

Hypothesis

Correlation between oral sex and a low incidence of preeclampsia: a role for soluble HLA in seminal fluid?

Carin A. Koelman ^a, Audrey B.C. Coumans ^b,
Hans W. Nijman ^b, Ilias I.N. Doxiadis ^a, Gustaaf A. Dekker ^b,
Frans H.J. Claas ^{a,*}

^a *Department of Immunohematology and Blood Bank, Leiden University Medical Centre,
PO Box 9600, 2300 RC Leiden, The Netherlands*

^b *Department of Obstetrics and Gynaecology, Free University Hospital, Amsterdam, The Netherlands*

Received 12 January 1999; received in revised form 20 October 1999; accepted 24 November 1999

Abstract

The involvement of immune mechanisms in the aetiology of preeclampsia is often suggested. Normal pregnancy is thought to be associated with a state of tolerance to the foreign antigens of the fetus, whereas in preeclamptic women this immunological tolerance might be hampered. The present study shows that oral sex and swallowing sperm is correlated with a diminished occurrence of preeclampsia which fits in the existing idea that a paternal factor is involved in the occurrence of preeclampsia. Because pregnancy has many similarities with transplantation, we hypothesize that induction of allogeneic tolerance to the paternal HLA molecules of the fetus may be crucial. Recent data suggest that exposure, and especially oral exposure to soluble HLA (sHLA) or HLA derived peptides can lead to transplantation tolerance. Similarly, sHLA antigens, that are present in the seminal plasma, might cause tolerance in the mother to paternal antigens. In order to test whether this indeed may be the case, we investigated whether sHLA antigens are present in seminal plasma. Using a specific ELISA we detected sHLA class I molecules in seminal plasma. The level

* Corresponding author. Tel.: +31-71-5263800; fax: +31-71-5216751.

E-mail address: ihbsecr@euronet.nl (F.H.J. Claas)

varied between individuals and was related to the level in plasma. Further studies showed that these sHLA class I molecules included classical HLA class I alleles, such as sHLA-A2, -B7, -B51, -B35 and sHLA-A9. Preliminary data show lower levels of sHLA in seminal plasma in the preeclampsia group, although not significantly different from the control group. An extension of the present study is necessary to verify this hypothesis. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Soluble HLA; sHLA-I; Preeclampsia; Sperm; Tolerance; Oral sex

1. Introduction

Presently, there is plenty of circumstantial, mostly epidemiologic evidence that immune mechanisms are involved in the etiology of preeclampsia. Genuine preeclampsia is primarily a disease of first pregnancies. A previous normal pregnancy is associated with a significantly decreased incidence of preeclampsia in subsequent pregnancies (Campbell, 1985). Even a previous abortion provides some protection (Strickland, 1986). The protective effect of multi parity, however, is lost with change of partner (Feeney, 1980; Ikedife, 1980; Chng, 1982; Campbell, 1985; Robillard et al., 1993; Dekker et al., 1998). Additionally, preeclampsia occurs more frequently in pregnancies induced by artificial insemination by a donor (Serhal and Craft, 1987). Moreover, the length of unprotected sexual cohabitation before conception appears to be inversely related to the incidence of pregnancy-induced hypertensive disorders (Marti and Herrmann, 1977; Klonoff-Cohen, 1987; Robillard, 1994). Thus, preeclampsia may be a problem of primipaternity rather than primigravidity (Robillard et al., 1993). These findings support the idea that males might tolerize the female partner prior to gestation.

It was shown before that sHLA class I molecules are detectable in seminal plasma by ELISA techniques using a monomorphic monoclonal antibody (Schaller et al., 1993), but it is not known whether these represent classical HLA class I molecules. Pregnancy has many similarities with tolerance induction to a transplanted graft whereby a certain tolerization state to foreign HLA molecules is important for graft survival. Blood transfusions (contact with foreign HLA molecules) have a beneficial effect in developing transplantation tolerance (Opelz et al., 1973) and is also associated with a decreased occurrence of preeclampsia (Feeney, 1977). In addition, it has been reported that sHLA molecules can induce specific tolerance by the induction of apoptosis in alloreactive T cells (Zavazava and Kronke, 1996).

