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# The Degenerative Effect on Rabbit Implantation Sites by Indomethacin. II. Changes in Estrogen and Progesterone Levels as a Possible Cause for Indomethacin Antifertility Effect

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ABSTRACT. The effect of Indomethacin (Id) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) alone or in combination on plasma estradiol-17B and progesterone levels during days 5-9 of pregnancy in the rabbit was determined. Id treatment prevented the rise in plasma progesterone which was observed to occur in the non-treated animals after day 7. Treatment with $PGF_{2\alpha}$ alone or together with Id resulted in a significant drop in plasma progesterone with subsequent disruption of the progesterone to estradiol ratio. Furthermore, there was only a limited success in reversing the antifertility effect of Id by $PGF_{2\alpha}$ supplementation. The results of this experiment suggest that the antifertility effect of Id is at least partially related to its disruptive action on progesterone levels.

Several studies have indicated that indomethacin (Id), a potent inhibitor of prostaglandin (PG) biosynthesis (Vane 1971, Flower 1974) increases the occurrence of embryonic death and resorption when given to rabbits early in pregnancy (El-Banna *et al.* 1976, Hoffman 1978, El-Banna 1980). The mechanism by which Id interferes with pregnancy is still uncertain. Although the major biological effects of Id seem to be related to its inhibition of PG synthesis (El-Banna 1980), it is possible that Id antifertility effect is mediated through an effect on the ovarian steroids and their circulating levels. Little has been done to investigate this possibility, and the limited work reported in the literature indicates that plasma estradiol and progesterone are unaffected by the Id treatment (Evans and Kennedy 1978, Hoffman *et al.* 1978). In these reports, however, no attempt was made to study the daily change in plasma estrogen and progesterone, in the same individual, during Id treatment. Such an attempt is necessary to

determine the effect of the treatment on the relationship between these two hormones at the critical stage of implantation.

The present study was designed a) to determine the effect of Id and $PGF_{2\alpha}$, alone or in combination, on peripheral estradiol and progesterone levels and b) to test the hypothesis that the inhibition of PG synthesis by Id causes disruption of the normal ovarian steroid levels and/or ratio and thus leads to unsuccessful implantation.

## Material and Methods

Female New Zealand white rabiits weighing 3.5-4 kg were used. Rabbits were isolated for at least 3 weeks prior to use. Each doe was mated to two fertile bucks and the day of mating was designated as day 0 of pregnancy. Id (Sigma) was prepared as a 300 mg/ml suspension in sesame oil, and the dose used was 8 mg/kg body weight twice daily. $PGF_{2\alpha}$ (Sigma) was prepared in 0.9% NaCl. Rabbits were randomly divided into 10 groups and subcutaneous injections were initiated on day 5 of pregnancy and continued twice daily until day 9 as follows: 1) control, received the same volume of vehicle, 2) Id alone, 3) Id + 10 $\mu$g/kg $PGF_{2\alpha}$, 4) Id + 50 $\mu$g/kg $PGF_{2\alpha}$, 5) Id + 100 $\mu$g/kg $PGF_{2\alpha}$, 6) Id + 150 $\mu$g/kg $PGF_{2\alpha}$, 7) Id + 200 $\mu$g/kg $PGF_{2\alpha}$, 8) 50 $\mu$g/kg $PGF_{2\alpha}$, 9) 200 $\mu$g/kg $PGF_{2\alpha}$, and 10) 250 $\mu$g/kg $PGF_{2\alpha}$.

The animals were bled from a lateral ear vein and blood samples were taken daily from each animal starting on day 5 until day 9 of pregnancy. The blood was collected in chilled heparinized tubes, centrifuged immediately at 4°C, and the plasma was stored at −18°C until analysis. All animals were sacrificed at day 9, and the number of corpora lutea (CL) on each ovary as well as the number of implantation sites were measured. Embryos were inspected under the stereoscopic microscope (10× to 30× magnification) to determine viability.

Estradiol-17B was determined by radioimmunoassay without chromatography using a sepcific antiserum for estradiol-17B-6-carboxy-*o*-methyl-oxime-bovine serum albumin as described by Elsaesser *et al.* (1978). The inter- and intra-assay coefficients of variation were 13.3 and 9.0%, respectively. The concentrations of progesterone were determined by radioimmunoassay without chromatography using a specific antiserum directed against progesterone-11$\alpha$-bovine serum albumin as described by Parvizi *et al.* (1976). The inter-and intra-assay coefficients of variation were 12.0 and 8.0%, respectively.

