats in which first estrus and ovulation was induced by injection of 4 IU PMS at 28 days of age.* Rats were on lighting schedule of 14 hours light : 10 hours dark, with lights on at 0700. Serum LH levels, measured by radioimmunoassay (RIA), are expressed in terms of NIAMD-Rat LH-RP1 reference preparation. Progesterone was measured in unfractionated petroleum ether extracts, of serum, by RIA using an antiserum against Progesterone-6-BSA supplied by Dr. Gordon NISWENDER. Prostaglandins of the F series were measured in unfractionated ethanolic extracts of whole ovaries using an antiserum prepared against a prostaglandin $F_{2\alpha}$-BSA conjugate donated by Dr. Harold R. BEHRMAN. Points on curves are means of at least 4 animals $\pm$ S. E.

levels became elevated, approximately in parallel with serum levels of LH or progesterone measured in the same rats ; ovarian PGF reached apparently maximal levels shortly before ovulation. Similar elevations of ovarian PGF levels were found following i.v. or i.p. injections of exogenous LH, after a lag period of about 2 hours (fig. 2) (ARMSTRONG, ROBINSON and DORRINGTON, unpublished observations). In the same animals, ovarian levels of progesterone and cyclic adenosine-3′, 5′-monophosphate (cAMP) were observed to increase much more rapidly. The time-courses of these three ovarian responses to LH are consistent with the well-established concept of cAMP being a mediator of the steroidogenic action of LH, but argue against PGF as an obligatory intermediate in either of the other responses.

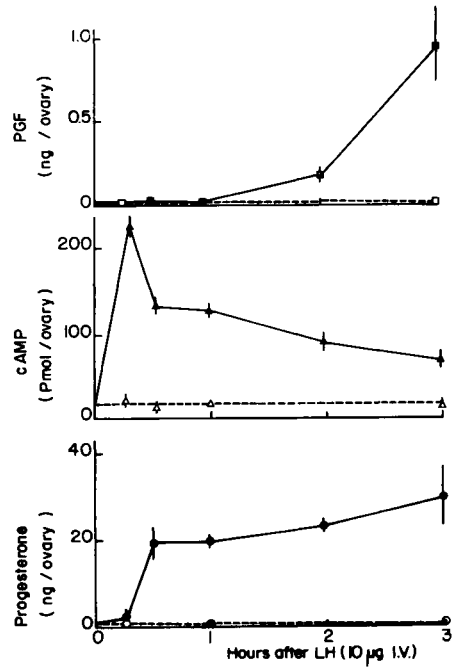

FIG. 2. — *Ovarian levels* (means ± S. E.) *of PGF, cAMP and progesterone on the second day after PMS treatment of prepubertal rats* (as in fig. 1), *at varying times after i.v. injection at 0730-0800 of 0.9 p. 100* NaCl (broken lines) *or 10 µg NIH-LH-S8* (solid lines). Cyclic AMP was measured by a modification of the protein-binding assay of GILMAN (1970).

B) *Rabbits.*

The effects of endogenous (coitus-induced) and exogenous LH on levels of PGF measured in rabbit Graafian follicles are summarized in figure 3 (ZAMECNIK and ARMSTRONG, 1973). As in rat ovaries, no significant elevation of PGF was noted one hour aftrer LH (exogenous or endogenous), whereas PGF levels were increased several-fold 8 hours later (*i. e.*, approximately one hour before expected ovulation). Similar elevations of PGF as well as prostaglandin E (PGE) in rabbit follicles following human chorionic gonadotropin (HCG) injection have been reported by LE MAIRE *et al.* (1973).

C) *Swine.*

Prepubertal gilts have been injected with a combination of PMS and HCG in order to synchronize estrus and induce ovulation. Follicular fluid was aspirated from large surface follicles, via laparotomies performed at varying times from 64 to 115 hours after the combined PMS-HCG treatment (AINSWORTH, BAKER and ARMSTRONG, 1975). As summarized in figure 4, follicular fluid concentrations of PGF became significantly elevated by 100 hours and reached levels 30 times basal values by 113 hours in follicles ascertained on the basis of morphologic characteristics as being pre-ovulatory. No significant elevations of PGF levels were noted in fluid from atretic follicles sampled at the same times. In this experimental model, ovulation normally occurs at 116 ± 8 hours after PMS-HCG treatment (R. D. BAKER, personal communication).