Because it is well known that especially oral exposure to antigens can induce tolerance (Sosroseno, 1995; Brandtzaeg, 1996) we wondered if this way of tolerance induction might play a role in pregnancies. Exposure of

sHLA molecules via this route would theoretically be the most potent way for tolerance induction to foreign HLA antigens. Therefore we investigated whether there is an inverse relation between oral sex and the incidence of preeclampsia by asking a selected group of preeclamptic woman and a control group if they practiced oral sex (fellatio) with their partner.

2. Materials and methods

2.1. Study design of questionnaires

Forty-one consecutive primiparous women with a history of proteinuric preeclampsia and a consecutive control group of 44 primiparous women were asked if they practised oral sex (oral ejaculation) with their partner before the index pregnancy. If the answer was positive, they were asked if they were swallowing the ejaculate or not. No information was asked on quantitative aspects of exposure to seminal plasma. The patients were informed first about the study and the type of questions, before they filled in the anonymous questionnaire.

Definitions used:

Preeclampsia: diastolic blood pressure (K IV) 90 mmHg or more and an increase of at least 20 mmHg compared to the diastolic blood pressure in the first trimester plus proteinuria of at least 300 mg/24 h.

Controls: uncomplicated normotensive pregnancy, birth weight > 3000 g.

Because of privacy reasons this group had to be completely different from the group of couples evaluated in the soluble HLA part of this study.

2.2. Material

Blood and semen was collected from 12 partners of women with preeclampsia in their medical history attending the department of Obstetrics and Gynaecology of the Academical Hospital, Vrije Universiteit, Amsterdam. Fathers having partners with proven normal pregnancy (seven) were used as control groups.

Isolated lymphocytes of 14 men (ten of preeclampsia group and four controls) were frozen in liquid nitrogen to perform an HLA typing for class I and class II by conventional serological methods (Darke and Dyer, 1993; Naipal et al., 1984). Plasma was collected from heparinized blood and centrifuged at $2200 \times g$ for 15 min. Semen was centrifuged for 25 min at $2200 \times g$ before seminal plasma was collected and stored at -20°C .

2.3. Measuring sHLA class I specific antigens

sHLA class I and sHLA class I specific antigens (sHLA-A2, -A9, -B7, -B51) were measured by different ELISAs. Used antibodies, number of tested samples and their dilutions are indicated in Table 1. Detailed information on the specific assays and the monoclonal antibodies used was published previously (Mulder et al. (1997), Liem et al. (1997), Koelman et al. (1998) and Koelman et al. (1999)).

2.4. Standards used in the ELISAs

Total levels of sHLA class I were related to the TSB7 standard as defined at the 1st International Workshop on soluble HLA (Pouletty et al., 1993).

For each HLA antigen specific ELISA a panel of ten positive sHLA plasma samples of HLA-typed healthy individuals (heterozygous for the specific antigen) were tested. The average absorbance value (O.D.) was normalized to 100%. All measured O.D. values are related to this value.

2.5. Statistics

To analyse the relation between sHLA in blood plasma and seminal plasma linear regression analysis forced through the point (0, 0) was used. This analysis was done for levels of sHLA class I and sHLA-A2.

For the analysis of the questionnaires a 2-sided Fishers exact test was used.

3. Results

3.1. Oral sex and the incidence of preeclampsia

Evaluation of the questionnaires in this small population shows that oral sex is associated with a lower incidence of preeclampsia; 41 primiparous women with a history of proteinuric preeclampsia and a control group of 44 women were asked if they had oral sex (intra-oral ejaculation) with their partner before the index pregnancy. In the 41 preeclamptic women 18 (44%) had oral sex with their partner before the index pregnancy versus 36 (82%) out of 44 in the control group ($P = 0.0003$). In addition, seven (17%) out of the 41 preeclamptic patients versus 21 (48%) out of the 44 control patients confirmed that they swallowed the sperm ($P = 0.003$).

