

Assessment of Tumor Necrosis Factor Alpha, Interleukin 1 Beta and Interleukin 6 Concentrations during Implantation and Placentation Period of Pregnancy in the Mouse

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Abstract

The present study was designed to assess tumor necrosis factor alpha, interleukin 1 beta and interleukin 6 concentrations in the mouse uterus in preimplantation, postimplantation and in dioestrus period. In this study, a total of twenty-three adult female Swiss Albino mice were separated into four groups. Group 1 (n = 5), group 2 (n = 6), group 3 (n = 6) was gone through ovariohysterectomy at preimplantation day 3, 10 and 16, respectively. Group 4 (n = 6) was confirmed to be in dioestrus after vaginal cytology and the same operation was performed for nonpregnant dioestrus mice as controls. Uterine tissues of all groups were snap frozen in liquid nitrogen and stored at -80°C until tumor necrosis factor alpha, interleukin 1 beta and interleukin 6 assay. Sera were removed and stored at -20°C for progesterone and oestradiol measurements. The concentration of tumor necrosis factor alpha was not statistically different among the groups (P > 0.05), whereas interleukin 1 beta concentration was lower in postimplantation day 10 uterus than dioestrus uterine tissues (P < 0.05). Interleukin 6 concentration decreased at postimplantation day 16 compared to postimplantation day 10 (P < 0.05) and dioestrus period (P < 0.05). However, the serum progesterone and oestradiol concentrations significantly increased during pregnancy when compared to dioestrus period (p < 0.05). There was a negative correlation between interleukin-6 concentration and oestradiol concentration in dioestrus period (r = -0.92; P < 0.05). Tumor necrosis factor alpha concentration was negatively correlated with interleukin 1 beta concentration in postimplantation day 16 (r = -0.83; P < 0.05). High interleukin 6 concentration during preimplantation period and low concentration of interleukin 1 beta might be related with maternal recognition of conceptuses and maintenance of pregnancy.

Keywords: Implantation; Placentation; Mice; Tumor Necrosis Factor Alpha; Interleukin 1 Beta; Interleukin 6

Introduction

Cytokines, growth factors and hormones are released during interactions between the embryo and the uterus that are necessary for establishment of pregnancy [1]. Shifting the immun respond toward type 2 T helper (Th2) cells secreting interleukin 6 (IL-6) might be beneficial, whereas toward pro-inflammatory Th1 cells secreting tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) might be harmful for the fetus [2]. Cytokines are proved to have important roles in the stages of canine pregnancy both in maternal uterine tissue and embryo during preimplantation and placentation in bitches [3,4]. TNF-α is a cytokine that involves in apoptosis and

cell survival in inflammation and immunity [5]. It is expressed by endometrial tissue and trophoblasts between day 30 and 55 of mare pregnancy and is thought to be participated in the placental growth and maternal leukocyte responses to trophoblasts [6].

Interleukin-1 β , IL-6 and TNF- α are amongst the cytokines that have roles in human trophoblast invasion and implantation. IL-1 β is known to be secreted by cytotrophoblastic (CTB) cells, endometrial glandular epithelium and stromal cells [7] and to upregulate leptin production by human CTB cells [8]. Leptin is considered to be a regulator of cytotrophoblast invasiveness during implantation and placentation [9]. IL-6 has also role in leptin secretion therefore implantation [10].

It has been postulated that IL-1 β , IL-6 and TNF- α were involved in the establishment of pregnancy in pigs [11,12] particularly by means of myometrial synthesis of prostaglandin F2 α and prostaglandin E2 during the first third of gestation [13] as well as during luteolysis in cyclic pigs [14]. In contrary, TNF- α and IL-6 gene expression were not determined during the implantation and placentation stages of canine pregnancy though detected at early dioestrous uterus [4].

In mice, expression of TNF- α and IL-6 was determined to increase during estrus and to decrease at metestrus and to be basal at diestrus [15]. In addition, the presence of IL-1, IL-6 and TNF- α in mouse preimplantation embryos [16] and in the preimplantation uterus [17] was detected. The aim of this study was to investigate whether TNF- α , IL-1 β and IL-6 concentrations differ in the mouse gravid uterus in preimplantation period postimplantation day 10 and 16 as well as in non-gravid diestrus uterus.