### *Statistical Analysis*

Student's t-test (Snedecor 1956) was used to detect significant differences between specific time points. Results were expressed as means ± S.E.M.

## Results

The percentages of preimplantation loss and of viable embryos in the different treatments are shown in Table 1. The complete data on the effects of Id and/or $PGF_{2\alpha}$ on

**Table 1.** Effect of indomethacin and/or $PGF_{2\alpha}$ on pregnancy in rabbits as observed on day 9 after mating.

| Treatment | No. of Animals | No. of C.L. | No. of Implantation Sites | No. of Viable Embryos | Preimplantation loss (%)+ | Viable Embryos (%) |
|---|---|---|---|---|---|---|
| Control | 7 | 62 (8.9±0.4) | 58 (8.3±0.7) | 58 (8.3±0.7) | 6.4 | 100.0 |
| Id | 14 | 140 (10.0±0.5) | 75 (5.4±0.9) | 6 (0.4±0.1)* | 46.4 | 8.0 |
| Id + 10 $\mu$g/kg $PGF_{2\alpha}$ | 6 | 63 (10.5±0.7) | 25 (4.2±0.8) | 3 (0.5±0.09)* | 60.3 | 12.0 |
| Id + 50 $\mu$g/kg $PGF_{2\alpha}$ | 5 | 42 (8.4±0.8) | 19 (3.8±1.0) | 8 (1.6±0.4)* | 54.8 | 42.0 |
| Id+100 $\mu$g/kg $PGF_{2\alpha}$ | 6 | 56 (9.3±0.7) | 35 (5.8±0.9) | 21 (3.5±0.8)* | 37.5 | 60.0 |
| Id+150 $\mu$g/kg $PGF_{2\alpha}$ | 4 | 37 (9.3±0.9) | 17 (4.3±1.0) | 6 (1.5±0.5)* | 54.0 | 35.3 |
| Id+200 $\mu$g/kg $PGF_{2\alpha}$ | 6 | 45 (7.5±0.8) | 7 (1.2±0.4) | 0 | 84.4 | 0 |
| 50 $\mu$g/kg $PGF_{2\alpha}$ | 6 | 57 (9.5±0.4) | 55 (9.2±0.8) | 41 (6.8±0.6) | 3.5 | 74.6 |
| 200 $\mu$g/kg $PGF_{2\alpha}$ | 5 | 36 (7.2±0.8) | 16 (3.2±0.7) | 12 (2.4±0.6)* | 55.5 | 75.0 |
| 250 $\mu$g/kg $PGF_{2\alpha}$ | 8 | 68 (8.5±0.6) | 28 (3.5±0.9) | 8 (1.0±0.2)* | 58.8 | 28.6 |

+ The preimplantation loss is the difference between the numbers of implantation sites and CL.

* Values significantly different from the control at $P < 0.05$ (paired test). Values in parenthesis represent the Mean ± S.E.M.

implantation and embryo viability has been reported previously (El-Banna 1980). Fig. 1 shows the changes in plasma estradiol and progesterone levels in the control and in the Id treated animals during days 5-9 of pregnancy. The pattern of change in plasma estradiol in the Id treated animals did not differ significantly from that of the control. In the control animals, there was a slight decline in estradiol concentration from an average value of 38.0 ± 2.4 pg/ml on day 5 to an average value of 31.6 ± 2.5 pg/ml on day 9. In the Id treated animals the decline was from an average of 42.4 ± 2.6 pg/ml on day 5 to an average value of 32.8 ± 2.5 pg/ml on day 9. Progesterone concentrations in the control animals increased significantly ($P < 0.05$) between days 5 and 9 of pregnancy. The average value of progesterone on day 5 was 8.8 ± 0.7 ng/ml and the average value on day 9 was 14.0 ± 1.1 ng/ml. In the Id treated animals progesterone concentrations remained about the same during the treatment period. The average value on day 5 was 8.8 ± 0.5 ng/ml and the average value on day 9 was 9.3 ± 0.7 ng/ml; the difference between the two values was not statistically significant. Progesterone concentrations on day 9 in the Id treated animals was significantly ($P < 0.05$) lower than the corresponding value in the control animals.