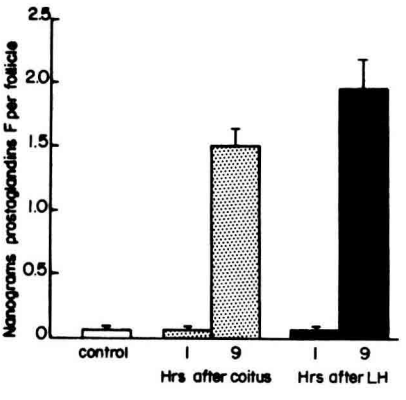

FIG. 3

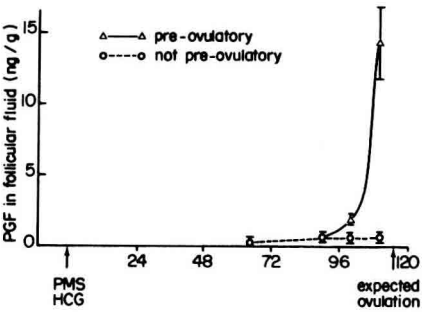

FIG. 4

FIG. 3. — *Elevation of PGF levels* (mean ± S. E.) *in follicles of estrous rabbits, induced by coitus and by exogenous LH* (50 µg NIH-LH-B7 i.v.) (data from ARMSTRONG, MOON and ZAMECNIK, 1974)

FIG. 4. — *Pre-ovulatory elevation of follicular fluid prostaglandins F* (mean ± S. E.) *in prepubertal gilts.* First estrus was synchronized by a single i. m. injection of 400 IU PMS combined with 200 IU HCG at 5-6 months of age. Follicles sampled at 99-101 hours and 110-113 hours after PMS-HCG could be classified according to gross morphological appearance and vascularity as either pre-ovulatory (△——△) or not pre-ovulatory (○ - - - - - ○) (data from AINSWORTH, BAKER and ARMSTRONG, 1975).

II. — *Blockade of pre-ovulatory elevations of ovarian prostaglandin F levels; effects on other ovarian responses to LH*

A) *Rats.*

Indomethacin, an established inhibitor of prostaglandin synthetase (VANE, 1971), was administered systemically to rats at 1445 on the second day following PMS treatment of prepubertal rats-*i.e.*, approximately 1-3 hours before the expected LH surge (ARMSTRONG and ZAMECNIK, 1975). Another group of animals was injected with barbital, an established inhibitor of LH secretion, at approximately the same time. As summarized in figure 5, both indomethacin and barbital completely prevented the expected increases in levels of PGF, in ovaries obtained at autopsies performed at 2030-2100. In the barbital-treated rats, the expected elevations of serum LH and serum progesterone were completely suppressed, whereas indomethacin caused no significant reduction in the levels of either of these hormones (fig. 6).

Meiosis in the large Graafian follicles of control rats autopsied at 2030-2100 was found to have proceeded to metaphase I or beyond. This resumption of meiosis (oöcyte maturation) was prevented by barbital treatment, but not by indomethacin treatment (fig. 5) (ARMSTRONG, unpublished observation). A similar failure of indomethacin to prevent oöcyte maturation in adult rats on proestrus has been reported by TSAFRIRI, KOCH and LINDNER (1972).

Both indomethacin and barbital effectively blocked ovulation. No tubal ova nor follicle rupture points were noted in rats killed at 0900-1100 on the third day after PMS treatment, whereas 8.7 ± 0.7 ova were found in the oviducts of control rats autopsied at the same time.

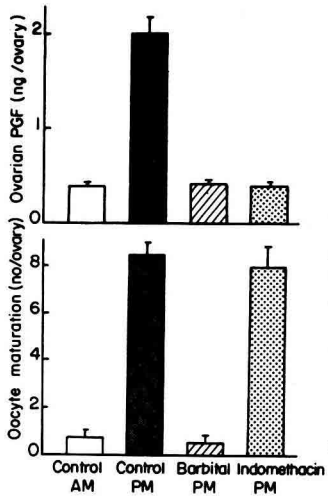

FIG. 5

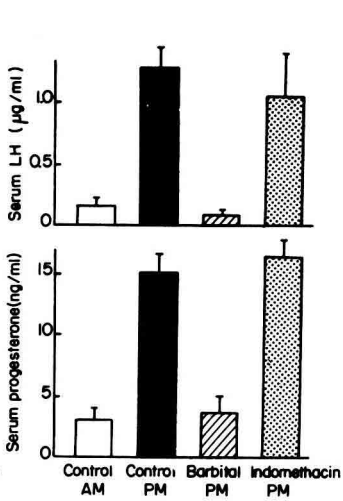

FIG 6.

