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# A practical approach to the prevention of miscarriage: Part 2 - active immunotherapy

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## Summary

**Purpose:** To present data suggesting that active immunization with lymphocyte immunotherapy is a treatment that has benefit in preventing miscarriage. **Methods:** Lymphocyte immunotherapy is given to women with a history of recurrent miscarriage or failure to achieve a successful pregnancy, despite several previous embryo transfers. Active immunization was combined with progesterone therapy. The lymphocytes were not refrigerated, but used fresh. **Results:** Compared to controls, i.e., progesterone therapy alone, the injection of paternal lymphocytes intradermally improved miscarriage rates and improved live delivered pregnancy rates per embryo transfer. **Conclusions:** The addition of progesterone treatment may act synergistically with lymphocyte immunotherapy, especially in primary aborters and tertiary aborters. However, it is important to use fresh – not refrigerated – stored lymphocytes.

**Key words:** Lymphocyte immunotherapy; Miscarriage; Refrigerated white cells; Progesterone induced blocking factor.

## Introduction

Part 1 of “A practical approach to the prevention of miscarriage” emphasized the importance of progesterone supplementation and suggested that one of its main effects may be on the immune system.

There are data supporting the concept that one of the mechanisms involved in escape from immune surveillance, especially by natural killer (NK) cells in normal pregnancy, is through the hormone progesterone (P) [1]. A 34 kDa protein has been identified in pregnant women which can block NK cell mediated lysis of K562 tumor cells [2]. Because the expression of this protein by CD8+ T-lymphocytes (specifically gamma/delta T cells) needs P exposure for its expression, it was called the progesterone induced blocking factor (PIBF) [3].

There is evidence that PIBF may induce a shift from TH1 to TH2 cytokines [3, 4]. Progesterone receptors have not been demonstrated in normal T lymphocytes, yet these receptors have been found at a lower density than other tissues with P receptors (e.g., endometrium) in healthy pregnant women [5-7]. Liver transplants and blood transfusions have been shown to induce P receptors on these gamma/delta T cells, even in male patients [8]. Injection of paternal lymphocytes prior to ovulation has been shown to increase PIBF secretion in mid to late luteal phase in women exposed to the allogeneic stimulus of embryos following embryo transfer [9].

These data have led to the following hypothesis, as to at least one way that the fetus escapes immune rejection by NK cells: The fetal semi-allograft induces P receptors in gamma/delta T cells following trophoblast invasion. The interaction of these receptors with a high concentration of P causes the expression of PIBF by these gamma/delta T cells with induced P receptors. The PIBF is only made at the maternal fetal interface because that is where there is an adequate P concentration. Progesterone receptors in gamma/delta T cells are made throughout the body but the P level is insufficient to cause PIBF expression by gamma/delta T cells not at the maternal-fetal interface.

PIBF inhibits NK cell cytotoxic activity at least partially, by inhibiting the release of perforin from storage granules of NK cells [10]. PIBF also inhibits TH1 cytokines and favors TH2 cytokines, thus inhibiting cellular immune response and promoting hormonal response [2]. The suppression of the cellular immune system is limited to the maternal-fetal interface and this constitutes selective immune tolerance.

Szekeres-Bartho *et al.*, by using an enzyme linked immunosorbent assay, measured PIBF in normal pregnancies, at the termination of pregnancy (at onset of labor, at time of miscarriage, and at the time of pre-term deliveries), and in the 10<sup>th</sup> week of women who subsequently spontaneously lost the pregnancy [11]. All women at pregnancy termination had sera PIBF levels lower than those of healthy pregnant women [11]. They also found that using a cut-off value of 197.5 ug/ml for PIBF, that 52 of 87 women who would eventually spontaneously abort, either immediately or up to 12 weeks later, had low PIBF levels [11].

The same group using an immunocytochemistry method compared PIBF expression in women between the 9<sup>th</sup> and 40<sup>th</sup> week of gestation [12]. They found that the percentage of PIBF expressing lymphocytes in the peripheral blood of 96 healthy pregnant women was 67% vs 6.5% in 62 women with pathological pregnancies [12]. This group found over 90% of pathological pregnancies had PIBF levels below the established cut-off for normal pregnancies [12].

Theoretically, a low PIBF level may be caused by insufficient progesterone, or insufficient development of progesterone receptors on gamma/delta T cells. There are data that favor that the most common problem is the lack of progesterone, rather than lack of development of P receptors on gamma/delta T cells. One study evaluated PIBF levels in women supplemented with extra progesterone according to whether they miscarried or not [13]. The PIBF expression was similar in those having a miscarriage vs those who were successful in delivering a live baby [13].

### *Lymphocyte immunotherapy*

Lymphocyte inoculation from the male partner's blood was given to women who had either failed to have a successful pregnancy following at least two in vitro fertilization (IVF) cycles with embryo transfers (ET), or had a history of recurrent (at least 3) miscarriages consecutively. The mean percentage of lymphocytes expressing PIBF was 2.9% before lymphocyte immunotherapy and 8.2% after the procedure. The percentage of women with PIBF expression in over 1% of the lymphocytes increased from 33.3% to 58%. There were 56% of the women without PIBF expression despite pregnancy and this decreased to 22% after lymphocyte injection [14].

There is controversy as to whether lymphocyte immunotherapy provides any benefit in preventing miscarriage with some studies finding a reduced rate [15-22], but others finding no benefit [23-25]. In fact, a meta-analysis concluded that there was no evidence that lymphocyte immunotherapy provides any benefit [26].

