STUDIES ON THE EFFECT OF PROSTAGLANDIN INHIBITION BY ASPIRIN AND INDOMETHACIN ON OVULATION AND PREGNANCY IN THE RAT.

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BY

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CERTIFICATION

This is to certify that the thesis titled:

"Studies on the effect of Prostaglandin inhibition by Aspirin and Indomethacin on ovulation and pregnancy in the rat".

Submitted to the School of Postgraduate studies, university of Lagos for the award of the degree of Ph.D in Physiology is a record of original research carried out by OLUFEYISIPE A. ADEGOKE in the Department of Physiology, College of Medicine, University of Lagos.

Signed

EXTERNAL EXAMINER

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ABSTRACT

The present study was conducted with the aim of determining the effect of prostaglandin inhibition on certain aspects of the reproductive system, namely ovulation and pregnancy, in young adult female Sprague - Dawley rats. Prostaglandin inhibition was achieved by the administration of two widely used prostaglandin inhibitors, Aspirin and Indomethacin.

In order to determine the probable mechanism as well as the site of action of the prostaglandin inhibitors, the effects of human chorionic gonadotropin (hCG) and luteinizing hormone (LH) on the action of the drugs were investigated. Morphometric studies on the ovaries were also carried out.

The experiments involved the testing of the effects of Aspirin and Indomethacin on (i) Spontaneous ovulation (ii) Superovulation, (iii) ovulation in the presence of exogenous hCG (iv) ovulation in the presence of exogenous LH, and (v) pregnancy. The parameters measured were the preovulatory plasma LH level, the preovulatory plasma PGF level, the rate of ovulation, the pattern of follicular development in the ovary, the rate of embryo implantation, the length of gestation, and the number of offsprings delivered at the end of gestation.

The results of the present study indicate that Aspirin and Indomethacin when given in doses that inhibit inflammatory reactions will inhibit the process of ovulation, including follicular development and ovum maturation. In this action indomethacin was a stronger inhibitor of ovulation than aspirin. Morphometric studies reveal that while indomethacin is effective in suppressing follicular rupture, aspirin mainly retards ovum maturation.

The two drugs also caused a significant reduction in plasma PGF level with indomethacin again being the more effective of the two. It therefore appears that aspirin and indomethacin may inhibit ovulation by inhibiting prostaglandin synthesis, implying that prostaglandins are involved in the process of ovulation. Since both aspirin and indomethacin are anti-inflammatory agents, the mechanism of prostaglandin action on ovulation may likely be an inflammatory one.

It was also observed that hCG and LH did not reverse the inhibition of ovulation by aspirin and indomethacin. It is also noteworthy that the administration of aspirin and indomethacin did not affect the preovulatory LH level. This indicates that aspirin and indomethacin exert their ovulation inhibitory action directly on the ovary and not on the hypothalamic - pituitary axis.

The results also revealed that while aspirin administration during pregnancy caused no adverse effects, indomethacin reduced the rate of implantation, prolonged the gestation period, and caused the resorption of some of the fetuses.

It is concluded that prostaglandins play a crucial role in the mechanisms involved in the processes of ovulation and pregnancy.

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CHAPTER ONE

INTRODUCTION

A. OVULATION AND PREGNANCY

The reproductive system of the mammalian female shows regular cyclic changes in the ovary and the uterus, and these changes may be regarded as periodic preparations for fertilization and pregnancy. These changes are controlled by interactions between the gonadotropic hormones, Follicle Stimulating hormone (FSH) and Luteinising hormone (LH) and the ovarian hormones estrogen and progesterone. One of the events which occur in the ovary during each cycle is the process of ovulation whereby the ovarian follicle ruptures and extrudes the ovum (oocyte) contained in it. As reviewed by Lipner (1988) ovulation marks the culmination of a series of events which are initiated by the surge of LH which occurs midway through the cycle. These events include final follicular growth and maturation, initiation of luteinization of the granulosa cells of the follicles, progesterone and secretion by the follicular cells, and the restructuring of the follicle wall, with resultant follicular rupture and release of a mature fertilizable ovum.

The LH surge is known to be initiated by a pre ovulatory rise in circulating estrogen (Ganong, 1987)) although the normal LH secretion is under the control of hypothalamic Luteinising hormone releasing hormone (LHRH) This implies

that the pre ovulatory elevated estrogen stimulates LHRH release from the hypothalamus which in turn stimulates the anterior pituitary to increase its secretion of LH.

After ovulation has occurred fertilization of the ovum results in the formation of a blastocyst (later called an embryo) which then implants in the uterus, and this marks the beginning of pregnancy. A lot of hormonal secretion occurs during pregnancy, all of which serve to enhance implantation as well as the survival of the implanted embryo.

The mechanisms of events which occur to terminate pregnancy are not quite clear but it is known that at the end of pregnancy prostaglandins and the hormone oxytocin are secreted in large quantities and they stimulate uterine contractions (Ganong, 1987).

B. REPRODUCTION IN THE RAT

The rat undergoes a sexual cycle called the Estrous cycle. It is now well known that in the rat the estrous cycle occurs every four or five days, the rat therefore being a polyestrous animal. The estrous cycle can be divided into four phases - the diestrous, proestrus, estrus and metestrus phases (Daniel, 1978). The proestrus and estrus are anabolic stages during which active growth occurs in various parts of the genital tract. They culminate in ovulation and, where mating occurs, in fertilization. Metestrus is a catabolic stage characterized by degenerative changes in the genital tract while diestrus is a period of

quiescence or slow growth. Several early workers (e.g. Snell, 1941; Thung et al, 1956) found that the estrous cycle may be conveniently charted by examining vaginal smears. Cellular characteristics of vaginal smears reflect changes in the structure of the vaginal epithelium which, in turn, are dependent upon estrogen and follow a regular and predictable course during the cycle.

Freeman (1988) in his review stated that the events of the estrous cycle are largely under photoperiodic control i.e the lighting periodicity plays a dominant role in the incidence and duration of the stages of the cycle. The period of estrus and the time of ovulation are normally controlled by the diurnal rhythm of light and darkness. According to Daniel (1978) reversing the time of light and darkness reverses the time of estrus and ovulation. He also reviewed the evidence supporting the conclusion that the midpoint of the ovulation period is determined by the midpoint of the dark phase.

As reviewed by Freeman (1988) ovulation occurs spontaneously in the rat during estrus whether or not mating has occurred. However, ovulation may not occur at every estrus, and estrus may not necessarily accompany ovulation since the two phenomena (estrus and ovulation have different hormonal bases. Estrus is dependent upon estrogen secretion while ovulation occurs in response to gonadotropin secretion by the anterior pituitary. Following initial stimulation of follicular growth by follicle stimulating hormone (FSH) Luteinising hormone (LH) begins to rise resulting in

drastically increased secretion of follicular fluid, and finally ovulation.

The pre ovulatory period of the estrous cycle is characterized by a growth of ovarian follicles and a concomitant enhanced secretion of estrogen (Shaikh, 1971). In the 4-day cycling rat peripheral plasma levels of estradiol are basal through estrus - but late on metestrus through early diestrus plasma levels begin to rise. This increase continues through diestrus and early proestrus to reach peak values and plateau by mid-proestrus. During the early evening, shortly before the dark interval, estradiol levels fall rapidly reaching basal values by the early morning hours of estrus.

In rats with 4-day cycles peripheral plasma levels of testosterone and androstenedione are found to be similar to those of estradiol (Dupon & Kim, 1973). This similar patterns of secretion for the androgens and estrogens suggest that the two classes of hormones come from the same organ, the ovary, and that their synthesis, secretion, and controls may be interrelated. Just like for estrogen, a large increase in progesterone secretion occurs during the afternoon and evening of proestrus. It reaches peak levels around the time of the LH peak in the early evening and returns to basal levels by the morning of estrus (Freeman, 1988). A second major peak (of luteal origin) begins about mid-day on metestrus and falls to basal levels during diestrus.

As pointed out by Schwartz and McCormack (1972) the

secretion of LH is low from late estrus to early proestrus because of the negative feedback provided by estrogen and progesterone from the ovaries. The level then begins to rise reaching a peak (LH surge) in the evening of proestrus. The pattern of FSH secretion bears some basic similarities to that of LH e.g. the control by ovarian hormones. However, some workers have reported a basic difference in the bimodal pattern of FSH secretion during proestrus through early estrus (Butcher et al, 1974; Ashiru and Blake, 1979).

When the rat cervix and vagina are stimulated during estrus either mechanically or by coitus, prolactin is released from the anterior pituitary to enable the corpus luteum to secrete progesterone (Whittingham, 1979). This secretion continues for about thirteen days when, if fertilization has occurred, the placenta is fully developed and takes over progesterone secretion.

As in all mammals the process of parturition in the rat is the result of the action of oxytocin on the myometrium of the uterus when it is sensitized by estrogen. Ganong (1987)reviewed that uterine contraction by oxytocin is facilitated by prostaglandins.

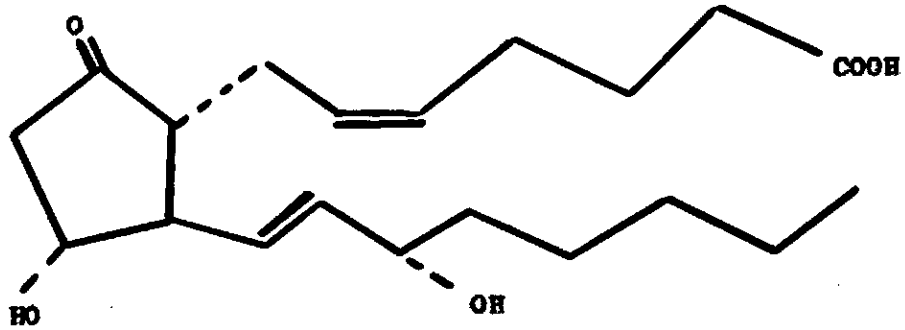
C. PROSTAGLANDINS AND THEIR GENERAL FUNCTIONS IN THE CONTROL OF REPRODUCTION

Prostaglandins (PGs) are biologically-active lipids that are believed to be synthesized in probably every tissue of the body, including the brain. They are a series of closely

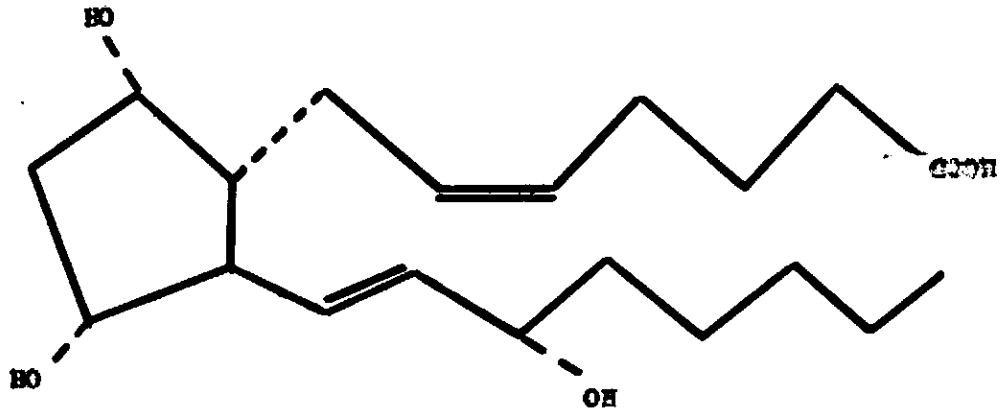
related 20-carbon unsaturated fatty acids containing a cyclopentane ring. PGs are divided into groups (E, F, etc) on the basis of the configuration of the cyclopentane ring. The number of double bonds in the side chains is indicated by subscript numbers (see fig.3) e.g PGE_2 is an E group PG with two double bonds. This number depends on which of the 3 precursor fatty acids has been utilized. Linoleic acid gives rise to PG_1 series, Arachidonic acid gives rise to PG_2 series and Pentanoic acid gives rise to PG_3 series.

C.1. Biosynthesis

PGs are synthesized from essential fatty acids which have been incorporated into the phospholipids of cell membranes (see fig.4). They are essentially local hormones acting at, or near, their site of synthesis, and are inactivated in the lungs during one circulation in the bloodstream. The family of PGs with the greatest biologic activity is that having two double bonds, derived fromarachidonic acid, which is also the main precursor of prostaglandins in humans. Arachidonic acid can be obtained from two sources - directly from the diet (from meats) or by formation from its precursor linoleic acid which is found in vegetables. As reviewed by Speroff et al (1984) the release of free arachidonic acid is the rate-limiting step in the formation of prostaglandins, and the prostaglandins which are of relevance to reproduction are PGE_2 and PGF_2, and possibly PGD_2 which are all derived from arachidonic acid.



PGE_2



PGF_2

Fig. 2. - STRUCTURE OF PGE_2 & PGF_2

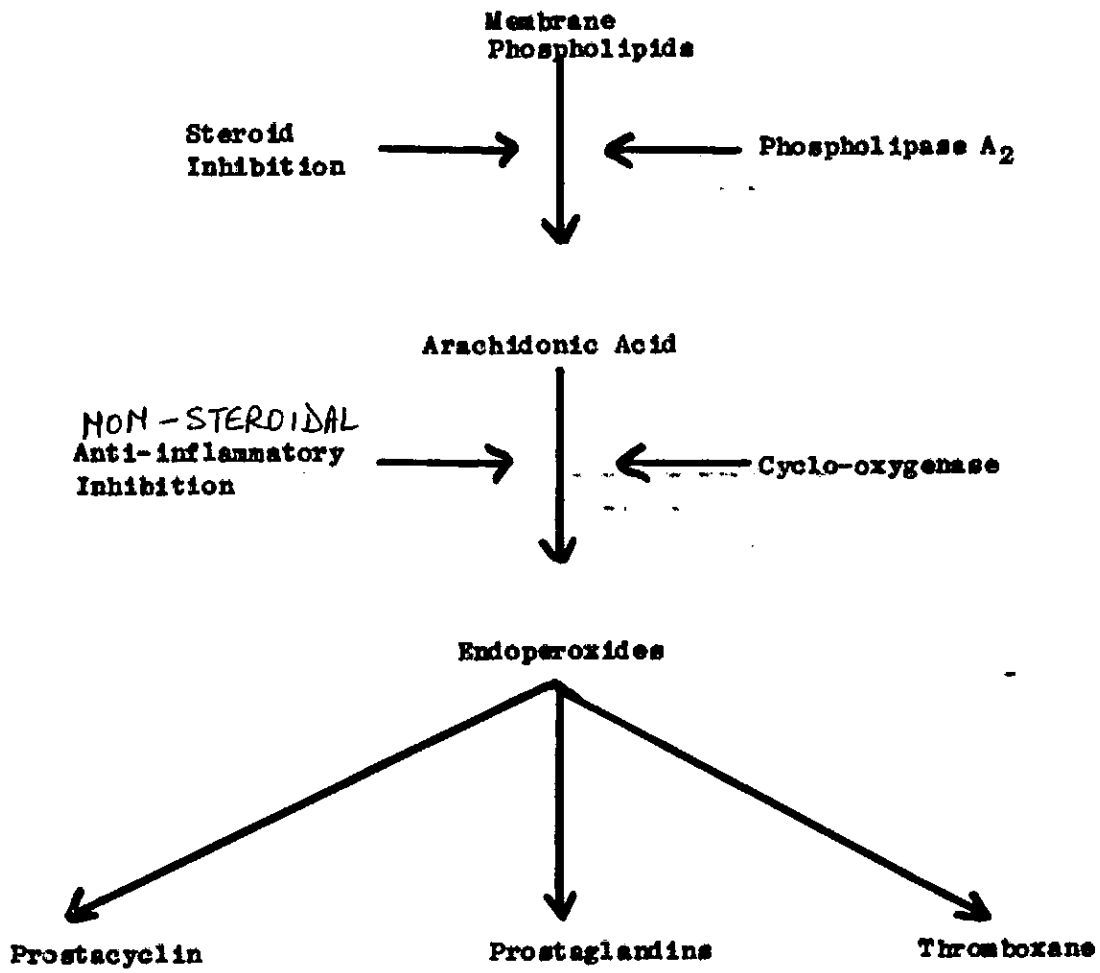


Fig. 3. - SCHEME OF PROSTAGLANDIN SYNTHESIS

Prostaglandins have attracted great attention because they have been found to have a wide variety of actions, even when administered in small doses. They seem to have some effect on almost all the systems of the body especially the gastro-intestinal system, reproduction and the endocrine system (Johnson & Everitt, 1984). Cyclic AMP is known to be involved in most if not all of these prostaglandin actions, and it has therefore been widely speculated that perhaps the various prostaglandins act to adjust the generation of cyclic AMP in response to various stimuli (Tsang et al, 1980; Armstrong, 1981).

C.2. Ovulation and Luteinization

Several workers (e.g Satoh et al., 1985) have found that there is a pre ovulatory increase in prostaglandins in the follicular fluid and ovarian venous blood indicating that PGs play important roles in the process of ovulation. Also ovulation is known to be initiated by an LHRH induced cyclicsurge of LH secretion from the pituitary. Likewise the synthesis of PGE and PGF compounds by ovarian tissue in several species is stimulated by LH and cyclic AMP. This field has been reviewed by other workers including Goldberg and Ramwell (1977), Johnson and Everitt (1984) and Rhodes et al (1985).

In non-primate mammals prostaglandins appear to have a mandatory role in the rupture of ovarian follicles although there is still some controversy as to whether this role is by a direct action or by modulating the actions of other

reproductive hormones. The involvement of prostaglandins in the regulation of ovarian follicular function was first postulated on the basis of the demonstration that inhibitors of prostaglandin synthesis (e.g. aspirin and indomethacin) were capable of blocking ovulation in rats. (Armstrong and Grinwich, 1972; Orczyk and Behrman, 1972; Lipner, 1988). These findings were soon confirmed in several other species including mice, goldfish and rabbits. In rabbits (Grinwich et al, 1972) and goldfish (Stacey and Pandey, 1975) the inhibitor was effective when applied locally to the follicle, indicating that the blockade was exerted directly upon the follicle rather than being mediated via some indirect mechanism, such as through the inhibition of gonadotropin secretion. Further evidence of a role of prostaglandins at the follicular level was provided by the findings that intra follicular levels of prostaglandins E and F increased markedly in several of these species shortlybefore ovulation (Ainsworth et al., 1975, 1979a). Indomethacin at dosages which prevented ovulation effectively prevented these increases in PG levels. It was also observed that injection of antiserum against prostaglandins blocked the LH-induced ovulation in estrous rabbits (Armstrong, 1981) and this added support to the concept of a role of PG$_s$ in ovulation.

It has been found that exogenous PGE$_2$ generally mimics the effect of LH on isolated follicles. Thus ovum maturation (Lindner et al, 1980) steroidogenesis (Holmes, 1983) and luteinization (Armstrong, 1981) increased

adenylate cyclase activity (Mason and Marsh, 1975) and many other changes in ovarian intermediary metabolism are stimulated by PGE_2 just like LH does. PGF_2 is generally less effective in mimicking the actions of LH in these processes. This implies that PGE_2 may be of greater importance than PGF_2. On the contrary Kabayashi et al (1981) observed that PGF_2 stimulated follicular maturation in the rabbit ovary whereas PGE_2 had little effect. Some earlier investigators (Channing, 1972; Kuehl et al., 1972) had suggested that the actions of LH were mediated via PGE_2, but later results suggest that LH and PGE_2 independently affect these processes via the adenylate cyclase system. The evidence for this includes blockade of ovarian prostaglandin synthesis by indomethacin which was found not to affect either the LH-induced ovum maturation or the increase in cyclic AMP concentrations(Lindner et al, 1980).

There is also the feeling that although LH and PGE act independently it is possible that prostaglandins may reinforce the actions of LH in these functions. LeMaire and Marsh (1975) studied follicles taken at intervals from hCG-treated rabbits and found that those follicles showing signs of ovulation had higher concentrations of PGE and PGF before ovulation compared with non-ovulating follicles. Moreover, cyclic AMP concentrations which may be expected to parallel increasing PGE and PGF concentrations did not increase as ovulation approached. Similarly LH loses its ability to stimulate cyclic AMP in this system as ovulation

approaches.

It is generally accepted that the process of follicular rupture requires the presence of prostaglandins, and here PGF_2 may be more important than PGE_2 as the latter has been found to actually reduce ovulation in the rabbit (Diaz-Infante et al, 1974). Several workers (e.g. Kobayashi, 1981) have found that the three processes of follicular ovulation can be brought about by exogenous prostaglandin in the absence of LH, yet only the final LH-response i.e follicular rupture, is consistently blocked by inhibition of prostaglandin synthesis.

In rats and many other laboratory animals, indomethacin or antiserum to PGF_2 given systemically or directly into the follicle inhibits ovulation, and this can be overcome by prostaglandins. Some studies (Tsafriri et al, 1973;Lindner et al, 1980) have indicated that PGE_2 can overcome the ovulation block caused by inhibition of the LH surge with pentobarbitone, and that in this situation the PGE_2 appeared to have a direct effect on the ovary in addition to stimulating pituitary LH release. Since PGE_2 was unable to effect ovulation in hypophysectomized rats (Sato et al, 1974) and LH cannot generally induce ovulation in rats treated chronically with indomethacin, it has been suggested that LH and PGE are both essential for ovulation, and that PGE may act in part by an effect on the hypothalamo-pituitary axis. Because PGF_2 is less effective than PGE_2 in stimulating hypothalamic gonadotropin-releasing hormone (LHRH) and subsequent

gonadotropin release it may not have this central component as part of its action.

In a review, Lindner et al (1980) indicated that administration of PGE_2 to phenobarbital-blocked rats will elicit an LH surge adequate to cause ovulation and that this response to LH is prevented by passive immunization against LHRH indicating that it depends on a PG effect on the hypothalamus. This view received further support from previous experiments showing that PGE_2 does not stimulate LH release from cultured hemipituitaries, and that indomethacin and aspirin fail to inhibit the LHRH induced LH release from hemipituitaries in vitro (Naor et al, 1975).

Prostaglandins have also been implicated in the process of luteinization. Armstrong (1981) reviewed that PGE_2 could induce morphological luteinization and stimulate progesterone secretion in cultured granulosa cells. He went further to say that the inhibitors of PG synthesis and action prevented LH-induced luteinization, and that this effect could be reversed by administration of exogenous PGE_2. Moreover PGE_2 levels remained elevated longer than PGF_2 levels after ovulation suggesting that while PGF_2 may be more important for follicular rupture PGE_2 may play a role in the luteinization process which normally follows ovulation.

To establish more firmly that prostaglandins play a physiologic role in the processes leading to follicular rupture, it had to be shown that they are formed in the follicle in response to the ovulatory surge of LH. This has

indeed been shown to be the case by earlier workers including Bauminger and Lindner (1975) who found that follicular prostaglandin content increases after exposure to LH after a lag of 2 to 4 hours and reaches a peak towards the time of ovulation with a marked (rat) or slight (rabbit) preponderance of PGE over PGF. This rise can be enhanced by the administration of LH in the morning of proestrus, or prevented by treatment with phenobarbital or anti-LH serum.

The synthesis of PG$_s$ in the ovarian follicle was also recently confirmed by Satoh et al (1985). These workers cultured immature rat ovarian follicles primed with 10 i.u of PMS for 48 hours and showed that exogenous hCG had dose-dependent stimulatory effects on the production of PG$_s$ but not that of progesterone. On the contrary inhibitors of protein synthesis like puromycin and actinomycin D inhibited production of prostaglandins as well as progesterone dose-dependently. This suggests that the action of LH on the matured follicle results in the stimulation of cyclooxygenase through activation of protein biosynthesis

Armstrong (1981) investigated prostaglandin production by the human follicle as well as the effects of prostaglandins on human follicular cells. His results indicated a significant production of PGF by cultured follicles when the gonadotropins HMG and hCG were added to the culture media. His investigations with isolated follicle cell types indicated that both the theca and granulosa cells have the ability to produce substantial

amounts of prostaglandins in culture.

