

قسم الفسيولوجيا والأقربالين  
كلية الطب البيطري - أدينا  
رئيس القسم : أ.د/ بهيج نعمة الله •

بعض الدراسات عن أهمية تخليق البروستاجلاندين  
أثناء عملية تثبيت الأجنة المبكر في الفئران الحوامل

سمير غنام ، بهيج نعمة الله ، محيي الدين عبد الله ، صبحي حداية

عولجت مجموعة من سبع فئران بالايندوميثاسين ( وهو مانع لتخليق البروستاجلاندين )  
في اليوم الخامس من الحمل ثم قتلت بعد ذلك بيومين • بالمقارنة بالمجموعة التحكم  
( والمكونة من 6 فئران ) فقد وجد أن حقن الايندوميثاسين ( 1 مجم لكل 100 جم من وزن  
الجسم تحت الجلد عند الساعة الخامسة والعاشرة والخامسة عشر ) لم يقلل من نسبة حدوث  
الحمل في الفئران المعالجة وكذلك لم يؤثر في عدد الأجنة المثبتة بالرحم •

وقد نتج عن حقن الايندوميثاسين نقصا معنويا في وزن القرون الرحمية ونقصا معنوياً  
في حجم الانتفاخات الجنينية • ولوحظ تجمع الانتفاخات الجنينية في طرف قرن الرحم  
القريب من المبيض في الفئران المعالجة بالايندوميثاسين وكذلك أحدث الايندوميثاسين  
زيادة معنوية في الأماكن الدالة على النفوق الجنيني المبكر في أرحام الفئران المعالجة به •  
ونتائج هذا البحث تدعم أهمية تخليق البروستاجلاندين أثناء المراحل المبكرة من  
الحمل في الفئران •

Dept. of Physiology, Biochemistry and Pharmacology,  
Faculty of Vet. Med., Alex. University,  
Head of Dept. Dr. B.R. Nemetallah.

**SOME STUDIES ON THE IMPORTANCE OF PROSTAGLANDIN  
SYNTHESIS DURING EARLY IMPLANTATION IN PREGNANT RATS**  
(With One Table & One Fig.)

By  
**S.A. GHANAM; B.R. NEMETALLAH; M.D. MOHAMED**  
**and S.A. HEDALIAH\***  
(Received at 10/11/1987)

**SUMMARY**

Rats were treated with indomethacin (prostaglandin synthesis inhibitor) on Day 5 of pregnancy then killed 2 days later. As compared to control pregnant rats, indomethacin treatment (1 mg/100 g body weight given S/C at 05:00, 10:00 and 15:00 hr) did not reduce the incidence of pregnancy among treated rats or the number of implantation swellings. However, indomethacin treatment significantly decreased ( $P/ < 0.01$ ) the weight of uterine horns and significantly reduced ( $P/ < 0.001$ ) the size of implantation swellings. Aggregation of the implantation swellings was observed near the ovarian end of the uteri of indomethacin treated rats. Besides, a significant increase ( $P/ < 0.05$ ) in the sites of early embryonic death was observed in uteri of indomethacin treated rats.

These data may confirm the importance of prostaglandin synthesis during early pregnancy in rats.

**INTRODUCTION**

Implantation of blastocyst involves a complex series of morphological and biochemical interactions between the blastocyst and the uterus. A bunch of evidence points to the possible involvement of prostaglandins in the implantation process. In this respect, a pre-implantation peak of uterine PGF was observed in rats (GARG, 1981) and PGE and F were found in the blastocysts of Day 5 pregnant rabbit (DICKMANN and SPILMAN, 1975) and in Day 13-15 pregnant cow (SHEMESH, MILAGUIR, AYALON and HANSEL, 1979) and are released from uterine tissues by decidual stimuli (KENNEDY and LUKASH, 1982). Besides, PGs are considered as mediators of the increased endometrial vascular permeability (EVANS and KENNEDY, 1978). Inhibition of PG synthesis following indomethacin treatment (FLOWER, 1974) delays the appearance of the uterine vascular reponse during implantation in rats (CHRISTINE, PHILLIPS and POYSER, 1981), mice (SAKSENA, LAU and CHANG, 1976) and rabbits (HOFFMAN, DIPIETRO and McKENNA, 1978).