FIG. 5. — *Effects of barbital* (10 mg/rat) *and indomethacin* (0.8 mg/rat) *on proestrous ovarian prostaglandin F levels and on oöcyte maturation in prepubertal rats.* Autopsies were performed at 1 000-1 030 (AM) and at 2030-2100 (PM) on the second day following PMS injection to synchronize first estrus (as in fig. 1). Barbital and indomethacin were administered by i.p. injection at 1345 and 1445 respectively, on the same day. Oöcyte maturation was determined in follicles of pre-ovulatory size by histological examination of serial sections of ovaries.

FIG. 6. — *Effects of barbital and indomethacin on proestrous elevations of serum LH and serum progesterone in prepubertal rats in which first estrus was synchronized with PMS* (as in fig. 1). Drug administrations and autopsies were as in figure 5.

TABLE I

Peripheral plasma progesterone (1) *in rabbits during pseudopregnancy following blockade of LH-induced ovulation by indomethacin*

Days after LH Treatment (3)	Treatment (2)	
	Controls	Indomethacin (20 mg/kg)
1	3.3 ± 0.6	2.7 ± 1.2
4	3.7 ± 0.9	6.6 ± 2.3
8	7.1 ± 2.8	7.1 ± 3.4
12	9.0 ± 3.3	13.0 ± 3.1
16	2.2 ± 1.3	4.5 ± 1.4
20	2.0 ± 0.6	1.8 ± 0.9

(1) Progesterone (Mean ng/ml plasma ± S. E.) measured by radioimmunoassay.

(2) Treatment administered i. v. in 0.1 M phosphate buffer, pH 8.0, 1/2 hour before injection. 5 rabbits per treatment.

(3) 50 µg NIH-LH-B7 i. v. to induce ovulation and/or pseudopregnancy.

B) *Rabbits.*

Indomethacin, when injected 1/2 hour before, or up to five hours after injection of an otherwise ovulatory dose of LH to estrous rabbits, has been reported to prevent ovulation (fig. 7) (GRINWICH, and KENNEDY ARMSTRONG, 1972). Despite the blockade of ovulation, corpora lutea formed which appeared normal histologically (ARMSTRONG, MOON and GRINWICH, 1973), and maintained normal serum levels of progesterone during the resulting pseudopregnancy (table 1).

Intrafollicular injection of a much smaller dose of indomethacin has been found almost equally as effective as 20 mg/kg administered systemically, in blocking ovulation (fig. 8) (ARMSTRONG *et al.*, 1974). Control follicles injected intrafollicularly with the phosphate buffer vehicle ovulated normally. As summarized in figures 7 and 8, the ovulation-blocking dosages of indomethacin administered via the i.v. or intrafollicular routes were effective in suppressing the elevations of follicular PGF.

Intrafollicular injections of antiserum prepared against $PGF_{2\alpha}$ have been found as effective as indomethacin in blocking ovulation when injected 5 hours after an ovulation-inducing dosage of LH (ARMSTRONG *et al.*, 1974). Most control follicles injected with either normal rabbit serum, or antiserum against PGE_2 underwent normal ovulation.

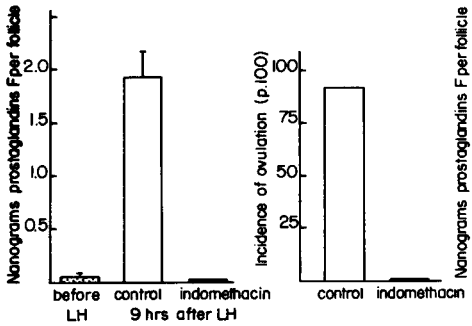

FIG. 7

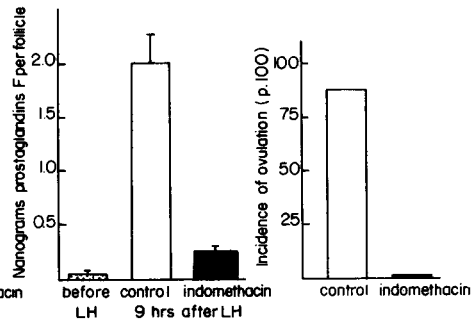

FIG. 8

FIG. 7. — *Blockade of elevation of prostaglandin F levels in rabbit follicles and of ovulation by systemic injection of indomethacin, 20 mg/kg s. c. immediately before i.v. injection of 50 μg NIH-LH-B7.* Rabbits were killed 9 hours after LH for PGF determinations and approximately 24 hours after LH to determine incidence of ovulation, as described by GRINWICH, KENNEDY and ARMSTRONG (1972).

