

## EFFECTS OF INDOMETACIN ON ALLOGENIC AND SYNGENIC PREGNANCY

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**Summary.** Factors of making and keeping immunotolerance in pregnancy mainly affect immunocompetent cells by immunosuppressive factors or moving of Th1/Th2 balance in favour of Th2 response. Errors made in making and keeping immunotolerance in pregnancy can lead to clinic entities such as resorption of fetoplacental unit, miscarriage and reduced placental and fetal weight. Besides, production of sex steroids in the yellow body of gravidity and later in the fetoplacental unit depends on the factors such as prostaglandins and cytokines. The significance of paternal and maternal MHC (in)compatibility of rats for the number of embryos, percentage of embryos in resorption, placental and fetal weight and the level of serum steroids were tested in our experiment. Specimens taken for this investigation were syngene Sprague Doley (SD) and Wistar (W). The rats of SD and W lineage were treated by indometacin of 2.1-2.8 mg/kg daily by giving them drinking water in the period of 6-18 day of pregnancy. Stimulation of Th1 response by indometacin in allogenic pregnancy led to resorption of 56% embryos while the rate of resorption in the control group of W rats was 12%. Syngene SD animals treated by indometacin did not show a significantly higher rate of embryos in resorption (8.14%) than the ones in the control group (5.13%) Acceleration of Th1 response by indometacin led to activation of decidual CTL and NK cells as well as to intensified expression of MHC antigens on the cells of trophoblast. In allogenic pregnancy this resulted in the increased resorption of embryos, while in SYNGENIC pregnancy acceleration of Th1 response did not result in increased resorption of embryos. Besides, indometacin leads to the significant fall of the level of serum progesterone and oestradiol notwithstanding the MHC compatibility of pregnancy However, the level of serum progesterone in W rats treated by indometacin is significantly lower than in SD rats also treated by indometacin. This result shows the possibility of greater damage of trophoblast in MHC incompatible pregnancies under the influence of indometacin or greater activities of cytokine of Th1 group, which inhibits synthesis of sex steroids.

**Key words:** Pregnancy, indometacin, progesterone, oestradiol, prostaglandin, immunomodulation, Th1 and Th2 response

### Introduction

From its early days the embryo shows a good expression of MHC antigens class II and I and from the point of view of MHC incompatibility, it can be defined as allogenic transplant (1,2). Placenta has embryonic origin and it is directly connected with maternal immunocompetent cells, but the character of expression of MHC antigens on the placental tissues is selective, restrictive and conditional. Varied limitations at the expression of MHC antigens impede the definition of placenta as allogenic transplant (1,2). Fetoplacental unit under certain conditions can become a target of aggression by maternal immune system. As a counterpoise to the potential immunodestructive mechanisms, the whole series of immunoprotective mechanisms of pregnancy immunotolerance have been developed (3). One of the main factors of making and keeping immunotolerance

in pregnancy is prostaglandine activity of decidua and trophoblast. Placenta mainly synthesizes immunomodulatory PGE2 whose major role is suppression of immunocompetent cells over the inhibition of secretion IL-2 and the inhibition of expression of receptors for IL-2 (2,3).

The whole series of research by Lala et al. shows in the best way a role of prostaglandins in pregnancy. These authors got the high percentage of resorption of fetoplacental unit and miscarriages, which raised to even 89% in experiments based on chronical treatment of allogravid female rats by indometacin or IL-2. The authors explained this phenomenon by immune mechanisms, which were mediated by rejection of fetoplacental unit as allogenic transplant (3). In the researches that appeared later the same group of authors confirmed that for the fetoplacental unit resorption stimulated by indometacin or IL-2 mainly responsible are decidual NK

cells, which are otherwise suppressed by decidual prostaglandins and Th2 cytokines. Decidual NK cells show a very low rate of activity to YAC-1 lymphoma cells. Decidual NK cells from allogenic pregnant animals treated by indometacin or IL-2 show a high degree of anti-YAC-1 activity (3,4). In reference to the IL-10 importance compared by the process of marring and keeping immunotolerance in pregnancy, it is significant to mention that PGE2 considerably increases IL-10 and IL-8 concentration in the maternal serum and decidual compartment (3,4).