Table 1
Materials and methods of the different sHLA-allotype ELISAs^a

Specific ELISA	sHLA-class I	sHLA-A2	sHLA-A9	sHLA-B7	sHLA-B51
Pre-coating	GαMIgG	GαM-IgG	GαHuIgM	GαMIgG	GαHuIgG
Coating (Origin)	TP25.99 (Mouse)	MA2.1, CR11-351 (Mouse)	BvK5C4 (Human)	MB40.2, ME1 (Mouse)	HDG8D9 (Human)
Number tested samples	<i>n</i> = 28	<i>n</i> = 8 (A2 pos), <i>n</i> = 6 (A2 neg)	<i>n</i> = 3 (A9 pos), <i>n</i> = 6 (A9neg)	<i>n</i> = 6 (B7 pos), <i>n</i> = 8 (B7 neg)	<i>n</i> = 4 (B51 pos), <i>n</i> = 4 (B51 neg)
Dilution blood plasma	1:160	1:8	1:10	1:10	1:4
Dilution seminal plasma	1:20	1:2	1:2	1:2	1:2
Reading out: step 1	Rαβ2m	Rαβ2m	Rαβ2m	Rαβ2m	Rαβ2m
Reading out: step 2 (Conjugate)	GαR-HRP	GαR-HRP	GαR-HRP	GαR-HRP	GαR-HRP

^a Antibodies used, number of samples and (seminal) plasma dilutions are indicated.

3.2. Soluble HLA in seminal fluid

Using a class I specific ELISA, soluble HLA class I molecules could be detected in blood plasma (average 6.4 ± 4.4 $\mu\text{g/ml}$) and in seminal plasma. Seminal plasma contained 1.05 ± 0.95 $\mu\text{g/ml}$ sHLA class I molecules. Fig. 1 shows that the level of soluble HLA molecules in the seminal plasma is related to the level in plasma. A linear regression analysis was done and the R^2 was found to be 0.70. In general, there is a 6-fold lower level of total soluble HLA in seminal plasma compared to blood plasma. Fig. 1 also shows that the sHLA levels in the preeclampsia group are generally lower, although not significantly, compared to the levels in the control groups. Elevated levels of sHLA are mainly found in men who carry the HLA-A9 HLA typing (data not shown).

To investigate whether the classical HLA class I antigens are detectable in seminal plasma, we used sHLA-A2, sHLA-B7, sHLA-A9 and sHLA-B51 specific ELISAs. Variable levels of sHLA-A2 antigens can be found in seminal plasma of HLA-A2 positive individuals (Fig. 2a). Similarly sHLA-B7, sHLA-A9 and sHLA-B51 can be detected in seminal plasma of positive individuals (Fig. 2b–d). In seminal plasma of an HLA-B35 positive individual sHLA-B35 could be detected in seminal plasma as well (data not shown). In individuals positive for these respective antigens, the levels of