The possibility exists that the lymphocyte stimulus is insufficient to stimulate an increased level of PIBF from the gamma/delta T cells with the theoretical increase in P receptors, unless an increase in progesterone is also given. In one of our studies primary aborters with a history of recurrent miscarriages (3 or more) were randomly assigned to either progesterone supplementation from the early luteal phase throughout the first trimester vs progesterone therapy with the addition of lymphocyte immunotherapy [27]. Miscarriage occurred in eight of 14 (57.1%) given progesterone alone, vs six of 22 (26.0%) given combined therapy (Fisher's exact test,  $p = .073$ ) [27].

When a couple has sex at the appropriate time or intrauterine insemination, failure to achieve a pregnancy may be related to failure to fertilize, failure of the zygote to cleave further, rejection of the embryo by the immune system, or other reasons for implantation failure. With in vitro fertilization and embryo transfer, failure to conceive is obviously not related to fertilization or embryo cleavage issues.

The efficacy of lymphocyte immunotherapy was evaluated in a group of women who averaged 4.3 previous embryo transfers without a live pregnancy and found in this matched pair study a live delivery rate of 16.2% (6/37) for those treated with progesterone only vs 51.3% (19/37) for those women who were given lymphocyte immunotherapy ( $p < 0.01$ , Fisher's exact test) [28]. This study evaluated only the first cycle of lymphocyte immunotherapy. In a larger retrospective study of all cycles receiving lymphocyte immunotherapy, the live delivery rate was 30.8% (39/94) per transfer vs 19.7% for controls [29]. For women with five previous failures the live delivery rate was 35.1% (13/37) vs 15.6% (10/64) [29].

Lymphocyte immunotherapy was evaluated in 20 embryo transfer cycles using donor oocytes, where the recipient was on estrogen/progesterone replacement having failed to have a live baby after three previous embryo transfers of embryos derived from donor oocytes. Six of the ten not receiving lymphocyte immunotherapy conceived, with one ectopic pregnancy and two miscarriages vs nine of ten demonstrating a clinical pregnancy with the addition of lymphocyte immunotherapy, and none aborted. The live delivery rate of 30% vs 90% was significantly different ( $p < 0.05$ , Fisher's exact test) [30]. This study is important because one concept is that the lack of progesterone receptors induced on gamma/delta T cells by the embryo/fetus may be related to a less immunogenic fetus due to sharing of histocompatibility antigens between the male and female couple. White blood cells are 100 to 1,000 times more immunogenic than in the fetus [29]. Using donor oocytes, theoretically if sharing of histocompatibility antigens played an important role, the foreign antigen from the donor egg source should overcome the problem. Thus these data favor more the concept that some women have a defective immune response to the relatively weak antigens stimulus of the fetus but may respond to a more potent white blood cell injection. Other data supports the conclusion that histocompatibility antigen sharing is not the reason for failure to suppress immune reaction of the fetus, especially DQ-alpha type II antigens [31].

One study has had a major impact on the use of lymphocyte immunotherapy in the United States. Unfortunately the study has also had a strong negative impact on its use [32]. Ober *et al.* found no reduction in the chance of a subsequent miscarriage, but actually found that there was a higher miscarriage rate in those females receiving paternal lymphocytes vs controls [32]. The miscarriage rate was 55.5% (37/68) for those receiving paternal lymphocytes vs 34.9% (22/63) for controls [32]. The patients from Ober *et al.*'s study, contributed to more than 20% of the patients used for a meta-analysis by Porter *et al.* published in 2006 [33]. The Cochrane review [33] found a miscarriage rate following lymphocyte immunotherapy of 35.5% (116/320) vs 41.0% (235/331) with an OR and 95% CI of 1.27 (0.92-1.74) which was not significantly different. However, if the data from Ober *et al.* was removed from the meta-analysis, the group

receiving lymphocyte immunotherapy would have had a miscarriage rate of 30.6% vs 42.1% in the controls [33]. These studies used for the meta-analysis were without any other therapy, e.g., progesterone, which facilitates lymphocyte therapy even more and thus our randomized controlled study was not included which would have further increased the benefit of active immunotherapy with lymphocyte injection.

One may question why remove the study by Ober *et al.* from the more recent meta-analysis? [32, 33]. Indeed, it is based on the study by Ober *et al.* showing a negative effect on a randomized double blinded study, that influenced the Food and Drug Administration in the United States to require an investigational new drug (IND) application to use immunization with paternal lymphocytes [32]. Since the cost of the application was close to a million dollars, most reproductive centers no longer use this procedure in the United States. This is the basis of my statement that the Ober *et al.* study is one of the most influential of the studies since it abruptly stopped this treatment in the United States.

There have been many objections to the study by Ober *et al.* [34, 35]. One of the main objectives is that the other studies used for the meta-analysis used fresh paternal white cells, whereas Ober *et al.* stored the white cell prep at 4°C [32]. Clark *et al.* found that storing leukocytes at 4°C overnight can cause loss of effectiveness, related to shedding of surface CD200 molecules into the supernatant [36]. The CD200 molecules may be important in the induction of progesterone receptors in gamma/delta T cells. Though fresh white cell injections have been found to help decrease miscarriage rates in animals, the use of refrigerated white blood cells would either not lower the miscarriage rate or even make it worse [34-36].

Measurement of PIBF by commercial assay is not possible as yet, though an ELISA assay has been developed and has been submitted for approval to the European FDA. In my opinion, no other testing that is available at present, including natural killer cell levels or activity before pregnancy in the blood or endometrium, sharing of histocompatibility antigens, measurement of tumor necrosis factor alfa, interferon gamma, and TH1 promoting interleukins can be relied on to detect those women who would benefit from lymphocyte immunotherapy [37-41]. Similarly, I do not think there is sufficient evidence to make decisions on whether to use lymphocyte immunotherapy, or determine the efficacy of treatment by the detection or lack of detection of lymphocytotoxic antibodies.