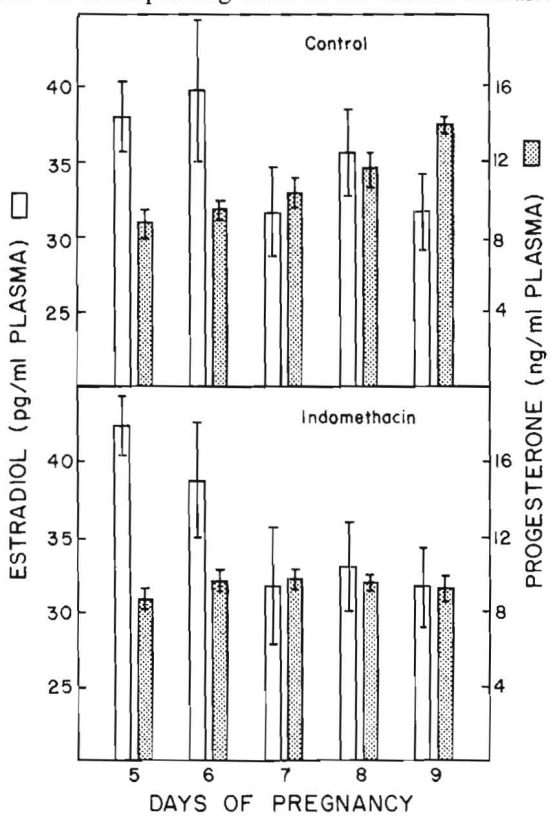


**Fig.1.** Changes in plasma estradiol and progesterone concentrations (Mean ± S.E.M.) in control and Id treated rabbits during days 5-9 of pregnancy.

Figure 2 shows the effect of treatment with Id plus increasing doses of $PGF_{2\alpha}$ on estradiol and progesterone levels. In all groups, except the group treated with Id + 10 $\mu$g/kg $PGF_{2\alpha}$, plasma estradiol and progesterone concentrations began to decline after the second day of treatment, and the decline in progesterone levels was more dramatic. On day 9 of pregnancy, the lowest plasma estradiol concentration was 28.0 $\pm$ 4.1 pg/ml in animals treated with Id + 200 $\mu$g/kg $PGF_{2\alpha}$, and the lowest plasma progesterone concentration was 0.7 $\pm$ 0.1 ng/ml in animals treated either with 150 $\mu$g or 200 $\mu$g/kg $PGF_{2\alpha}$.