C.3. Luteolysis

In most mammals (with the notable exception of primates) it is found that luteal life can be prolonged considerably by removing the uterus. If the endometrium of the excised uterus is homogenized and injected, then luteolysis does occur. These results led to the suggestion that a humoral factor passes from the endometrium to the ovary and causes luteolysis. The identity of the endometrial substance responsible has now been established as PGF_2 and it causes luteal regression in many species. Speroff et al(1984) reviewed the evidence that PGF_2 is the agent responsible for terminating the life span of the corpus luteum if fertilization fails to take place. The PGF_2 originates in the endometrium, and its synthesis is stimulated by the estrogen being produced in the growing follicles. According to these authors PGF_2 is transported directly to the corpus luteum through the vasculature connecting the ovary and the uterus, thus achieving an effective concentration at the corpus luteum and avoiding a systemic level with widespread actions.

The mechanism of PGF_2-induced luteolysis is two-fold : a rapid anti-LH action followed by a slower loss of LH receptors in the corpus luteum (Behrman, 1979). The rapid action is expressed only in intact cells and appears to be the result of some mediator that blocks LH receptor activation of adenylate cyclase. The slower response is an

indirect action, interfering with prolactin are necessary for maintenance of the corpus luteum species. Both LH and prolactin have been shown (Grinwich et al, 1976) to protect the early corpus luteum from PGF_{2} -induced luteolysis.

The control of luteolysis in primates does not involve uterine prostaglandins. The levels of PGs secreted do rise in the late luteal phase but neither hysterectomy nor antibodies to prostaglandins prolong luteal life. Moreover injections of prostaglandins are without effect on the corpus luteum unless very high doses are used and even them only a transient drop in progesterone output occurs (Johnsonand Everitt, 1984).

C.4. Implantation

The involvement of prostaglandins in implantation is poorly understood. Lau et al (1974) showed that Indomethacin treatment in early pregnancy prevented implantation, and that this could be overcome by administration of PGE_{2} or PGF_{2}. Moreover it was found that implantation that is blocked in ovariectomized mice could be overcome by hormone replacement with progesterone and estradiol but this reversal was prevented by Indomethacin (Saksena et al, 1974). As shown by several other workers, there is evidence to suggest an involvement of prostaglandins in implantation and subsequent fetal development. Phillips and Poyser (1980) showed that in the rat there is an increase in the synthesizing capacity of the uterus for PGE_{2} and PGF_{1} on day 5 of pregnancy,

suggesting a role for these PG$_s$ in the process of implantation. They also observed that implantation is delayed by treatment with Indomethacin (a further evidence for the role of PG$_s$ in implantation), and that Indomethacin also interferes with subsequent development of the blastocyst, again suggesting that PG$_s$ are important during the early stages of pregnancy.

C.5 Parturition

In as much as prostaglandins are necessary for implantation to occur they are also known to enhance uterine contractions in the latter stages of pregnancy. As reviewed by Johnson and Everitt (1984) it is now known that towards the end of pregnancy there is an increase in the estrogen/progesterone ratio and this stimulates the synthesis and release of PGF$_2$ in the uterus. PGF$_2$ is the activator of the mechanical events at parturition i.e myometrial contractions and cervical ripening. It is also suggested that oxytocin may further enhance the synthesis and release of PGF$_2$ as parturition proceeds. According to Speroff et al. (1984) the evidence for a role of prostaglandins in parturition includes the following:-

(a) prostaglandin levels in maternal blood and amniotic fluid increase in association with labour.

(b) arachidonic acid levels in the amniotic fluid also rise during labour.

(c) patients taking high doses of aspirin have a highly significant increase in the average length of gestation, incidence of post-maturity, and duration

of labour.

(d) Indomethacin prevents the normal onset of labour in monkeys, and stops premature labour in human pregnancies

(e) stimuli known to cause the release of prostaglandins (e.g cervical manipulation, rupture of membranes) augment or induce uterine contractions

(f) prostaglandins induce labour.

The presence of prostaglandins in uterine tissues of several laboratory animals (e.g rat, mouse, guinea pig, hamster) has been established, and intravenous administration of PGE_2 and PGF_2 is known to stimulate uterine activity in these animals. However, as reviewed by Labhsetwar (1975) the response of the uterus to PGs depends upon the steroidal environment to which it is exposed. For example under the influence of progesterone, isolated uterine segments of the guinea pig and rabbit are known to show decreased motility in response to prostaglandins. This author also observed that PGF_2 -induced parturition is associated with a rapid fall in peripheral plasma progesterone level. Another worker, Karim (1978), observed that drugs which inhibit prostaglandin synthesis (e.g aspirin, indomethacin and fenamic acids) can delay parturition in laboratory animals and women.

The response of the human uterus to prostaglandins is known to be dependent upon several factors. These include the type of prostaglandin used, route of administration,

whether the subject is pregnant or non-pregnant, and whether the studies are carried out in vitro or in vivo. PGE compounds are known to relax the non-pregnant human uterus but generally stimulate the pregnant uterus in vitro. Karim and Hillier (1975) showed that PGF compounds stimulate boththe non-pregnant and pregnant uterus in vitro and in vivo, while Klem et al (1982) successfully induced parturition in the mare with PGF_2

The route of administration of prostaglandins is known to affect uterine response. Toppozada et al (1977) found that the intact non-pregnant uterus is usually stimulated by PGE_2 when given intravenously. They also found that intra-uterine administration of PGE_2 during the secretory and proliferative phases of the uterine cycle causes uterine stimulation but at mid-cycle it inhibits uterine contraction. In contrast Martin et al (1978) found a marked decrease in sensitivity of the non-pregnant uterus to PGE_2 and PGF_2 at the time of ovulation, but did not observe an inhibitory response to PGE_2. They suggested that the lowest sensitvity to PGE_2 is correlated with the highest estrogen concentration.

According to Karim & Hiller (1979) PGE compounds always stimulate the activity of the pregnant human uterus in vivo and in vitro. The sensitivty of the pregnant uterus to PGE and PGF compounds increase with the progression of pregnancy although this increase in sensitivity is known to be slight. They reviewed that in lower animals uterine contractility is generally stimulated by the addition of

prostaglandin, and suggested that the effects of exogenous oxytocin in stimulating the rat uterus may be mediated by prostaglandins because this stimulation can be reduced byindomethacin in doses that do not inhibit prostaglandin-induced contractions.

D PROSTAGLANDIN INHIBITORS

The process of ovulation has been likened to that of inflammation. In fact Espey (1982) hypothesised that follicular rupture is brought about by an inflammatory reaction in mature follicles. In comparing ovulation and inflammation many workers have reported a number of similarities in the two processes e.g (a) the occurrence of vasodilatation and vascular permeability (Cherney et al, 1975; Lewis, 1977) (b) the presence of leucocytes and macrophages (Espey, 1974; Bonta and Parnham, 1978) (c) the synthesis of cAMP (Hunzicker-Dunn et al, 1979; Lamprecht et al, 1979; Lindgren et al, 1978) (d) the release of histamine (Wallach et al, 1978; Knox et al, 1979; Kitai et al 1985) (e) increased prostaglandin synthesis (Wallach et al, 1975; Tsang et al, 1979; Lewis, 1977).
It therefore stands to reason that an anti-inflammatory agent may be expected to be an anti-ovulatory agent as well as an inhibitor of prostaglandin synthesis.

According to Espey (1982) anti-inflammatory agents can be divided into a number of categories:-

(1) the steroidal anti-inflammatory agents e.g

dexamethasone, hydrocortisone, and other corticosteroids (2) the non-steroidal anti-inflammatory drugs e.g aspirin, indomethacin, diclofenac, flufenamic acid, naproxen and similar drugs

(3) the anti-neoplastic drugs with anti-inflammatory properties e.g colchicine and other immunosuppressants which commonly inhibit the metaphase stage of cell division.

(4) a wide variety of other agents that suppress inflammation to some extent e.g chloroquine (an antimalarial agent) and acetaminophen (an antipyretic agent).

As far as their effect on prostaglandin synthesis is concerned, the non-steroidal anti-inflammatory drugs are believed to act by inhibiting the biosynthesis of prostaglandins from arachidonic acid (see fig. 2), and this they probably do by inhibiting the enzyme cyclo-oxygenase at the nociceptor level (Girdwood, 1984). On the other hand cortico-steroids are believed to produce their anti-inflammatory and analgesic effects by interfering with the production of arachidonic acid from phospholipids by inhibiting phospholipase A$_2$.

Non-sterodial anti-inflammatory drugs may act by preventing prostaglandin from sensitizing pain receptors to other endogenous pain-producing substances, and since it is believed that prostaglandins acting on the brain may cause fever, this may explain why aspirin and indomethacin are antipyretics as well as analgesics (Girdwood, 1984). All the

above tend to suggest that one of the steps in the mechanism of ovulation is the inflammation process. However Epsey et al (1981) found that oedema and follicle enlargement (some normal preovulatory occurrences) occur when ovulation is blocked with indomethacin. They therefore concluded that the determinants of ovulation are neither oedema nor inflammation per se, but instead, synthetic mechanisms inherent to the preovulatory follicle and initiated by LH.

Aspirin is known to be an irreversible inhibitor which selectively acetylates the fatty acid dioxygenase involved in prostaglandin synthesis, while indomethacin is a reversible agent forming a reversible bond with the active site of the enzyme cyclo-oxygenase (Speroff et al, 1984). The analgesic, antipyretic and anti-inflammatory actions of these agents are mediated by inhibition of cyclooxggenase. While indomethacin is a very potent inhibitor of cyclooxygenase aspirin is only a mild inhibitor (Espey, 1983). Espey showed that indomethacin was more effective than aspirin in inhibiting ovulation in the rabbit, and the degree of inhibition of ovulation paralleled the degree of inhibition of prostaglandin synthesis. He suggested that prostaglandin inhibitors must completely abolish the preovulatory elevation of prostaglandins in mature follicles in order to totally inhibit ovulation. However, Satoh et al (1985) reasoned that since cyclooxygenase inhibitors like indomethacin block not only cyclooxygenase but also otherenzymes, their action in suppressing ovulation cannot be attributed solely to their inhibition of prostaglandin

production. They therefore compared the action of antiserum to cyclooxygenase with that of indomethacin. They found that administration of the antiserum inhibited ovulation with concommitant decrease in prostaglandin production. Conclusive proof was obtained by demonstrating that the ovulation that had been inhibited by suppression of prostaglandin production would be recovered by administration of prostaglandins. In an earlier work Espey (1982) revealed that indomethacin (and other non-steroidal anti-inflammatory agents) can interrupt the ovulatory process even after the follicle has begun producing substantial amounts of prostaglandins. This suggests that prostaglandins need to be produced continuously in the follicle up to the time of actual rupture.

E SUPEROVULATION

Superovulation is the process of inducing ovulation by stimulating the ovaries such that there is enhanced follicular growth and maturation and subsequent increase in the normal ovulation rate. According to Greenwald and Terranova (1988) various methods have been used for superovulation and they all act by affecting either exogenous or endogenous levels of gonadotropins. These workers reviewed three mechanisms of action which have been suggested for the effects of gonadotropins on follicular development and these are as follows:- (a) follicles already undergoing early atresia are presumably "rescued" as a result of vigorous mitotic activity in

agranulosa and/or thecal compartments (b) smaller healthy follicles are recruited into a more active growth phase, and (c) the rate of follicular atresia is reduced.

In laboratory animals and large domestic species superovulation is usually induced by injection of Pregnant Mare's Serum gonadotropin (PMSG), followed by Human Chorionic Gonadotropin (hCG), and it yields about 3 to 4 times the number of ova shed during spontaneous ovulation (Cole, 1975). PMSG is structurally similar to hCG (Moore et al, 1980) and is known to function almost exclusively as an LH-like hormone in the mare and the stallion but in other species like the rodents, however, PMSG has both FSH and LH like activity (Stewart and Allen, 1979; Licht et al, 1979) and it promotes follicular growth. hCG is biologically similar to LH and therefore simulates the mid-cycle LH ovulatory surge, causing final follicular maturation and ovulation.

In humans superovulation is achieved by giving a combination of Human Menopausal Gonadotropins (HMG) and hCG, and the system serves as a very important step in the process of In-Vitro Fertilization and Embryo Transfer which is seen by many as a last step in the treatment of infertility. It is also used to treat anovulation in women. HMG is a purified preparation of gonadotropins FSHand LH, extracted from the urine of post-menopausal women. It stimulates follicular growth and maturation.

The interval between the injection of hCG and ovulation varies according to species. In rats and mice ovulation

occurs about 12 hours after hCG injection while in humans ovulation occurs about 36 hours after hCG injection.

According to Speroff et al, (1984) the events which occur in the ovary finally culminating in spontaneous ovulation or superovulation are the results of hypothalamic-pituitary-gonadal interactions. The hypothalamic releasing hormone, LHRH stimulates the anterior pituitary causing three positive actions (a) synthesis and storage of gonadotropins LH and FSH (b) activation and movement of stored gonadotropins for direct secretion and (c) immediate release of gonadotropins. FSH in turn is known to stimulate follicular growth within the ovary along with follicular estrogen secretion while LH is responsible for the final maturation of ova within the follicles, and ovulation. The interplay among these substance is governed by feedback effects, both positive stimulatory and negative inhibitory. Workers like Satoh et al (1985) have found that PGs of the E and F series increase markedly in the preovulatory follicular fluid reaching a peak concentration at ovulation, and that inhibition of PG synthesis blocks follicular rupture. These authors have shown that the mechanism by which prostaglandins induce follicular rupture is by acting on the prostaglandin receptors and activatingproteolytic enzymes like plasmin to rupture the apex of the follicle thus discharging the oocyte.

Because of the complex interaction of the hypothalamus-pituitary-gonadal axis and the implication of PGs in the ovulation process it might be a worthwhile effort

to investigate further on the mechanisms by which PGs affect ovulation by looking at the interactions between PG inhibitors and the hypothalamic-pituitary-gonadal secretions. This will help in determining the level at which prostaglandins exert their effect.

F PURPOSE OF STUDY

The aims of the present study were:-

(1) to determine the effect of two widely used prostaglandin inhibitors Aspirin and Indomethacin on (a) ovulation (b) superovulation, and (c) implantation, length of gestation, and number of offsprings delivered at the end of gestation.

(2) to determine the effect of Human Chorionic Gonadotrophin (hCG) and LH on the action of Aspirin and Indomethacin on ovulation and PG secretion

(3) to carry out morphometric studies on the ovaries of rats which have been subjected to the above treatments.

Results from 2 and 3 will help to establish the site of action of the inhibitors, and suggest probable mechanisms of action.

Investigations on the inhibitors of prostaglandin synthesis cannot be overemphasised because of the importance of prostaglandins to fertility, and especially in the treatment of infertility including early trimester abortion. Knowledge in this area will therefore help in the control of fertility.

CHAPTER TWO

RESEARCH METHODS

Animals

All experiments were conducted on female Sprague-Dawley rats (in-bred at the animal house of the College of Medicine, University of Lagos). The rats were 12-20 weeks old and weighed between 120 and 200gms. They were fed with rat pellets (Pfizer Nig. Ltd), and water ad libitum, and were kept in a temperature-controlled ($27^{o}c$-$30^{o}c$) and light-controlled room with 12 hours lighting (from 07.00 hrs.-19.00hrs.)and 12 hours darkness. This was to ensure proper and regular cycling. The rats were kept for 14 or more days before the estrous cycle was monitored by daily vaginal lavage. This way the rats were allowed to adapt to their new environment, a factor which also prevents irregular cycling.

A daily (morning) lavage was done to determine the epithelial cells predominantly present in the vaginal lavage. The predominant presence of nucleated cells is an indication of the proestrus stage, cornified cells indicate estrus (ovulation having occurred) while the predominant presence of leucocytes with or without epithelial cells signifies the metestrus/diestrus stages. Only rats which exhibited two or more consecutive normal 4-day estrous cycles were used. The male rats which were used to mate the female rats were also in-bred Sprague-Dawley species of proven fertility.

LH - induced ovulation (Superovulation)

Rats were superovulated by the injection (intra-peritoneal) of 10 I.U PMSG (Sigma Chemical Co., U.S.A) in the afternoon of diestrus followed by 10 I.U hCG (Sigma Chemical Co., U.S.A) 48 hours later, as described by Quinn and Harlow, 1978.

Ova Recovery

Ova were recovered from female rats by sacrificing them about 12 hours after ovulation, and then removing both oviducts into petri dishes containing normal saline solution. The ampullae of the oviducts were punctured with a 26 - gauge disposable needle while the rest of the oviduct was teased out with the needle (Hoppe & Pitts, 1973). This resulted in the release of ova surrounded by cumulus cells. The ova were pipetted into a clean petri dish, counted and examined properly. The whole procedure was carried out under a stereoscopic dissecting microscope at a total magnification of x20.

According to Biggers et al (1971) and Roblero and Riffo (1986) ovulation occurs about 12 hours after hCG treatment, and in rats and nice the ova move into the oviduct about 4 hours later (i.e. 16 hours after hCG treatment) where they remain for at least 24 hours before moving into the uterine horns (fig 1). Therefore in the present study ova recovery in superovulated rats was done by sacrificing the rats 20 hours after hCG injection.

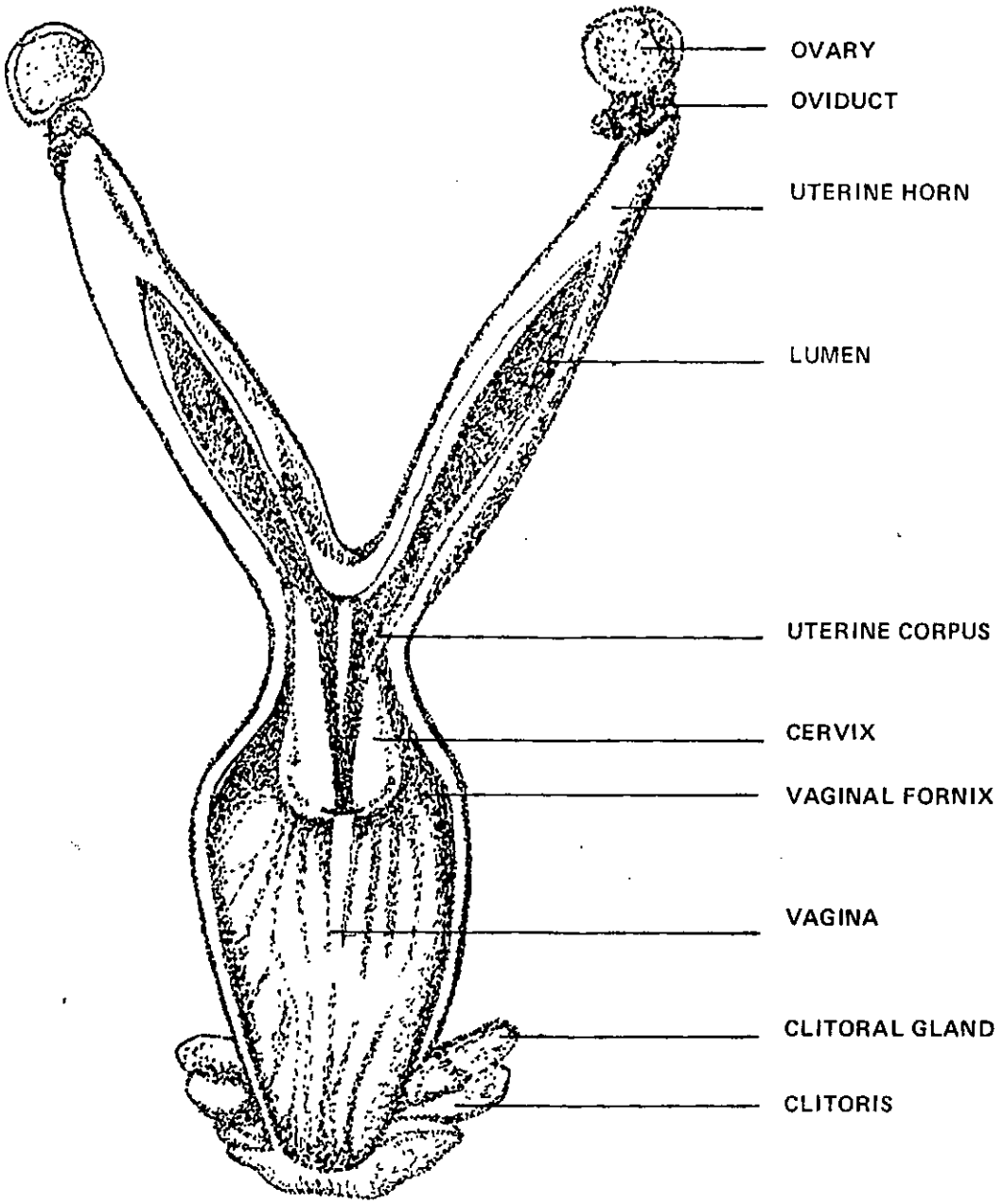


FIG. 1 - Drawing of the female genital system in the rat. (x 20; after Daniel, 1978).

In spontaneously ovulating rats, ova recovery was done by sacrificing the rats in the afternoon of estrus whereafter the oviducts were removed and then recovered by teasing out.

Radioimmunoassay

Radioimmunoassay of serum FSH and LH was performed according to instructions contained in the Amerlex - M FSH and LH radioimmunoassay kits which were purchased from Amersham International (U.K).

The radioimmunoassay method depends on the competition between FSH/LH in serum and ^{125}I-labelled FSH/LH for a limited number of binding sites on an FSH/LH specific antibody. The proportion of the ^{125}I-labelled FSH/LH bound to the antibody is inversely related to the concentration of unlabelled FSH/LH present in the sample.

Standard FSH/LH samples (containing known amounts of FSH/LH) and the unknown serum samples were mixed thoroughly with the corresponding anti-serum (anti- FSH serum / anti-LH serum) and the mixture was incubated at 37 ^{o}c for 1 hour. The ^{125}I-labelled FSH/LH was then added, and the mixture was then incubated at 28 ^{o}c for 2 hours. The antibody bound FSH/LH was reacted with the Amerlex - M second antibody reagent and the mixture was centrifuged at 8,000 r.p.m. for 15 minutes. Following decantation of the supernatant the proportion of ^{125}I-labelled FSH/LH bound in the presence of reference standard solution was determined by counting the amount of radioactive compound,

and a graph of ^{125}I counts against FSH/LH concentrations was plotted. The concentration of FSH/LH present in the unknown samples was then interpolated from the graph.

Serum FSH/LH is expressed in terms of mI.U/ml. Measurements were done in duplicates and the results were averaged. Radioactivity was determined by using a gamma scintillation counter (mini- assay type 6-20, mini - instruments Ltd. Essex, England).

Bioassay

A bioassay of plasma prostaglandin was performed according to the method of Miller et al, 1984. This was done by recording the contraction of the rat stomach strip when suspended in the different plasma samples. The rat stomach strip contracts in response to PGI2 as well as to PGE_2 and $PGF_{2\alpha}$ (Omini et al, 1977) and therefore reflects changes in the concentration of these prostaglandins. This musculotropic activity was quantified as $PGF_{2\alpha}$ equivalents by finding the experimental response of the rat stomach strip to standard concentrations of $PGF_{2\alpha}$ (fig 2). Contractions were measured with a Harvard isotonic transducer (type FT 10) and recorded on a Grass Model 7D polygraph (Quincy , M.A, U.S.A). The magnitude of contraction of the stomach strip when suspended in a plasma sample was thus used to determine the concentration of $PGF_{2\alpha}$ in the particular plasma sample (fig 2).