FIG. 8. — *Blockade of elevation of prostaglandin F levels in rabbit follicles and of ovulation by intrafollicular injections of 5 μg indomethacin in 1 μl of 0.15 M phosphate buffer 5 hours after i.v. injection of 50 μg NIH-LH-B7.* Control follicles on the opposite ovaries of the same rabbits were injected with 1 μl of the phosphate buffer vehicle. Autopsy times were as for figure 7. Data from ARMSTRONG, GRINWICH, MOON and ZAMECNIK, 1974.

III. — *Effect of intrafollicular injections of LH and prostaglandins on ovulation and oocyte maturation*

Luteinizing hormone has been reported previously (JONES and NALBANDOV, 1972) to induce ovulation when injected directly into the follicles of estrous rabbits. In order to confirm this action, and to determine whether this response could be mimicked by exogenous prostaglandins, LH, PGE_2 and $PGF_{2\alpha}$ were injected intrafollicularly. Ovaries were removed 4, 8, 12 or 24 hours later and examined grossly and histologically for evidence of ovulation, and histologically for evidence of oöcyte

maturation. As shown in table 2 (ARMSTRONG, MOON and ZAMECNIK, 1974), LH was highly effective in inducing what appeared to be normal follicular rupture at the apices of 6 of the 13 follicles injected. The oöcytes from 2 additional LH-injected follicles were found, on serial sectioning of the ovaries, outside the follicles, within the ovarian stroma adjacent to the points of the micro-injections of the follicles.

TABLE 2

Effects of intrafollicular injections of prostaglandins and LH on ovulation and oöcyte maturation

Material injected	Hours between injection and autopsy	No. of follicles injected	No. of follicles « ovulated (1) »	Nuclear Stage of oöcyte (2)		
				Dictyate	Metaphase I	Metaphase II
LH (200 ng)	24	9	7	—	—	3
LH (200 ng)	4	4	1	1	3	—
$PGF_{2\alpha}$ (10 µg)	24	8	3	1	2	3
$PGF_{2\alpha}$ (10 µg)	12	6	2	—	4	—
$PGF_{2\alpha}$ (10 µg)	8	4	2	—	3	1
$PGF_{2\alpha}$ (10 µg)	4	5	2	1	3	—
PGE_2 (10 µg)	24	8	0	4	1	—
PGE_2 (10 µg)	12	7	0	5	—	—
Saline	4	9	0	9	—	—

(1) Oöcytes extruded either through apical rupture point and not recovered, or extruded through point of injection and recovered within ovarian stroma (see text).

(2) Oöcytes recovered either in ovarian stroma (outside follicles) or retained within injected follicles.

TABLE 3

Effects of intrafollicular injection of prostaglandin $F_{2\alpha}$ in varying amounts on ovum extrusion in rabbits

ng PGF injected per follicle	No. of follicles injected	No. of ova estradiol 24 hrs after injection	Percentage response
0	4	0	0
10	10	4	40
100	5	4	80
1 000	10	4	40
10 000	4	3	38

Meiosis had resumed in these oöcytes, as well as in those recovered within 5 non-ruptured follicles which had been injected with LH. Of the 23 follicles injected with $PGF_{2\alpha}$, 9 were observed to have extruded their oöcytes through the points of injec-

tions, in a manner similar to that of the latter two LH-injected follicles. None underwent « normal » ovulation through apical stigmata. Meiosis had resumed in eight of these extruded ova, as well as in eight of those still retained within the remaining $PGF_{2\alpha}$-injected follicles. None of the oöcytes was extruded from 15 PGE_2-injected follicles or from 9 saline-injected follicles, and only one oöcyte in a PGE_2-injected follicle had progressed beyond the dictyate stage of meiosis.

A comparison of varying dosages of $PGF_{2\alpha}$ (table 3), revealed that intrafollicular injection of as little as 10 ng were approximately as effective in causing ovum extrusion as were the high dosages used in the experiments summarized in table 2.

DISCUSSION

In the studies reviewed above, four types of evidence have been obtained, suggesting a role of $PGF_{2\alpha}$ in the process of ovulation as follows : 1) Levels of PGF have been observed to increase markedly in rat ovaries and in rabbit and pig follicles shortly before ovulation. 2) Pharmacologic inhibition of prostaglandin synthesis in rats and rabbits, thereby preventing the expected pre-ovulatory increases in follicular PGF, was associated with blockade of ovulation. 3) Antiserum against $PGF_{2\alpha}$ blocked ovulation when administered via micro-injection directly into pre-ovulatory follicles of estrous rabbits. 4) Intrafollicular injection of $PGF_{2\alpha}$, in the absence of LH treatment, caused extrusion of ova from a significant number of injected follicles of estrous rabbits.