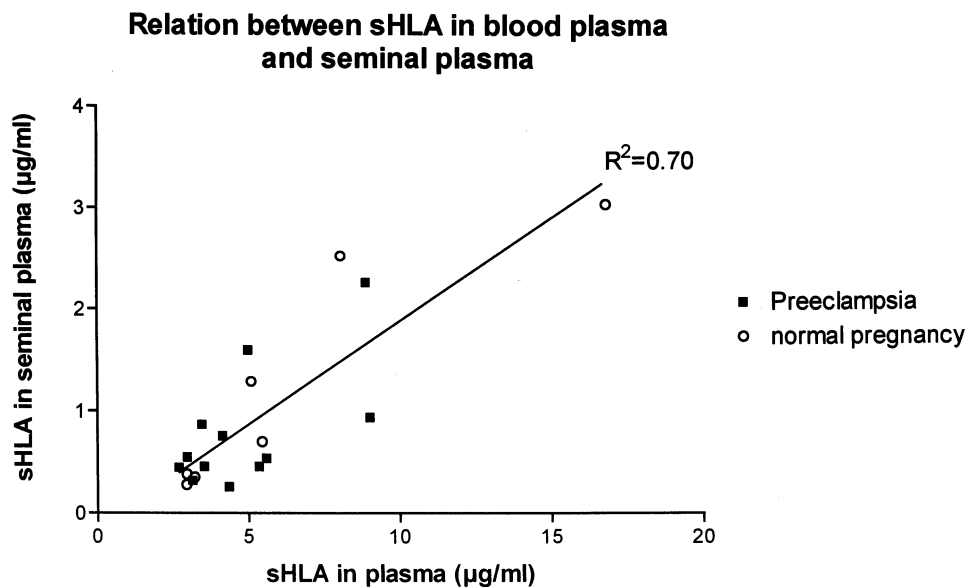


Fig. 1. Total sHLA levels in seminal plasma related to the levels of sHLA in blood plasma. Males of the different groups (preeclampsia and normal pregnancy) are indicated.

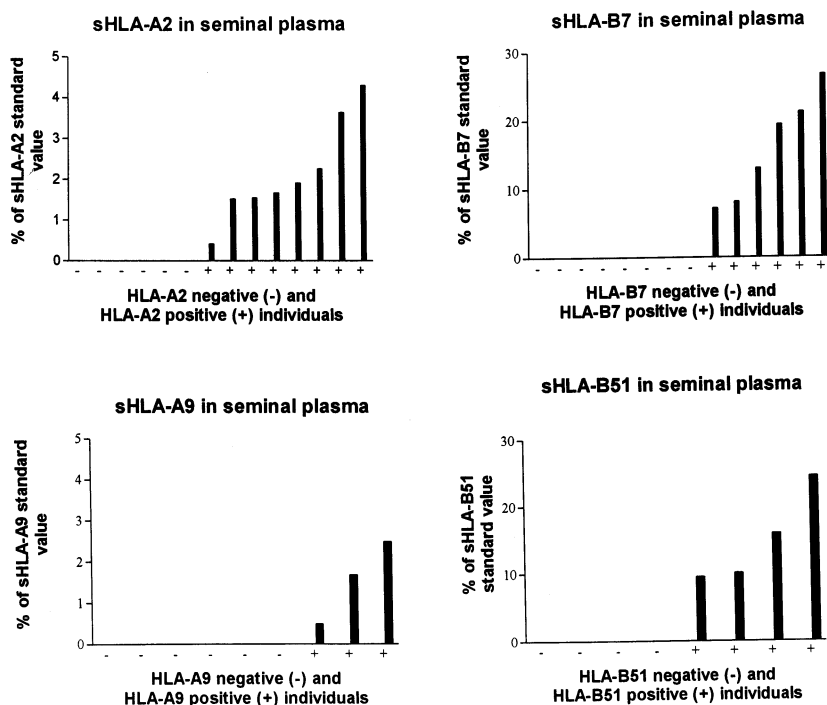


Fig. 2. Levels of different sHLA specific antigens in seminal plasma of HLA typed men. (a) sHLA-A2; (b) sHLA-A9; (c) sHLA-B7; and (d) sHLA-B51.

sHLA-A2, sHLA-A9, sHLA-B7 and sHLA-B35 in seminal plasma were compared with the levels in blood plasma. Fig. 3 shows that blood plasma contains ≈ 6 -fold more sHLA-A and -B compared to seminal plasma ($R^2 = 0.61$). For HLA-A2, this ratio seems to be even higher.

4. Discussion

In preeclampsia, there are many indications that immunological features might play a role in the occurrence of this disease (Vinatier and Monnier, 1995) and that a paternal factor might play an important role in the induction preeclampsia. These indications are: (1) The protective effect of multiparity in preeclampsia is lost with change of partner (Feeney, 1980; Ikedife, 1980; Chng, 1982; Robillard et al., 1993; Dekker et al., 1998); (2) the length of unprotected sexual cohabitation is influencing the incidence of pregnancy-induced hypertensive disorders (Marti and Herrmann, 1977; Klonoff-Cohen, 1987; Robillard, 1994), included the finding that donor

insemination and oocyte donation shows relatively more cases of preeclampsia (Serhal and Craft, 1987, 1989); (3) some men 'induced' preeclampsia in more than one woman (Astin et al., 1981); and (4) The incidence of preeclampsia is significantly lower in primigravidae who previously received blood-transfusions (Feeney, 1977).