These tests add a considerable amount of cost to the patient, especially since they are considered experimental and many third party payers do not reimburse for these tests. I think that the best indication is simply the history, i.e., recurrent pregnancy loss. Along these lines, the therapy to primary aborters with three consecutive losses does not have to be restricted. The treatment is safe (though a blood product is being injected without quarantine), blood is taken from a male partner with whom biological fluids are exchanged and the male partner is checked first for infectious diseases. The treatment should be inexpensive – how much can it cost to draw blood from the male partner, separate the lymphocytes and give intradermal injections? I actually think it would be appropriate to offer lymphocyte immunotherapy to a woman with her first pregnancy ending in a miscarriage, if the loss occurred despite progesterone therapy and testing of the fetus found a normal chromosomal constitution, especially if the fetus was a male with no risk of maternal contamination.

However, even if a woman had a chromosomal explanation for her first loss, if she indicated that psychologically she could not take another miscarriage unless she thought she had done everything to prevent one, she could be given active immunotherapy since it is not certain that the immune system caused the loss before organ abnormalities related to the chromosome abnormality of the fetus caused the death.

My general policy is if a woman presents with one or two miscarriages and has never been treated and no chromosome studies have been performed on the fetus, I would assume the stance that there was not a chromosome cause. I would offer progesterone therapy first, since it is inexpensive with minimal side-effects and would not compromise a new pregnancy, if it was not needed. If another miscarriage occurred and chromosome analysis was normal, or inconclusive, I would offer lymphocyte immunotherapy, if it was available. The group least likely to benefit from lymphocyte immunotherapy is secondary aborters, with the most efficacy for primary and tertiary aborters.

I think that once an ELISA assay for PIBF is available, management of women with a history of miscarriage will utilize this assay to determine the proper therapy. If progesterone therapy is not allowing the serum PIBF to attain a level found normal for non-aborting women at a certain stage of pregnancy, the dosage of progesterone would be increased. If the increase failed to improve the PIBF level, then lymphocyte immunotherapy would be given (if available) and PIBF remeasured.

Of course it would be beneficial if women who had a defect in the NK allorecognition system could be identified. Some studies are evaluating the possibility of a limited repertoire of InH Kir receptors [42, 43]. Possibly future studies will hopefully identify women less likely to respond to the relatively weak allogeneic stimulus of the fetus.

The United States FDA funded Ober and his group over two million dollars for their study. Perhaps their decision to require such an expensive IND to use lymphocyte immunotherapy may be their belief that they should support the conclusions from the study [32]. They may not want to admit that they supported a flawed study, i.e., the use of refrigerated white cells. It is unlikely that in the present economy, the FDA will support a multicenter study performed using fresh lymphocytes, to give a true evaluation of the efficacy of this procedure. Hopefully, appropriate studies will be conducted outside of the United States. However, I hope such studies include a treatment arm of progesterone with the lymphocyte immunotherapy.