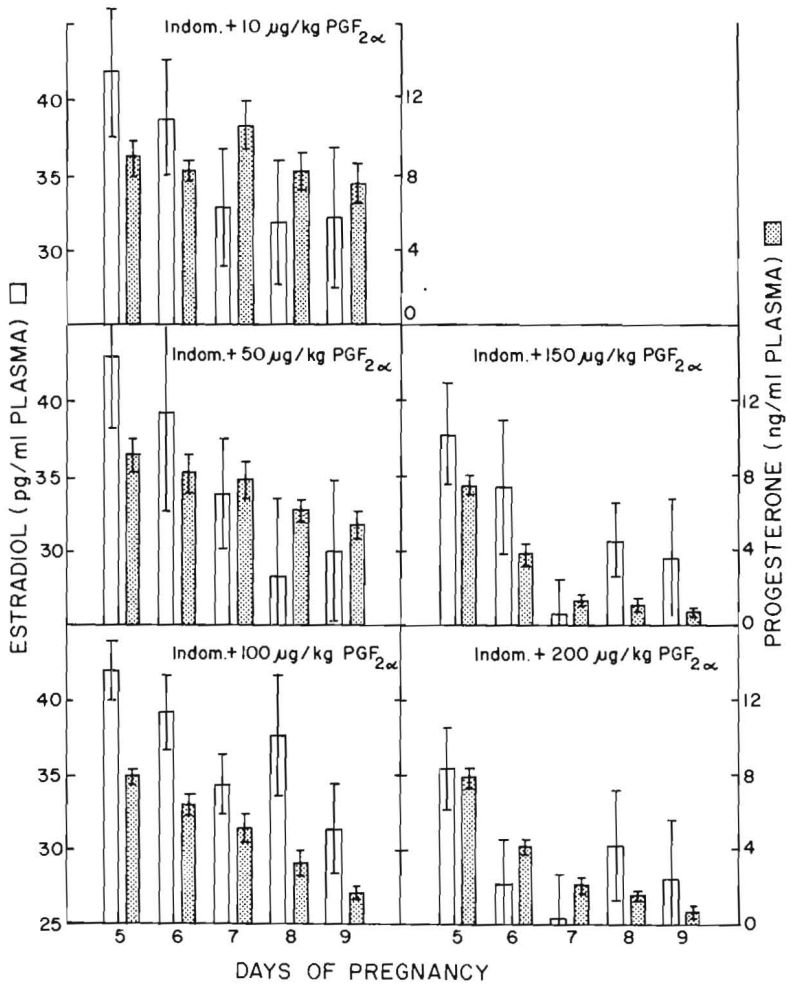


**Fig.2.** Effect of treatment with Id plus increasing doses of $PGF_{2\alpha}$ on plasma estradiol and progesterone concentrations (Mean $\pm$ S.E.M.) in rabbits during days 5-9 pregnancy.

The effect of treatment with $PGF_{2\alpha}$ alone on estradiol and progesterone levels is shown in Fig. 3. $PGF_{2\alpha}$ alone had a similar effect to that of Id plus $PGF_{2\alpha}$, and there was a decline in the concentrations of both steroids as the treatment continued. The decline in progesterone concentrations was more pronounced as $PGF_{2\alpha}$ dose was increased.

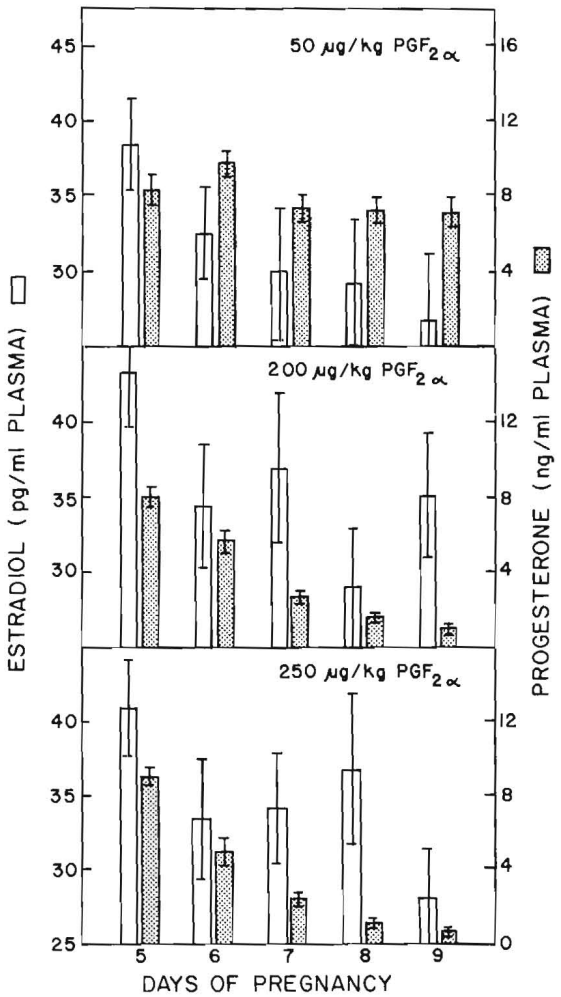


**Fig.3.** Effect of treatment with different doses of $PGF_{2\alpha}$ on plasma estradiol and progesterone concentrations (Mean ± S.E.M.) in rabbits during days 5-9 of pregnancy.

The changes in progesterone to estradiol ratio in the different treatment groups are shown in Fig. 4. In the control group, this ratio climbed steadily from 232:1 on day 5 to 444:1 on day 9 of pregnancy. In groups treated with Id alone, or Id + 10 or 50 $\mu$g/kg $PGF_{2\alpha}$, or with 50 $\mu$g/kg $PGF_{2\alpha}$ alone, progesterone to estradiol ratio remained about constant with little variations. In all other groups, the progesterone to estradiol ratio showed a rapid decline.