Materials and Methods

A total of twenty-three adult female Swiss Albino mice weighing 30 - 35g and aged 2.0 - 2.5 months were randomly divided into four groups. Animals were maintained with a 12-h light/dark schedule and fed standard mice diet (Korkuteli Food Industry, Turkey) ad libitum. The estrus cycle phase of the mice was identified by vaginal cytology. Group 1 (n = 5), group 2 (n = 6) and group 3 (n = 6) were mated and when spermatozoites were seen on the smears, the mice was diagnosed as pregnant (Day 0) [18]. Group 1 was gone through ovariectomy (OHE) at preimplantation period (Day 3) and identified as early pregnant by embryo flushing immediately after the operation. Group 2 and 3 were gone through OHE at postimplantation day 10 and 16, respectively. Group 4 (n = 6) was confirmed to be at diestrus after vaginal cytologic examination. The same operation was performed for group 4 that were nonpregnant diestrus mice as controls. Median laparotomy was performed in all groups under xylazine HCl (5 mg/kg, im) and ketamine HCl (45 mg/kg, im) anaesthesia. Uterine tissues of all groups together with placenta in Group 1, 2 and 3 were excised from the middle of the left horn. Surrounding tissues, fats and fetuses were removed. Tissues were snap frozen in liquid nitrogen and stored at -80°C until TNF- α , IL-1 β and IL-6 assay. Intravenous blood samples from tail vein were obtained preoperatively into plain tubes and were immediately centrifuged at 1550g for 10 minutes. Sera were removed and stored at -20°C for progesterone and oestradiol measurements.

All procedures including the use of animals were approved by the Gazi University Animal Experiments Local Ethics Committee (Approval no:16.33; Turkey) and were performed at Laboratory Animals Breeding and Experimental Researches Center of the same university.

Uterine tissue extraction

Uterine tissue samples were homogenized using a homogenizer (Sonics Vibra Cell VCX 130, USA) in phosphate buffered saline (5 ml/g tissue). After centrifugation at 13.000 x g for 15 minutes at 4°C, supernatants were stored at -80°C until assay [19].

Cytokine assays

The cytokine concentrations of uterine tissue extracts were determined using mouse specific enzyme-linked immunosorbent assay (ELISA) kits (IL-1 β ; MBS175967, IL-6; MBS730957, TNF- α ; MBS825075, MyBioSource, USA) following the procedure as described by the manufacturer's. Absorbance was measured at 450 nm using a microplate reader (Infinite F50, Tecan Austria GmbH, Grödig, Austria). All standards and samples were measured in duplicates. The concentrations of assayed cytokines were expressed as ng/g.

Progesterone and estradiol assays

Serum progesterone and oestradiol concentrations were measured using commercial ELISA kits (progesterone; MBS703963, oestradiol; MBS733304, MyBioSource, Inc. San Diego, CA 92195-3308, USA) following the manufacturer's protocol. Assays of standards and samples were performed concurrently in duplicate.

Statistical analyses

Statistical analysis was carried out using SPSS version 13.0 software program (SPSS Inc., Chicago, Illinois USA). The significances of cytokine and hormonal concentrations were performed by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test. Data are expressed as means \pm standard deviation and P values less than 0.05 were considered to be statistically significant.

Results and Discussion

The mean concentrations of TNF- α , IL-1 β and IL-6 in gravid uterus and blood serum progesterone and oestradiol at preimplantation day 3 (group 1), postimplantation day 10 (group 2) and 16 (group 3) and in diestrus (group 4) are given in table 1. The concentration of TNF- α was not statistically different among the groups ($P > 0.05$) whereas, IL-1 β concentration was lower in Group 1, 2 and 3 (pregnant groups) than Group 4 (diestrus uterus) ($P < 0.05$). In addition, IL-6 concentration decreased at postimplantation day 16 compared to postimplantation day 10 ($P < 0.05$) and diestrus period ($P < 0.05$). However, both serum progesterone and oestradiol concentrations significantly increased during pregnancy when compared to diestrus group ($p < 0.05$) (Figure 1). Negative correlation was verified between IL-6 concentration and oestradiol concentration in diestrus ($r = -0.92$; $P < 0.05$). Similarly, TNF- α concentration was negatively correlated with IL-1 β concentration in postimplantation day 16 ($r = -0.83$; $P < 0.05$).