## References

- [1] Pence H., Petty W.M., Rocklin R.E.: "Suppression of maternal responsiveness to paternal antigens by maternal plasma". *J. Immunol.*, 1975, 114, 525.
- [2] Szekeres-Bartho J., Kilar F., Falkay G., Csernus V., Torok A., Pacsa A.S.: "The mechanism of the inhibitory effect of progesterone on lymphocyte cytotoxicity: I. Progesterone-treated lymphocytes release a substance inhibiting cytotoxicity and prostaglandin synthesis". *Am. J. Reprod. Microbiol.*, 1985, 9, 15.
- [3] Szekeres-Bartho J., Autran B., Debre P., Andreu G., Denver L., Chaouat G.: "Immunoregulatory effects of a suppressor factor from healthy pregnant women's lymphocytes after progesterone induction". *Cell. Immunol.*, 1989, 122, 281.
- [4] Szekeres-Bartho J., Barakonyi A., Polgar B., Par G., Faust Z., Palkovics T., Szeresay L.: "The role of gamma/delta T cells in progesterone-mediated immunomodulation during pregnancy: a review". *Am. J. Reprod. Immunol.*, 1999, 42, 44.
- [5] Szekeres-Bartho J., Csernus V., Hadnagy J.: "The blocking effect of progesterone on lymphocyte responsiveness is receptor-mediated". *Biol. Immunol. Reprod.*, 1989, 15, 36.
- [6] Szekeres-Bartho J., Szekeres G., Debre P., Autran B., Chaouat G.: "Reactivity of lymphocytes to a progesterone receptor specific monoclonal antibody". *Cell Immunol.*, 1990, 125, 273.
- [7] Chiu L., Nishimura M., Ishii Y., Nieda M., Maeshima M., Takedani Y., Shibata Y., Tadokoro K., Juji T.: "Enhancement of the expression of progesterone receptor on progesterone treated lymphocyte after immunotherapy in unexplained recurrent spontaneous abortion". *Am. J. Reprod. Immunol.*, 1996, 35, 552.
- [8] Szekeres-Bartho J., Weill B.J., Mike G., Houssin D., Chaouat G.: "Progesterone receptors in lymphocytes of liver-transplanted and transfused patients". *Immunol Lett.*, 1989, 22, 259.
- [10] Check J.H., Arwitz M., Gross J., Peymer M., Szekeres Bartho J.: "Lymphocyte immunotherapy (LI) increases serum levels of progesterone induced blocking factor". *Am. J. Reprod. Immunol.*, 1997, 37, 17.
- [10] Faust Z., Laskarin G., Rukavina D., Szekeres-Bartho J.: "Progesterone induced blocking factor inhibits degranulation of natural killer cells". *Am. J. Reprod. Immunol.*, 1999, 42, 71.
- [11] Szekeres-Bartho J., Varga P., Pejtsik B.: "ELISA test for the detection of an immunological blocking factor in human pregnancy serum". *J. Reprod. Immunol.*, 1989, 16, 19.
- [12] Szekeres-Bartho J., Faust Z., Varga P.: "The expression of a progesterone-induced immunomodulatory protein in pregnancy lymphocytes". *Am. J. Reprod. Immunol.*, 1995, 34, 342.
- [13] Check J.H., Ostrzenski A., Klimek R.: "Expression of an immunomodulatory protein known as progesterone induced blocking factor (PIBF) does not correlate with first trimester spontaneous abortions in progesterone supplemented women". *Am. J. Reprod. Immunol.*, 1997, 37, 330.
- [14] Check J.H., Arwitz M., Gross J., Peymer M., Szekeres-Bartho J.: "Lymphocyte immunotherapy (LI) increases serum levels of progesterone induced blocking factor". *Am. J. Reprod. Immunol.*, 1997, 37, 17.
- [15] Mowbray J.F.: "Effect of immunization with paternal cells on recurrent spontaneous abortion". In: Clark D.A., Croy B.A. (eds.): Reproductive Immunology, Amsterdam, Elsevier, 1986, 269.
- [16] Mowbray J.F.: "Genetic and immunological factors in human recurrent abortion". *Am. J. Reprod. Immunol. Microbiol.*, 1987, 15, 138.
- [17] Mowbray J.F.: "Success and failures of immunization for recurrent spontaneous abortion". In: Beard R.W., Sharp F. (eds.): Early Pregnancy Loss, Mechanisms and Treatment. Ashton-under-Lyme, Peacock Press, 1988, 325.
- [18] Beer A.E.: "Pregnancy outcome in couples with recurrent abortion following immunological evaluation and therapy". In: Beard R.W., Sharp F. (eds.): Early Pregnancy Loss, Mechanisms and Treatment. Ashton-under-Lyme, Peacock Press, 1988, 337.
- [19] Reznikoff-Etievant M.F., Durieux I., Huchet J., Slamon C., Netter A.: "Human MHC antigens and paternal leukocyte injections in recurrent spontaneous abortions". In: Beard R.W., Sharp F. (eds.): Early Pregnancy Loss, Mechanisms and Treatment. Ashton-under-Lyme, Peacock Press, 1988, 375.
- [20] Smith J.B., Cowchock F.S.: "Immunological studies in recurrent spontaneous abortion: Effects of immunization of women with paternal mononuclear cells on lymphocytotoxic and mixed lymphocyte reaction blocking antibodies and correlation with sharing of HLA and pregnancy outcome". *J. Reprod. Immunol.*, 1988, 14, 99.
- [21] Carp H.J.A., Toder V., Gazit E., Orgad S., Mashiach S., Nebel L., Serr D.M.: Immunization by paternal leukocytes for prevention of primary habitual abortion. Results of a matched controlled trial". *Gynecol. Obstet. Invest.*, 1990, 29, 16.
- [22] Takakuwa K., Toto S., Hasegawa I., Ueda H., Kanazawa K., Takeuchi S., Tanaka K.: "Result in immunotherapy on patients with unexplained recurrent abortion: A beneficial treatment for patients with negative blocking antibodies". *Am. J. Reprod. Immunol.*, 1990, 23, 37.
- [23] Cauchi M.N., Lim D., Young D.E., Kloss M., Pepperell R.J.: "Treatment of recurrent aborters by immunization with paternal cells: Controlled trial". *Am. J. Reprod. Immunol.*, 1991, 25, 16.
- [24] Ho H.N., Gill T.J. III, Hsieh H.J., Jiang J.J., Lee T.Y., Hsieh C.Y.: "Immunotherapy for recurrent spontaneous abortions in a Chinese population". *Am. J. Reprod. Immunol.*, 1991, 25, 10.
- [25] Hwang J.L., Ho H.N., Yang Y.S., Hsieh C.Y., Lee T.Y., Gill T.J.: "The role of blocking factors and antipaternal lymphocytotoxic antibodies in the success of pregnancy in patients with recurrent spontaneous abortion". *Fertil. Steril.*, 1992, 58, 691.
- [26] Fraser E.J., Grimes D.A., Schulz K.F.: "Immunization as therapy for recurrent spontaneous abortion: A review and meta-analysis". *Obstet. Gynecol.*, 1993, 82, 854.
- [27] Check J.H., Tarquini P., Gandy P., Lauer C.: "A randomized study comparing the efficacy of reducing the spontaneous abortion rate following lymphocyte immunotherapy and progesterone treatment versus progesterone alone in primary habitual aborters". *Gynecol. Obstet. Invest.*, 1995, 39, 257.
- [28] Check J.H., Liss J.R., Check M.L., Diantonio A., Duroseau M.: "Lymphocyte immunotherapy can improve pregnancy outcome following embryo transfer (ET) in patients failing to conceive after two previous ET". *Clin. Exp. Obstet. Gynecol.*, 2005, 32, 21.
- [29] Check J.H., Liss J., Check M.L., Diantonio A., Choe J.K., Graziano V.: "Leukocyte immunotherapy improves live delivery rates following embryo transfer in women with at least two previous failures: A retrospective study". *Clin. Exp. Obstet. Gynecol.*, 2005, 32, 85.
- [30] Check J.H., Liss J.R., Diantonio A., Check M.L., Duroseau M.: "Matched control study of efficacy of lymphocyte immunotherapy in donor oocyte recipients to support or refute the concept of need for histocompatibility antigen sharing of the couple for benefits". *Clin. Exp. Obstet. Gynecol.*, 2005, 32, 155.
- [31] Check J.H., Choe J.K., Ubelacker L., DiAntonio A.: "Maternal/paternal sharing of DQ-Alpha type II histocompatibility antigens not associated with pregnancy outcome following in vitro fertilization (IVF)-embryo transfer (ET)". *Clin. Exp. Obstet. Gynecol.*, 2001, 28, 142.
- [32] Ober C., Karrison T., Odem R.R., Barnes R.B., Branch D.W., Stephenson M.D. et al.: "Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial". *Lancet*, 1999, 354, 365.
- [33] Porter T.F., La Coursiense Y., Scott J.R.: "Immunotherapy for recurrent miscarriage". *Cochrane Database Syst. Rev.*, 2006, CD 000112.
- [34] Clark D.A.: "Shall we properly re-examine the status of allogeneic therapy for recurrent early pregnancy failure". *Am. J. Reprod. Immunol.*, 2004, 51, 7.