The epidemiological indication that oral sex and swallowing sperm might have a protective effect in the occurrence of preeclampsia, fits with the concept that exposure to paternal antigens prior to gestation has a beneficial effect towards normal pregnancies.

Because pregnancy has many similarities with transplantation, we hypothesize that the lack of tolerance to HLA molecules can also play a role in the aetiology of preeclampsia. Therefore we investigated the presence of soluble HLA in seminal plasma.

Results from our study show that seminal plasma contains soluble HLA class I molecules that have the same characteristics as sHLA in blood plasma; classical HLA antigens like sHLA-A2, -A9, -B7, and sHLA-B51 antigens could be detected in seminal plasma. The levels of total sHLA molecules differ between individuals (average 1.05 ± 0.95 $\mu\text{g/ml}$) and are related to the levels in blood plasma (average 6.4 ± 4.4 $\mu\text{g/ml}$). In general, the total levels in plasma are ≈ 6 -fold higher than in seminal plasma. This is in the same range as reported by Schaller et al. (1993). They found a

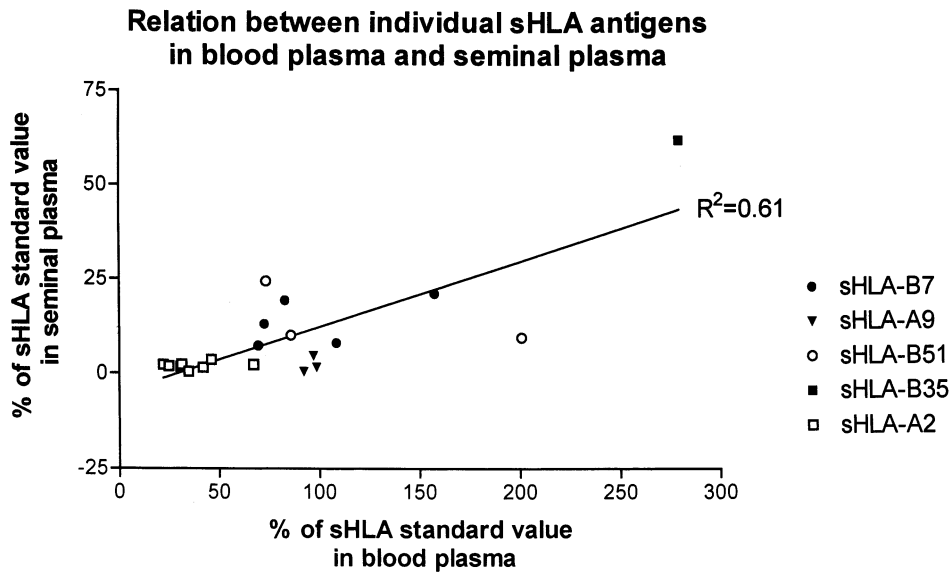


Fig. 3. Individual sHLA antigen levels in seminal plasma related to the levels in blood plasma.

4-fold higher concentration in blood plasma compared to the level in seminal plasma.

Similarly, by combining the data of the individual sHLA antigens HLA-A2, HLA-A9, HLA-B7 and HLA-B51 a 6-fold lower concentration of these classical HLA molecules was found in seminal plasma compared to blood plasma. When sHLA-A2 was analysed separately, the ratio was even 20-fold lower. These data clearly show that the classical HLA-A and -B molecules are present in seminal plasma and therefore, oral exposure to these molecules may indeed lead to a state of tolerance. Of course, other HLA class I molecules, like for instance the classical HLA-C molecules and the non-classical HLA class I molecules HLA-E, F and G, may even be present in higher concentrations. However, so far, these molecules have not been demonstrated in seminal plasma, probably because of the lack of specific monoclonal antibodies recognizing these non-classical HLA molecules. Considering the lack of polymorphism of these non-classical HLA class I molecules and the low immunogenicity and expression level of HLA-C, we do not see an important role of these molecules, in the induction of tolerance to paternal antigens. Significantly elevated levels of sHLA are detected in men who carry the HLA-A9 typing, both in serum and seminal plasma. This increased level of sHLA-A9 confirms the study of Doxiadis et al. (1989) who showed elevated levels of sHLA in serum of HLA-A9 positive individuals.