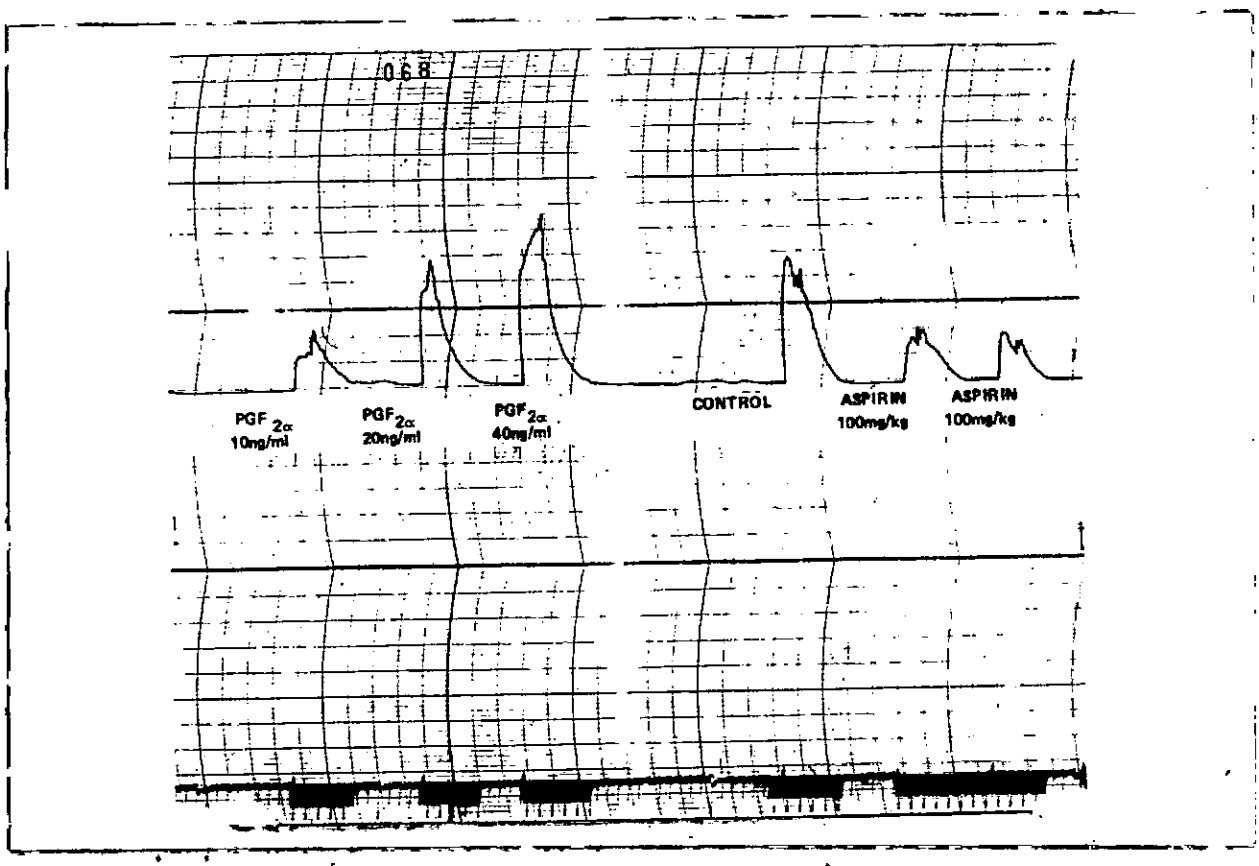
Prior to recording a calibration of the transducer was done by attaching known weights of 500 mg and recording the amplitude of the deflection.

The rat stomach strip was inserted into the inner chamber of the organ bath apparatus with tyrode solution in it. The polygraph was then left to equilibrate with tissue for about 1 hour before recordings were made.

Morphometric Studies

Morphometric studies on the ovary were carried out as described by Fagbohun et al (1987). Ovaries from control and treated rats were removed immediately after cervical dislocation and fixed in Bouin's fluid, a fixative that is known to keep well, penetrate rapidly and evenly, and which causes little shrinkage of the specimen (Culling, 1974). The ovaries were then dehydrated in ascending grades of ethanol (70%, 80%, 90% and 100%). Thereafter they were cleared in chloroform. This was followed by tissue infiltration in molten paraffin wax in the oven at 60 ^{o}C, and then embedding and blocking out the tissues in molten paraffin wax. The tissue blocks were trimmed and fixed onto wooden block for sectioning.

Serial sections of the ovaries were cut at 10 micrometer (um) thickness using a microtome (Leitz Wetzlar Minot - Mikroton Type 1212). Every 5th section was mounted on slides and dried on a hot plate. The day mounted sections were then deparaffined by passing them through xylene, and rehydrated in descending grades of ethanol (100%, 90%, 80%,



CONTROL - Plasma sample from untreated rats.

ASPIRIN (100mg/kg) - Plasma sample from rats treated with 100mg/kg Aspirin.

FIG. 4 - Pen deflection of standard $PGF_{2\alpha}$ solutions and some unknown plasma samples during PGF bioassay.

The mounted sections were stained in haematoxylin, rinsed off with distilled water and then differentiated in acid alcohol. They were then counterstained in eosin, rinsed in distilled water, finally dehyrated in ascending grades of ethanol (70%, 80%, 90% and 100%) and cleared by passing them through xylol, whereafter they were mounted by inverting each slide ovar a drop of xylene placed on a cover slip. The stained sections were air dried and kept for light microscopic examination.

The stained sections were observed under the light microscope at a total magnification of x100 for the number of follicles and more importantly the type of follicles (fig .....) present in the sections. Both the atretic and non-atretic follicles were counted. The criteria used for the determination of atresia includes granulosa cell degeneration, divided and fragmented ovum, collapsed or absent zona pellucida. The non - atretic follicle were futher classified into preantral, antral and preovulatory follicles (Ross and Vande Wiele, 1981; Speroff et al, 1982). In addition corpora lutea in each section were also counted.

Drugs and Injections

(1) The anti-inflammatory prostaglandin inhibitors used were Aspirin,acetyl salicylic acid, (Aspegic, Laboratoires Synthelabo, France) and 2) Indomethacin - (1-p-chlorobenzoyl - 5 - methoxy - 2 - methylindol - 3 - acetic acid), Sigma chemical Company, U.S.A.

FIG. 5 - Photomicrograph showing a preantral follicle (arrowed) **(x 400)** within the rat ovary.

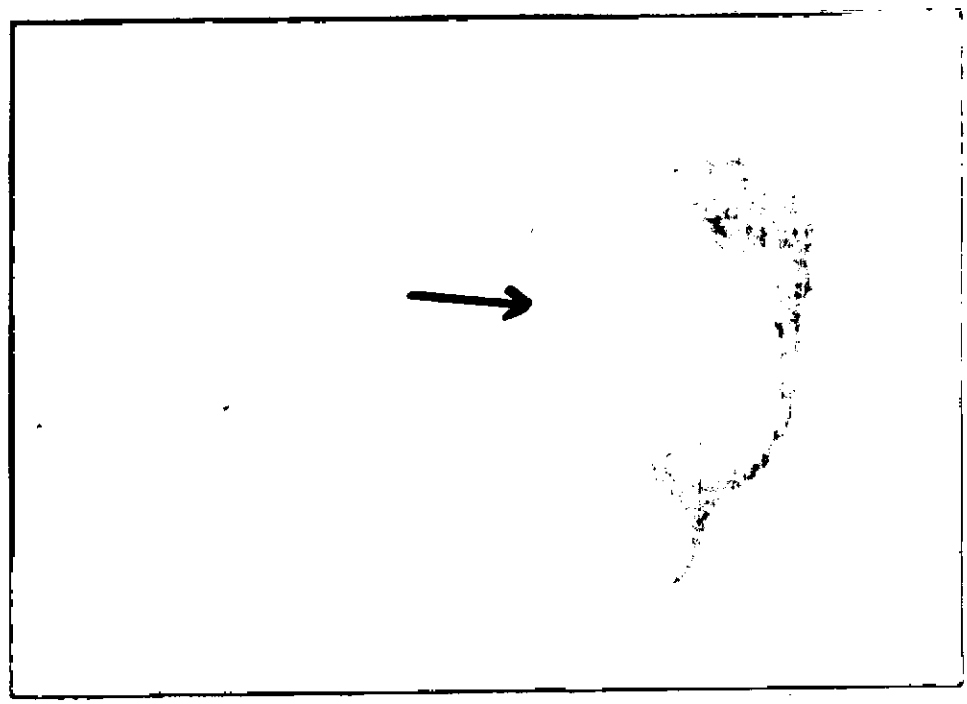


FIG. 6 - Photomicrograph showing an antral follicle (arrowed) (x 400) within the rat ovary.

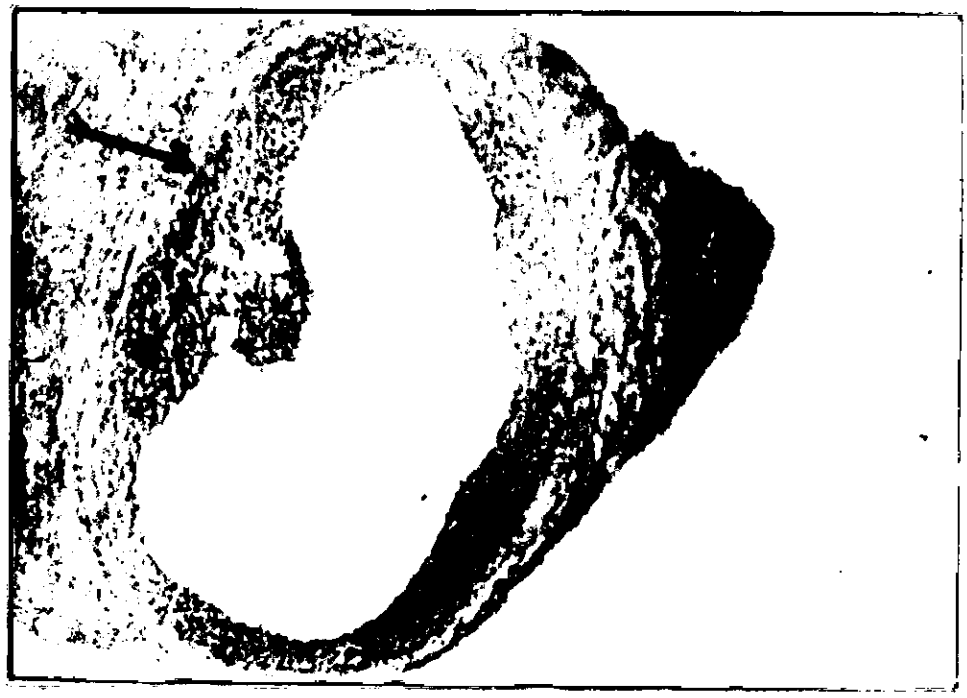


FIG. 7 - Photomicrograph showing a preovulatory follicle (arrowe
(x 400) within the rat ovary.

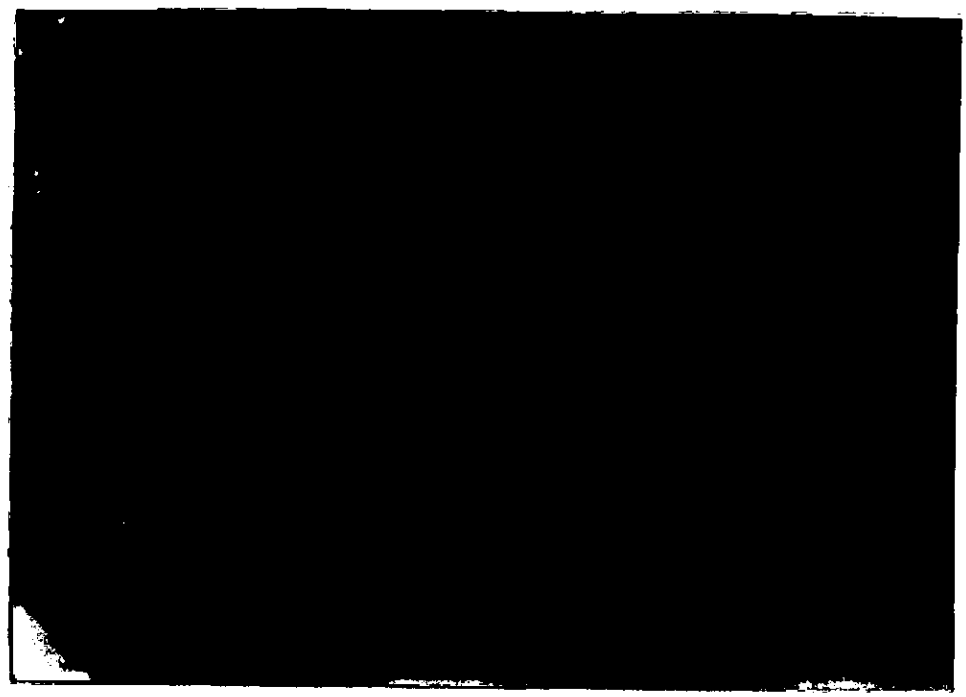


FIG. 8 - Photomicrograph showing an atretic follicle (arrowed) **(x 400)** within the rat ovary. Note the degeneration of ovum and granulosa cells.

Other drugs used were

(a) Pregnant Mare Serum Gonadotropin, PMSG (Sigma Chemical Company, U.S.A.)

(b) Human Chorionic Gonadotropin, hCG (Sigma Chemical Company, U.S.A

(c) PGF_2 (Sigma Chemical Company, U.S.A)

(d) Rat LH (National Institute of Arthritis and Metabolic Diseases, NIAMD, - rat LH - I - 4, U.S.A).

All the drugs were prepared by reconstituting with sterile normal saline (0.9% Nacl) which served as the drug vehicle. $PGF_{2\alpha}$ was reconstituted with methanol to ensure stability.

Aspirin was used at a dose of 100mg/kg body weight while indomethacin was used at a dose of 50 mg/kg body weight. These doses were observed to inhibit ovulation during preliminary experiments. According to Girdwood (1984), The anti-inflammatory dose of Aspirin in humans is about 20 mg/kg body wt (400 mg per tablet) while the anti-inflammatory dose of indomethacin is about 1 mg/kg body weight (50 mg per capsule). All injections were given intraperitoneally with a 26-gauge needle, and in the case of indomethacin a $23\frac{1}{2}$ - gauge needle was used Control rats were injected with the drug vehicle only.

Mating technique

To achieve pregnancy in the rats, proestrus rats were caged overnight with males of proven fertility. As much as possible only i female was caged with one male (but

sometimes 2 females were caged with 2 males) to enhance the chances of mating.

Rats are known to be nocturnal breeders and heat normally begins in the evening of proestrus and lasts for about 12 hours (Biggers et al, 1978). During this period the female is quite receptive to the male. ovulation is known to occur between 00:00 hrs and 03:00 hours of the following day, and this falls during the period of heat.

Successful mating was confirmed the following morning by the presence of a copulation plug in the Vagina (day 0 of pregnancy in spontaneous ovulation/day 1 of pregnancy in superovulation) and by a vaginal lavage which revealed the presence of sperms. The copulation plug is formed by a mixture of the secretions of the vesicular and coagulating glands of the male, and it usually fills the vagina from cervix to vulva (Biggers et al, 1971). From about day 3 of pregnancy progesterone secretion causes the vaginal lavage to show a typical diestrus or luteal picture of leucocyte invasion, and all cycling is abolished. The pregnant rats were therefore monitored by performing a daily vaginal lavage to ensure the abolition of cycling. Any rats which resumed cycling was taken to have had a resorption of embryos, and were not used in the experiments.

Sometimes there was the need to get several rats pregnant at the same time or to get them to be at the same phase of the estrous cycle. Since this is difficult to achieve naturally, the cycles of the different rats, were synchronized using a method described by Whittingham

(1979). This procedure involved the injection of several female rats with low doses of gonadotrophins. The rats were injected with 2 I.U PMSG and 48 hours later with 2 I.U hCG. About 80% of the rats were found to be in proestrus on the day of hCG injection.

Photomicrography

Photomicrographs shown in this study were taken using the Zeiss universal research microscope combined with an MC 63 photomicrographic camera at a magnification of × 400.

Statistical Analysis

Statistical comparison of data between different test groups were performed on the IBM Personal Computer using the Student's t - test.The t-test is normally performed to show whether the difference between two means is significant or is just due to chance. Significance was tested at the 95% confidence level and a P value < 0.05 was therefore considered significant.

Results are expressed as means ± standard error of mean ($\bar{x} \pm S.E$).

One way analysis of variance (ANOVA) was also performed on the IBM Personal Computer to compare data between several different experimental groups.

The ANOVA test was done to compare means from more than two experimental samples i.e as an extension of the t-test to accomodate more than just two groups.

CHAPTER THREE

EFFECT OF ASPIRIN AND INDOMETHACIN ON SPONTANEOUS OVULATION

Experiments were carried out to see if Aspirin and Indomethacin had any inhibitory effects on spontaneous ovulation.

Proestrus rats (n = 30) were used for these experiments. The rats were divided into three groups A, B, C. They were treated at 3.00 p.m. in the afternoon of proestrus.

Group A rats were injected with Aspirin (100 mg/kg). Group B rats were injected with Indomethacin (50 mg/kg) while Group C rats served as the control and were injected with the drug vehicle (sterile normal saline) only.

4 rats from each group were sacrificed by cervical dislocation at 6.00 p.m. on the day of proestrus. Blood was immediately collected through a cardiac puncture, and serum from the blood was stored at -20 C for subsequent hormone (LH and FSH) and prostaglandin assay.

The following morning all the remaining rats whose vaginal smears consisted of cornified cells were assumed to have ovulated. They were sacrificed by cervical dislocation and blood was again immediately collected by cardiac puncture for hormone assay. The ovaries were excised and fixed in Bouin's solution while the oviducts were teased out and observed for ova.

RESULTS

Ovulation

The pattern of spontaneous ovulation when Aspirin and Indomethacin are injected is shown in Table 1 and Figure.10.

Both Aspirin and Indomethacin significantly ($P<0.05$) reduced the rate of ovulation as well as the number of ova shed by the ovulating rats. The effect of Indomethacin was more remarkable in that it did not only suppress ovulation more effectively than Aspirin, it did so at a much lower dose (Indomethacin 50 mg/kg, Aspirin 100 mg/kg). Many of the ova shed during Aspirin treatment were observed to be immature (fig. 15)

PG level

Measurement of preovulatory levels of plasma PGF_2 in these rats revealed that PG level is significantly reduced ($P<0.05$) with Aspirin administration and further reduced with Indomethacin administration (table 2).

LH & FSH

Preovulatory plasma LH levels did not change with Aspirin or Indomethacin treatment (table 3) at doses which normally inhibit ovulation i.e 100 mg/kg and 50 mg/kg respectively.

In preliminary experiments post-ovulatory plasma FSH was the same in control and aspirin-treated rats, and it was not significantly different in control and indomethacin - treated rats. Post-ovulatory plasma LH was low in both

control and experimental rats. These results confirm that FSH and LH concentrations return to basal levels after ovulation has occurred, and that FSH and LH levels are basal where ovulation did not occur.

Morphometry

Morphometric studies showed that the ovaries of aspirin-treated rats and control rats contained similar numbers of the same types of follicles, including the corpus luteum In the indomethacin - treated ovaries it was seen that apart from the fact that many of the follicles were atretic the number of preovulatory follicles and corpora lutea were significantly reduced ($P<0.05$) (fig.12).

DISCUSSION

The results of the present experiments clearly indicate that Aspirin and Indomethacin (both anti-inflammatory drugs) when given in anti-inflammatory doses can inhibit the process of ovulation, with indomethacin being a stronger inhibitor (fig.10). The two drugs are known to be inhibitors of prostaglandin synthetase (Espey, 1982; Chatterjee and Chatterjee, 1982), therefore their inhibition of ovulation implies a strong role for prostaglandins in the process of ovulation.

The effect of indomethacin on ovulation and its mechanism of action have been studied by several workers like Tsafriri et al (1972, 1973) who administered indomethacin into rats in the afternoon of proestrus and

TABLE 1

EFFECT OF ASPIRIN AND INDOMETHACIN ON SPONTANEOUS OVULATION

	Treatment	Proportion of Rats Ovulating	Mean No. of Ova Shed/ovulating rat
A	Aspirin (100mg/kg)	4/6	2.1 ± 0.9
B	Indomethacin (50mg/kg)	2/6	0.5 ± 0.2
C	- (control)	6/6	4.5 ± 0.6

Means of control and aspirin groups are significantly different ($P < 0.05$)

Means of control and indomethacin groups are significantly different ($P < 0.001$)

ANOVA - means of samples are significantly different.

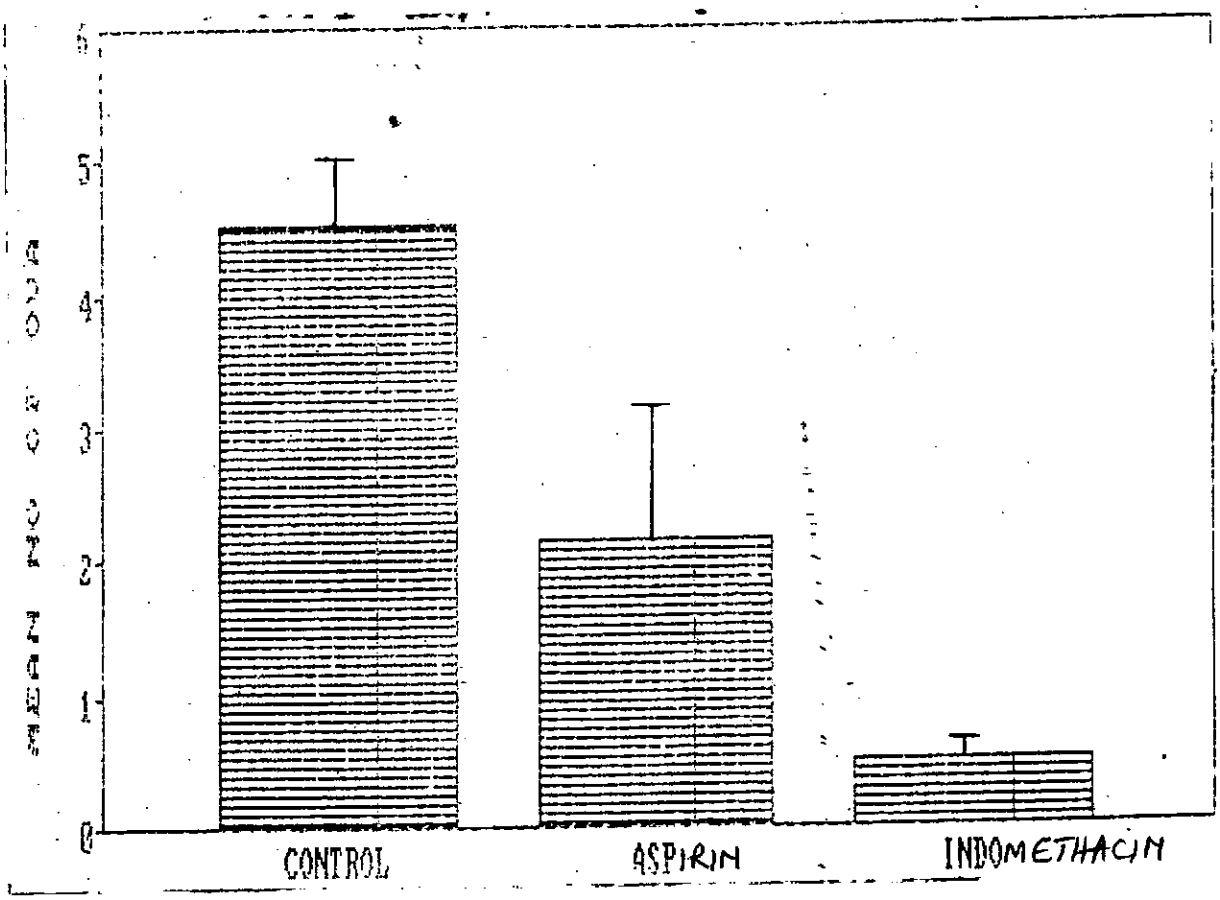


FIG. 10 - Effect of Aspirin and Indomethacin on spontaneous ovulation.