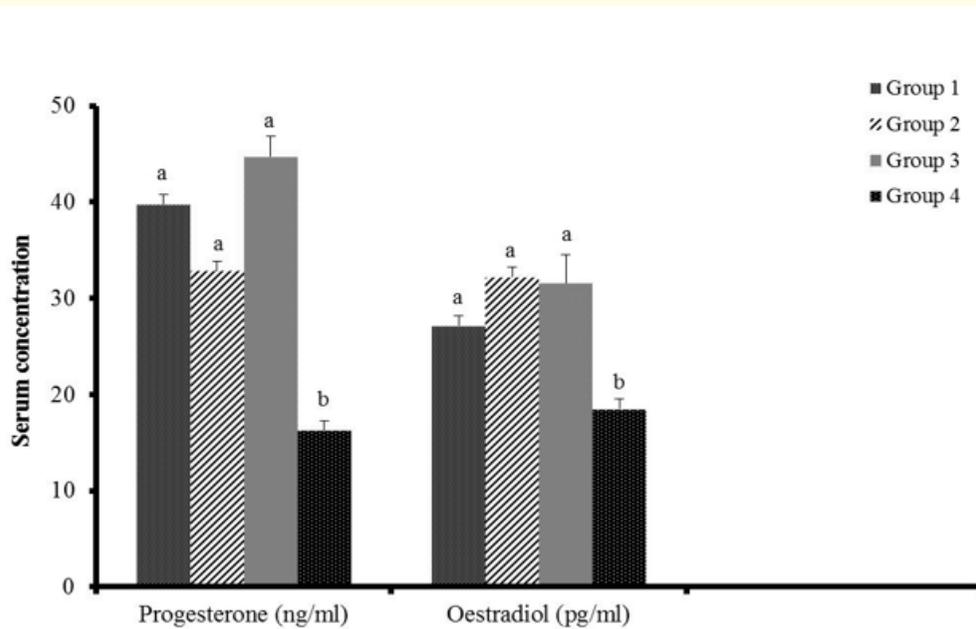


Figure 1: Mean concentrations of serum progesterone (ng/ml) ve oestradiol (pg/ml) among the groups, a,b Different letters indicate statistically different values ($P < 0.05$).

Groups	TNF- α (ng/g)	IL-1 β (ng/g)	IL-6 (ng/g)
Group 1	2.37 \pm 0.39	2.39 \pm 0.76 ^{a,b}	0.94 \pm 0.17 ^{a,b}
Group 2	3.13 \pm 0.18	1.29 \pm 0.17 ^b	0.48 \pm 0.06 ^a
Group 3	2.75 \pm 0.11	2.03 \pm 0.07 ^{a,b}	0.19 \pm 0.01 ^b
Group 4	2.55 \pm 0.30	4.85 \pm 1.31 ^a	0.69 \pm 0.19 ^a

Table 1: Mean concentrations of TNF- α , IL-1 β and IL-6 in uterine tissue in groups.

^{a, b}: Mean values with different letters within the same column are statistically different ($P < 0.05$).

Inflammatory events in the uterus involve the production of proinflammatory cytokines, chemokines and growth factors [4] that are essential for the preparation of endometrium for pregnancy and for the development of placenta and fetus in mammals [15]. In humans and mice, increased Th1 cytokines such as TNF- α and IL-1 have been reported to be related with pregnancy failure or pregnancy complications in the second or third term of pregnancy [20]. Furthermore, it was postulated that TNF- α and IL-1 play a role in the pathogenesis of stress-triggered abortions, and may lead a compensatory physiological increase in suppressive activity in normal pregnancy counteracting pro-inflammatory cytokine [21]. In the present study, expression of IL-1 β , IL-6 and TNF- α were detected in the mouse uterus at days 3, 10 and 16 of pregnancy and at dioestrus.