On the other hand, pregnancy is the condition when a great concentration of sex steroids serum occurs. This is the consequence of increased synthesis of these hormones, first in the yellow body of pregnancy and later in placenta or more precisely in the fetoplacental unit (5).

Hypothalamic-hypophysis hormones and chorionic gonadotropin are other mediators that have influence on the intensity of biosynthesis of sex steroids, first of all those which have influence on the level of cAMP, such as prostaglandins and histamine (1,2,6).

Indometacin efficiently suppresses luteolytic characteristics of prostaglandins so that it is proved that the yellow body persists, even in non-pregnant animals, until there is a significant suppression of synthesis of prostaglandins by indometacin (7,8). As for ovarian steroidogenesis more significant is the lipooxygenic way of metabolism of arachidonic acid than the cyclooxygenic way. Inhibition of the lipooxygenic way results in decrease of intensity of ovarian steroidogenesis, while the inhibition of cyclooxygenase (COX) by indometacin, except antiluteolytic effects, has no significant effect on ovarian steroidogenesis (1,9).

The other mechanisms of including prostaglandins in the regulation of biosynthesis of sex steroids is more significant for pregnancy and is based on stimulation of adenylyl-cyclase and the increase of concentration of cAMP in trophoblast cells, that results in intensifying of the process of basic steroidogenesis and synthesis of all steroid hormones. Placenta is a place of very intensive synthesis of prostaglandins that by all means take part in the stimulation of the process of biosynthesis of placental sex steroids. Sendrani et al. affirm that PGE2 stimulates synthesis of all steroid hormones (2,7,9). Treatment of female rabbits by indometacin in the early pregnancy makes a significant slow down of steroidogenesis and decrease of the level of serum progesterone and oestradiol. Parallel treatment of female rabbits by indometacin and PGE2 restore the level of all products of steroidogenesis to the physiological level (7,9).

Cytokines are very often mentioned as factors that can have influence on the level of sex steroids, as well as on ovarian and placental production of these hormones. Ohno et al. in their investigations *in vitro* got the suppression of production of progesterone from granular cells cultivated with IL-2, while in their research IL-1 did not show such effects. They came to conclusion that the inhibition of production of progesterone depends on the dosage related to IL-2 concentrations in medium (2,10,11).

In contrast to granular cells, trophoblast cells intensify steroidogenesis when IL-1 stimulates them. This cytokine stimulates aromatase of steroidogenic enzymatic system of trophoblast cells and in that way intensifies secretion of progesterone and estrogenic hormones. This is explained by intensified production of prostaglandins in cultured trophoblast cells stimulated by IL-1. By adding anti-IL-1 antibody, the level of progesterone and estrogenic hormones is restored to the level of control. The effects of IL-2 on trophoblast cells and their steroidogenic potential are negative. It is proved that IL-2 inhibits production of sex steroids (2,10).

One of the aims of this research was to establish the significance of MHC incompatibility on the number of embryos, the percentage of embryos in resorption, placental and fetal weight. As the aim of the research we also set the observation of these parameters in the conditions of acceleration Th1 response by indometacin in allogenic and syngenic pregnancy and establishing the effects of indometacin on synthesis of sex steroids in pregnancy. As the aim of the research we also set a comparison of effects of indometacin as an accelerator of Th1 type immune response on synthesis of sex steroids in allogenic and syngenic pregnancy.

## Materials and Methods

The animals have been divided into 4 groups, two of them control ones for W and SD rats and two experimental groups of W and SD rats, which have been treated by indometacin. Animals for experiment have been female, 12-16 week old and weighed  $190 \pm 10$  g. Female rats from all four groups have been mated in harem. Indometacin has been included in the drinking water to the experimental groups of W and SD rats from the 6th day of pregnancy. Regarding the fact that a rat of 200 g weight drinks 30-40 ml water daily, concentration of indometacin is adapted to  $14 \mu\text{g/ml}$ , so that a daily dosage of indometacin, which animals consumed, was 2.1-2.8 mg/kg. The animals were sacrificed on the 18th day of pregnancy in Nesdonal anesthesia by heart puncture. The blood got from the heart was kept at room temperature for 2 hours in order to form a blood cake and to separate the serum. After that it was centrifuged at 3000 rpm in the period of 5 minutes. This serum was taken by an automatic pipette and put aside at  $-20^\circ\text{C}$  till the moment of further treatment. The level of serum progesterone and oestradiol has been determined by RIA methods on the RIA System-Junior F-520, by means of original kits of monoclonal antibodies (INEP-Zemun).