T A B L E 2

EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY $PGF_{2\alpha}$ DURING SPONTANEOUS OVULATION

	Treatment	Mean Plasma $PGF_{2\alpha}$ (ng/ml)
A	Aspirin (100mg/kg)	18.58 ± 2.2
B	Indomethacin (50mg/kg)	4.54 ± 0.5
C	- (Control)	38.06 ± 0.5

Means of control and treatment groups are significantly different ($P < 0.001$)

ANOVA - means of samples are significantly different

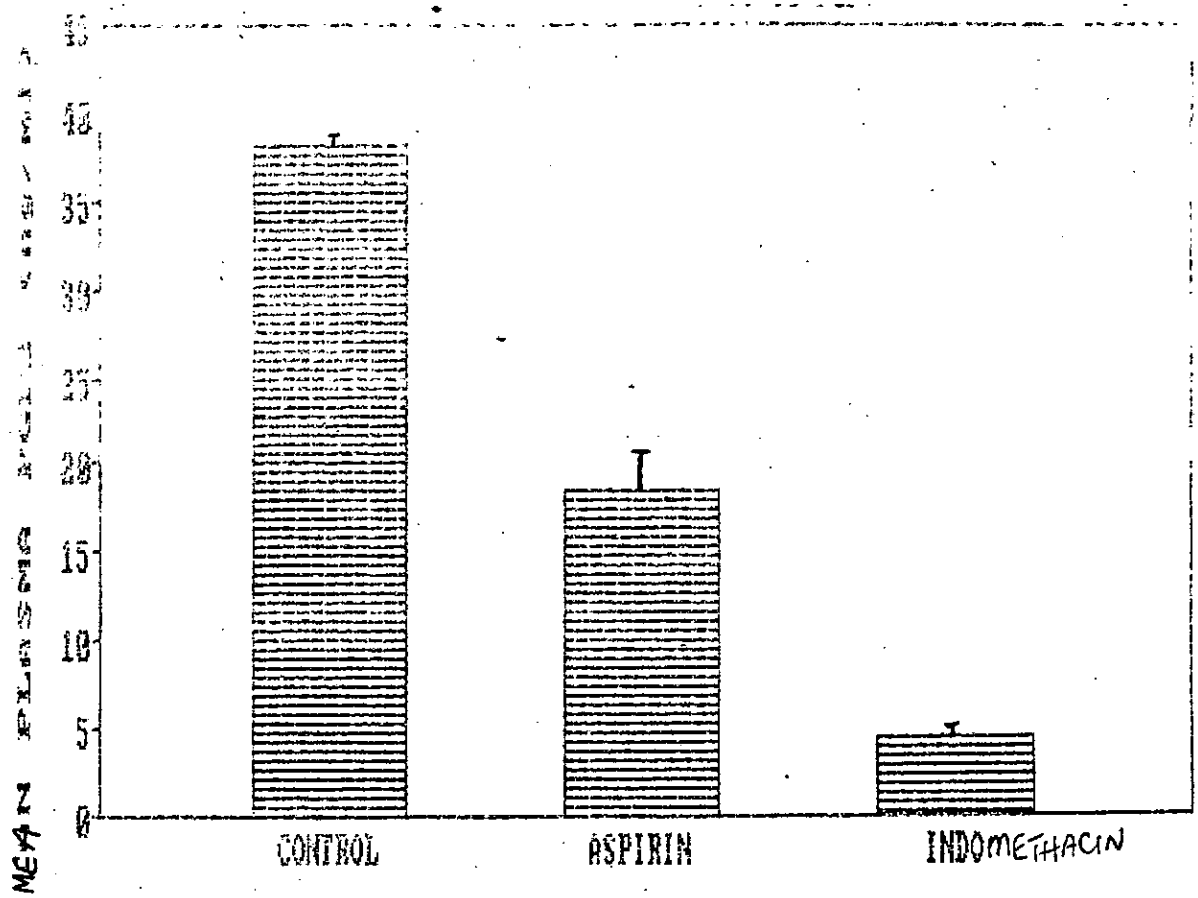


FIG. 11 - Effect of Aspirin and Indomethacin on pre-ovulatory PGF_2 during spontaneous ovulation.

TABLE 3

EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY LH LEVEL DURING SPONTANEOUS OVULATION

	Treatment	Mean Plasma LH (mI.U/ML)
A	Aspirin (100mg/kg)	11.00 ± 0.45
B	Indomethacin (50mg/kg)	10.75 ± 0.52
C	- (Control)	9.25 ± 0.63

No significant different between means of control and treatment groups ($P > 0.05$)

ANOVA - means of samples are significantly different

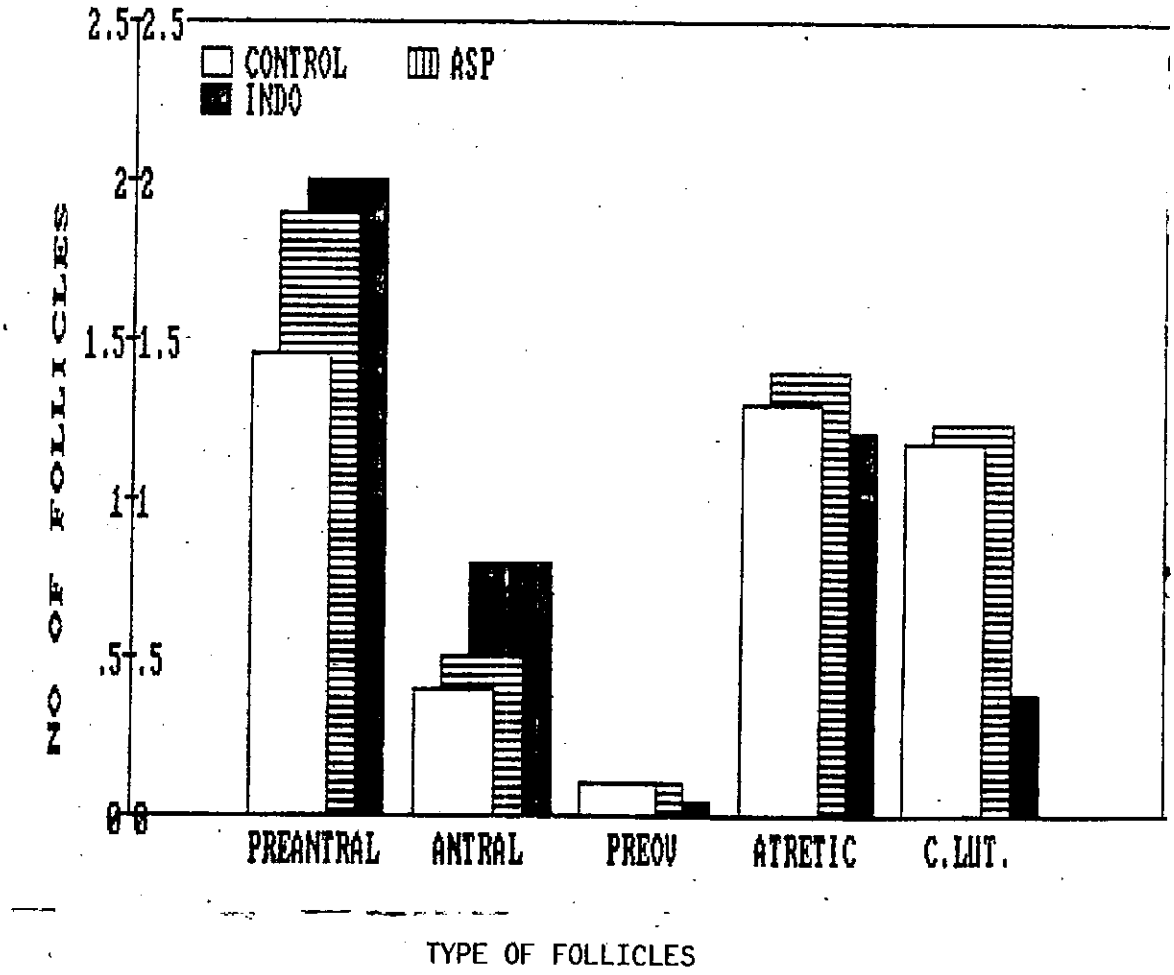


FIG. 12 - Effect of Aspirin and Indomethacin on follicular development during spontaneous ovulation.

found that it prevented follicular rupture in about 90% of the animals. Because LH administration did not overcome the indomethacin block of ovulation they concluded that indomethacin does not block LH release but exerts its anti-ovulatory action directly on the follicle. Likewise Espey (1982) report that indomethacin inhibits ovulation by interfering with the synthesis of prostaglandins in preovulatory follicles. He showed that indomethacin can inhibit ovulation when administered even as late as 1 hour before the expected time of follicular rupture. Indomethacin has also been found to block ovulation in perfused rabbit ovaries (Hamada at al, 1978; Holmes et al, 1983). Based on this observation there is a strong indication of an ovarian level of action for indomethacin since the experiments were carried out on isolated perfused ovaries.

In the present work aspirin was observed to cause only partial inhibition of ovulation and many of the ova shed were immature. This observation is in line with the work of Espey (1983) who found that aspirin when administered 8 hours after the ovulatory process was stimulated by hCG did not inhibit ovulation.

Relatively few workers have investigated the effect of aspirin on ovulation. Such workers include Orczyk and Behrman (1972) who found that both aspirin and indomethacin blocked ovulation in the rat, and that indomethacin was effective at 1/30 the dose of aspirin. These workers suggested that the site of the blocking action of aspirin

and indomethacin may be either at the hypothalamic or/and pituitary level. However Behrman et al (1972) later reported that aspirin blocks ovulation at the hypothalamic level since the block was consistently reversed by LH and LHRH administration.

It is thus apparent that the site of action of the two drugs, indomethacin and aspirin, seems to be controversial with some workers suggesting an ovarian level of action while others advocate a hypothalamic - pituitary level.

Results of the present work suggest that aspirin and indomethacin may act by reducing the secretion of PGF_2 (fig. 10) and indomethacin is seen to be much more effective in this action. The two drugs are also seen to have no effect on plasma LH level (table 3) which seems to imply that the action of aspirin and indomethacin is really at the ovarian level and not on the hypothalamic - pituitary axis. This is to say that the two drugs act by suppressing PGF_2 synthesis by the ovarian follicles. The fact that aspirin significantly reduced plasma $PG_{2\alpha}$ implies that a reduction in PGF_2 level must occur for ovulation to be at least partially blocked. Indomethacin caused further reduction of plasma PGF_2, an indication that PGF_2 level must be very low for complete blockade of ovulation to occur. This shows that ovulation is not an "all - or - none" phenomenon in the sense that even when conditions are not conducive ovulation is not necessarily completely abolished. Depending on the circumstances ovulation rate may be merely reduced. This finding is in line with the

work of Behrman et al (1972) who also reported a fall in peripheral PGF_2 after aspirin and indomethacin administration. It also agrees with the finding of Espey et al (1986) who reported a significantly reduced follicular prostaglandin production with indomethacin administration whether given early or late during the ovulation process in spite of the fact that there was no significant correlation between follicular prostaglandin levels and ovulation rate i.e different levels of prostaglandin were sometimes observed to give the same rate of ovulation.

Chatterjee and Chatterjee (1982) also found that indomethacin blocks ovulation in rats, and that the blockade was almost completely abolished when PGF_2 was given concurrently with indomethacin indicating that indomethacin acts by inhibiting the synthesis of prostaglandins. In like manner Holmes et al (1983) reported an indomethacin - blocked ovulation which was reversed when PGF_2 was added. These findings support the observations in the present study. The finding in the present study that indomethacin and aspirin did not affect the preovulatory LH level suggests that their action is at the ovarian level not at the hypothalamic or pituitary level. It therefore becomes doubtful that prostaglandins are essential for the action of LHRH or LH release as suggested by earlier workers like Makino (1973) and Labhsetwar (1973). Also since it has been established that prostaglandins are formed in the rat ovarian follicle in response to the ovulatory LH surge (Lindner et al, 1974 Bauminger and Lindner, 1975; Hamada et

al, 1978), the present study shows that while not disturbing the LH surge indomethacin and aspirin prevent the follicle from responding to any stimulation by LH to form prostaglandins, or the response is at least reduced.

Contrary to the report of Lindner et al (1980) the present study does not indicate an effective blockade of ovulation by aspirin, and many of the ova shed in these instances had not undergone maturation (fig. 14). It may be concluded that although aspirin does not reduce the PGF_2 to a level that will effectively block ovulation, it has a specific action of inhibiting the effect of LH in causing ovum maturation. This is in contrast to the action of indomethacin which has been proved to inhibit prostaglandin synthesis and thereby prevent follicle rupture but not ovum maturation (Lindner et al, 1980; Kobayashi et al, 1981).

According to a report by Espey (1983) any potent non-steroidal anti-inflammatory agent should inhibit ovulation in the same manner as indomethacin. Perhaps it is the fact that aspirin is only a weak non-steroidal anti-inflammatory agent that makes it inhibit ovulation less effectively. These results implicate prostaglandin synthesis (and therefore inflammation, since prostaglandins are known to cause inflammation) as a probable mechanism involved in the process of ovulation as suggested by Espey (1980).

Morphometric studies on the ovaries of control and treated (experimental) rats show that indomethacin causes an arrest of follicular growth between the preantral and antral

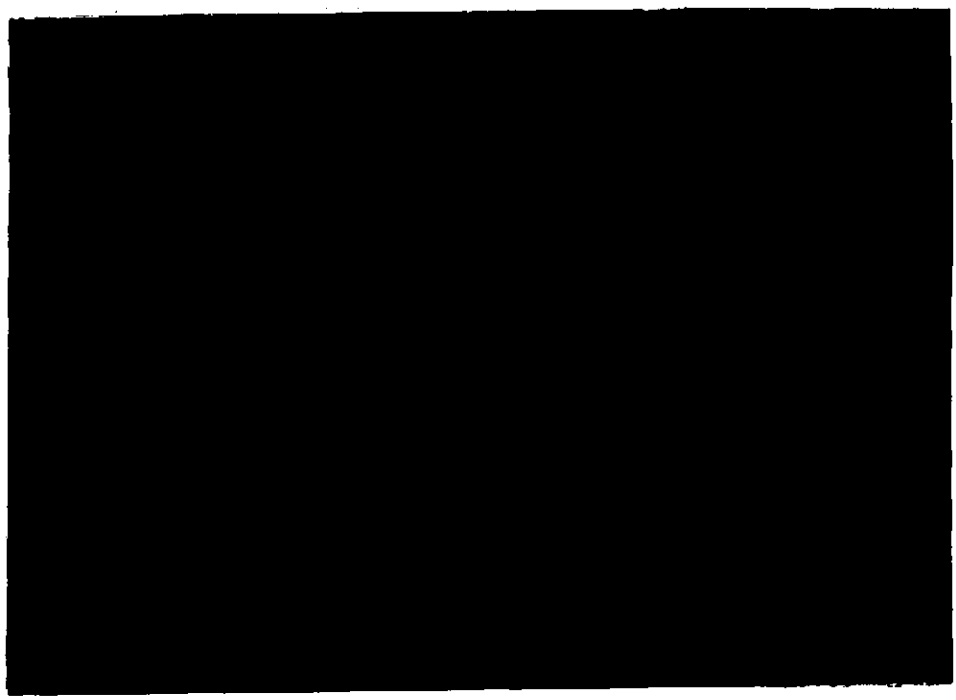


FIG. 14 - **Photomicrograph showing** a mature ovum (arrowed) (x 400) which was recovered from the rat oviduct. Ovum is surrounded by the zona pellucida and the cumulus cells.

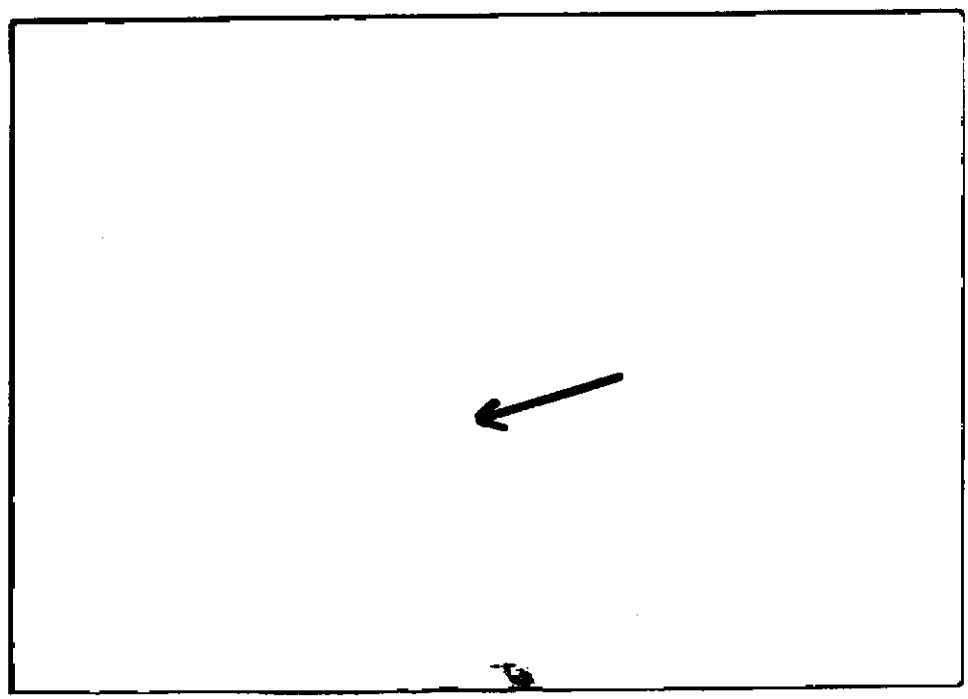


FIG. 15 - Photomicrograph showing an immature ovum (arrowed) (x 400) which was recovered from the rat oviduct. Note the absence of the zona pellucida.

stages, and it is also very effective in suppressing follicular rupture. This is demonstrated in the low number of corpora lutea observed in the indomethacin - treated rats when compared with aspirin - treated rats and control rats (fig. 9). Perhaps PGF_2 is necessary for the conversion of follicles from preantral to antral stage as well as for follicular rupture. Since indomethacin reduces PGF_2 secretion it follows that an arrest of follicular growth will occur. Aspirin does not seem to appreciably affect follicular rupture judging by the relatively high number of corpora lutea seen after aspirin treatment. This seems to confirm that aspirin does not suppress PGF_2 to a level that is low enough to inhibit follicular rupture effectively even though the level of suppression may retard follicular growth and ova maturation. The above findings are in agreement with a previous study by Downs and Longo (1982) who investigated the action of indomethacin and reported that prostaglandins seem to be involved in meiotic maturation of ova as well as the changes in ovarian follicular wall which accompany ovulation.

Similarly, in an in vitro study on the rabbit ovary Holmes et al (1983) reported the complete blockade of ovarian follicular rupture when indomethacin was added to the perfusate, and that addition of PGF_2 simultaneously with the indomethacin restored follicular rupture. This is an indication that indomethacin exerts its inhibitory action on ovulation at a time when follicular synthesis of prostaglandins is known to increase. In an earlier study

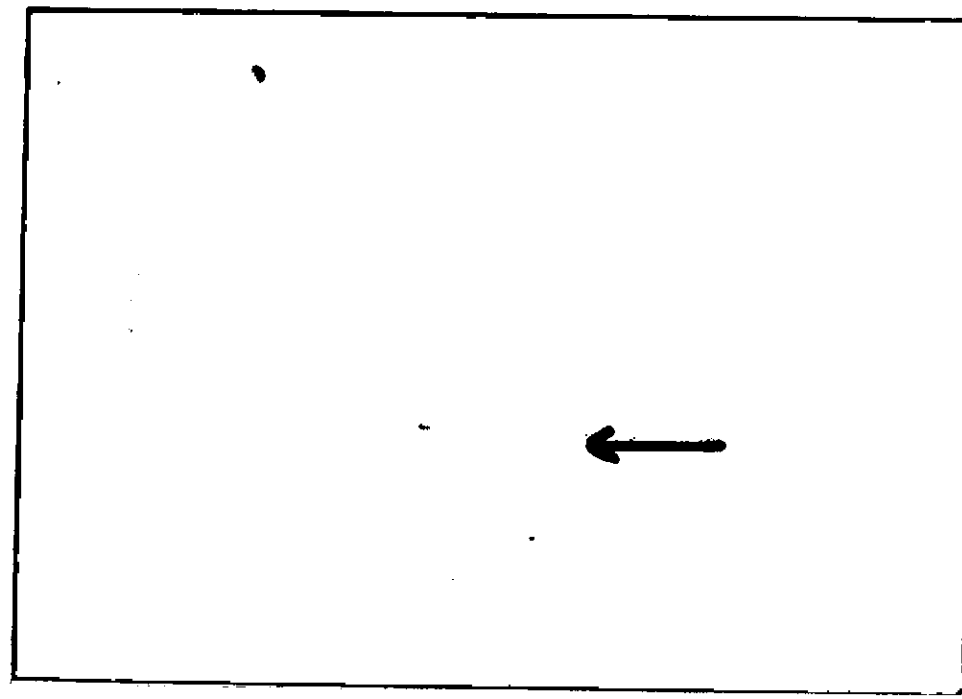


FIG. 9 - Photomicrograph showing a corpus luteum (arrowed) **(x 400)** within the ovary of the rat.

Parr (1974) did a histological examination of the rat ovarian follicle wall prior to ovulation. He concluded that in the inhibition of ovulation by aspirin and indomethacin, a process which involves prostaglandin synthesis is compromised and that this step may possibly be the early vascular phase of an inflammatory response.

Kobayashi et al (1981) using an in vitro perfused rabbit ovary studied the effect of prostaglandin synthesis inhibition by indomethacin on ovulation and ovum maturation. Contrary to the report of Downs and Longo (1982) they did not observe any inhibition of ova maturation with indomethacin treatment. However they found that increased degeneration of metaphase II (final phase of ovum maturation before ovulation) ova was associated with indomethacin treatment, and claimed that this degeneration could be prevented by supplementing the perfusate with PGF_2. These findings imply that although inhibition of prostaglandin synthesis by indomethacin may prevent follicular rupture it may not affect ovum maturation, at least in vitro

There is no doubt that like Espey (1986) mentioned, questions about the specific role of prostaglandins in the ovulation process have not been totally answered. However it has been established in the present study that prostaglandins are important for the phenomenon of follicular rupture.

CHAPTER FOUR

EFFECT OF ASPIRIN AND INDOMETHACIN ON LH-INDUCED OVULATION (SUPEROVULATION)

Experiments were carried out to find out if Aspirin and Indomethacin would inhibit superovulation. Rats were selected in diestrus and divided into 3 groups, A, B and C.

Group A rats (n = 19) served as the control group. They were superovulated and given the drug vehicle immediately after.

Group B rats (n = 42) were superovulated and injected with Aspirin immediately after superovulation.

Group C rats (n = 26) were also superovulated and injected with Indomethacin immediately after superovulation.

Preliminary experiments involved comparison of spontaneous ovulation and superovulation in rats to confirm the higher yield of ova during superovulation. A dose - response study of the effects of the two drugs was also carried out on some of the rats in groups B and C. For this Indomethacin was used in graded doses of 5, 10, 25 and 50 mg/kg body weight while Aspirin was used in doses of 50, 100, 140 and 160 mg/kg body weight.

4 rats from each group (A, B, C) were sacrificed by cervical dislocation 5 hours after hCG (± Drug) injection and blood was immediately collected by cardiac puncture for later measurement of preovulatory level of FSH, LH and PGF_{2}. The remaining rats were sacrificed 20 hours

after hCG (± Drug) injection, and blood was again collected for later measurement of post-ovulatory level of FSH and LH. The serum obtained from the blood samples was stored at - 20 °C until needed.