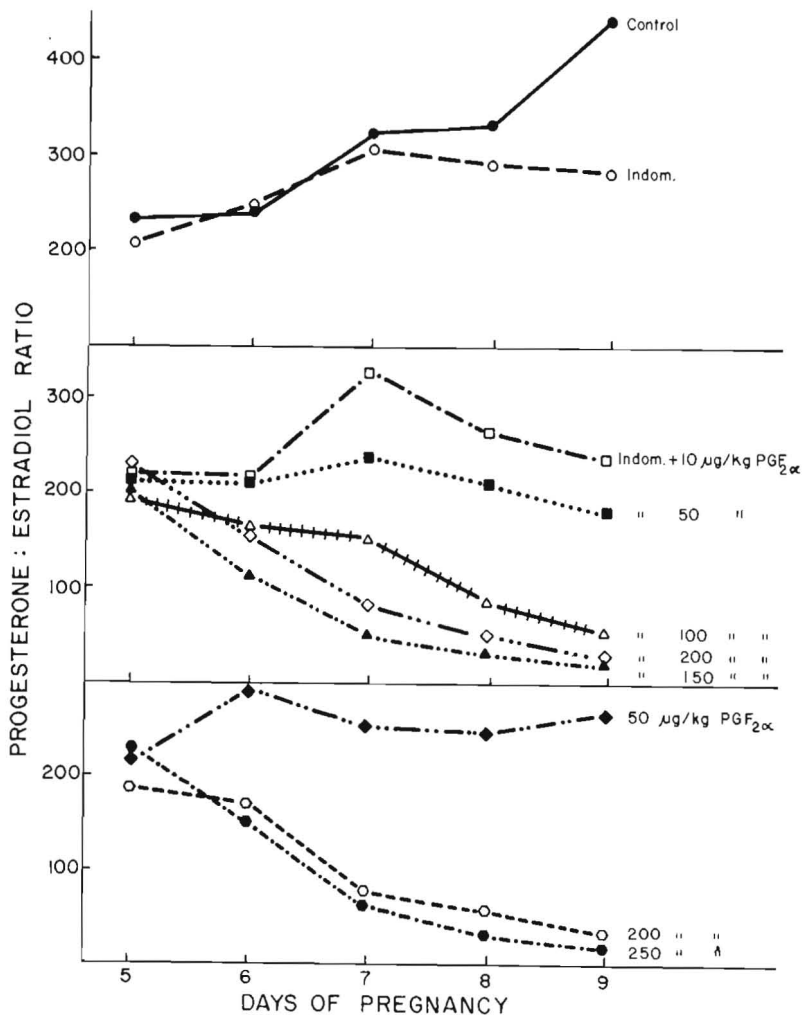


**Fig.4.** Progesterone to estradiol ratio in the different treatment groups as calculated from the average concentrations on each day of treatment.

## Discussion

The author has recently reported (El-Banna 1980) that the antifertility effect of Id in rabbits is most effective when Id is administered (8 mg/kg) twice daily during days 5-7 of pregnancy, and that persisting levels of Id during this critical stage are required to induce the effect. In the present study, the results indicate that Id treatment has no effect on plasma estradiol levels, but the treatment significantly altered the pattern of plasma progesterone. The gradual increase in progesterone levels observed in the control group on days 8 and 9 of pregnancy did not occur in the Id treated animals, and progesterone levels remained about constant during the period of Id treatment. In both the control and Id treated animals, the progesterone to estradiol ratio was about the same until day 7 of pregnancy. After day 7 the ratio continued to increase in the control but remained unchanged in the Id treatment animals. It is possible that the failure of progesterone to increase in Id treated animals had contributed to the embryonic death and resorption observed on day 9 of pregnancy. Progesterone is the hormone of pregnancy as it appears to be essential for maintenance of pregnancy in all mammals (Dickmann *et al.* 1976, Siiteri *et al.* 1977). Reduction or disruption of progesterone levels or of the progesterone to estradiol ratio could interfere with implantation (Dickmann *et al.* 1976) or could lead to abnormal implantation with subsequent embryonic death and resorption, as seen in this experiment. Recent observations (El-Banna, unpublished) suggest that Id treatment alters the electrophoretic pattern of uterine luminal proteins in pseudopregnant rabbits. This alteration, which first appeared on day 7 and persisted through day 9 of pseudopregnancy, could be because of a disruption of progesterone levels in the Id treated rabbits and could create unfavourable conditions for maintaining pregnancy. Progesterone is known to regulate the production of certain rabbit uterine proteins (Arthur and Daniel 1972). Lau *et al.* (1973) observed a reversal of Id-induced infertility in pregnant mice with progesterone. In rabbits, Hoffman (1978) reported that concurrent administration of progesterone with Id did not reverse the antifertility effect. In that report, however, it was noticed that progesterone administration was discontinued after day 7 of pregnancy which, according to the present study, is the time when the effect of Id on progesterone levels starts to appear. Therefore, continued administration of progesterone beyond day 7 could have produced different effect.