The present study was planned to reveal the importance of PG synthesis during early implantation process in rats.

---

\* Part of M.V.Sc. Thesis presented to Alexandria Univ.

## MATERIAL and METHODS

Mature female albino rats, weighing 225-250 g, were placed with males of proven fertility. Vaginal smears were examined each morning for the presence of spermatozoa, the first appearance of which was considered Day- 1 of pregnancy. The pregnant rats were either injected with indomethacin (Sigma chemical Co.) which is (1- [p-chorobenzayl] -5- methoxy -2- methylindole -3- acetic acid) or received the vehicle. Indomethacin was administered subcutaneously to 7 female rats at 05:00, 10:00 and 15:00 (3 PM) hr Day 5 of pregnancy in 0.2 ml 0.5% tween (Merck Schuchardt) in sterile 0.9% NaCl at dose level of 1 mg/100 g body weight (KENNEDY, 1977; EVANS and KENNEDY, 1978). Control animals (6 female rats) received an equal volume of vehicle. Experimental rats were decapitated at about 10:00 hr on Day 7 of pregnancy. After laparotomy, the uteri were rapidly dissected out, trimmed free from fat and then weighed to the nearest mg. The uterine horns were pinned out with mapping pins on filter paper moistened with saline and laid on a cork board. The pins were inserted at the junction of the two horns and at the ovarian ends. The size of each implantation swelling was measured by using divider and ruler (graduated from 0.5 mm to 20 cm) and recorded in mm (O'GRADY and HEALD, 1969). The number of implantation swellings was counted in each uterine horn.

### Detection of early embryonic death:

Uterine horns from indomethacin treated and control rats were cut longitudinally with a fine pointed scissor, each horn was then immersed in 10% ammonium sulphide for about 10 min. The uteri were examined for the presence of black spots which indicates embryonic resorption sites (KOPF, LORENZ and SALEWSKI, 1964).

## RESULTS

As shown in table 1, indomethacin treatment on Day 5 of pregnancy significantly reduced ( $P/$  0.01) the weight of the uterine horns of treated rats. However, the incidence of pregnancy among the indomethacin treated rats was not significantly different from that in control group. The total number of implantation swellings was not significantly reduced after indomethacin treatment of pregnant rats. Indomethacin treatment significantly decreased ( $P/$  0.001) the size of implantation swellings. In three pregnant rats treated with indomethacin the implantation swellings showed a benching or crowding manner near the ovarian end of the uteri (incidence 50%). A significant increase ( $P/$  0.05) in the number of black spots representing sites of early embryonic death (Fig. 1) was observed in uteri of indomethacin treated rats as compared to those of vehicle treated rats.

## DISCUSSION

In the present study, indomethacin treatment of pregnant rats on Day 5 of pregnancy did not affect the incidence of pregnancy or the number of implantation swellings as compared to controls. This finding was previously reported by KENNEDY (1977) in rats and by EVANS and KENNEDY (1978) in hamsters. In rabbits, SAKSENA and HARPER (1974) reported that indomethacin treatment of donors had no effect on subsequent implantation of their blastocysts transferred to normal recipients. Besides, in the present study, indomethacin treatment of pregnant rats significantly reduced the weight of uterine horns of treated rats. This effect may be subsequent to inhibition of increased endometrial permeability following indomethacin

## PROSTAGLANDIN SYNTHESIS DURING IMPLANTATION

treatment (CASTRACANE, SAKSENA and SHAIKH, 1974; HOFFMAN, *et al.* 1978). The significant reduction in the size of the implantation swellings observed in indomethacin treated rats is consistent with the previous findings of KENNEDY (1977) and EVANS and KENNEDY (1978) who reported that indomethacin causes decrease in size of implantation swellings, delays implantation and prolong gestation periods in rats and hamsters respectively. RECENTLY, JONES, CAO, ANDERSON, NORRIS and HARPER (1986) provided evidence that indomethacin causes a delay rather than a complete inhibition of implantation in rabbits. These authors reported that blastocysts from indomethacin-treated donors are depleted of PGs however, they can become replenished and then release these PGs in a recipient rabbit. In the present study, overcrowding of implantation swellings near the ovarian end of the uterine horns was observed in 50% of indomethacin treated pregnant rats. Similar findings were observed by KENNEDY (1977) in rats and HOFFMAN, *et al.* (1978) in rabbits. The spacing of blastocysts in the uterus of the rat is determined by myometrial activity (O'GRADY and HEALD, 1969) which is under the control of prostaglandins (VANE and WILLIAMS, 1972; LABHSETWAR, 1974). Thus in the present study the observed uneven distribution of implantation swellings following indomethacin treatment may be due to inhibition of prostaglandin synthesis.