- [35] Carp H.J.A.: "Immunization with refrigerated paternal cells did not prevent recurrent miscarriage". *Evidence Based. Obstet. Gynecol.*, 2000, 2, 49.
- [36] Clark D.A., Chaovat G.: "Loss of surface CD200 on stored allogeneic leukocytes may impair anti-abortion effect in vivo". *Am. J. Reprod. Immunol.*, 2004, 53, 13.
- [37] King A., Wheeler R., Carter N.P., Francis D.P., Like Y.W.: "The response of human decidual leukocytes to IL-2". *Cell. Immunol.*, 1992, 141, 409.
- [38] King A., Wheeler R., Carter N.P. *et al.*: "The response of human decidual leukocytes to IL-2". *Cell Immunol.*, 1992, 141, 409.
- [39] Kwak J.Y., Beer A.E., Kim S.H., Mantouvalos H.P.: "Immunopathology of the implantation site utilizing monoclonal antibodies to natural killer cells in women with recurrent pregnancy losses". *Am. J. Reprod. Immunol.*, 1999, 41, 91.
- [40] Coulam C.B., Goodman C., Roussev R.G., Thomason E.J., Beaman K.D.: "Systemic CD56+ cells can predict pregnancy outcome". *Am. J. Reprod. Immunol.*, 1995, 33, 40.
- [41] Aoki E., Kajivra S., Matsumoto Y., Ogasawara M., Okada S., Yagami Y. *et al.*: "Preconceptual natural killer cell activity as a predictor of miscarriage". *Lancet*, 1995, 345, 1340.
- [42] Varla-Leftherioti M., Spyropoulou-Vlachou M., Niokou D., Keramitsoglou T., Darlamitsou A., Tsekoura C. *et al.*: "Natural killer cell receptors nepertoine in couples with recurrent spontaneous abortions". *Am. J. Reprod. Immunol.*, 2003, 49, 183.
- [43] Varla-Leftheriotti M., Spyropoulou-Vlachou M., Keramitsoglou T., Papadimitropoulos M., Tsekoura C., Graphou O. *et al.*: "Lack of the appropriate natural killer cell inhibitory receptors in women with spontaneous abortion". *Hum. Immunol.*, 2005, 66, 65.

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# Misoprostol for second trimester abortion in women with prior uterine incisions

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## Summary

*Purpose:* Termination of pregnancy in the second trimester with misoprostol is safe and effective, but there is very limited published experience of its use in women with one or more previous cesarean sections. Uterine rupture might occur when misoprostol and oxytocin are used for pregnancy termination at the second trimester in women with previous uterine scars. In the English literature there are some case-series of studied women with a history of previous cesarean sections, in which misoprostol was used for second trimester termination of pregnancy. However, many different protocols have been used with different doses of misoprostol and different intervals between doses and it is difficult to draw definite conclusions. Therefore, the decision to attempt pregnancy termination in the second trimester in cases with previous uterine scar should be made on a case-by-case basis, after consideration of the number of previous cesarean sections and gestational age, and careful labor monitoring of these patients.

*Key words:* Pregnancy termination; Second trimester; Misoprostol; Uterine rupture; Cesarean section.

## Misoprostol and prior uterine incision for second trimester termination of pregnancy

The reasons for pregnancy termination in the second trimester include congenital anomalies, chromosomal defects, metabolic diseases or other genetic syndromes [1]. Serious complications during second trimester abortions are blood loss requiring transfusion, infections, cervical lacerations, cervicovaginal fistulas, major unintended surgery and even maternal death [2, 3]. The usual agents employed to induce a medical termination of pregnancy in the second trimester are prostaglandin preparations. Misoprostol is a synthetic prostaglandin E<sub>1</sub> analog widely used for the prevention and treatment of gastroduodenal ulcers. Also, misoprostol promotes myometrial contractility and causes cervical softening and dilation. Misoprostol can be given orally, intravaginally, rectally or sublingually [4]. Termination of pregnancy in the second trimester with misoprostol is safe and effective, with a success rate of more than 90% [5]. However, there is very limited published experience of the use of misoprostol for termination of pregnancy in the second trimester in women with one or more previous cesarean sections.

Uterine rupture is a serious complication in cases for termination of pregnancy with a previous uterine scar and may occur either in the mid-trimester or the third trimester. The risk of rupture has been reported to be higher when oxytocin is associated with prostaglandins [6]. Some clinical studies examined the complications of second trimester medical pregnancy terminations in women with previous cesarean section using prostaglandins PGF<sub>2a</sub> and PGE<sub>2</sub>, before the wide use of misoprostol for pregnancy termination. Atienza *et al.* [7] reported one case of uterine rupture among 76 patients with a previous cesarean section managed with amnioinfusion and PGF<sub>2a</sub>. Also, Boulot *et al.* [8] reported one case of uterine rupture among 23 women with a history of caesarean section managed with a combination of mifepristone (Roussel Laboratories) and gemeprost (PGE<sub>2</sub>). In addition, Chapman *et al.* retrospectively reviewed 606 second trimester medical pregnancy terminations using prostaglandins PGE<sub>2</sub> or PGF<sub>2a</sub>, of which 79 were in women with at least one previous cesarean, and found that the risk of uterine rupture was nearly 21 times higher in women with a scarred uterus than in women without this history (3.8% vs 0.2%,  $p < 0.01$ ; OR = 20.8, 95% CI = 14.1-104) [9].