The mechanism by which Id interferes with progesterone levels is not clear. Several lines of evidence indicate that Id is most likely to act through its inhibition of PG synthesis (Sananes *et al.* 1976, Tobert 1976, Hoffman *et al.* 1977, Kennedy 1977, Hoffman *et al.* 1978). Recent reports (Hoffman 1977, Kennedy 1977, Kennedy and Zamecnik 1978) suggest that the absence of prostaglandins at the time of implantation prevents the endometrial vascular permeability that precedes, and apparently is required for, successful blastocyst implantation. The absence of prostaglandins, however could also have an effect on steroidogenesis, particularly on progesterone synthesis. There is a considerable amount of evidence which shows that prostaglandins act as mediators in steroidogenesis (Speroff and Ramwell 1970, Marsh 1971, Kuehl 1974,

Silver and Smith 1975), and the author believes that the reduction in progesterone levels observed in the present study is related to such action. This effect on progesterone levels is apparently a delayed effect, since it was first observed after two days of Id treatment. The effect is also selective, since the stage of pregnancy most sensitive to the antifertility effect of Id in the rabbit is during days 5-7 of pregnancy (El-Banna 1980).

There was a limited success in reversing the antifertility effect of Id with the simultaneous administration of $PGF_{2\alpha}$ in the present study. Replacement doses of 50 $\mu$g/kg, 100 $\mu$g/kg or 150 $\mu$g/kg $PGF_{2\alpha}$, resulted in an improvement in the number of viable embryos, but not in the amount of preimplantation loss. Also, replacement doses of $PGF_{2\alpha}$ in the present study failed to restore the steroid levels to normal. To the contrary, the effect of such treatment further reduced both estradiol and progesterone levels, particularly with higher doses of $PGF_{2\alpha}$. This latter effect could be due to the luteolytic action of $PGF_{2\alpha}$ (Scott and Rennie 1970). The failure of $PGF_{2\alpha}$ to reverse the antifertility effect of Id could also be due to several reasons such as; the short half-life of $PGF_{2\alpha}$ (Raz 1972), the need for prostaglandins other than $PGF_{2\alpha}$, or the need for a combination of prostaglandins. Remaining, however, is the possibility that Id may have a direct effect on the ovaries in a way that is not related to the inhibition of prostaglandin synthesis. For example, a direct effect on the enzyme system for progesterone synthesis.

The author concludes, from these observations, that the antifertility effect of Id is, at least partially, related to the hormonal imbalance caused by the disruption of plasma progesterone levels. Further studies are in progress to investigate the mechanism by which Id affects progesterone synthesis and/or release.

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# ضمـــور الأجنـة في الأرانب نتيجـــة المعـاملة بالإندوميثاسين.
# ٢ ـ الـتغـــير في مـســتـوى الاســتـــروجـــين والبروجستيرون كسبب محتمـل للتأثـير المضاد للخصوبة للإندوميثاسين

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في هذا البحث تمت دراسة تـأثير المعـاملة بالإنـدوميثاسـين والبروستاجلاندن ( ف٢ الفا )، كل على حدة أو مع بعضهما البعض، على مستوى الإستروجين والبروجستيرون في بلازما الدم في الأرانب خلال الفترة من اليوم الخامس إلى اليوم التاسع من الحمل. وقد أدت المعاملة بالإندوميثاسين إلى منع الزيادة في البروجستيرون التي تحدث عادة بعد اليوم السابع من الحمـل في الأرانب غـير المعـاملة. أمـا المعـاملة بالبروستاجلاندن ( ف٢ الفا ) على حدة أو مع الإندوميثاسين فقد تسببت في انخفاض معنوي في بروجستيرون البلازما، وبالتالي في نسبة البروجستيرون إلى الإستروجين في الدم. ولم ينتج عن المعاملة بالبروستاجلانـدن ( فـ٢ الفا ) إلا نجاح محـدود في التغلّب عـلى التـأثـير المضـاد للخصـوبـة لمـادة الإندوميثاسين.

وتدل نتائج هذه الدراسة على أن التأثير المضاد للخصوبة لمادة الإندوميثاسين قد يكون نتيجة عمل هـذه المادة عـلى إحداث خلل في مستوى هرمون البروجستيرون.