Also, in the present study it was found that the mean number of embryonic resorption sites/rat was significantly increased in indomethacin treated rats as compared to values in control rats which indicate that the incidence of early embryonic death was increased in indomethacin treated rats and this may be due to insufficient blood supply to the implanted blastocysts, as a result of inhibiting prostaglandin synthesis by indomethacin. This suggestion agrees with the previous findings of O'GRADY, CALDWELL, AULETTA and SPEROFF (1972); SAKSENA and HARPER (1974) who found that indomethacin causes substantial fetal mortality and resorption of foeti when administered to rabbits in early days of pregnancy.

In summation, the results of the present study support the possibility that prostaglandin synthesis during early pregnancy do have a role in the implantation process.

## REFERENCES

- Castracane, V.D.; Saksena, S.K. and Shaikh, A.A. (1974): Effect of IUD's, prostaglandins and indomethacin on decidual cell reaction in the rat. *Prostaglandins* 6: 397.
- Christine, A.; Phillips, L. and Poyser, N.L. (1981): Studies on the involvement of prostaglandins in the rat. *J. Reprod. Fert.* 62: 73-81.
- Dickmann, Z. and Spilman, C.H. (1975): Prostaglandins in rabbit blastocysts. *Science*. 190: 997-998.
- Evans, C.A. and Kennedy, J.G. (1978): The importance of prostaglandin synthesis for the initiation of blastocyst implantation in the hamster. *J. Reprod. Fert.* 54: 255-261.
- Flower, R.J. (1974): Drugs which inhibit prostaglandin biosynthesis. *pharmacol. Rev.* 26: 33-67.
- Garg, S.K. (1981): Uterine prostaglandins E and F levels during pregnancy in the rat. *Indian. Med. Res.* 73: 637-640.
- Hoffman, L.H.; Dipietro, U.L. and McKenna, T.J. (1978): Effect of indomethacin on uterine capillary permeability and blastocyst development in rabbits. *Prostaglandins* 15: 823-829.
- Jones, M.A.; Cao, Z.; Anderson, W.; Norris, C. and Harper, J.K. (1986): Capillary permeability changes in the uteri of recipient rabbits after transfer of blastocysts from indomethacin-treated donors. *J. Reprod. Fert.* 78: 261-273.
- Kennedy, T.G. (1977): Evidence for a role for prostaglandins in the initiation of blastocyst implantation in the rat. *Biol. Reprod.* 16: 286-291.

- Kennedy, T.G. and Lukash, L.A. (1982): Induction of decidualization in rats by the intrauterine infusion of prostaglandins. *Biol. Reprod.* 27: 253-260.
- Kopf, R.; Lorenz, D. and Salewski, E. (1964): Der Einfluss von Thalidomid auf die fertilitat von Ratten in Generation seversuch uber Zwei Generationen. Nauny, Schmiedeberg's. *Arch. Exp. Path. Pharmac.* 247: 121.
- Labhsetwar, A.P. (1974): Prostaglandins and the reproductive cycle. *Fed. Proc.* 33: 61-77.
- O'Grady, J.P.; Caldwell, B.V.; Auletta, F.J. and Speroff, L. (1972): The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation, pregnancy and pseudopregnancy in the rabbit. *Prostaglandins.* 1: 97.
- O'Grady, J.P. and Heald, P.T. (1969): The position and spacing of implantation sites in the uterus of the rat during early pregnancy. *J. Reprod. Fert.* 20: 407-412.
- Saksena, S.K. and Harper, M.K. (1974): Prostaglandin-mediated action of intrauterine devices: F-PGs in the uterine horns of pregnant rabbits with unilateral intrauterine devices. *Fert. Steril.* 25: 121.
- Saksena, S.K.; Lau, I.F. and Chang, M.C. (1976): Relationship between oestrogen,  $PGF_2$  and histamine in delayed implantation in the mouse. *Acta. Endocr.* 81: 801-807.
- Shemesh, M.; Milaguir, F.; Ayalon, N. and Hansel, W. (1979): Steriogenesis and prostaglandin synthesis by cultured bovine blastocysts. *J. Reprod. Fert.* 56: 181-185.
- Vane, J.R. and Williams, K.I. (1972): Prostaglandin production contributes to the contractions of the rat isolated uterus. *Br. J. Pharmac.* 45: 146.