During the first half of pregnancy, progesterone is secreted by the ovary whereas, during the second half of pregnancy it is also secreted by the placenta. Additionally, maternal oestrogen is produced throughout gestation to activate the corpus luteum in the rat [22]. Similarly, in our study, both serum oestradiol and progesterone concentrations significantly increased during pregnancy when compared to dioestrus period ($P < 0.05$). The decrease in the mean concentration of progesterone at day 10 compared to day 3 and 16 was consistent with the findings of Murr, et al. [23] that has been evaluated as a transient period until the placenta is able to produce steroid hormones. Accordingly, Group 3 mice (day 16) had higher progesterone concentration. In the mammalian uterus, the activity of Th1 cytokines decreases while progesterone concentration increases [24]. Progesterone leads the development of a Th2 response, whereas antagonizes the formation of Th1 cells [25]. The results of our study revealed that IL-1 β concentrations in Group 1, 2 and 3 were lower compared to diestrus control mice that had a lower progesterone concentration. This supports the fact that the activity of Th1 cytokines decreases when progesterone increases. Conversely, TNF- α concentration, another proinflammatory cytokine, did not differ among the groups. TNF- α has been determined to reach the basal expression at diestrus in the uterus of mice while at estrus there is significant increase [15]. Inflammatory cytokines are suggested to prevent gonadotropin production at the level of the hypothalamus and pituitary in the event of chronic or acute illness, and to prevent progesterone synthesis by the corpus luteum and induce luteal regression as part of the normal ovulatory cycle in mammals [25-27]. Therefore their expression are expected to be low during pregnancy. Though estrus mice were not evaluated in the study, it has been assumed that both TNF- α and IL-1 β was low during pregnancy consistent with the increase in progesterone concentration.

Cytokine production attended the intrauterine acute inflammatory response after mating. It was thought that IL-1 increase was temporarily related with the preimplantation surge in oestrogen [17,28]. Furthermore, IL-1 highly expressed particularly at implantation day and dramatically decreased at postimplantation period in mice [17]. In our study, IL-1 β concentration was lower in Group 2 (day 10) uterus than Group 1 (day 3) that were in preimplantation period and than dioestrus uterine tissue. In a study of ovariectomized mice, IL-1 mRNA expression were undetectable whereas, IL-6 and TNF- α expression delayed in uterus. However, systemic administration of oestrogen or progesterone to these mice resulted in production of IL-1, IL-6 and TNF- α expression significantly [29]. There was a negative correlation between IL-6 concentration of uterus and blood oestradiol concentration in dioestrus mice in our study. Thus, it was thought that concentration of cytokines might be associated with concentrations of steroid hormones ($r_s = -0,92$; $P < 0.05$). This negative correlation is of particular interest for further studies to elucidate the association between oestradiol and the cytokines in mice. Oestrogen is known to take part in Th1 and Th2 cell maturation and activation [30,31]. Additionally, Galien and Garcia (1997) determined that oestrogen recep-

tor impairs IL-6 expression by preventing protein binding. Therefore, physiological alterations during oestrus cycle and pregnancy still seems to deserve new research approaches in the future.

Luteal insufficiency was related with the actions of the proinflammatory cytokine TNF- α [25]. It was demonstrated that increased TNF- α expression occurs in the immune-mediated, abortion-prone CBA/J X DBA/2J mouse model and also leads to miscarriage due to inadequate luteal progesterone support [19,25]. Similarly, high concentrations of TNF- α , IL-1 and IL-6 were found in the mouse uterus on days 1 and 2 of normal pregnancy [29]. Additionally, TNF- α and IL-6 concentrations at postimplantation day 9 were higher than preimplantation day 3 [17]. In our study, TNF- α concentrations were not statistically significant among the groups, whereas IL-6 concentration gradually decreased while pregnancy exceeded (Table 1). IL-6 is known to stimulate the production of proteases and complement inhibitors and thought to have a role in placentation [33]. Additionally, Mathialagan, et al. [34] determined the presence of IL-6 mRNA in elongation period of preimplantation sheep, pig and cow conceptuses. Therefore, in the study, increased concentration of IL-6 in preimplantation period might be a part of preparation for implantation and placentation.