## Results

The results of this research show that the number of embryos in allogenic pregnancy is significantly larger ( $p < 0.05$ ) than in syngenic pregnancy, while the treat-

ment by indometacin did not significantly have any influence on the total number of embryos of SD and W rats (Table 1). The percentage of embryos in resorption of allogravid female rats treated by indometacin is significantly larger ( $p < 0.05$ ) than the percentage and the number of embryos in resorption of untreated allogravid animals and syngenic pregnant rats treated by indometacin (Table 2, Fig. 1).

Table 1. Average values of the number of embryos found in the uterus of gravid rats.

	Sprague Doley rats		Wistar rats	
	Treated by Control (n = 8)	Treated by indometacin (n = 9)	Treated by Control (n = 8)	Treated by indometacin (n = 9)
Altogether	78	86	104	109
Average	9.75	9.56	13.00	12.11
St.Dev.	0.85	1.24	2.56	1.96

Table 2. Average values of the number of embryos in resorption found in the uterus of gravid rats.

	Sprague Doley rats		Wistar rats	
	Treated by Control (n = 8)	Treated by indometacin (n = 9)	Treated by Control (n = 8)	Treated by indometacin (n = 9)
Altogether	4	7	13	61
Average	0.5	0.78	1.63	6.78
St.Dev.	0.53	0.67	1.19	2.11

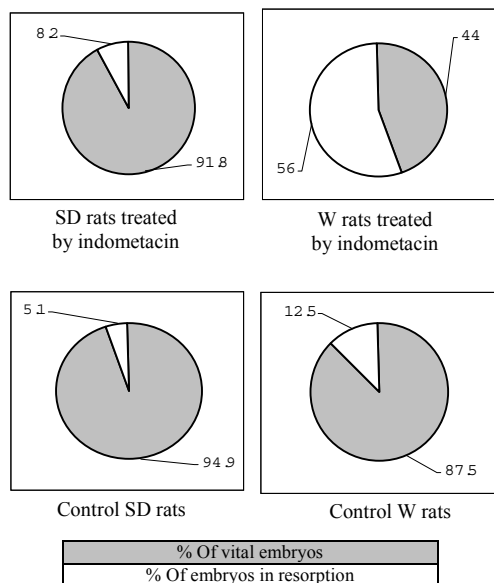


Fig. 1. Percentage of the vital embryos and the embryos in resorption at all groups of rats.

The values of serum progesterone in allogenic pregnancy do not significantly differ ( $p < 0.05$ ) from the values of serum progesterone of syngene pregnancy. Treatment by indometacin had a significant influence ( $p < 0.05$ ) on the low value of serum progesterone in allogenic and syngenic pregnancy. Recorded fall of concentration of serum progesterone under the influence of indometacin in allogenic pregnancy is more significant

( $p < 0.05$ ) than the one in syngenic pregnancy (Fig. 2).

The values of serum oestradiol in allogenic pregnancy do not significantly differ ( $p < 0.05$ ) from the values of serum oestradiol of syngenic pregnancy. Treatment by indometacin had a significant influence ( $p < 0.05$ ) on the fall of value of serum oestradiol in allogenic and syngenic pregnancy (Fig. 2).

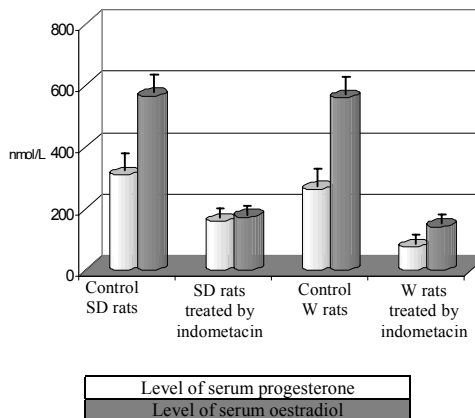


Fig. 2. The level of serum progesterone and oestradiol at all four groups of rats.