The ovaries of the rats were removed and fixed in Bouin's fluid for histolpgical examination while the oviducts were teased out and examined for ova. The number of ova shed per treated rat was recorded and compared with the number of ova shed per control rat.

Results

Ovulation Studies

Results of the present experiments revealed that at a dose of 100 mg/kg body weight Aspirin significantly reduces the rate of ovulation in rats. (table 4; fig. 13). The mean number of ova at ovulation was reduced by over 50%. Indomethacin at a dose of 50 mg/kg body weight was found to virtually completely suppress ovulation (table 4; fig. 13).

In the Aspirin - treated rats 11 out of 15 rats still ovulated although the mean number of ova was drastically reduced. In comparison all the control rats ovulated and the mean number of ova shed per each ovulating rat was quite high (table 4). With Indomethacin treatment only 2 out of 15 rats (not significant) were able to ovulate and the mean number of ova shed per ovulating rat was negligible. Results from preliminary studies showed that Indomethacin (50 mg/kg)completely suppressed superovulation while

Indomethacin (25 mg/kg) also greatly (80% reduction) suppressed it. Indomethacin (10 mg/kg and below) had no effect (fig. 16).

Aspirin (100 mg/kg) almost completely suppressed ovulation (85% reduction) but a higher dose of 140 mg/kg caused little suppression (32% reduction). A still higher dose of 160 mg/kg however appreciably suppressed superovulation (86% reduction) (fig. 16). The lowest dose of Aspirin used, 50 mg/kg had no effect. The results from the Aspirin study seem to suggest that the action of Aspirin in suppressing ovulation may be a biphasic one.

PGF level

Measurement of plasma PGF_2 showed that Aspirin (100 mg/kg) causes a significant reduction in preovulatory PGF_2 level while Indomethacin (50 mg/kg) causes an even greater reduction in preovulatory PGF_2 level (table 5; fig. 17).

LH & FSH levels

Preovulatory LH levels were found to be slightly higher in superovulated rats than in spontaneously ovulating rats. In addition while the LH levels in control and Indomethacin - treated rats were not significantly different, the level was higher in the Aspirin - treated rats (table 6). Post - ovulatory plasma FSH and LH measurements in preliminary experiments again showed no significant different between control rats and Aspirin - treated or Indomethacin - treated rats.

Morphometry

Morphometric analysis of the rat ovaries revealed that in comparison with the control rats, rats treated with 100 mg/kg Aspirin gave rise to fewer numbers of the different types of follicles (fig. 19) which suggests an inhibition of the whole process of follicle development. In Indomethacin - treated (50 mg/kg) rats most of the follicles were either in the early preantral stage or atretic There was a significant absence of corpora lutea. This suggests that ovulation did not occur in this group of rats.

DISCUSSION

The results of the present experiments confirm that both Indomethacin and Aspirin can suppress the LH - induced ovulation. Again indomethacin is seen to be more effective in this action as it almost completely blocks ovulation even when given at half the dose of aspirin (fig. 13)

The suppression of ovulation by indomethacin in superovulated rats has been previously reported by Armstrong and Grinwich (1972). These workers observed ovulation in immature rats pre-treated with Pregnant Mare Serum (PMS) and later given indomethacin (40 - 50 mg/kg). They found that indomethacin completely blocked ovulation and that whenadministered before the "critical period" for LH secretion, follicular luteinization and signs of progesterone secretion were also prevented; thus suggesting that indomethacin might be acting on the hypothalamic - pituitary axis at the ovarian level. Mori et al (1980) also

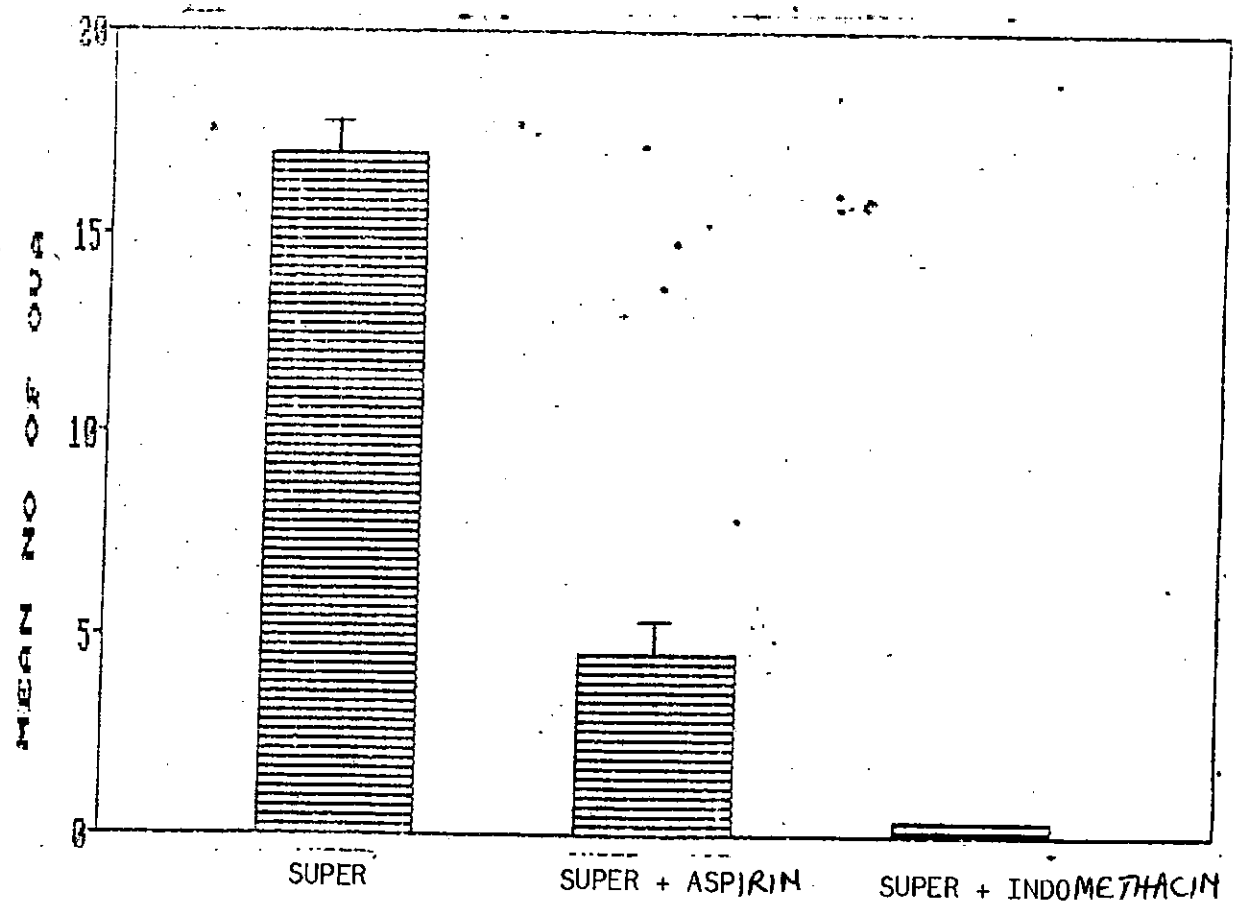
T A B L E 4

EFFECT OF ASPIRIN AND INDOMETHACIN ON LH - INDUCED OVULATION (SUPEROVULATION)

	Treatment	Proportion of Rats Ovulating	Mean No. of Ova Shed/
A	Superovulation only (Control)	15/15	16.9 ± 1.0
B	Superovulation + Aspirin (100mg/kg)	11/15	4.5 ± 0.9
C	Superovulation + Indomethacin (50mg/kg)	2/15	0.3 ± 0.2

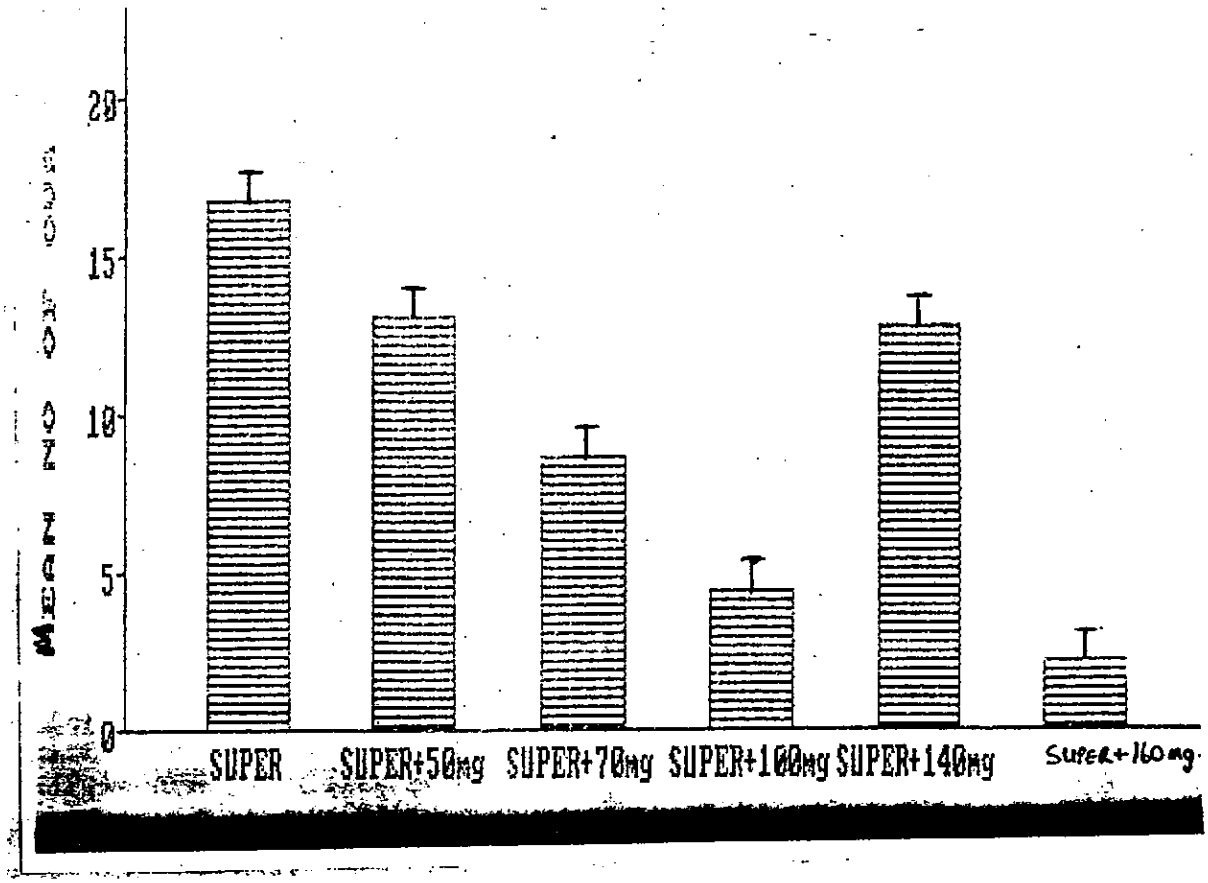
Means of control and treatment groups are significantly different ($P < 0.001$)

ANOVA - means of samples are significantly different



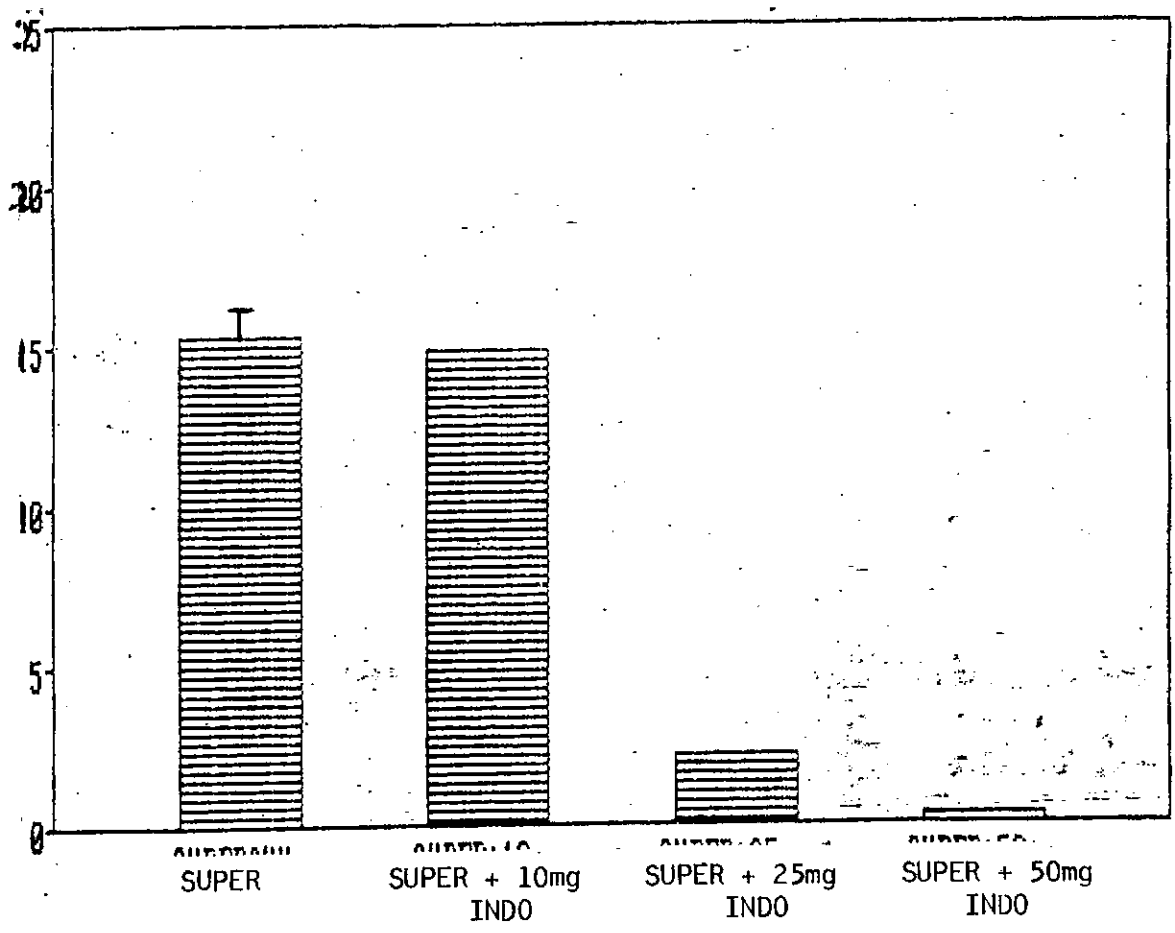
KEY: SUPER = SUPEROVULATION

FIG. 13 - Effect of Aspirin and Indomethacin on LH-induced ovulation (Superovulation)



KEY: SUPER = SUPEROVULATION

FIG. 16 - Dose-response effect of Aspirin on Superovulation.



KEY: SUPER = SUPEROVULATION
INDO = INDOMETHACIN

FIG. 17 - Dose-response effect of Indomethacin on superovulation.

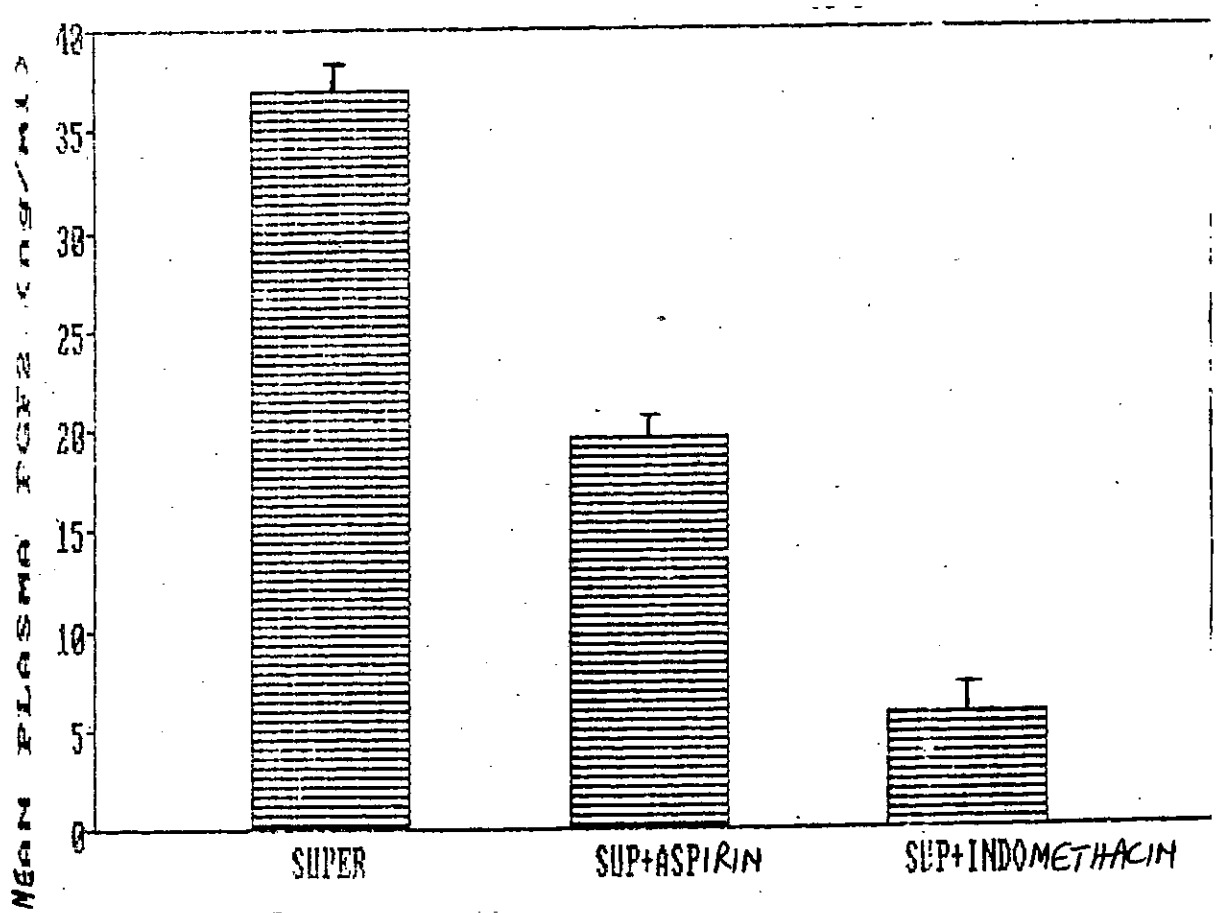
TABLE 5

EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY $PGF_{2\alpha}$ DURING SUPEROVULATION

	Treatment	Mean Plasma $PGF_{2\alpha}$ (ng/ml)
A	Superovulation only (Control)	37.01 ± 1.2
B	Superovulation + Aspirin (100mg/kg)	19.53 ± 1.0
C	Superovulation + Indomethacin (50mg/kg)	5.60 ± 1.2

Means of control and treatment groups are significantly different ($P < 0.001$)

ANOVA - means of samples are significantly different



KEY: SUP) / SUPER) = SUPEROVULATION

FIG. 18 - Effect of Aspirin and Indomethacin on preovulatory PGF_2 in superovulation.

TABLE 6

EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY LH LEVEL DURING SUPEROVULATION

	Treatment	Mean Plasma LH (mI.U/ml)
A	Superovulation only (Control)	11.25 ± 0.75
B	Superovulation + Aspirin (100mg/kg)	17.75 ± 0.25
C	Superovulation + Indomethacin (50mg/kg)	15.25 ± 0.48

Means of control and treatment groups are significantly different ($P < 0.01$)

ANOVA - means of samples are significantly different

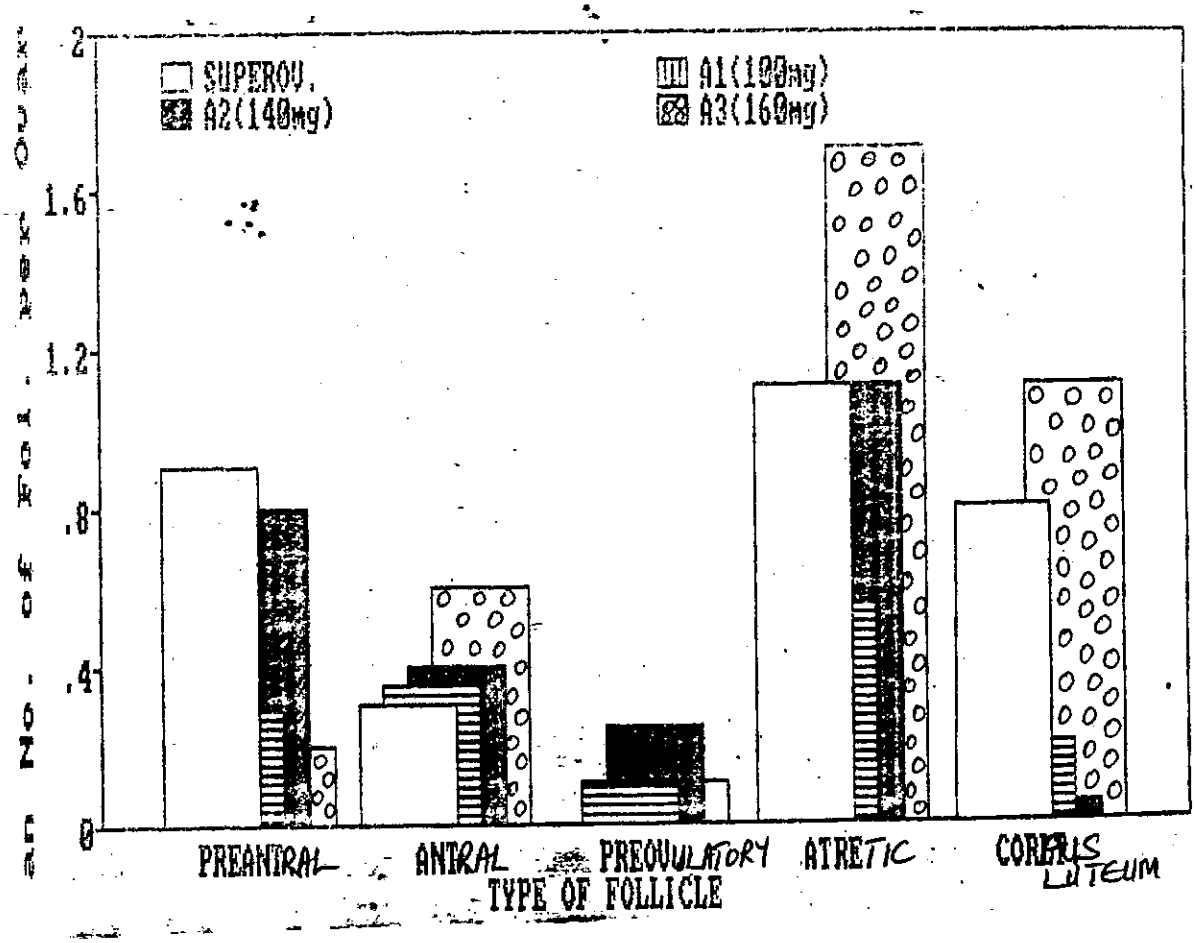


FIG. 19 - Pattern of follicular development after administration of Aspirin during superovulation.

treated immature rats with PMS and hCG to induce ovulation, and indomethacin was given concurrently with hCG as was done in the present study. They observed that indomethacin greatly reduced, in a dose-dependent manner, the mean number of ova shed. But when the interval between injections of hCG and indomethacin was increased the mean number of ova shed (for each dose) also increased suggesting that prostaglandins act differently on ovulation at different stages of the preovulatory process. These workers therefore concluded that prostaglandins probably mediate the action of hCG on ovulation through two mechanisms which operate separately, one at the earlier stages and the other at the later stages of the preovulatory process.