The answer to the question "is misoprostol safe when used to induce labor in the second trimester in a surgically scarred uterus" is of great importance, since the cesarean rate is escalating rapidly and most obstetricians would do everything possible to avoid another repeat cesarean when such women require second trimester pregnancy termination of a nonviable fetus [10]. Some case reports describe uterine rupture with the use of misoprostol in both the scarred and unscarred uterus. For example, Phillips *et al.* reported a case of uterine rupture at 18 weeks of pregnancy in a patient with a history of cesarean section managed with a combination of oral mifepristone (Roussel Laboratories) and intrav-

aginal misoprostol [11]. Oral mifepristone 48 hours prior to misoprostol was administered followed by 600 µg misoprostol vaginally. Six hours later, further misoprostol (600 µg) was administered vaginally in the absence of uterine contractions. Four hours later, painful uterine contractions had been established with vaginal hemorrhage and the fetus was delivered with manual assistance, but the placenta was retained. Because of the fall in hemoglobin and the severe abdominal pain, emergency manual removal of the placenta was performed under general anesthesia and when the uterine cavity was checked digitally it was evident that there was a large defect in the uterine wall. Laparotomy was performed and the findings of a 8 cm uterine rupture with substantial hemorrhage into the broad ligament and abdominal cavity were confirmed. Hysterectomy with right salpingo-oophorectomy, were required to control the hemorrhage [11]. Also, Chen and Shih [12] reported a case of a woman with two previous cesareans who had separation of the uterine scar after a single 200 µg dose of intravaginal misoprostol for second-trimester termination. This dose stimulated strong, regular uterine contractions three hours after the misoprostol. Seven hours later, the cervix had dilated to 2 cm with engagement of the fetal head and after five hours she had sudden lower quadrant pain with vaginal blood clots, regression of the cervical dilation, no engagement of the fetal head and lessening of fetal movement. At emergency laparotomy, separation of the cesarean scar with intact fetal membranes was found, and the fetus and placenta were partially protruding into the peritoneal cavity. A 660 g, stillborn female was delivered and the uterus was repaired [12]. In addition, Berghahn *et al.* reported a woman with two prior cesareans who experienced uterine rupture after misoprostol was used for cervical ripening before second-trimester dilation and evacuation [13]. However, with case reports the absolute risk for uterine rupture remains essentially unknown. There have been only a few case series with a small number of studied women with a history of previous cesarean sections in which misoprostol was used for second trimester termination of pregnancy. However, many different protocols have been used with different doses of misoprostol and different intervals between doses and therefore it is difficult to draw definite conclusions. Daskalakis *et al.* examined 108 women with previous cesarean sections and 216 women without such a history (controls) who underwent pregnancy termination between 17 and 24 weeks of gestation because of fetal anomalies [1]. The first dose included 400 µg misoprostol per os together with 400 µg of intravaginal misoprostol. The same dose of 400 µg of intravaginal misoprostol was repeated every six hours for a maximum of five doses. One uterine rupture was found in the control group. It seems that this treatment protocol does not affect the incidence of complications when women with a previous uterine scar undergo mid-trimester pregnancy termination with misoprostol [1]. Mazouni *et al.* reported a significant incidence (4%) of uterine rupture after treatment using misoprostol among 13 patients at more than 15 weeks' gestation and with a history of cesarean section managed with a combination of oral mifepristone (Roussel Laboratories) and intravaginal misoprostol [14]. Three tablets of mifepristone 36 hours prior to prostaglandin analogue were administered. Women with previous caesarean sections and women without known uterine scars received 1 x 200 µg and 2 x 200 µg misoprostol tablets vaginally, respectively, every three hours until the onset of labor or uterine contractions. If no evidence of either event was observed after three treatments, the procedure was stopped and repeated 24 hours later [14]. Moreover, Bhattacharjee *et al.* examined 80 women who had at least one previous cesarean section and 80 women without such history who had undergone termination of pregnancy between 13 and 26 weeks of gestation for various indications. The standard regimen for misoprostol in all the cases was 400 µg up to 20 weeks of gestation and 200 µg for pregnancies longer than 20 weeks, either vaginally or sublingually every six hours (up to maximum of 24 hours). Misoprostol was found to be safe in the cohort of post-cesarean women and there was only one case of dehiscence [10]. It seems that this proposed regimen is safe in women with previous cesarean sections treated with misoprostol for pregnancy termination.

In conclusion, the decision to attempt pregnancy termination in the second trimester in cases with a previous uterine scar should be made on a case-by-case basis, after consideration of the number of previous cesareans, the gestational age and the placentation. These patients should be carefully monitored during labor. In addition, the combination of misoprostol and oxytocin should be avoided in such patients.