### Discussion

Result related to comparison of number of embryos in allogenic and syngenic pregnancy are in accordance with some citations from literature that alloimmunization can be a factor that will contribute to a larger number of descendants (12). There is an opinion that activation of Th2 response in allogenic pregnancy contributes to a more successful implantation and keeping a large number of embryos in the conditions of multifetal pregnancy (13). Mechanisms that induced a significant rate of embryos in resorption in allogravid animals treated by indometacin are probably related to pushing out of PGE2 synthesis. Inhibition of placental production of prostaglandin induces activation Th1 response (2,3). As a result of the intensified Th1 response, an intensified secretion of cytokine such as IL-2, TNF, and IFN- $\gamma$  is induced. These cytokines contribute to the intensified resorption of fetoplacental unit activating decidual NK cells and Tc cells. Above mentioned cytokines stimulate the expression of MHC antigens on the trophoblast cells and in that way emphasize the immune reaction focused against fetoplacental unit (1,13).

Lala et al. in parenteral treatment of allogravid mice by indometacin got the percentage of embryos in resorption of 89%, and they got the same percentage of embryos in resorption in the groups of mice treated by IL-2. This group of authors affirms that NK cells are the main effector cells in the process of fetoplacental resorption mediated by immune mechanisms (3,4).

For the phenomenon of fetoplacental unit resorption mediated by immune mechanisms two prerequisites are necessary and they are: (i) the presence of paternal MHC alloantigens and (ii) acceleration of Th1 re-

sponses. One fact shows that there is no statistically significant difference in the percentage of embryos in resorption of control group of SD rats and in the percentage of embryos in resorption of the group of SD rats treated by indometacin. Namely, besides the treatment by indometacin of gravid syngenic SD rats and the factor of acceleration of Th1 response it did not come to the significant increase in the percentage of resorption of embryos, and that is so because of compatibility of paternal and maternal MHC phenotypes of the trophoblast cells (Table 1, Table 2, Fig. 1). The fact that there is no statistically significant difference in the resorption between control groups W and SD rats, shows that in the conditions of normal pregnancy when there is a balance of Th1 and Th2 response, MHC incompatibility of allogene pregnancy of W rats does not have any significance for developing the phenomenon of fetoplacental unit resorption. Judging by the percentage of fetal resorption, it can be said that allogenic and syngenic pregnant animals in the condition of balanced Th1 and Th2 response behave in the same way. This proves that trophoblast shows a very low rate of expression of MHC antigens, but only to the moment of imbalance between Th1 and Th2 responses in favor of Th1 response (1,2).

The results of our research show that MHC incompatibility in allogene pregnancy has no significance for the synthesis of progesterone and its serum level. We come to this conclusion by observation that in syngene pregnant animals treated by indometacin, the level of serum progesterone is significantly lower than in non-treated syngene animals and this decrease of the level of progesterone is proportionate to the decrease of the level of progesterone in treated out bred animals.

Prostaglandins have the role to regulate the biosynthesis of sex steroids, which is included in the stimulation of adenylcyclase enzyme, so that by the increase of concentration of cAMP, they activate protein kinase and the process of phosphorylation of ester-cholesterols, and as consequence of that intensifying of the process of basic steroidogenesis and synthesis of all steroid hormones (1,2,15). The inhibition of synthesis of prostaglandins by indometacin could be responsible for the decrease of steroidogenic potential of trophoblast and for the decrease of value of concentration of serum progesterone, as well as in allogenic and syngenic pregnancy.

The level of serum progesterone of allogravid animals treated by indometacin is significantly lower than the level of serum progesterone of syngene animals treated by indometacin as well, and that is so because of greater damage of placental tissue in relation to treated syngene animals. That is why we come to the conclusion that the significance of MHC incompatibility in terms of the level of serum progesterone can be emphasized in the conditions of immunostimulation of

maternal immune system and probably intensified expression of MHC antigen on the trophoblast. This might show that MHC incompatibility itself is not of essential significance for some pregnancy parameters, while in the condition of immunostimulation and likely intensified expression of MHC antigens on the placental cells result in disordered homeostasis in pregnancy.