Other workers who have studied the effect of Indomethacin during superovulation include Downs and Longo (1982) who investigated the possible role of prostaglandins in preovulatory follicular development. These workers induced ovulation in immature mice by treating them with PMS followed later by LH and indomethacin. Their results showed that indomethacin (a) significantly inhibited ovulation (b) suppressed ova maturation, and (c) prevented the dissociation and thinning of the apical follicular wall which is necessary for follicular rupture. Their results also suggested that all these processes are regulated by prostaglandins since previous studies had established that indomethacin inhibits ovulation by reducing prostaglandin secretion. Previous studies on the effect of Aspirin on superovulation include the work of Orczyk and Behrman (1972)

who used both aspirin and indomethacin on PMS - primed immature rats to assess the role of prostaglandins in gonadotropin secretion. They found that both drugs reduced plasma PGF content, and they also blocked ovulation. Their results also revealed that injection of either LH or a mixture of PGE_2 and PGF_2 at the time of the expected ovulatory LH surge reversed the blockade of ovulation caused by indomethacin suggesting that prostaglandins may play a functional role in regulating the necessary ovulatory LH.

In his own study Espey (1983) tested some non-steroidal anti-inflammatory agents including aspirin and indomethacin to determine how well they inhibit the preovulatory elevation of prostaglandin production. Ovulation was induced by the administration of hCG. His results indicated that while ovulation was completely blocked by indomethacin, it was unaffected by aspirin. In like manner the inhibition of preovulatory PGE_2 and PGF_2 was much more pronounced in indomethacin - treated animals than in the aspirin - treated ones. Espey's results clearly show that in order for an agent to totally inhibit ovulation it must completely abolish the preovulatory elevation of prostaglandins in mature follicles. The present study reveals an inhibitory effect on preovulatory PGF_2 by both aspirin and indomethacin (fig. 18). Indomethacin almost completely suppressed PGF_2 production while aspirin significantly reduced it. This then implies that suppression of PGF_2 just before ovulation leads to inhibition of ovulation, and the greater the suppression of

PGF_2 the greater the degree of inhibition of ovulation. This direct relationship between ovulation and PGF level, as obtained in the present experiments, is in line with the findings of the previous workers mentioned above, and it shows that even in the presence of excess LH (as occurs during superovulation) the PGF_2 must be up to a certain minimum level for the LH to cause follicular rupture.

Karim and Hillier (1979) suggested in their review that LH, PGE and PGF are all essential for ovulation, and that the prostaglandins may act in part by an effect on the hypothalamic - pituitary axis. They also suggested that part of the mechanism of follicular rupture might be the induction of ovarian contractility by PGF_2. The Administration of $PGF_{2\alpha}$ in vivo and in vitro has been reported to stimulate increases in the tone and amplitude of contraction of ovaries (Kobayashi et al, 1981; Lipner, 1988). Kobayashi et al (1981) carried out in vitro studies on the perfused rabbit ovary and reported that PGF promotes follicular rupture through enhanced ovarian contractile activity. Addition of $PGF_{2\alpha}$ was seen to increase ovarian contractility as well as follicular rupture. If this is so, results from the present study may imply that the $PGF_{2\alpha}$ must be up to a certain minimum level for it to enhance ovarian contraction which subsequently leads to follicular rupture. However results of the present experiments also indicate a significant rise in preovulatory LH level with both aspirin and indomethacin,

and aspirin causing a higher elevation of LH (table 6). This seems to imply that the anti-inflammatory agents aspirin and indomethacin actually act on $PGF_{2\alpha}$ release and not on LH release even though the LH effect of causing follicular rupture is compromised. Thus LH level may remain high even when PGF level is dwindling It is however not clear why the LH level increased above the control (i.e rats superovulated but not treated with aspirin or indomethacin) value. Perhaps the anti-inflammatory agents have a slight stimulatory effect on LH release from the pituitary or on LHRH release from the hypothalamus. This would seem to be at variance with some previous reports like those of Karim and Hillier (1979) who said that it had been established that a variety of prostaglandins will stimulate FSH and LH secretion in vivo, suggesting a direct LH - releasing action of prostaglandins on the pituitary. However it is in line with the results of Naor et al (1975) who reported that indomethacin and aspirin fail to inhibit the LHRH - induced LH release from hemipituitaries in vivo. Likewise Lindner et al (1980) also reported that PGE_2 does not stimulate LH release from cultured hemipituitaries suggesting that an anti - PG agent is not neccessarily an anti - LH agent. For clarification other aspects of the ovulatory process need to be further investigated and these include (i) the possibility of an interaction between hCG (as given during superovulation) aspirin or indomethacin which could lead to a stimulation of LH release, and (ii) a possible mechanism of aspirin/indomethacin action which involves blocking of LH

receptors in the ovary with subsequent elevation of peripheral LH.

Morphometric studies on the ovary have given further insight into the ovarian activities during the ovulation blockade by aspirin and indomethacin. In comparison with the control, rats treated with 100 mg/kg Aspirin gave rise to fewer numbers of the different type of follicles (fig. 18) which suggests an inhibition of the whole process of follicle development. In indomethacin - treated (50 mg/kg) rats most of the follicles were either in the early preantral stage or were already atretic. There was a significant absence of corpora lutea which buttresses the reported inhibition of ovulation. This implies that indomethacin prevents follicular development and maturation as well as follicular rupture. This finding is in agreement with the report of Downs and Longo (1982) who suggested that alterations in the morphology of the follicle prior to ovulation (specifically meiotic maturation) are regulated by prostaglandins. However another report by Kobayashi et al (1981) says that inhibition of prostaglandin synthesis by indomethacin in vitro prevents follicle rupture but does not affect ovum maturation, thus providing evidence that these two processes are distinct phenomena in vitro. Further work may need to be done in this area to establish if the mechanism of prostaglandin action on the ovarian follicles differs or not in vivo and in vitro; if the mechanism differs it will be necessary to ascertain if any local or systemic factors acting in vivo is responsible for the difference in prostaglandin actvity.

C H A P T E R F I V E

THE INFLUENCE OF hCG ON THE INHIBITORY ACTION OF ASPIRIN AND INDOMETHACIN ON OVULATION

These series of experiments were carried out to investigate if the presence of hCG would affect the action of Aspirin and Indomethacin in inhibiting ovulation.

Rats were monitored from the day of proestrus when they were divided into six groups, A,B,C,D,E, and F.

Groups A rats (n=6) served as the control and were allowed to ovulate spontaneously without any treatment.

Group B rats (n=6) were given 100mg/kg aspirin in the afternoon of proestrus and were then left to ovulate spontaneously.

Group C rats (n=6) were given 50mg/kg Indomethacin in the afternoon of proestrus and then left to ovulate spontaneously.

Group D rats (n=8) received 10 I.U hCG in the afternoon of proestrus and were allowed to ovulate spontaneously thereafter.

Group E rats (n=8) received 10 I.U hCG plus 100mg/kg Aspirin in the afternoon of proestrus and were left to ovulate spontaneously.

Group F rats (n=8) were given 10 I.U hCG plus 50mg/kg Indomethacin and were left to ovulate spontaneously.

4 rats from each group were sacrificed by cervical dislocation at 6.00 p.m on the day of proestrus, and blood was immediately taken through a cardiac puncture, for

subsequent LH and prostaglandin assay. The plasma obtained from the blood was stored at -20°c until required.

The remaining rats had vaginal smears carried out on the following morning, and those with cornified cells in the vagina were sacrificed by cervical dislocation. The ovaries were immediately excised and fixed in Bouin's solution for histological studies. The oviducts were teased out and the number of ova was estimated.

Results

Ovulation Studies

In these series of experiments it can be seen that administration of hCG did not cause ovulation but actually inhibited it (fig.20). hCG also did not reverse the inhibition of ovulation by indomethacin but seemed to potentiate the inhibitory action of indomethacin (table 7; fig. 19).

Aspirin when administered alone was observed to inhibit ovulation but when it was administered along with hCG this inhibitory action was completely reversed (fig.20). There was in fact a significant increase in the rate of ovulation although many of the ova were seen to be immature.

PGF level

Measurement of preovulatory plasma PGF_{2} revealed that when administered separately, aspirin and indomethacin significantly reduced PGF level with indomethacin being morepotent (table 8). hCG when administered alone also

significantly reduced PGF level. However when aspirin was given along with hCG the level of PGF remained the same as for control rats, but indomethacin, when administered along with hCG again significantly reduced PGF to a level that was even lower than that obtained with hCG alone (fig. 21). This again seems to imply a synergistic action between hCG and Indomethacin.

LH level

When preovulatory plasma LH was measured it was found that LH level was elevated with administration of hCG alone (table 9). However when Aspirin and Indomethacin were administered along with hCG there was no further elevation in LH level, suggesting that the two agents (aspirin and indomethacin) have no effect on the secretion of LH.

Morphometric Studies

Morphometric studies on the rat ovaries showed that in control rats and those treated with Aspirin there was development of preantral follicles to antral follicles, while in the indomethacin treated ones there was an arrest of development (fig. 22). corpora lutea were very few in both control and aspirin-treated rats while they were not present at all in indomethacin - treated rats.

This corroborates the results of the earlier experiments on hCG and ovulation (table 7, fig. 20), and confirms that hCG does not reverse the inhibitory action of indomethacin on ovulation, or the immaturity of the ova produced during

TABLE 7

INFLUENCE OF hCG ON THE INHIBITORY ACTION OF ASPIRIN AND INDOMETHACIN ON OVULATION

	Treatment	Proportion of Rats ovulating	Mean No. of Ova Shed/
A	- (Control)	6/6	5.5 ± 0.5
B	Aspirin (100mg/kg)	4/6	2.1 ± 0.9
C	Indomethacin (50mg/kg)	2/6	0.5 ± 0.2
D	hCG (10 I.U)	4/8	1.0 ± 0.4
E	hCG (10 I.U) + Aspirin (100mg/kg)	8/8	7.5 ± 0.3
F	hCG (10 I.U) + Indomethacin (50mg/kg)	0/8	0.0

Difference between means of Ova shed in groups B and E is significant ($P < 0.01$)

Difference between means of Ova shed in groups C and F is not significant ($P > 0.05$)

Difference between means of control (A) and HCG -treated (D) groups is significant ($P < 0.001$)

ANOVA - means of samples are significantly different

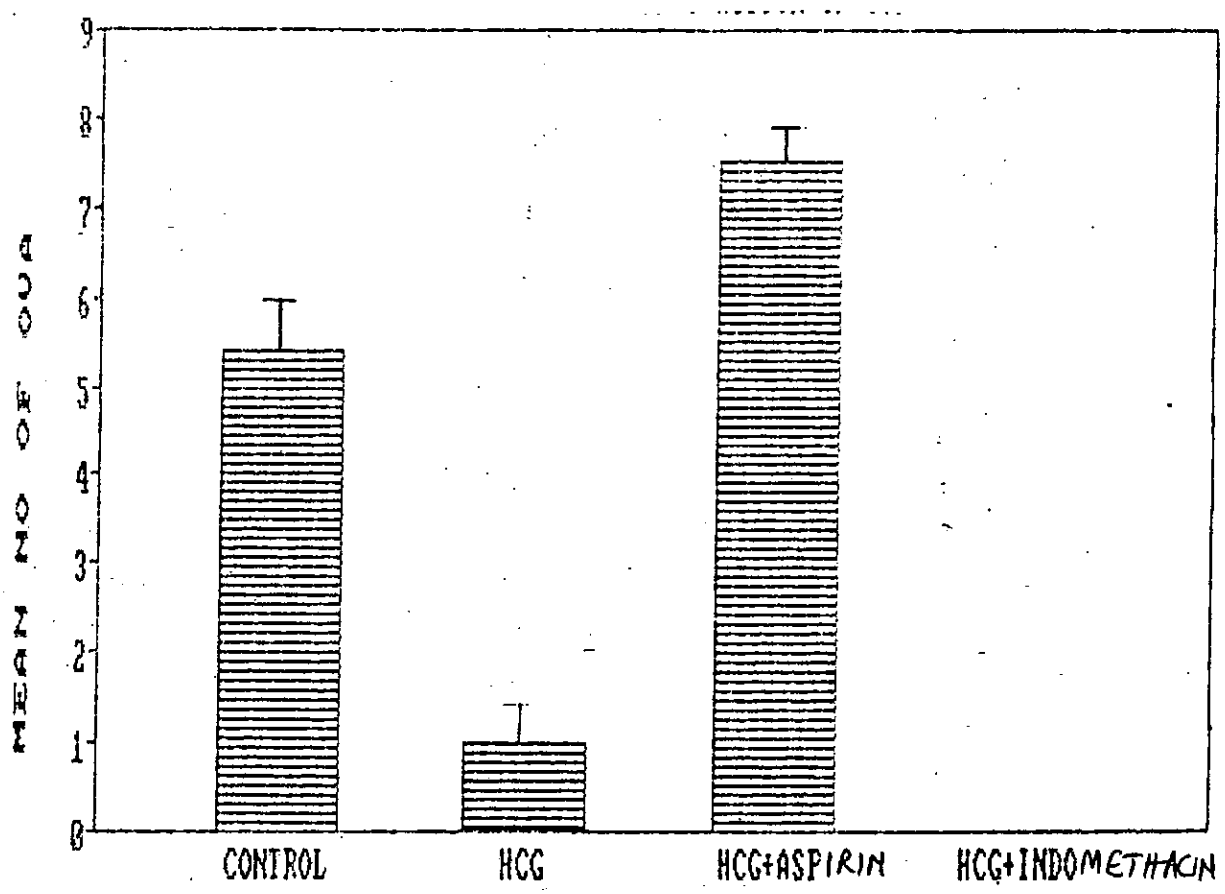


FIG. 20 - hCG influence on inhibitory action of Aspirin and Indomethacin on ovulation.

TABLE 8

INFLUENCE OF HCG ON THE EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY $PGF_{2\alpha}$

	Treatment	Mean Plasma PGF_2
A	- (Control)	38.06 ± 0.5
B	Aspirin (100mg/kg)	18.58 ± 2.2
C	Indomethacin (50mg/kg)	4.54 ± 0.5
D	hCG (10 I.U)	12.91 ± 0.6
E	hCG (10 I.U) + Aspirin (100mg/kg)	39.07 ± 1.1
F	hCG (10 I.U) + Indomethacin (50mg/kg)	6.84 ± 0.7

Mean values in groups B and E are significantly different ($P < 0.001$)

Mean values in groups C and F are not significantly different ($P > 0.05$)

Means of control (A) and HCG - treated (D) groups are significantly different ($P < 0.001$)

ANOVA - means of samples are significantly different

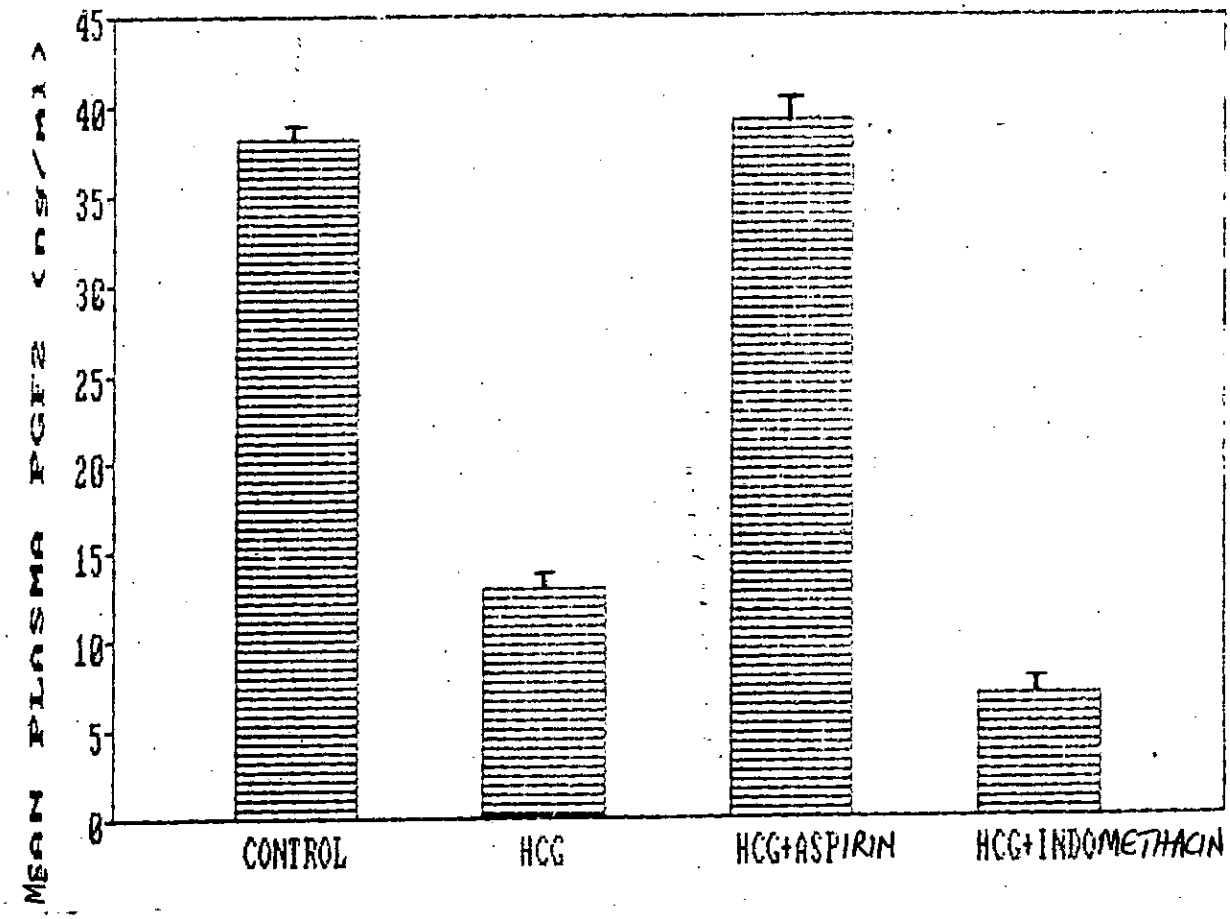


FIG. 21 - hCG influence on Aspirin and Indomethacin effect on preovulatory PGF_2

TABLE 9

INFLUENCE OF HCG ON THE EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY LH

	Treatment	Mean Plasma LH (mI.U/ML)
A	- (Control)	8.75 ± 0.63
B	Aspirin (100mg/kg)	11.00 ± 0.45
C	Indomethacin (50mg/kg)	10.75 ± 0.52
D	hCG (10 I.U)	11.75 ± 0.48
E	hCG (10 I.U) + Aspirin (100mg/kg)	12.25 ± 0.63
F	hCG (10 I.U) + Indomethacin (50mg/kg)	13.00 ± 0.70

Mean values in groups B and E are not significantly different ($P > 0.05$)

Mean values in groups C and F are significantly different ($P < 0.05$)

Means of control (A) and HCG - treated (D) groups are significantly different ($P < 0.01$)

ANOVA - means of samples are significantly different

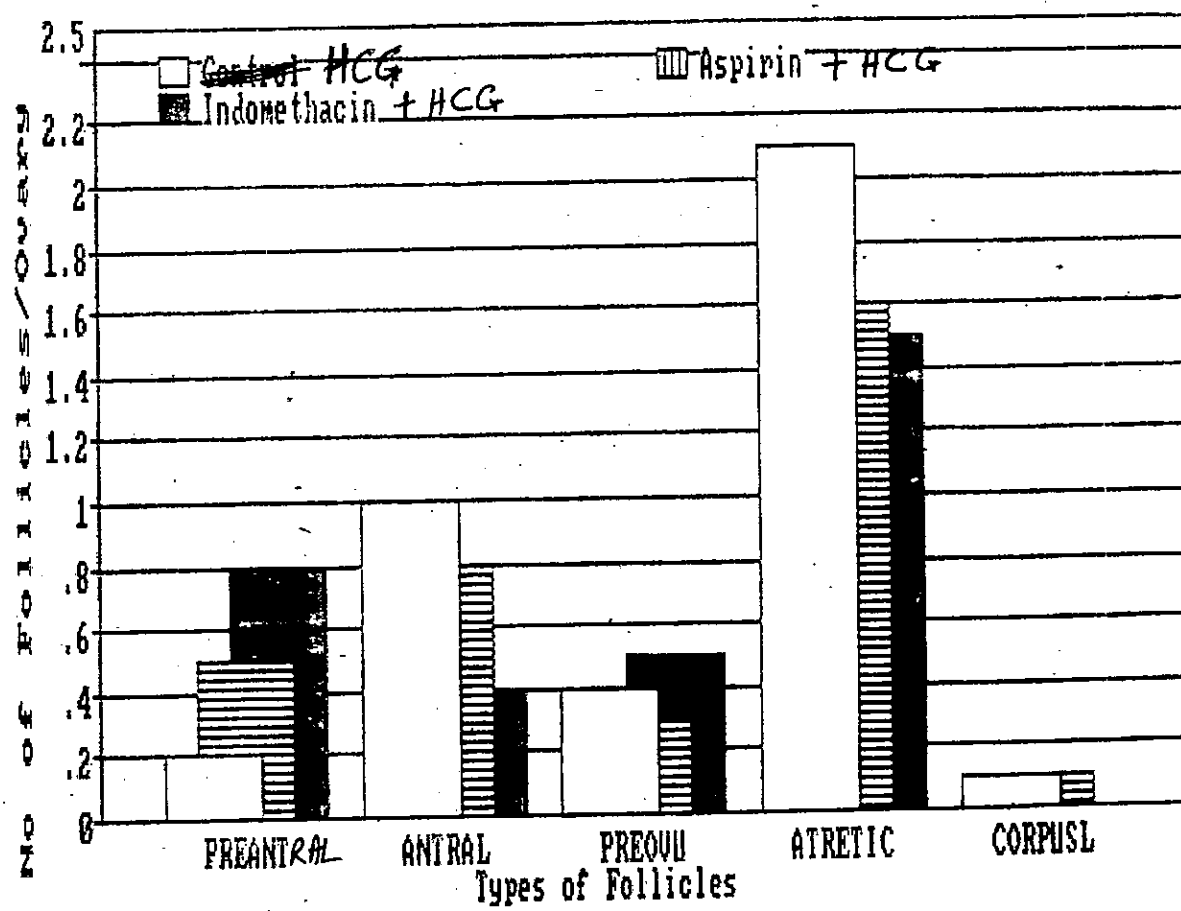


FIG. 22 - hCG influence on Aspirin and Indomethacin effect on follicular development.

DISCUSSION

hCG is widely used as an agent for ovulation induction in most animal species. It is known to be particularly effective for follicular rupture after necessary priming by PMS. In their review Greenwald and Terranova (1988) stated that PMS causes recruitment of preantral follicles i.e. PMS is crucial for the conversion of primary follicles to preantral ones (an essential step in the process of superovulation) while hCG is speculated to cause final follicular maturation and rupture.

It would therefore appear that administration of hCG to proestrus rats should enhance ovulation. However results of the present study indicate an inhibition of ovulation when hCG was administered alone without prior PMS priming (table 7, fig. 20). This further supports the above suggestion that hCG might not be required for follicular growth and maturation, but only for follicular rupture. This means that when hCG is present without previous PMS priming the ovarian follicles do not grow or mature and therefore do not reach a stage where hCG will effect a rupture. It may also be that the follicular cells may be too immature to have acquired hCG receptors. Perhaps for hCG to cause follicular growth and maturation all on its own it would have to be used in a much higher dose than the ovulatory dose (10 I.U hCG) which was used in the present study. Hamada et al (1978) reported that hCG alone at a dose of 100 I.U was used for induction of ovulation in rabbits. This is similar to what was done by Kobayashi et al (1981) who perfused the

of ovulation. Furthermore when hCG and aspirin were administered together it was observed that PGF_2 level did not differ from the control level. On the other hand the combination of hCG and indomethacin caused a drastic reduction in PGF_2 level. This again confirms the importance of PGF_2 in ovulation. This is in agreement with the report of Kobayashi et al (1981) that even though a high dose (100 I.U) of hCG caused ovulation in their in vitro experiments on the perfused rabbit ovary, addition of PGF_2 to the perfusate increased the rate of ovulation. This suggests that rate of ovulation increases with increase in PGF level, and that the hCG may have caused ovulation by stimulating some PGF synthesis within the ovary.