Conclusion

In conclusion, it has been well known that embryo development and and placental growth are disturbed if profile in favor of Th-1 cytokines [35]. Thus, it can be concluded from the study that increased IL-6 concentration in gravid uterus during preimplantation period and decreased concentration of IL-1 β might be associated with maternal recognition of conceptuses and maintenance of mouse pregnancy.

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Bibliography

1. Duc-Goiran P, et al. "Embryo-maternal interactions at the implantation site: a delicate equilibrium". *European Journal of Obstetrics and Gynecology and Reproductive Biology* 83.1 (1999): 85-100.
2. Kanellopoulos-Langevin C., et al. "Tolerance of the fetus by the maternal immune system: role of inflammatory mediators at the fetomaternal interface". *Reproductive Biology and Endocrinology* 1 (2003): 121.
3. Schafer-Somi S., et al. "Expression of genes in the canine pre-implantation uterus and embryo: implications for an active role of the embryo before and during invasion". *Reproduction in Domestic Animals* 43.6 (2008): 656-663.
4. Beceriklisoy HB., et al. "Cytokines, growth factors and prostaglandin synthesis in the uterus of pregnant and non-pregnant bitches: the features of placental sites". *Reproduction in Domestic Animals* 44.2 (2009): 115-119.
5. Locksley RM., et al. "The TNF and TNF receptor superfamilies: integrating mammalian biology". *Cell* 104.4 (2001): 487-501.
6. Grunig G and Antczak DF. "Horse trophoblasts produce tumor necrosis factor alpha but not interleukin 2, interleukin 4, or interferon gamma". *Biology of Reproduction* 52.3 (1995): 531-539.
7. Schafer-Somi S. "Cytokines during early pregnancy of mammals: a review". *Animal Reproduction Science* 75.1-2 (2003): 73-94.
8. Chardonnnes D., et al. "Modulation of human cytotrophoblastic leptin secretion by interleukin-1 α and 17 β -oestradiol, and its effect on human chorionic gonadotropin secretion". *Molecular Human Reproduction* 5.11 (1999): 1007-1082.