It is well known that cytokines can have some influence on the level of sex steroids, on the ovarian as well as on the placental production of sex steroids hormones (1,2,10). The results related to the serum level of oestradiol in allogenic and syngenic pregnancy show the little importance of MHC incompatibility for the intensity of synthesis and level of this hormone in pregnancy. The mechanisms of the inhibitory effect of indometacin on the synthesis of estrogen hormones in pregnancy are the same as in the relation of indometacin and progesterone. It might be said that indometacin generally inhibits all steroidogenic processes by the inhibition of prostaglandins synthesis and by the significant changes in the network of cytokines, above all trophoblast-decidual union.

## Conclusion

Based on the given data from literature and analyzing the given results, we could draw the following conclusions:

MHC incompatibility in allogene pregnancy is a factor that has a considerable influence on the total number of embryos.

The treatment by indometacin can considerably increase the percentage of embryos in the resorption, but only in case of incompatibility between maternal and embryonic MHC phenotype, while in syngenic pregnancy there is not a significant influence in the percentage of embryos in the resorption.

MHC incompatibility of allogenic pregnancy does not have a considerable influence on the average values of serum progesterone.

Indometacin considerably decreases the average values of serum progesterone regardless of the fact whether pregnancy is allogenic or syngenic.

Indometacin efficiently inhibits the synthesis of progesterone in allogene pregnancy that is probably related to MHC incompatibility and acceleration of Th1 response.

MHC incompatibility of allogene pregnancy does not have a considerable influence on average values of serum oestradiol.

Indometacin considerably decreases average values of serum oestradiol regardless of the fact whether pregnancy is allogenic or syngenic.

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## EFEKAT INDOMETACINA NA ALOGENU I SINGENU TRUDNOĆU

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Kratak sadržaj: *Faktori uspostavljanja i održavanja gravidarne imunotolerancije uglavnom deluju direktno imunosupresivno na imunokompetentne ćelije ili na pomeranje Th1/Th2 balansa u korist Th2 odgovora. Greške u uspostavljanju i održavanju gravidarne imunotolerancije dovode od kliničkih entiteta kao što su resorpcija fetoplacentne jedinice, spontani pobačaj, smanjena placentna i fetalna masa. Osim toga, produkcija polnih steroida u žutom telu trudnoće, a kasnije u fetoplacentnoj jedinici je zavisna od faktora kao što su prostaglandini i citokini. U našem eksperimentu je ispitivan značaj paternalne i maternalne MHC (in)kompatibilnosti pacova na brojnost plodova, procenat plodova u resorpciji, placentnu i fetalnu masu i nivo serumskih steroida. Kao eksperimentalni model su poslužili singeni Sprague Doley (SD) i alogeni Wistar (W) pacovi. Pacovi soja SD i W su tretirani indometacinom 2,1-2,8 mg/kg dnevno kroz pijaću vodu od 6. do 18. dana trudnoće. Stimulacija Th1 odgovora indometacinom je u alogenoj trudnoći dovela do resorpcije 56% plodova dok je stopa resorpcije u kontrolnoj grupi W pacova bila 12,5%. Singene SD životinje tretirane indometacinom nisu pokazale značajno veću stopu plodova u resorpciji (8,14%) od kontrolne grupe (5,13%). Akceleracija Th1 odgovora indometacinom je dovela do aktivacije decidualnih Tc i NK ćelija, kao i do pojačane ekspresije MHC antigena na ćelijama trofoblasta. U slučaju alogene trudnoće, ovo je rezultovalo povećanom resorpcijom plodova, dok u singenoj trudnoći akceleracija Th1 odgovora nije rezultovala povećanjem resorpcije plodova. Osim toga, indometacin dovodi do značajnog pada nivoa serumskog progesterona i estradiola bez obzira na MHC kompatibilnost trudnoće. Međutim, nivo serumskog progesterona kod W pacova tretiranih indometacinom je značajno niži nego kod SD pacova takođe tretiranih indometacinom, što ukazuje na mogućnost većeg oštećenja trofoblasta u MHC inkompatibilnim trudnoćama pod uticajem indometacina ili veće aktivnosti citokina Th1 grupe koji inhibišu sintezu polnih steroida.*

Ključne reči: *Trudnoća, indometacin, progesteron, estradiol, imunomodulacija, Th1 i Th2 odgovor*