The effectiveness of $PGF_{2\alpha}$ to induce oöcyte maturation suggests that this prostaglandin may be involved in the resumption of meiosis, in response to LH. Prostaglandin E_2 was essentially ineffective in this regard, in contrast to its effectiveness in the organ culture studies of TSAFRIRI *et al.* (1972), using rat follicles. These observations are difficult to reconcile with the observations reported here, as well as with those of TSAFRIRI, KOCH and LINDNER (1972), that LH-induced ovum maturation *in vivo* is not blocked by indomethacin at dosages effective in suppressing PGF synthesis and in blocking ovulation. It is possible that considerably smaller increments in follicular prostaglandin levels are required for ovum maturation than for ovulation, and that indomethacin does not completely prevent the required small increases from occurring.

The time-course of elevations of ovarian PGF, cAMP and progesterone following exogenous LH administration to proestrous rats indicates that the latter two responses occur considerably earlier than does the increased PGF synthesis. The rapid increase in cAMP levels, followed promptly by elevation of ovarian progesterone concentration, supports the « second messenger » role of this cyclic nucleotide in stimulation of steroidogenesis. The delayed elevation of ovarian PGF concentration makes it unlikely that PGF is involved in the mechanism by which LH activates adenylate cyclase ; an alternative possibility is that increased PGF synthesis may be a consequence of elevated cAMP and/or steroid levels.

The mechanism by which prostaglandins lead to ovulation remains uncertain. One possibility is that a contractile proces mediated by $PGF_{2\alpha}$, contributes to rupture of the follicle wall after it has become distended and weakened by proteolytic

digestion in response to LH. This explanation receives support from the observations, reported here, that oöcytes from a significant percentage of follicles injected with $PGF_{2\alpha}$ were apparently extruded through the sites of injection, as though forced through this path of least resistance by contractions of the follicle. Additional support for such an explanation has been provided by the recent report of VIRUTAMASEN and FUCHS (1974) describing increased contractile activity of rabbit ovaries beginning 8-9 hours after HCG injection. Failure of $PGF_{2\alpha}$-injected follicles in the present studies to ovulate in the normal manner, *i.e.*, through stigmata on their surfaces, could be explained by lack of proteolytic digestion and weakening of the follicle wall due to absence of LH. RONDELL (1970) has provided evidence that ovarian steroids, e. g., progesterone, may be mediators of this latter response to LH, acting upon proteolytic enzyme (s) within the follicle wall. Further research will be required to unravel the interactions between ovarian steroids, prostaglandins, and possibly cAMP, which lead ultimately to normal ovulation in response to LH.

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RÉSUMÉ

RÔLE DES PROSTAGLANDINES DANS LES RÉPONSES FOLLICULAIRES A LH

Il a été trouvé que les niveaux de prostaglandines de la série F augmentent plusieurs fois pendant la période préovulatoire dans les follicules de Lapines, de Rattes et de Truies ; les niveaux maximum sont atteints peu avant l'ovulation. Chez la Lapine et la Ratte, l'augmentation peut être provoquée par de l'hormone lutéinisante exogène (LH). Cette augmentation chez la Ratte est précédée par un accroissement des niveaux ovariens d'AMP cyclique et de progestérone. L'administration systémique d'indométhacine empêche l'augmentation de prostaglandine F au niveau ovarien et bloque l'ovulation chez la Lapine et la Ratte. L'injection intrafolliculaire d'indométhacine chez la Lapine à des doses inefficaces par voie générale bloque l'augmentation des prostaglandines folliculaires ainsi que l'ovulation. L'antisérum anti-$PGF_{2\alpha}$ bloque aussi l'ovulation quand il est injecté dans le follicule. L'injection intrafolliculaire de $PGF_{2\alpha}$ chez la Lapine provoque, en l'absence de LH, la libération de l'ovocyte chez 40 p. 100 des follicules injectés et une reprise des divisions méiotiques de l'ovocyte dans 90 p. 100 des follicules injectés. La prostaglandine E_2 est beaucoup moins efficace. Ces résultats sont en faveur d'un rôle physiologique de la $PGF_{2\alpha}$ folliculaire dans les processus d'ovulation et de maturation ovocytaire en réponse à LH.

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