The results of the present experiments are comparable with those of Mori et al (1980) who also found that concurrent administration of indomethacin with hCG reduced the mean number of ova shed. Kohda et al (1983) shed some light on the inhibitory action of the simultaneous administration of hCG and Indomethacin. They reported that contrary to popular opinion hCG is necessary for both early and late preovulatory stages. The results of their experiments showed that while progesterone mediates the action of hCG on the earlier stages of ovulation prostaglandins mediate the action of hCG on the later stages. When preovulatory LH was measured in the present set of experiments it was found to be significantly higher in hCG - treated rats than in the control (untreated) rats (table 9). This was probably due to the presence of

additional exogenous hCG which could have been measured as LH. However LH level did not differ between the hCG and hCG + Aspirin or hCG + Indomethacin groups implying that the assumed synergistic action of hCG and Aspirin in causing ovulation is not mediated by LH and most likely does not affect the hypothalamic - pituitary axis. Neither does the combined action of hCG and indomethacin in inhibiting ovulation affect LH secretion. These results again support the view that the action of these three agents is at the ovarian level.

Results of the morphometric studies show that hCG (10 i.u) arrests follicular development at the antral stage and causes many follicles to become atretic (fig.22). The combination of hCG and Aspirin also had the same effect. It is possible that this combination causes rupture of immature follicles and turns them into atretic follicles. With hCG and Indomethacin there was complete absence of corpora lutea, an indication of absolute suppression of follicular rupture. This lends further credence to the earlier result of complete abolition of ovulation when hCG and Indomethacin were administered together (fig.20).

The results of this set of experiments confirm that hCG at the dose used (10 i.u) does not reverse the actions of aspirin and indomethacin on follicular growth and maturation, as well as follicular rupture.

CHAPTER SIX

THE INFLUENCE OF EXOGENOUS LH ON THE INHIBITORY ACTION OF ASPIRIN AND INDOMETHACIN ON OVULATION

These series of experiments were performed to find out if the administration of LH could reverse the inhibitory action of Aspirin and Indomethacin on ovulation.

Rats were selected in proestrus and divided into six groups A,B,C,D,E and F.

Group A rats (n=8) served as controls and were allowed to ovulate spontaneously without any treatment.

Group B rats (n=8) were given 100mg/kg Aspirin and were then left to ovulate spontaneously.

Group C rats (n=8) received 50mg/kg Indomethacin and were then left to ovulate spontaneously.

Group D rats (n=8) received 100mg/kg aspirin + 20 µg LH and were allowed to ovulate spontaneously thereafter.

Group E rats (n=8) received 50mg/kg Indomethacin + 20µg LH and were also left to ovulate spontaneously.

Group F rats (n=8) received 20ugLH and were also left to ovulate spontaneously.

All injection were given in the afternoon of proestrus.

4 rats from each group were sacrificed by cervical dislocation the evening of proestrus. Blood was immediately collected by cardiac puncture, and plasma obtained was stored at -20^{o}c pending later hormone and prostaglandin assay. The remaining rats had vaginal smears carried out on the following morning and those with cornified cells in

the vagina were sacrificed, and their oviducts were excised and teased out. The number of ova in each rat was then estimated.

RESULTS

OVULATION STUDIES

The pattern of Ovulation in rats treated with aspirin, indomethacin, Aspirin + LH, indomethacin + LH is shown in table 10 and fig. 23. The results indicate an inhibition of ovulation by both indomethacin and aspirin but more significantly by indomethacin. Ova produced by aspirin-treated rats were found to be immature (fig. 15). Neither of these events (ovulation inhibition and immaturity of ova) was reversed by LH administration.

The rate of ovulation as well as the mean number of ova shed were not significantly different in the Aspirin + LH group when compared to the Aspirin group. Likewise the two indices are exactly the same in the indomethacin and indomethacin + LH groups (fig. 23).

PGF Level

The results of the measurement of plasma preovulatory PGF $_{2\alpha}$ showed that PGF level in aspirin + LH treated rats was significantly higher than in the aspirin-treated rats (table 11). PGF level in indomethacin + LH treated rats wasalso significantly higher than in indomethacin-treated rats. These results suggest that PGF level rises in the presence of LH.

LH Level

When plasma preovulatory LH was measured it was found that LH level was higher in the groups that had exogenous LH administered (table 12). Aspirin and Indomethacin had been observed earlier in the study to have no effect on preovulatory LH level (table 3), but when they were given along with LH there was a significant rise in LH level, an obvious consequence of the additional exogenous LH.

Discussion

The actions of LH and prostaglandins appear to be critical factors in the ovulatory process. Their action during inhibition of ovulation by indomethacin and aspirin is therefore worthy of attention.

In the present study LH was not able to reverse the inhibition of ova maturation by aspirin or the suppression of ovulation by indomethacin (table 10 & fig. 23). This suggests that these actions of the two drugs on the ovulatory process occur at the ovarian level and not the hypothalamic - pituitary axis.

Aspirin on its own did not inhibit ovulation but the ova were mainly immature implying an inhibition of the maturation process. The fact that aspirin did not reduce the rate of ovulation may be the reason why no significant increase in the rate of ovulation was observed in the presence of LH. This means that in the present circumstances the normal incidence of follicular rupture occurred with Aspirin, therefore additional LH would not

T A B L E 10

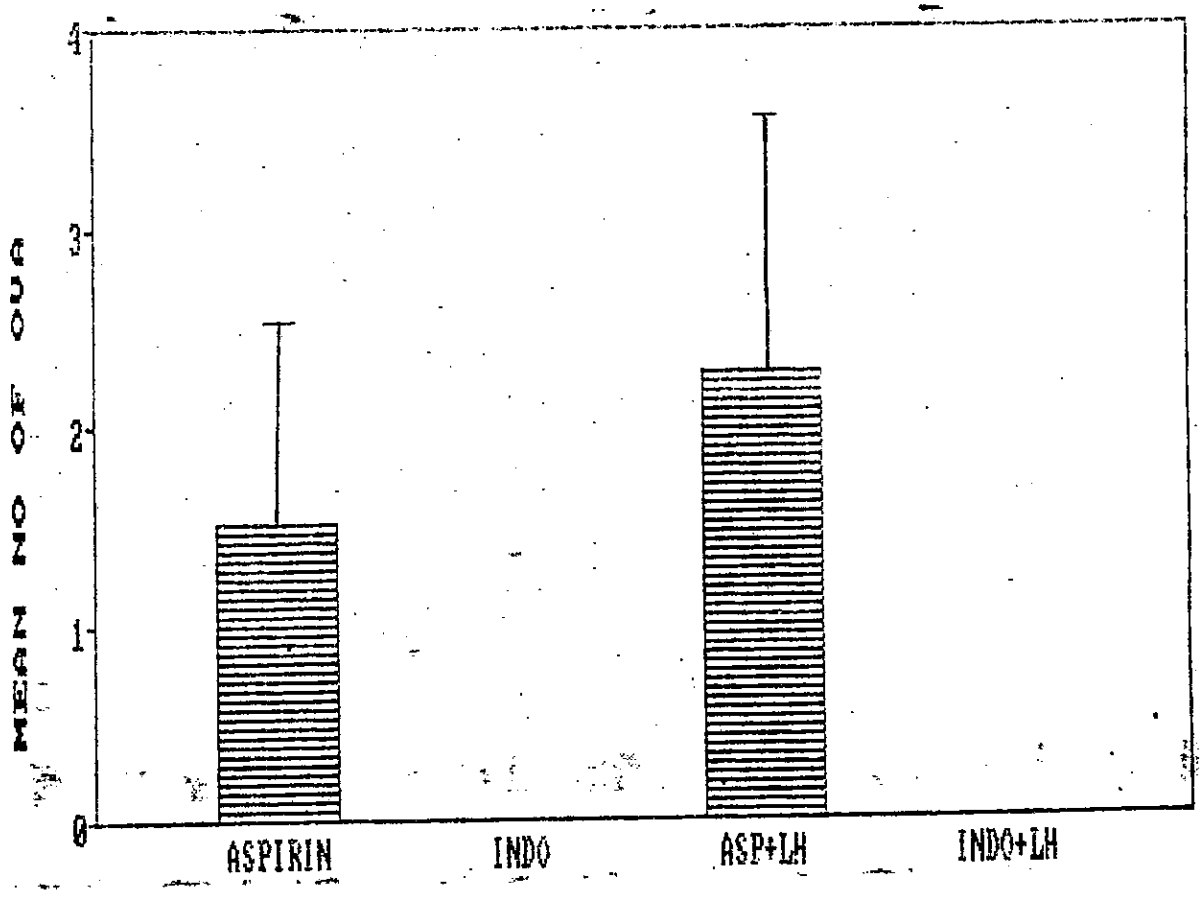
INFLUENCE OF LH ON THE INHIBITORY EFFECT OF ASPIRIN AND INDOMETHACIN ON OVULATION

	Treatment	Proportion of Rats Ovulating	Mean No. of Ova Shed/
A	- (Control)	4/4	4.5 ± 0.6
B	Aspirin (100mg/kg)	2/4	1.5 ± 0.9
C	Indomethacin (50mg/kg)	0/4	0.0
D	Aspirin (100mg/kg) + LH (20ug)	2/4	2.2 ± 1.3
E	Indomethacin (50mg/kg) + LH (20ug)	0/4	0.0
F	LH (20ug)	4/4	5.5 ± 0.7

Difference between means of Ova shed in groups B and D is not significant ($P > 0.5$)

Means of Ova shed in groups C and E are not different.

ANOVA - means of samples are not significantly different



KEY: ASP = ASPIRIN
INDO = INDOMETHACIN

FIG. 23 - LH influence on inhibitory action of Aspirin and Indomethacin on ovulation.

T A B L E 11

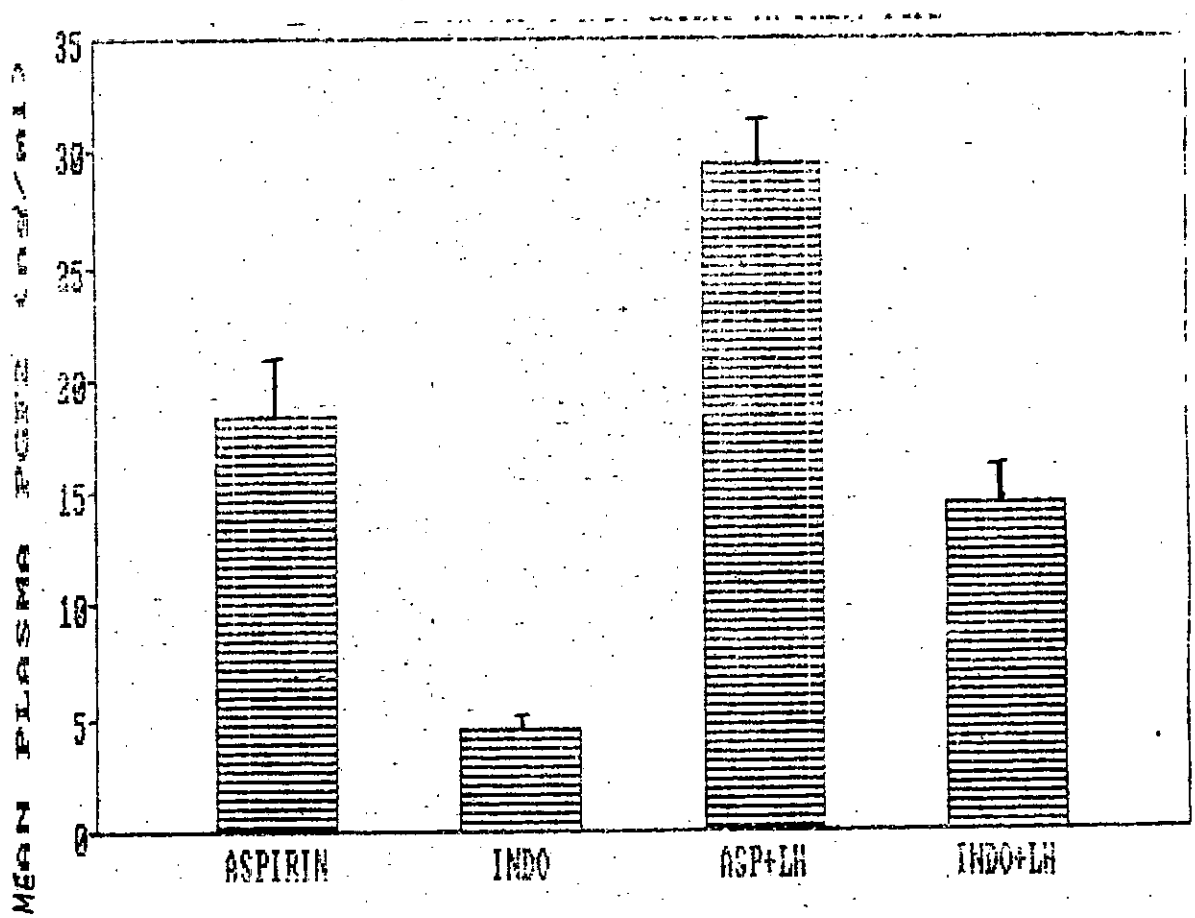
INFLUENCE OF LH ON THE EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY $PGF_{2\alpha}$

	Treatment	Mean Plasma $PGF_{2\alpha}$ (ng/ml)
A	- (Control)	38.06 ± 0.5
B	Aspirin (100mg/kg)	18.29 ± 2.3
C	Indomethacin (50mg/kg)	4.61 ± 0.5
D	Aspirin (100mg/kg) + LH (20ug)	29.39 ± 1.9
E	Indomethacin (50mg/kg) + LH (20ug)	14.33 ± 1.6
F	LH (20ug)	39.42 ± 0.4

Mean values in groups B and D are significantly different ($P < 0.02$)

Mean values in groups C and E are also significantly different ($P < 0.01$)

ANOVA - means of samples are significantly different



KEY : ASP = ASPIRIN
INDO = INDOMETHACIN

FIG. 24 - LH influence on Aspirin and Indomethacin effect on preovulatory PGF_2 .

T A B L E 12

INFLUENCE OF LH ON THE EFFECT OF ASPIRIN AND INDOMETHACIN ON PREOVULATORY LH

	Treatment	Mean Plasma LH (mI.U/ml)
A	- (Control)	8.75 ± 0.63
B	Aspirin (100mg/kg)	13.5 ± 0.65
C	Indomethacin (50mg/kg)	11.75 ± 0.48
D	Aspirin (100mg/kg) + LH (20ug)	16.0 ± 0.41
E	Indomethacin (50mg/kg) + LH (20ug)	19.0 ± 0.41
F	LH (20ug)	28.0 ± 0.56

Mean values in groups B and D are significantly different ($p < 0.02$)

Mean values in groups C and E are also significantly different ($P < 0.001$)

ANOVA - means of samples are significantly different

further increase the occurrence of follicular rupture. Behrman et al (1972) had reported that when they gave LH(10µg i.v) to aspirin-treated rats they observed that blockade of ovulation by aspirin was consistently reversed by LH administration. Their observation points to a hypothalamic level of action for aspirin blockade of ovulation. One reason for the difference in observation between the present study and the of Behrman et al (1972) may be due to the very high dose of Aspirin (400mg/kg) that was used by these workers when compared with the 100mg/kg aspirin that was used in the present study. This means that the higher dose of Aspirin might have been better able to inhibit ovulation than the lower dose.

Indomethacin-induced block of ovulation in the present study was not reversed by LH. This gives additional support for an ovarian site of action. If the site of action of indomethacin was the hypothalamic-pituitary axis the indomethacin-treated rats should have ovulated in response to exogenous LH. This non-reversal of indomethacin-blocked ovulation by LH that was observed in the present experiments is in agreement with the report of previous workers like Armstrong and Grinwich (1972) who reported ovulation blockade by indomethacin even in the presence of exogenous LH. Likewise Holmes et al (1983) when they injected indomethacin directly into rabbit ovarian follicles reported that ovulation was effectively blocked in these follicles even though the ovaries had been treated with LH. However, studies by Garza et al (1984) have shown that LH effect on

ovulation may be species dependent. For example they found that LH infusion into guinea pigs doubled the ovulation rate whereas similar treatment of cyclic mice and rats was ineffective.

LH initiates the ovulatory process in all mammalian species. Experiments with rats have shown that LH causes an elevation in prostaglandin levels in preovulatory follicles. For example Le Maire et al (1973) and Bauminger and Lindner (1975) demonstrated a marked increase in follicular content of PGE and PGF 8-10 hours after the LH surge The present experiments reveal the same observation. Aspirin significantly reduced plasma preovulatory PGF, and indomethacin had an even more potent reduction effect (fig. 24). But on administration of exogenous LH the plasma preovulatory PGF level was remarkably elevated in both instances. This shows that aspirin and indomethacin reduced PGF synthesis by the ovarian follicles but this action was reversed in the presence of LH. It also implies that the inhibitory action of aspirin and indomethacin on preovulatory PGF synthesis can be abolished if the LH level is high enough. These observation also suggest that LH may cause ovulation by elevating the prostaglandin level in preovulatory follicular rupture. The results of the present experiments are in agreement with the previous reports cited above. Lindner et al (1980) in their review also confirmed this when they stated that LH induces prostaglandin synthetase in ovarian follicles. To establish more firmly that prostaglandins play a physiological role in the

processes leading to follicular rupture they showed that follicular prostaglandin content increases after exposure to LH, and reaches a peak towards the time of ovulation with a preponderance of PGE over PGF.

Even though LH has been shown to cause an elevation in prostaglandin level, Karim and Hillier (1979) reviewed that many studies have also established that a variety of prostaglandins will stimulate FSH and LH secretion in vivo. They reported that when pentobarbitone is used to block LH release, administration of PGE_2 infact overcomes this blockade and causes LH secreation. Generally PGE compounds release LH in vivo and in vitro, and $PGF_{2\alpha}$ releases LH in vitro. In this prostaglandins may act by modulation of the ovarian feedback i.e the steroidal feedback effect on the pituitary, as well as by having a direct effect on the hypothalamic-pituitary axis (Lindner et al, 1974; Ojeda et al, 1977). The evidence for this is in the fact that administration of antisera to LHRH prevented the PGE_2-induced LH surge. It is therefore possible that the relatively high ovulation rate observed in the control rats for the present experiments was in past due to the presence of prostaglandins (since the control rats were not treated with the PG inhibitors, aspirin and indomethacin). The prostaglandins could in turn have caused an elevation of LH level which subsequently enhances ovulation rate.

CHAPTER SEVEN

EFFECT OF ASPIRIN AND INDOMETHACIN ON PREGNANCY

Pregnancy was achieved in female rats by selecting those rats that were in proestrus, and caging them overnight with proven males in the evening of proestrus. There was one male to one female rat, and the rats were housed 2 or 4 per cage depending on the size of the cage. This was to increase the chance of mating. The rats were separated the following morning and the females were examined for the presence of a vaginal plug (an indication of mating). The vaginal smear was also done to observe the presence of sperm cells as well as the presence of cornified epithelial cells which is an indication of estrus. This day is referred to as day zero of pregnancy and gestation period is counted from the following day.

For this set of experiments rats were divided into 5 groups, A,B,C,D and E.

Group A rats (n=8) were mated and were injected with the drug vechicle (normal saline) on days 4 and 5 of pregnancy (i.e. in early pregnancy).

Group B rats (n=8) were mated and injected with 100mg/kg Aspirin on days 4 and 5 of pregnancy.

Group C rats (n=8) were mated and injected with 50mg/kg Indomethacin on days 4 and 5 of pregnancy.

Group D rats (n=4) were mated and injected with 100mg/kg Aspirin from day 18 to day 21 of pregnancy. Group E rats (n=4) were mated and then injected with 50mg/kg Indomethacin

from day 18 to day 21 of pregnancy. Some rats in groups A,B and C were sacrificed on day 9 of pregnancy, and the number of implantation sites was observed and recorded.

The point in giving aspirin and indomethacin in some of these rats in early pregnancy was to see if the drugs, by reducing prostaglandin level, could disturb implantation of the blastocyst (which normally occurs around day 5 of pregnancy). The drugs were also administered in late pregnancy to see if by inhibiting prostaglandin synthesis they could extend the gestation period i.e by inhibiting uterine contractions which are purportedly initiated by prostaglandins.

Rats which successfully mated were weighed and smeared daily for 14 days. A consistent smear of leucocytes (caused by the presence of progesterone) was taken as an indication of pregnancy. From the 15th day these rats were weighed daily to confirm pregnancy which should result in an increase in weight.

The time of parturition as well as the size of the litter (number of offsprings) were observed and recorded in all the groups.

Results

Implantation of Embryo

Results from the present experiments show that aspirin treatment did not appear to affect the implantation of embryo as the number of implantation sites recorded was about the same in control rats and the aspirin-treated rats

(table 13). However, in Indomethacin-treated rats implantation sites were significantly reduced from 8 to 5.

Gestation Period

The gestation period in normal untreated (control) pregnancy rats was observed to be between 21 and 23 days with an average of 22 days (table 14). Pregnant rats treated with Aspirin in early (days 4 & 5) or late (days 18-21) pregnancy also had their length of gestation within this range. In the case of indomethacin administration, the length of gestation was within the normal range when given early (days 4 & 5) in pregnancy but it was slightly but significantly elevated by 2.0 when given late (days 18 -21) in pregnancy (table 14).

Number of Offsprings

The results obtained from this set of experiments were similar to those obtained during the study on gestation period. In normal control rats the number of offsprings ranged between 6 and 8 with an average of 7. The aspirin-treated (late or early pregnancy) rats had offsprings within the normal range, but with late pregnancy indomethacin-treated rats the number of offsprings was significantly reduced to an average of 4 (table 15). Some of the foetuses were observed to have resorbed. When indomethacin was administered in early pregnancy, the number of offsprings was not significantly different from that of control rats.