Currently, it is not known whether or not tolerance to paternal classical MHC antigens plays a role in fetal survival. The invading trophoblast does not express classical HLA-A and -B antigens. HLA-G, which is low polymorphic, and HLA-C, which is low immunogenic, appear to provide a set of trophoblast HLA class I molecules, which are required for optimal NK function. However, the induction of alloantibodies by pregnancy (Regan et al., 1991) indicates that the mothers' immune system is confronted with the paternal HLA-A and -B antigens of the child. It might well be that tolerance originally induced by soluble HLA-A and -B antigens spreads to epitopes of non-classical HLA antigens expressed on the trophoblast (Yang et al., 1999). Recently it has been described that pregnancy is an inflammatory state, whereby preeclampsia appears to represent an exaggeration of this inflammation (Sacks et al., 1998). A generally accepted phenomenon of inflammation is an increased expression of classical HLA class I molecules. For this reason, together with the immunological features mentioned earlier, we believe that immune responses to classical HLA class I molecules can not be excluded in the occurrence of preeclampsia.

Until now, the function of sHLA molecules in sperm is unknown. It might play a role in the protection of spermatozoa against the female

immune system, although spermatozoa do not express HLA molecules on their membranes (Castilla et al., 1993; Schaller et al., 1993). The data suggesting a paternal role in maternal tolerance to the fetus could be due to the presence of sHLA in seminal plasma and might indicate that tolerance induction can take place both via vaginal exposure to the soluble HLA antigens and via the oral route. In general oral tolerization to antigens is more easily to establish because the gut is a powerful tolerization site (Sosroseno, 1995; Brandtzaeg, 1996). Induction of tolerance by MHC peptides has been described before (Krensky and Clayberger, 1994; Buelow et al., 1995), and additionally, oral tolerance induction to MHC molecules has been shown in a rat model (Sayegh et al., 1996). The recent observation that renal transplants from siblings mismatched with the patients for the non-inherited maternal HLA antigens have a significantly better survival than grafts from siblings mismatched for the non-inherited paternal HLA antigens (Burlingham et al., 1998) may also be based on tolerance induction by oral exposure of the child to non-inherited maternal HLA antigens via breast feeding (Campbell et al., 1984). Furthermore, soluble HLA molecules can specifically induce apoptosis in human cytotoxic T cells (Zavazava and Kronke, 1996). Induction of apoptosis may be a mechanism in inducing specific tolerance against HLA molecules of the male partner. In preeclampsia the beneficial effects of blood transfusions (Feeney, 1977) has similarities with the blood transfusion effect in transplantation and the treatment of recurrent abortions by infusions of paternal leukocytes (Taylor and Faulk, 1981; Mowbray, 1988). The mechanism of this tolerizing effect is still unknown. Clonal deletion, clonal anergy, T cell suppression and the formation of anti-idiotypic antibodies can all play a role.

We would like to suggest that soluble HLA molecules in seminal fluid are relevant for the induction of tolerance. Hereby, sexual behaviour (e.g. oral exposure) can play an important role. Although our preliminary data do not show significantly higher levels of classical sHLA molecules in controls compared to patients, this does not exclude that oral exposure to these molecules is important. Quantitative aspects i.e. frequency of exposure to sHLA may be important for the induction of a state of tolerance. If the tolerizing state is not reached, problems like preeclampsia can occur. In this light, preeclampsia can indeed be expected to develop after limited exposure to sHLA. This may be caused by a limited number of cohabitations before the index pregnancy. Low levels of sHLA in sperm may also be a contributing factor. In fact, our preliminary data show slightly lower levels of sHLA in seminal plasma in the preeclampsia group, although not significantly different from the control groups. An extension of the study is necessary to verify this hypothesis.

Acknowledgements

We wish to thank Dr A. Mulder for critical reading the manuscript. This work was supported by a grant from the Dutch Heart Foundation and by the J.A. Cohen Institute for Radiology and Radiation Protection.