## References

- [1] Daskalakis G.J., Mesogitis S.A., Papantoniou N.E., Mouloupoulos G.G., Papapanagiotou A.A., Antsaklis A.J.: "Misoprostol for second trimester pregnancy termination in women with prior cesarean section". *BJOG*, 2005, 112, 97.
- [2] Cates W.J., Grimes D.A.: "Deaths from second trimester abortion by dilatation and evacuation: causes, prevention, facilities". *Obstet. Gynecol.*, 1981, 58, 401.
- [3] Grimes D.A., Kafriksen M.E., O'Reilly K.R., Binkin N.J.: "Fetal haemorrhage from legal abortion in the United States". *Surg. Obstet. Gynecol.*, 1983, 157, 461.
- [4] Tahg O.S., Ho P.C.: "Pilot study on the use of sublingual misoprostol for medical abortion". *Contraception*, 2001, 64, 315.
- [5] Dickinson J.E., Evans S.F.: "The optimization of intravaginal misoprostol dosing schedules in second-trimester pregnancy termination". *Am. J. Obstet. Gynecol.*, 2002, 186, 470.
- [6] Wiener J.J., Evans A.S.: "Uterine rupture in mid trimester abortion: a combination of gemeprost, vaginal pessaries and oxytocin: case report". *Br. J. Obstet. Gynecol.*, 1990, 97, 1061.

- [7] Atienza M.F., Burkman R.T., King T.M.: "Midtrimester abortion by hyperosmolar urea and prostaglandin F2 alpha in patients with previous caesarean section: clinical course and potential for uterine rupture". *Am. J. Obstet. Gynecol.*, 1980, 138, 55.
- [8] Boulot P., Hoffet M., Bachelard B., Lefort G., Hedon B., Laffargue F., Viala J.L.: "Late vaginal abortion after previous caesarean birth: potential for uterine rupture". *Gynecol. Obstet. Invest.*, 1993, 36, 87.
- [9] Chapman SJ, Crispens M, Owen J., Savage K.: "Complications of midtrimester pregnancy termination: The effect of prior cesarean delivery". *Am. J. Obstet. Gynecol.* 1996, 175, 889.
- [10] Bhattacharjee N., Ganguly R.P., Saha S.P.: "Misoprostol for termination of mid-trimester post-cesarean pregnancy". *Aust. N. Z. J. Obstet. Gynaecol.*, 2007, 47, 23.
- [11] Phillips K., Berry C., Mathers A.M.: "Uterine rupture during second trimester termination of pregnancy using mifepristone and a prostaglandin". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1996, 65, 175.
- [12] Chen M., Shih J.C., Chiu W.T., Hsieh I.J.: "Separation of cesarean scar during second-trimester intravaginal misoprostol abortion". *Obstet. Gynecol.*, 1999, 94, 840.
- [13] Berghahn L., Christensen D., Droste S.: "Uterine rupture during second-trimester abortion associated with misoprostol". *Obstet. Gynecol.*, 2001, 98, 976.
- [14] Mazouni C., Provensal M., Porcu G., Guidicelli B., Heckenroth H., Gamberre M., Bretelle F.: "Termination of pregnancy in patients with previous cesarean section". *Contraception*, 2006, 73, 244.

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**Reproductive Biology Section**

# Increasing the dosage of progesterone (P) supplementation from the mid-luteal phase in women not attaining a mid-luteal homogeneous hyperechogenic (HH) pattern with sonography improves pregnancy rates (PRS) following frozen embryo transfer (ET)

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**Summary**

*Purpose:* To determine if a mid-luteal phase non-homogeneous hyperechogenic (HH) endometrial echo pattern may lower pregnancy rates following frozen embryo transfer and to determine if raising the dosage of progesterone improves pregnancy outcome. *Methods:* Women not attaining an HH pattern at the mid-luteal phase following estrogen-progesterone replacement were randomly given (or not) an increase in progesterone dosage. *Results:* Increasing the progesterone dosage in those not attaining an HH pattern significantly improved the pregnancy rate relative to controls not attaining an HH pattern and showed a trend for higher pregnancy rates than those with an HH pattern. *Conclusions:* The mid-luteal phase echo pattern should be evaluated for a non-HH pattern so that an increase in progesterone dosage could be provided possibly resulting in higher pregnancy rates.

*Key words:* Mid-luteal phase; Echo patterns; Frozen embryo transfer.

**Introduction**

Sonographically, three echo patterns of the endometrium have been described in the literature; triple line (TL), isoechogenic (IE), and homogeneous hyperechogenic (HH). In the secretory phase, the secretion of progesterone (P) by the corpus luteum causes the sonographic appearance of the endometrium to change from a TL pattern seen at mid-cycle, which results from elevated estrogen levels, to an HH pattern.

Some studies have found lower pregnancy rates (PRs) when an HH echo pattern is not observed by transvaginal sonography in the mid-luteal phase following IVF-ET with controlled ovarian hyperstimulation (COH) and non-IVF cycles using luteal phase P, with or without follicle maturing drugs [1, 2]. The aim of the present study was to determine if failing to attain an HH pattern in frozen ET cycles in women who are on graduated estrogen therapy followed by P in the luteal phase have lower PRs than those attaining an HH pattern. Furthermore, the study would determine if raising the dosage of P could have a positive effect on pregnancy outcome.

**Materials and Methods**

During a two-year period women who were being prepared for frozen embryo transfer with a graduated estrogen protocol

followed by progesterone (while the estrogen was continued) were evaluated on the 7<sup>th</sup> day of progesterone replacement with transvaginal ultrasound to evaluate endometrial echo patterns.

On a fixed four days of the week if the echo pattern showed either a triple-line pattern or an isoechogenic pattern, the dosage of progesterone was increased. The increase in progesterone did not occur if these patterns were found on the other three days of the week or the woman had a homogeneous hyperechogenic pattern.

All women were initially treated with 200 mg progesterone vaginal suppositories twice daily plus 100 mg IM progesterone once daily. If an increase in progesterone dosage was made at the mid-luteal phase either 200 mg oral micronized progesterone was added or the evening suppository was increased to 400 mg.

