Indomethacin inhibition of ovulation in the cow

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Summary. Indomethacin or saline was administered via intramuscular, intrauterine or intraovarian routes to dairy cows, within 24 h after standing oestrus was first observed. The incidence of ovulation was determined at slaughter. All of the saline-treated cows (18/18) ovulated. Ovulation was not blocked after intramuscular injection (0/6) or intrauterine infusion (0/6) of indomethacin. In all cows, ovulation was blocked after intraovarian injection (6/6) of indomethacin. These findings add support to the hypothesis that prostaglandins play an essential role in ovulation in the cow as in many other mammalian species.

Introduction

Cystic ovaries are a serious cause of infertility in the dairy cow. Once formed these cysts fail to ovulate and persist for at least 10 days (Roberts, 1971). The ovulatory failure may be confined to the preovulatory follicle because after the LH surge, prostaglandin (PG) synthesis within the preovulatory follicle is critically involved in ovulation (Espey, 1980; Armstrong, 1981) and if PG synthesis does not occur then the preovulatory follicle would not rupture and possibly remain as a cyst. Ovulation has been successfully blocked by indomethacin, an inhibitor of PG synthesis, in the mouse (Lau, Saksena & Chang, 1974), rat (Armstrong & Grinwich, 1972), rabbit (Grinwich, Kennedy & Armstrong, 1972), monkey (Wallach, de la Cruz, Hunt, Wright & Stevens, 1975), sow (Ainsworth *et al.*, 1979) and ewe (Murdoch & Dunn, 1983), but there is no evidence that PGs play a definitive role in ovulation in the cow. The objective of the present investigation was, therefore, to determine whether indomethacin could block ovulation in the cow.

Materials and Methods

Animals. Non-lactating Holstein cows were injected with 25–35 mg PGF-2 α (Upjohn Co., Kalamazoo, MI, U.S.A.) to induce a synchronized oestrus. At 24 h after injection, cows were observed for oestrous behaviour every 6 h until 100 h. Cows observed in standing oestrus were randomly assigned to receive either indomethacin (Sigma Chemical Co., St Louis, MO, U.S.A.) in saline (9 g NaCl/l) or saline only. Indomethacin was administered as a suspension by intramuscular injection, intrauterine infusion or intraovarian injection.

Experiment 1: intramuscular injection of indomethacin. In a pilot study indomethacin was administered at doses ranging from 5 to 15 g after first observed standing oestrus. These treatments failed to block ovulation consistently. We assumed that indomethacin may not have been injected in sufficient concentrations or at the appropriate times during the preovulatory period. Therefore, in the present experiment, indomethacin was administered at a dose of 5 g per injection at first observed standing oestrus, and 6, 12, 18 and 24 h later for a total dose of 25 g in a total volume of 50 ml (N = 6). Another 6 cows (controls) were injected with saline only at the same times. Injections were given at multiple sites. Cows were slaughtered on Days 4 through 8 after treatment and the ovaries were examined for incidence of ovulation (ovulation in the cow occurs about 30 h after beginning of oestrus; Hawk & Bellows, 1980).

Experiment 2: intrauterine infusion of indomethacin. Indomethacin (720 mg) in 20 ml saline (N = 6) or saline only (N = 6) was infused into the lumen of each uterine horn at first observed standing oestrus and also 14 h later (total dose = 1440 mg per horn). Cows were slaughtered on Day 7 after infusion and the ovaries examined for incidence of ovulation.

Experiment 3: intraovarian injection of indomethacin. At first observed oestrus, food and water were removed from cows. About 16 h after first observed standing oestrus, high lumbar laparotomy was performed, using local anaesthesia, to enable intraovarian injection of indomethacin or saline into the ovary containing the preovulatory follicle (determined before surgery by palpation *per rectum*). Ovaries were also visually inspected through a Plexiglass speculum with a light source to confirm the presence of the preovulatory follicle. The ovary containing the preovulatory follicle received indomethacin (20 mg) in 1 ml saline (N = 6) or 1 ml saline only (N = 6). Injections were administered into the ovarian stroma near the base of the preovulatory follicle using a syringe fitted with a 20-gauge hypodermic needle. Cows were slaughtered on Days 3 through 12 after injections and treated ovaries were examined for incidence of ovulation.

Statistical methods. The incidence of ovulation after indomethacin or saline treatment was statistically evaluated with Fisher's Exact test, using the hypergeometric distribution (Steel & Torrie, 1960).

Results

All of the saline-treated cows ovulated. Ovulation was not blocked after intramuscular injection or intrauterine infusion of indomethacin. In all cows, ovulation was blocked after intraovarian injection of indomethacin (Table 1). The follicles from indomethacin-treated cows in which ovulation had been blocked were smooth and round on the surface and were greatly luteinized. In contrast the corpora lutea from the saline-treated cows clearly showed an indentation on the surface with rough edges representing the site of rupture of the follicle.

Table 1. Incidence of ovulation (no. of cows ovulating/no. treated) after intramuscular, intrauterine or intraovarian administration of indomethacin or saline into cows during oestrus

Route	Treatment	
	Saline	Indomethacin
Intramuscular	6/6	6/6
Intrauterine	6/6	6/6
Intraovarian	6/6	0/6*

*Different from saline-treated cows by Fisher's Exact test ($P=0.001$)

Discussion

Despite the fact that intramuscular administration of indomethacin can successfully block ovulation in several species, we were unable to block ovulation in the cow even after repeated injections throughout oestrus. Intrauterine infusion, considered to be a more desirable route to deliver the indomethacin to the ovaries because of the anatomical associations of uterine veins and ovarian arteries in the cow (Ginther, 1974), also failed to block ovulation in any of the cows treated. In the ewe, intrauterine infusion of low doses of indomethacin did not consistently block ovulation (W. J.

Murdoch, personal communication). In the rabbit (Armstrong, Grinwich, Moon & Zamecnik, 1974) and the ewe (Murdoch & Dunn, 1983) indomethacin blocked ovulation when administered directly into the antrum of the preovulatory follicle. With the technique we used, it was difficult to reach the antrum of the preovulatory follicle of the cow without rupturing it and so injections were made into the ovarian stroma near the base of the preovulatory follicle. Nevertheless, indomethacin blocked ovulation in all cows when administered in this manner. It appears, therefore, that indomethacin administered by the intramuscular or intrauterine routes may not have reached the ovaries in sufficient concentrations to block ovulation.

Several previous investigators (Tsafiriri, Lindner, Zor & Lamprecht, 1972; Hamada, Bronson, Wright & Wallach, 1977) have provided evidence that the blockade of ovulation with indomethacin is due to a local effect. The results of our investigation add further support to this contention. Although indomethacin blocked ovulation the follicles became luteinized. Ainsworth *et al.* (1979) and Murdoch & Dunn (1983) also noted luteinization of the follicles that had not undergone ovulation after indomethacin treatment. Two previous studies (Shemesh & Hansel, 1975; Shemesh, 1979) showed that bovine follicular tissue synthesized PGs under in-vitro conditions and that synthesis was enhanced by LH. We conclude that PGs may be a critical component in the chain of events leading to ovulation in the cow.

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