THE EFFECTS OF PROSTAGLANDINS AND INDOMETHACIN ON OVULATION IN THE PERFUSED FOWL OVARY

K. Tanaka[1], T. Higuchi, M. Tawarazumita and F. Hertelendy[2]

Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan

Summary

The effects of prostaglandins (PG) and indomethacin, a PG synthesis inhibitor, on ovulation in the perfused fowl ovary were studied. Laying hens were killed by cutting the jugular vein 18-19 h before expected ovulation of the second follicle of a clutch sequence. The cannulated ovary was dissected free and connected with a recycling perfusion apparatus. Agents to be studied for their effects on ovulation were added to the perfusion fluid. $PGF_{2\alpha}$ at 0.1 and 1 mg/l induced ovulation, with a success rate of 25% and 30%, respectively. The same doses of PGE_2 were effective at 60% and 63%, respectively. Indomethacin partially blocked gonadotrophin-induced ovulation. It is suggested that PGs may play a supportive role in the process of follicular rupture in the domestic fowl.
(**Key Words :** Fowl Ovary, Perfusion, Prostaglandin, Indomethacin)

Introduction

Prostaglandins (PG) are involved in the ovulatory process of several mammalian species (Goldberg and Ramwell, 1975), but such action has not been documented in the domestic fowl, since massive doses of indomethacin failed to inhibit spontaneous ovulation (Day and Nalbandov, 1977). Similary local application of PGE_2 and $PGF_{2\alpha}$ to ovarian follicles, either *in vivo* or *in vitro*, did not induce ovulation (Samsonovitch and Lague, 1977). However, Hertelendy et al. (1984) and Saito et al. (1987) suggested that the high concentrations of PGs in ovarian follicles may play a part in regulating follicular rupture in the domestic fowl.

Recently a system has been developed for the *in vitro* perfusion of the fowl ovary (Tanaka et al., 1987). This system is considered to be useful for studying the sequence of events leading to follicular rupture, since it permits isolation of the ovary from systemic influences, and continual observation of the ovary during perfusion. The present studies were designed to test the effectiveness of PGs and indomethacin on ovulation in the perfused fowl ovary.

Materials and Methods

Birds from a commercial hybrid egg-laying stock were used. They were maintained in individual laying cages and were exposed to a 14 h photoperiod. All hens selected for study were laying in clutches of more than three eggs with a pause of only a single day between clutches. Hens were injected intravenously with heparin sulphate (100 U/hen) 18-19 h before expected ovulation of the second follicle of a clutch sequence, and killed by cutting the jugular vein. After exposure of the ovary, a cannula was inserted into the dorsal aorta. The cannulated ovary was dissected free and connected with a recycling perfusion apparatus. The detailed technique and perfusion system have been described previously (Tanaka et al., 1987).

$PGF_{2\alpha}$ (5 mg) or PGF_2 (5 mg) was dissolved in 95% ethanol (0.5 ml), followed by mixing with 0.9% NaCl (4.5 ml). Two concentrations of these PGs, 0.1 and 1.0 mg/l, were evaluated by adding to the perfusion fluid.

To study the effect of indomethacin on gonadotrophin-induced ovulation, 20 or 40 mg of indomethacin, dissolved in 1 ml of 95% ethanol followed by mixing with 1 ml of 7% $NaHCO_3$, were added to 1 litre of perfusion fluid. Ovine

LH (NIAMDD-OLH-22, 2.3 U/mg) plus FSH (NIAMDD oFSH-13, 15 U/mg), 1 U each, dissolved in 2 ml of 0.9% NaCl were added 30 min after perfusion of the ovary with indomethacin. Two solutions, 0.1 ml of 95% ethanol plus 0.9 ml of 0.9 % NaCl, and 1 ml of 95% ethanol plus 1 ml of 7% $NaHCO_3$, served as control for PG groups and indomethacin groups, respectively. Routinely, two separate perfusion systems, containing 500 ml perfusion fluid/ovary, were operated simultaneously. Significance levels for the percentage of the largest follicles ovulating were determined by χ^2 test.

Results

Both doses of 0.1 and 1.0 mg $PGF_{2\alpha}$ were effective in inducing ovulation of the largest follicles, in 2 out of 8 (25%) and 3 out of 10 (30%) perfusion experiments, respectively. PGE_2 applied also at 0.1 and 1.0 mg/l caused ovulation in 60% and 63% of F_1 follicles, respectively, but the difference between $PGF_{2\alpha}$ and PGE_2 groups was not statistically significant. By comparison, the efficacy of gonadotrophins was significantly ($p < 0.05$) greater (table 1). Controls failed to ovulate.