9. Gonzalez RR., *et al.* "Effects of leptin, interleukin-1, interleukin-6, and transforming growth factor- β on markers of trophoblast invasive phenotype". *Endocrine* 15.2 (2001): 157-164.
10. Meisser A., *et al.* "Effects of interleukin-6 (il-6) on cytotrophoblastic cells". *Molecular Human Reproduction* 5.11 (1999): 1055-1058.
11. Blitek A., *et al.* "Regulation of expression and role of leukemia inhibitory factor and interleukin-6 in the uterus of early pregnant pigs". *Theriogenology* 78.5 (2012): 951-964.
12. Franczak A., *et al.* "The effect of tumor necrosis factor α (tnf α), interleukin 1 β (il1 β) and interleukin 6 (il6) on endometrial pgf2 α synthesis, metabolism and release in early-pregnant pigs". *Theriogenology* 77.1 (2012): 155-165.
13. Jana B., *et al.* "The influence of interleukin 1b (il)-1b and il-6 and tumor necrosis factor- α on prostaglandin secretion from porcine myometrium during the first third of pregnancy". *Acta Veterinaria Brno* 79 (2010): 559-569.
14. Blitek A. and Ziecik AJ. "Role of tumor necrosis factor alpha in stimulation of prostaglandins f (2alpha) and e (2) release by cultured porcine endometrial cells". *Reproduction in Domestic Animals* 41.6 (2006): 562-567.
15. Sierra-Mondragón E., *et al.* "Low expression of IL-6 and TNF- α correlates with the presence of the nuclear regulators of Nf-Kb, Ikbns and Bcl-3, in the uterus of mice". *Molecular Immunology* 68 (2015): 333-340.
16. Gerwin N., *et al.* "Interleukin gene expression in mouse preimplantation development". *Developmental Immunology* 4.3 (1995): 169-179.
17. De M., *et al.* "Expression of interleukin 1, interleukin 6 and tumour necrosis factor α in mouse uterus during the peri-implantation period of pregnancy". *Journal of Reproduction and Fertility* 97.1 (1993): 83-89.
18. Lohmiller JJ and Swing PS. "Reproduction and breeding: the laboratory rat". 2nd Edition, (Elsevier Academic Press, UK) (2006).
19. Robertson SA., *et al.* "Interleukin 10 regulates inflammatory cytokine synthesis to protect against lipopolysaccharide-induced abortion and fetal growth restriction in mice". *Biology of Reproduction* 76.5 (2007): 738-748.
20. Knackstedt MK., *et al.* "Th1 cytokines and the prothrombinase Fgl2 in stress-triggered and inflammatory abortion". *American Journal of Reproductive Immunology* 49.4 (2003): 210-220.
21. Arcka PC., *et al.* "Soluble receptors neutralizing Tnf-A and Il-1 block stress-triggered murine abortion". *American Journal of Reproductive Immunology* 37.3 (1997): 262-266.
22. Gibori G., *et al.* "Placental derived regulators and the complex control of luteal cell function". *Recent Progress in Hormone Research* 44 (1998): 377-429.
23. Murr SM., *et al.* "Plasma progesterone during pregnancy in the mouse". *Endocrinology* 94.4 (1974): 1209-1211.
24. Piccinni MP and Romagnani S. "Regulation of fetal allograft survival by hormone-controlled Th 1- and Th2-type cytokines". *Immunologic Research* 15.2 (1996): 141-150.
25. Erlebacher A., *et al.* "Ovarian insufficiency and early pregnancy loss induced by activation of the innate immune system". *Journal of Clinical Investigation* 114.1 (2004): 39-48.

26. Rivest S and Rivier C. "The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions". *Endocrine Reviews* 16.2 (1995): 177-199.
27. Davis JS and Rueda BR. "The corpus luteum: an ovarian structure with maternal instincts and suicidal tendencies". *Frontiers in Bioscience* 7 (2001): D1949-D1978.
28. De M., et al. "Determination of the number and distribution of macrophages, lymphocytes and granulocytes in the mouse uterus from mating through implantation". *Journal of Leukocyte Biology* 50.3 (1991): 252-262.
29. De M., et al. "Interleukin-1, Interleukin-6, and tumor necrosis factor α are produced in the mouse uterus during the estrous cycle and are induced by estrogen and progesterone". *Developmental Biology* 151.1 (1992): 297-305.
30. Wang F, et al. "Comparison of chlordecone and estradiol effects on splenic t-cells in (Nzbxnzw) F (1) mice". *Toxicology Letters* 183.1-3 (2008): 1-9.
31. Yu Y, et al. "Effects of steroid hormones on morphology and vascular endothelial growth factor expression in female bladder". *Urology* 73.6 (2009): 1210-1217.
32. Galien R. and Garcia T. "Estrogen receptor impairs interleukin-6 expression by preventing protein binding on the Nf-Kappab Site". *Nucleic Acids Research* 25.12 (1997): 2424-2429.
33. Martal J., et al. "Recent developments and potentialities for reducing embryo mortality in ruminants: the role of Ifn-Tau and Other cytokines in early pregnancy". *Reproduction, Fertility and Development* 9.3 (1997): 355-380.
34. Mathialagan N., et al. "Expression of interleukin-6 in porcine, ovine, and bovine preimplantation conceptuses". *Molecular Reproduction and Development* 32.4 (1992): 324-330.
35. Hill JA and Choi BC. "Immunodystrophism: evidence for a novel alloimmune hypothesis for recurrent pregnancy loss involving Th 1-type immunity to trophoblast". *Seminars in Reproductive Medicine* 18.4 (2000): 401-405.

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