T A B L E 13

EFFECT OF ASPIRIN AND INDOMETHACIN ADMINISTRATION ON IMPLANTATION OF EMBRYO

	Treatment	Average No. of Implantation sites on day 9 of Pregnancy
A	- (Control)	8 ± 0.62
B	Aspirin (100mg/kg)	7 ± 0.40
C	Indomethacin (50mg/kg)	5 ± 0.57

Drugs were administered on day 4 of pregnancy

Means of Control (A) and aspirin - treated (B) groups are not significantly different ($P > 0.1$)

Means of control (A) and indomethacin - treated (C) groups are significantly different ($p < 0.02$)

ANOVA - means of samples are significantly different

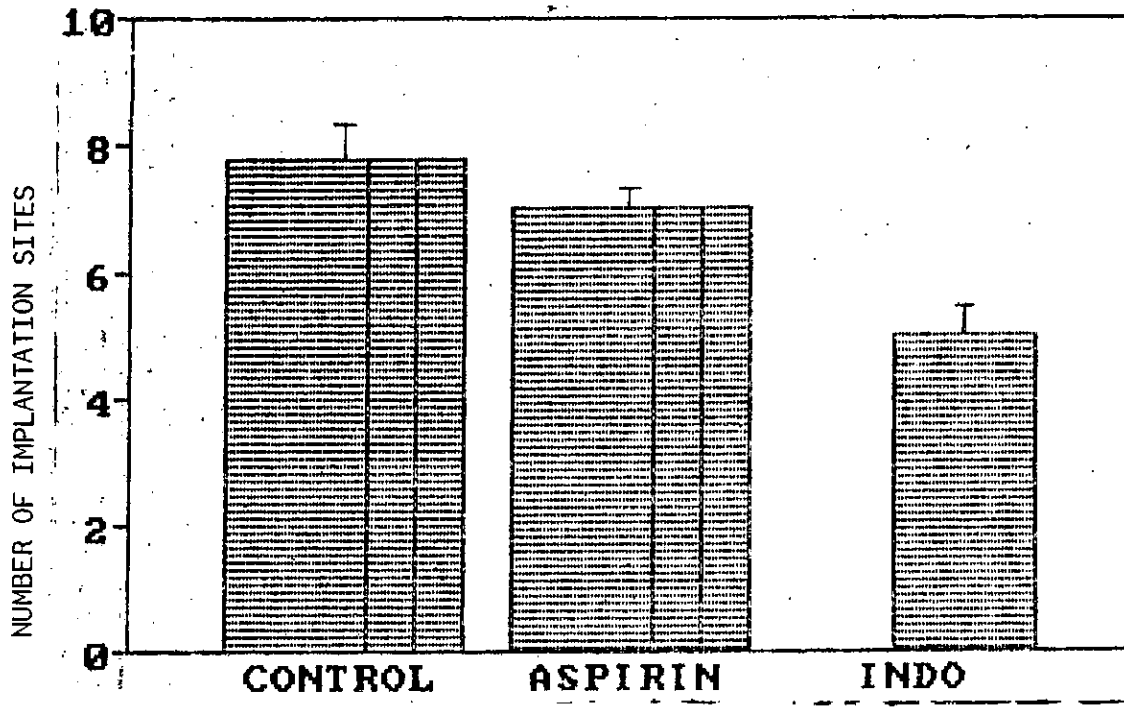


FIG. 25 - Effect of Aspirin and Indomethacin on Implantation of embryo.

T A B L E 14

EFFECT OF ASPIRIN AND INDOMETHACIN ADMINISTRATIOM IN EARLY AND LATE PREGNANCY ON LENGTH OF GESTATION

	Treatment	Average Length of Gestation (Days)
A	- (Control) *+	22 ± 0.40
B	Aspirin (100mg/kg) *	22 ± 0.40
C	Indomethacin (50mg/kg) *	22 ± 0.25
D	Aspirin (100mg/kg) +	23 ± 0.25
E	Indomethacin (50mg/kg) +	24 ± 0.25

* Drugs administered in early (days 4 & 5) pregnancy

+ Drugs administered in late (days 18 - 21) pregnancy

* Mean values between control (A) and treated groups (B & C) not significantly different ($P > 0.5$)

+ Means of control (A) and aspirin - treated (D) groups are not significantly different ($P > 0.1$)

Means of control (A) and indomethacin - treated (E) groups are significantly different ($P < 0.02$)

ANOVA - means of samples are significantly different

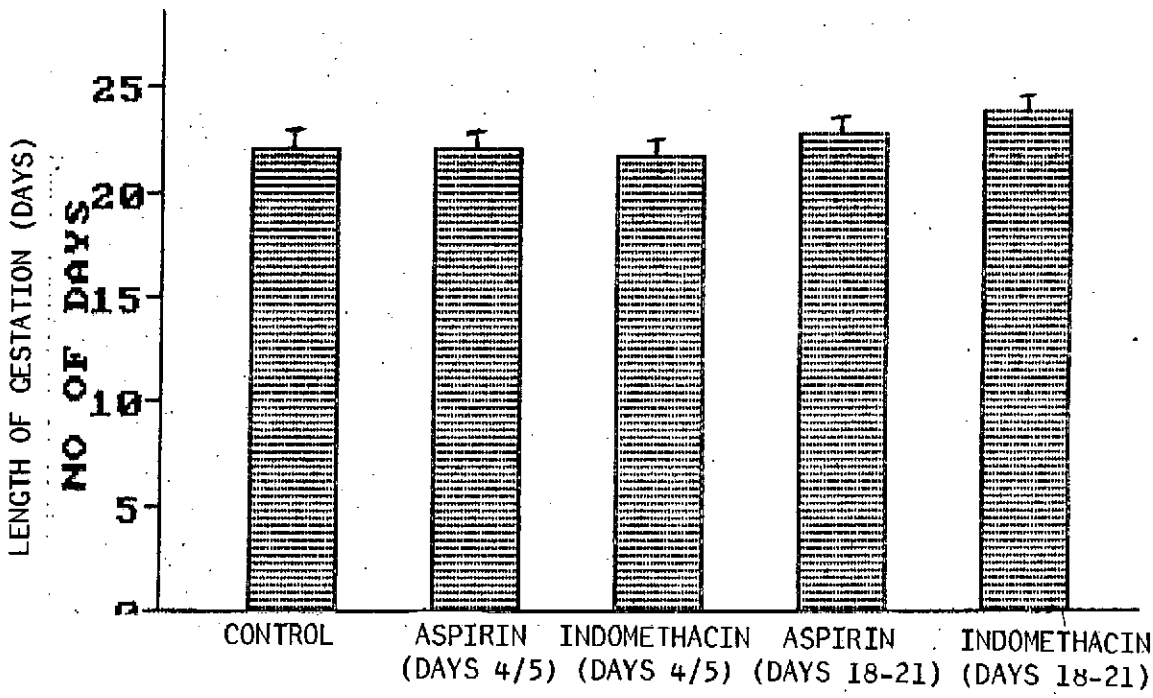


FIG. 26 - Effect of Aspirin and Indomethacin on the length of gestation.

TABLE 15

EFFECT OF ASPIRIN AND INDOMETHACIN ADMINISTRATION IN EARLY AND LATE PREGNANCY ON NUMBER OF OFFSPRINGS

	Treatment	Average No. of Offsprings
A	- (Control) *+	7 ± 0.40
B	Aspirin (100mg/kg) *	7 ± 0.25
C	Indomethacin (50mg/kg) *	7 ± 0.25
D	Aspirin (100mg/kg) +	6 ± 0.47
E	Indomethacin (50mg/kg) +	4 ± 0.25

* Drugs administered in early (days 4 & 5) pregnancy

+ Drugs administered in late (days 18 -21) pregnancy

* No difference between control and treated groups

+ Mean values between control and aspirin groups not significantly different ($P > 0.1$)

+ Means of control and indomethacin - treated groups are significantly different ($P < 0.01$)

ANOVA - means of samples are significantly different

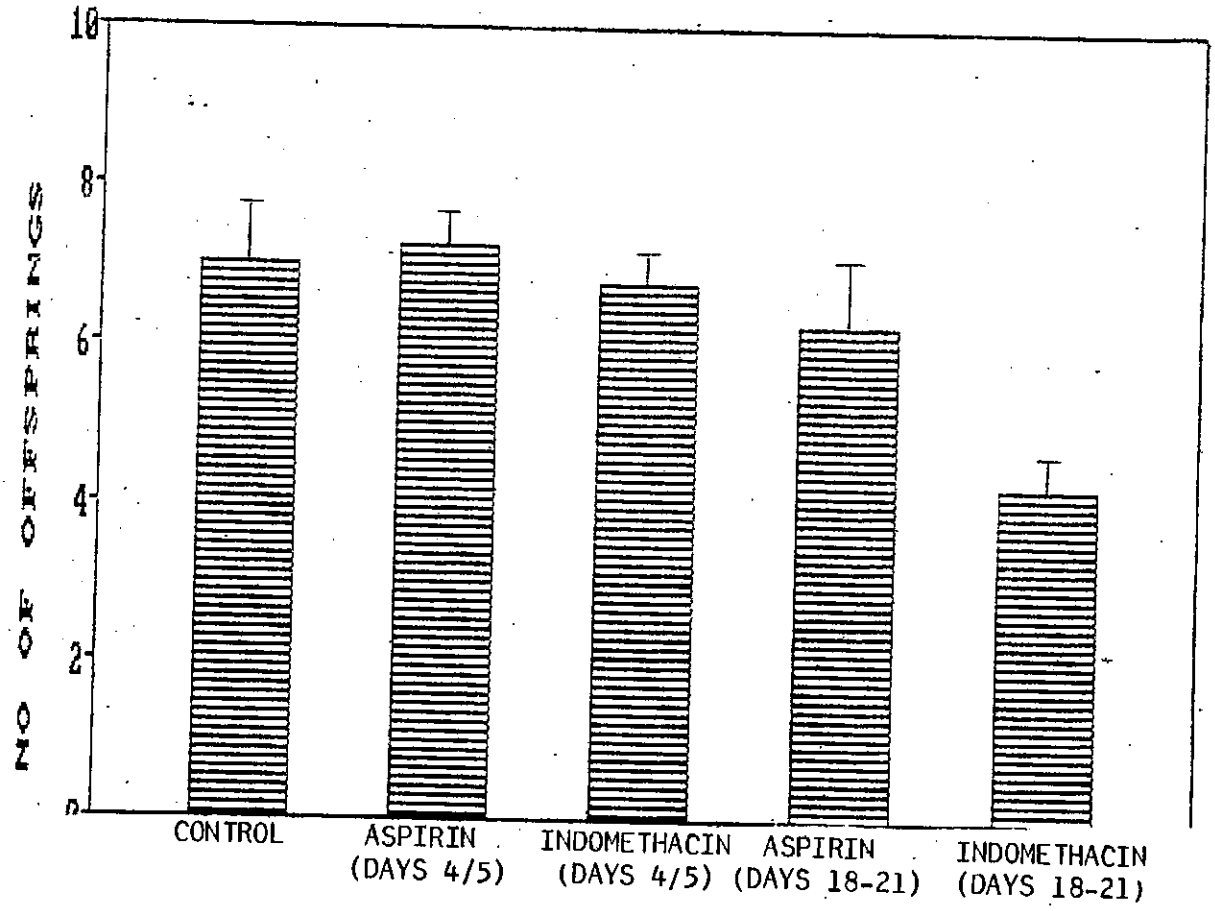


FIG. 27 - **Effect of Aspirin and Indomethacin on the number of offsprings delivered at the end of gestation.**

Discussion

Several studies have implicated prostaglandins as being necessary for the early part of pregnancy as well as for the termination pregnancy. Speroff et al (1984) noted that during pregnancy a decrease in local levels of progesterone combined with an increase in estrogen level leads to prostaglandin synthesis and increased myometrial activity. This is normally associated with the initiation of labour at the end of pregnancy. They also noted that prostaglandin levels in maternal blood and amniotic fluid begin to increase sharply a few hours to the onset of labour and reach a peak during delivery. Studies by Kooistra and Ginther (1976) also revealed that administration of $PGF_{2\alpha}$ in early pregnancy resulted in loss of the pregnancy.

If prostaglandins act to cause uterine contraction towards the end of gestation then one would expect that inhibitors of prostaglandin synthesis will do the opposite i.e delay or inhibit uterine contraction.

The present study has investigated any possible effects of prostaglandin inhibition on the implantation of embryos at the beginning of pregnancy. Results indicate that prostaglandin inhibition by aspirin did not adversely affect implantation sites (table 13; fig. 25). The dose of aspirin used was similar to that which caused suppression of ovulation as well as reduction of $PGF_{2\alpha}$ earlier in the study (tables 1 & 2). It therefore appears that the prostaglandin level needed for implantation to take place isrelatively low and a more drastic inhibition (probably by

a much higher dose) would be needed for implantation to be reduced or inhibited.

In the indomethacin-treated animals there was a significant reduction in the number of implants (table 13; fig. 25), an indication that prostaglandins may be quite important in the process of implantation. The dose of indomethacin used has been found in the present study to reduce PGF concentration to a much lower level than the experimental dose of Aspirin (table 2; fig. 11). It is therefore not surprising that an adverse effect on implantation was observed with indomethacin but not with aspirin. This gives more credence to the earlier suggestion that a drastic reduction or complete inhibition of prostaglandin level might be necessary for implantation to be inhibited.

According to Karim and Hillier (1979) the involvement of prostaglandins in implantation is poorly understood. However several studies have implicated prostaglandins in the process of implantation in the rat was delayed by treatment with indomethacin. Likewise Inskeep and Murdoch (1980) and Niswender et al (1985) reported that prostaglandins are luteotropic effectors and are therefore capable of eliciting an acute increase in steroidogenesis in luteal cells, or of maintaining progesterone secretion. Progesterone and estrogen are the luteal hormones and they enhance the process of implantation. Therefore if prostaglandins are indeed luteotropic they might be necessary for implantation to take place. In a study by

Chatterjee & Chatterjee (1982) it was observed that indomethacin not only interfered with the physiology of ovulation but effectively prevented blastocyst, implantation without obviously altering the hormonal profile.

The results from the present experiments show that prostaglandin inhibition by aspirin had no effect on the length of gestation. Whether aspirin was injected in early or late pregnancy the gestation period remained within the normal range (table 14; fig. 26). The non-interference of aspirin with the gestation period recorded in this work may again be due to the dose used, such that a higher dose might have reduced prostaglandin synthesis further and thereby prolonged the gestation period. This in effect is saying that prostaglandin level does not have to be very high for it to induce uterine contraction and labour.

In rats treated with indomethacin in early pregnancy the gestation period was the same as in control rats. However when indomethacin was administered in late pregnancy there was a slight but significant increase in the length of gestation (table 14; fig. 26). It is not surprising that indomethacin treatment in early pregnancy did not increase the gestation period because even though the indomethacin might have inhibited prostaglandin synthesis at the time of administration, the synthesis of prostaglandins must have resumed as soon as the inhibitory effect of indomethacin was lost. This means there must have been enough prostaglandin by late pregnancy to cause uterine contraction at the normalexpected time, thereby giving rise to a normal length of gestation.

In the case of indomethacin treatment in late pregnancy, the drug was administered during the crucial period when prostaglandin level normally rises. Speroff et al (1984) reported that in primates prostaglandin level is constantly low throughout pregnancy and only begins to rise just before labour. The prolongation of gestation which was observed suggests that indomethacin can reduce prostaglandin secretion to a level that is too low to initiate the process of labour or uterine contraction. The fact that the prolongation of labour was only for 2 days might be due to the resumption of prostaglandin synthesis after the effect of the last dose of indomethacin was lost. It will appear that with further injection of indomethacin the length of gestation might have been further prolonged.

Previous studies have shown that the response of the uterus to prostaglandins seems to depend upon the steroidal environment to which it is exposed (Labhsetwar, 1975; Martin et al, 1978; Klem et al, 1982). The reports from these studies suggest that low uterine sensitivity to prostaglandins correlates with high progesterone levels and low estrogen levels. Therefore the level of estrogen and progesterone must be taken into consideration in analysing the effect of prostaglandins on uterine contractility.

When the effect of aspirin and indomethacin on the number of offsprings delivered was investigated in the present study it was found that while aspirin injected in early or late pregnancy did not significantly affect the number of offsprings there was a significant reduction when

rats were treated with indomethacin in late pregnancy (table 15; fig. 26). Since the administration of indomethacin in early pregnancy did not reduce the final number of offsprings then it means that indomethacin did not inhibit implantation. On the other hand the reduction in the number of offsprings with indomethacin treatment in late pregnancy would appear to have been caused by a resorption of some fetuses. This was indeed confirmed when the rats were sacrificed after delivery. This finding is in agreement with an earlier work by O Grady et al (1972) who a.so administered indomethacin (16mg/kg/day) during pregnancy and observed a resorption of foetuses in all the rabbit. They however could not conclude whether the resorption was a toxic effect of indomethacin or whether it was due to inhibition of prostaglandin synthesis.

The non-effectiveness of aspirin in changing the number of offsprings delivered in the present experiments is again an indication that aspirin is only a mild inhibitor of prostaglandin synthesis and might need to be used in very high doses for any adverse effect to be observed. it can be concluded from the results of this set of experiments that prostaglandins may be vital to fetal survival, because a low level as would occur with indomethacin treatment caused a resorption of some of the fetuses.

GENERAL DISCUSSION AND CONCLUSIONS

Effect of Aspirin and Indomethacin on ovulation:

The present study has revealed that in both spontaneous and LH - induced ovulation, Aspirin and Indomethacin can suppress ovulation when given in anti-inflammatory doses. In this action indomethacin is seen to be a stronger inhibitor of ovulation than aspirin. Aspirin did not block ovulation completely but most of the ova shed during its administration were immature. The fact that these two drugs have been established as prostaglandin inhibitors (Espey, 1982; Chatterjee & Chatterjee, 1982) suggests that aspirin and indomethacin may be suppressing ovulation by inhibiting prostaglandin synthesis. This in turn implies that prostaglandins play a crucial role in the process of ovulation.

The results of the present work corroborate the implied role of prostaglandins in the process of ovulation (as mentioned above). The two drugs aspirin and indomethacin caused a reduction in PGF synthesis as observed in the circulating plasma PGF level. Again indomethacin was found to be more effective in this action. These results suggest a direct relationship between PGF level and the rate of ovulation. The lower the PGF level the greater the suppression of ovulation.

Measurement of preovulatory LH level in the present

study revealed that there was no change in LH concentration during the administration of aspirin and indomethacin. This is an indication that the absence or inhibition of prostaglandins (as would occur during aspirin and indomethacin administration) does not inhibit LH release as suggested by some previous workers (Makimo, 1973; Karim & Hillier, 1979). This means that prostaglandins may not be essential for the action of hypothalamic LHRH on pituitary LH release. The result also implies that the site of action of aspirin and indomethacin during their inhibition of ovulation is not the hypothalamic - pituitary axis but the ovary itself. If the action were on the hypothalamic - pituitary axis administration of aspirin and indomethacin would have caused a reduction in the LH level which could then have been responsible for the reduced rate of ovulation.

Morphometric studies in the present experiments also reveal that while indomethacin is effective in suppressing follicular rupture as was demonstrated by the low number of corpora lutea, aspirin retards ova maturation and possibly follicular growth.

It is therefore clear from the present findings that aspirin and indomethacin inhibit ovulation by inhibiting prostaglandin synthesis in the ovary. Although the actual mechanism by which prostaglandins bring about ovulation is still not clear, it does not appear to be via pituitary LH release. One thing that is certain however is that a low level of prostaglandin will prevent ova maturation while a

very low level will suppress or inhibit follicular rupture.

Aspirin and Indomethacin action in the presence of hCG:

The present study has revealed that hCG on its own can suppress ovulation rather than enhance it. This shows that even though hCG is widely used as an agent for induction of ovulation, it does not perform this function without prior priming of the ovarian follicles by other ovulation induction agents (Greenwald & Terranova, 1988). There is indication that for hCG to cause ovulation on its own it will have to be used in relatively high doses (50 i.u or higher), and that the main effect of hCG on follicles is to cause follicular rupture, and not follicular growth and ovum maturation.

Administration of hCG and Indomethacin was seen to block ovulation completely and this seems to suggest that there is a synergistic action between the two drugs to suppress ovulation, or at least confirms that hCG does not enhance ovulation without prior follicular priming. However when hCG was administered along with aspirin the rate of ovulation was seen to rise slightly. Since both hCG and aspirin had in earlier experiments individually suppressed ovulation, the observed enhancement of ovulation may mean that in the presence of aspirin hCG is able to exert its effect of enhancing follicular rupture. This may suggest that aspirin may act as a mediator of hCG action on the follicles. The experiments therefore suggest that although hCG is not able to reverse the inhibitory action of

indomethacin on ovulation it reverses that of aspirin. This was also observed in the morphometric studies, where it was shown that when HCG was administered with indomethacin therewas complete absence of corpora lutea. The reason for this difference in action again may lie in the fact that aspirin is only a mild anti-inflammatory agent while indomethacin is a much stronger one. If this suggestion is true it implies that ovulation is an inflammatory process, and that hCG has the ability to overcome mild anti-inflammatory reactions.

The present experiments also confirm the importance of prostaglandins in the process of ovulation. When there was a drastic reduction in PGF_2 level (during hCG and indomethacin administration) ovulation rate was found to be at its minimum. When preovulatory LH was measured, experimental values did not differ from control values, a pointer to an ovarian site of action for both aspirin and indomethacin.

Aspirin and indomethacin action in the presence of exogenous LH

In the present study Aspirin was observed to cause a slight inhibition of ovulation but mainly in the form of inhibition of ova maturation. Indomethacin caused suppression of ovulation rate by inhibiting follicular rupture. Administration of exogenous LH was not able to reverse these actions of aspirin and indomethacin, even though measurement of plasma LH revealed an elevation in

concentration of LH. This again corroborates the suggestion of an ovarian site of action for aspirin and indomethacin rather than a hypothalamic site as has been suggested by some workers. Reports by some previous workers (Bauminger & Lindner, 1975 Lindner et al, 1980) that LH causes an elevation in prostaglandin levels were confirmed in the present studies. Aspirin and Indomethacin caused a significant reduction in plasma preovulatory PGF level. However, on administration of exogenous LH plasma preovulatory PGF level was remarkably elevated in both instances. This is an indication that the mechanism by which LH causes ovulation may be that of elevation of PG synthesis which then causes follicular rupture.

Aspirin and Indomethacin action during pregnancy:

The present study has shown that prostaglandin inhibition by Aspirin does not adversely affect the rate of implantation, length of gestation or the number of offsprings delivered at the end of gestation. Since aspirin has been shown to reduce PGF_2 level as part of this study, the explanation for the above observation may be that aspirin at the dose used was not strong enough to reduce the PGF_2 to a level low enough to disturb any of the processes of pregnancy. This means that perhaps only when aspirin is administered at much higher doses can pregnancy be adversely affected.

Indomethacin has been seen to have adverse effects on pregnancy whether it was administered in the early or late

stages of pregnancy. It was observed that it reduced the rate of implantation when given during the early stages of pregnancy, and in late pregnancy it prolonged the gestation period and reduced the number of offsprings produced. These results indicate that prostaglandin synthesis must have been hindered during indomethacin administration leading to the delay in uterine contraction which would initiate the process of labour and delivery of offsprings. Since indomethacin administration did not inhibit implantation rate, a reduction in number of offsprings delivered as observed in the present experiments would mean that resorption of some fetuses occurred when indomethacin was given in late pregnancy, and this was in fact confirmed at the end of delivery when the rats were sacrificed and their uterine horns examined.

CONCLUSIONS

From the results of the present study the following conclusions can be arrived at:-

(1) Indomethacin and Aspirin can suppress both the spontaneous ovulation and superovulation when given in doses higher than the minimum anti-inflammatory dose. The two drugs seem to act by inhibiting prostaglandin synthesis. In their inhibitory action on ovulation indomethacin is seen to be much stronger and more potent. While indomethacin inhibits follicular rupture in particular, aspirin mainly inhibits ova maturation. Morphometric studies indicate that aspirin probably inhibits the whole process of follicle development.

(2) hCG at low doses cannot induce ovulation if the follicles have not been primed by an agent which enhances follicle development and recruitment e.g. PMS. hCG does not reverse the inhibition of ovulation by indomethacin, nor does it reverse the inhibition of ova maturation by aspirin.

(3) Although LH causes a significant elevation in prostaglandin level it does not reverse the inhibition of ovulation by aspirin and indomethacin. This is an indication that some other factors besides the presence of prostaglandins are necessary for ovulation to occur.

(4) Both Indomethacin and aspirin seem to exert their ovulation inhibitory action directly on the ovary rather

than the hypothalamic - pituitary axis.

(5) Aspirin (in high anti-inflammatory doses) whether administered in early or late pregnancy does not adversely affect implantation rate, length of gestation or the number of offsprings delivered. On the other hand indomethacin when administered in high anti-inflammatory doses in early pregnancy can reduce the rate of implantation. When administered in late pregnancy this high dose of indomethacin can prolong the gestation period, and may cause resorption of fetus, leading to a reduced number of offsprings. These results imply that prostaglandins (the synthesis of which is inhibited by aspirin and indomethacin) are quite important in these three aspects of pregnancy, namely, embryo implantation, length of gestation, and the number of offsprings produced at the end of gestation.

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