No apparent suppression of gonadotrophin-induced ovulation was observed using 20 mg/l of indomethacin, whereas 40 mg significantly reduced the rate of ovulation ($p < 0.05$).

The interval between the onset of perfusion and ovulation was about 4-5 h. In general, the follicles which failed to ovulate became atretic by 8 h after the start of perfusion. Once initiated, atresia rapidly spread to the entire surface of the follicle, usually within 1 h.

Discussion

The fact that either $PGF_{2\alpha}$ or PGE_2 was partially effective for the induction of ovulation in the perfused fowl ovary suggests some involvement of PGs in follicular rupture. Our initial concept was that PGs may exert their action quickly, if at all, through the stimulation of ovarian contractility, since concentrations of plasma and follicular PGs have been observed to rise around the time of ovulation (Olson et al., 1986; Saito et al., 1987). However, about 4-5 h were required until actual follicular rupture occurred following the onset of ovarian perfusion with $PGF_{2\alpha}$ or PGE_2. This might be associated with the second peak of plasma PGs occurring 5-7 h before ovulation (Hammond et al., 1980).

It is also likely that PGs act directly on the preovulatory follicle by both affecting its blood supply (Scanes et al., 1982), which may result in so called 'physiological atresia' (Nalbandov, 1961), and by stimulating the contraction of follicular smooth muscle to facilitate rupture of

TABLE 1. EFFECTS OF PROSTAGLANDINS AND INDOMETHACIN ON OVULATION IN THE PERFUSED FOWL OVARY

Agent (per liter)	No. of ovaries[1]	No. of follicles	
		Ruptured	%
$PGF_{2\alpha}$ (0.1 mg)	8	2	25[b]
$PGF_{2\alpha}$ (1.0 mg)	10	3	30[b]
PGE_2 (0.1 mg)	10	6	60[bcd]
PGE_2 (1.0 mg)	8	5	63[bcd]
Vehicle control[2]	8	0	0[a]
Indomethacin (20 mg) + LH(1U) + FSH(1U)	14	10	71[cd]
Indomethacin (40 mg) + LH(1U) + FSH(1U)	12	4	33[bc]
LH(1U) + FSH(1U) + vehicle[3]	8	7	88[d]
Vehicle control[3]	9	0	0[a]

[1] Ovaries were isolated 18-19 h before the expected ovulation of C2 follicle of a clutch sequence.
[2] Ethanol (95%, 0.1 ml) + NaCl (0.9%, 0.9 ml).
[3] Ethanol (95%, 0.1 ml) + $NaHCO_3$ (7%, 1 ml).
Values with different superscripts are significantly different, $p < 0.05$.

the stigma. In mammals, PGs activate ovarian adenylate cyclase to enhance steroidogenesis (Marsh and LeMaire, 1974), and LH promotes prostaglandin synthesis (Clark et al., 1978), leading to ovulation. In addition, indomethacin or anti-prostaglandin serum can block LH - induced ovulation (Zor and Lamprecht, 1977; Armstrong et al., 1974). However, no such inhibitory action of indomethacin on ovulation (Day and Nalbandov, 1977; Hertelendy and Biellier, 1978; Hammond and Ringer, 1978; Olson and Hertelendy, 1981) and on gonadotrophin-induced steroidogenesis (Hertelendy and Hammond, 1980) has been observed in the fowl.

In the present study, however, indomethacin partially blocked gonadotrophin-induced ovulation. From these results it seems more likely that PGs play a permissive or complementary role in the complex physiological mechanism(s) of ovulation. This hypothesis does not contradict earlier works concerning the role of PGs in the avian ovulatory process, since it is considered that under the *in vitro* perfusion system hormones or drugs act directly on the ovary, whereas *in vivo* studies are complicated by uncontrollable variables, including endocrine, neural, humoral, vascular and metabolic factors. In addition, it is assumed that the ovulatory event in the fowl may not be achieved by a sole mechanism, since in mammals PGs are involved in a variety of metabolic processes, which are essential to reproductive function, through pathways that are separate from the action of gonadotrophins (Marsh, 1975; Zor and Lamprecht, 1977). Further efforts are necessary to elucidate the possible role and mode of action of prostaglandins in ovulation in the domestic fowl.

Acknowledgements

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