References

- Astin, M., Scott, J.R., Worley, R.J., 1981. Pre-eclampsia/eclampsia: the fatal father factor. *Lancet* II, 533.
- Brandtzaeg, P., 1996. History of oral tolerance and mucosal immunity. *Ann. NY Acad. Sci.* 13, 1–27.
- Buelow, R., Burlingham, W.J., Clayberger, C., 1995. Immunomodulation by soluble HLA class I. *Transplantation* 59 (5), 649–654.
- Burlingham, W.J., Grailer, A.P., Heisey, D.M., Claas, F.H.J., Norman, D., Mohanakumar, T., et al., 1998. The effect of tolerance to noninherited maternal HLA antigens on the survival of renal transplants from sibling donors. *New Engl. J. Med.* 339, 1657–1664.
- Campbell, D.A., Lorber, M.I., Seeton, J.C., Turcotte, J.G., Niederhuber, J.G., Beer, A.E., 1984. Breast feeding and maternal-donor renal allografts. *Transplantation* 37, 340–344.
- Campbell, D., 1985. Pre-eclampsia in second pregnancy. *Br. J. Obstet. Gynaecol.* 92, 131–135.
- Castilla, J.A., Gil, T., Rodriguez, F., Molina, J., Samaniego, F., Vergara, F., Herruzo, A.J., 1993. Lack of expression of HLA antigens on immature germ cells form ejaculates with antisperm antibodies. *Castilla J. A.* 30, 9–14.
- Chng, P.K., 1982. Occurrence of preeclampsia in pregnancies to three husbands: case report. *Br. J. Obstet. Gynaec.* 89, 862–863.
- Darke, C., Dyer, P., 1993. Clinical typing by cytotoxicity. In: Dyer, P., Middleton, D. (Eds.), *Histocompatibility Testing, A Practical Approach*. Oxford University Press, Oxford, pp. 51–80.
- Dekker, G.A., Tubbergen, P., Valk, M., Althuisius, S.M., Lachmeijer, A.M.A., 1998. Change in paternity: a risk factor for preeclampsia in multiparous women. *Am. J. Obstet. Gynecol.* 178, S120.
- Doxiadis, I., Westhoff, U., Grosse-Wilde, H., 1989. Quantification of soluble HLA class I gene products by an enzyme linked immunosorbent assay. *Blut* 59, 449–454.
- Feeney, J.G., 1977. Influence of previous blood transfusion of incidence of pre-eclampsia. *Lancet* i, 874–875.
- Feeney, J.G., 1980. Pre-eclampsia and changed paternity. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 11, 35–38.
- Ikedife, D., 1980. Eclampsia in multipara. *Br. Med. J.* 280, 985–986.
- Klonoff-Cohen, H.S., 1987. An epidemiologic study of contraception and pre-eclampsia. *J. Am. Med. Assoc.* 262, 3143–3146.
- Koelman, C.A., Mulder, A., Jutte, N.H.P.M., Vaessen, L.M.B., Balk, A., Weimar, W., et al., 1998. The application of human monoclonal antibodies for monitoring donor derived soluble HLA class I molecules in the serum of heart transplant recipients. *Hum. Immunol.* 59, 106–114.
- Koelman, C.A., Ensink, W., Mulder, A., Tanke, J., Doxiadis, I.I.N., Claas, F.H.J., 1999. Anti-HLA antibodies interfere in the detection of soluble HLA class I molecules. *Hum. Immunol.* 60, 414–423.
- Krensky, A.M., Clayberger, C., 1994. The induction of tolerance to alloantigens using HLA-based synthetic peptides. *Curr. Opin. Immunol.* 6, 791–796.