**Table (1)**  
**Effect of indomethacin on early implantation on Day 7 of pregnancy in rats**

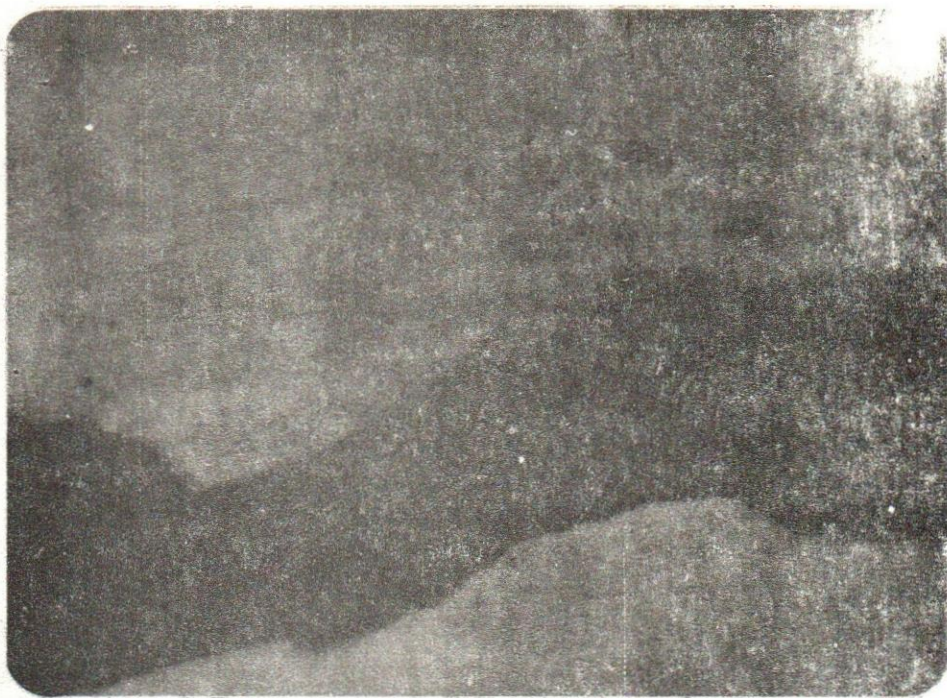
Item	Control	Indomethacin treated rats
1) Weight of uterine horns (mg).	457.5±12.9	364.3±20.8 <sup>a</sup>
2) Proportion of pregnant rats	5/6	6/7
3) Total number of implantation swellings in both uteri.	10.2± 0.49	9.4± 0.93 <sup>o</sup>
4) Size of implantation swellings in both uteri (mm).	3.3± 0.06	2.8± 0.08 <sup>b</sup>
5) Number of embryonic resorption sites in both uteri.	3.4± 0.51	5.6± 0.68 <sup>c</sup>

- Values are mean ± S.E.M.

- Values are significantly different from control at: a (P/ 0.01), b: (P/ 0.001), c: (P/ 0.05). (t-test).

<sup>o</sup> Overcrowding of implantation swellings near the ovarian end of the uterine horns was observed in 3 animals.





**Fig. (1)**

Embryonic resorption sites (black spots) in uterine horn of indomethacin treated rat on day 7 of pregnancy showed by immersing the open uteri in 10% amm. sulphide for 10 min.



1912  
The above is a list of the names of the persons who were  
admitted to the office of the Secretary of the  
State of New York on the 1st day of January, 1912.