- Liem, L.M., Koelman, C.A., Doxiadis, I.I.N., Houwelingen, J.C., Goulmy, E., Claas, F.H.J., 1997. Elevated serum HLA class I levels coincide with acute and chronic graft-versus-host disease. *Bone Marrow Transpl.* 20, 227–234.
- Marti, J., Herrmann, U., 1977. Immunogestosis: a new etiologic concept of essential EPH gestosis, with special consideration of the primigravid patient. *Am. J. Obstet. Gynaecol.* 128, 489–491.
- Mowbray, J.F., 1988. Controlled trial of treatment of recurrent spontaneous abortion by immunization with paternal cells. *Lancet* 17, 297–302.
- Mulder, A., Kardol, M.J., Niterink, J.G.S., Parlevliet, J.H., Marrari, M., Tanke, J., et al., 1997. Successful strategy for the large scale development of HLA-human monoclonal antibodies. In: Charron, D. (Ed.), *Genetic Diversity of HLA: Functional and Medical Implications*. EDK Publishers, Sevres, pp. 354–356.
- Naipal, A., D'Amato, J., Bruning, J.W., van Leeuwen, A., van Rood, J.J., 1984. Automated reading of propidium iodide lymphocytotoxicity tests for HLA-DR, MB, and MT typing. *Tissue Antig.* 24, 302.
- Opelz, G., Sengar, D.P.S., Mickey, M.R., Terasaki, P.I., 1973. Effect of blood transfusions on subsequent kidney transplants. *Transplant Proc.* 5, 253.
- Pouletty, P., Ferrone, S., Amesland, F., Cohen, N., Westhoff, U., Charron, D., et al., 1993. Summary report from the first international workshop on soluble HLA antigens. Paris, August 1992. *Tissue Antig.* 42, 45–54.
- Regan, L., Braude, P.R., Hill, D.P., 1991. A prospective study of the incidence, time of appearance and significance of anti-paternal lymphocytotoxic antibodies in human pregnancy. *Hum. Reprod.* 6, 294–298.
- Robillard, P.Y., 1994. Association of pregnancy-induced hypertension with duration of sexual cohabitation before conception. *Lancet* 344, 973–975.
- Robillard, P.Y., Hulsey, T.C., Alexander, G.R., Keenan, A., de Caunes, F., Papiernik, E., 1993. Paternity patterns and risk of preeclampsia in the last pregnancy in multiparae. *J. Reprod. Immunol.* 24, 1–12.
- Sacks, G.P., Studena, K.M.D., Sargent, I.L., Redman, C.W.G., 1998. Normal pregnancy and preeclampsia both produce inflammatory changes in blood leukocytes akin to those of sepsis. *Am. J. Obstet. Gynecol.* 179, 80–86.
- Sayegh, M.H., Khoury, S.J., Hancock, W.W., Weiner, H.L., Carpenter, C.B., 1996. Mechanisms of oral tolerance by MHC peptides. *Ann. NY Acad. Sci.* 13, 338–345.
- Schaller, J., Glander, H.J., Ladusch, M., Westhoff, U., Grosse-Wilde, H., 1993. Lack of HLA-molecules on human spermatozoa and in seminal plasma. *Andrologia* 25, 77–81.
- Serhal, P.F., Craft, I., 1987. Immune basis for pre-eclampsia: evidence from oocyte recipients. *Lancet* i, 744–746.
- Serhal, P.F., Craft, I.L., 1989. Oocyte donation in 61 patients. *Lancet* I, 1185–1187.
- Sosroseno, W., 1995. A review of the mechanisms of oral tolerance and immunotherapy. *J. F. Soc. Med.* 88, 14–17.
- Strickland, D.M., 1986. The relationship between abortion in the first pregnancy and development of pregnancy-induced hypertension in the subsequent pregnancy. *Am. J. Obstet. Gynecol.* 154, 146–148.
- Taylor, C., Faulk, P.W., 1981. Prevention of recurrent abortion with leucocyte transfusions. *Lancet* II, 68–69.
- Vinatier, D., Monnier, J.C., 1995. Preeclampsia: physiology and immunological aspects. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 61, 85–97.
- Yang, L., DuTemple, B., Gorczynski, R.M., Levy, G., Zhang, L., 1999. Evidence for epitope spreading and active suppression in skin graft tolerance after donor-specific transfusion. *Transplantation* 67, 1404–1410.
- Zavazava, N., Kronke, M., 1996. sHLA class I molecules induce apoptosis in alloreactive cytotoxic T cells. *Nat. Med.* 2, 1005–1011.