**Results**

A total of 408 women met the inclusion criteria; 66.7% (272/408) had an HH pattern in the mid-luteal phase, 21.3% (87/408) had an IE pattern, and 12% (49/408) had a TL pattern. Triple-line and IE echo patterns were then combined for the remainder of the analyses (non-HH).

None of the 272 women with an HH echo pattern and 77 of 136 women with a non-HH pattern were given an increase in their P dosage in the mid-luteal phase. Although a trend was observed for higher PRs in women with an HH pattern vs non-HH pattern where the P dosage was not increased, the difference was not significant (Table 1).

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Table 1. — Pregnancy rates in women with no increase in P dosage according to attaining or not attaining a homogeneous hyperechogenic pattern of their endometrium in the mid-luteal phase.

	HH	Non-HH	p value
Clinical PR	37.1% (101/272)	28.6% (22/77)	.2
Ongoing/delivered PR	30.9% (84/272)	23.4% (18/77)	.26
Implantation rate	18.3% (146/798)	14.4% (33/229)	.2

Table 2. — Comparison of pregnancy rates in women with a non-HH echo pattern by whether progesterone supplementation was increased or not.

	Increased P Dosage	No Increase	p value
Clinical PR	47.5% (28/59)	28.6% (22/77)	.037
Ongoing/delivered	42.4% (25/59)	23.4% (18/77)	.03
Implantation rate	21.1% (36/171)	14.4% (33/229)	.08

Table 3. — Patient characteristics by increase in progesterone dosage.

	Increase	No increase	p value
Age	34.9 ± 5.0	35.1 ± 5.6	.8a
Number of days of E2 therapy	14.7 ± 1.3	15.0 ± 1.8	.3a
E2 mid-cycle (ng/ml)	1121.6 ± 798.7	1387.2 ± 940	.08a
Mid-cycle endometrial thickness (mm)	9.7 ± 1.4	9.9 ± 1.8	.5a
Mid-cycle echo pattern %			
TL	94.9% (56/59)	96.1% (74/77)	.7 <sup>b</sup>
IE	5.1% (3/59)	3.9% (3/77)	.7 <sup>b</sup>
Number of embryos transferred	2.9 ± .9	3.0 ± .9	.5a
Mid-luteal serum P (pg/ml)	69.5 ± 29.3	70.8 ± 32.5	.8a
Mid-luteal endo thickness (mm)	10.3 ± 2.6	10.4 ± 2.6	.8a

<sup>a</sup>Independent t-test; <sup>b</sup>Chi-square analysis.

Since the objective of the study was to determine if increasing P dosage in the mid-luteal phase of women with a non-HH echo pattern would be effective, pregnancy rates were then compared in those not attaining an HH pattern by whether the dosage was increased or not. Clinical and ongoing pregnancy rates were significantly higher when P was increased (Table 2).

There were no confounding variables to explain the outcome according to adjusting P dosage (Table 3). Contributing factors were similar between the two groups.

## Discussion/Conclusions

With frozen ET, there was a non-significant trend for higher pregnancy rates with a mid-luteal HH echo pattern similar to the findings of fresh ETs and ovulating women without IVF [1, 2]. The data were consistent with another study of pregnancy rates in frozen embryo transfers according to whether the lining converts to an HH pattern in the mid-luteal phase with no adjustment of progesterone dosage [3].

The importance of evaluating the parameters was shown by a significant increase in pregnancy rates after increasing the progesterone dosage from the mid-luteal phase to pregnancy test. However, there was a trend for higher ongoing/delivered PRs (42.4%) in women with a poorer endometrial echo pattern but given a higher P dosage in the mid to late luteal phase than in women with the expected HH echo pattern and constant P dosage (30.9%).

This raises the question as to whether the P dosage should be increased either from the start or in the mid to later portion of the luteal phase in all women. Further study would be needed to answer this question.

## References

- [1] Check J.H., Dietterich C., Lurie D.: "Non-homogeneous hyperechogenic pattern 3 days after embryo transfer is associated with lower pregnancy rates". *Hum. Reprod.*, 2000, 15, 1069.
- [2] Check J.H., Gandica R., Dietterich C., Lurie D.: "Evaluation of a nonhomogeneous endometrial echo pattern in the midluteal phase as a potential factor associated with unexplained infertility". *Fertil. Steril.*, 2003, 79, 590.
- [3] Check J.H., Dietterich C., Nazari A., Lurie D., Choe J.K., Check M.L.: "Non-homogeneous hyperechogenic echo pattern three days after frozen embryo transfer is associated with lower pregnancy rates". *Clin. Exp. Obstet. Gynecol.*, 2005, 32, 15.

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# Successful pregnancies following embryo transfer despite very thin late proliferative endometrium

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## Summary

**Purpose:** To determine if successful pregnancies are possible following fresh or frozen embryo transfer despite a maximal endometrial thickness of only  $\leq 5$  mm. **Methods:** A retrospective review of all fresh and frozen embryo transfers over a seven-year period was performed. The maximum thickness either on the day of human chorionic gonadotropin injection during fresh embryo transfer or the day before the initiation of progesterone in frozen embryo transfer was performed. All embryo transfers performed with a maximum endometrial thickness of 5 mm were identified and the pregnancy rates were determined. **Results:** There were 35 embryo transfers performed with a maximum endometrial thickness of  $< 6$  mm. There were three clinical pregnancies (8.5% per transfer), two live delivered babies (5.7% pregnancy rates per transfer). One of the live births was a fresh transfer using a minimal stimulation protocol and the endometrial thickness was 5.8 mm and the other a frozen embryo transfer with a maximum thickness of 5.0 mm (1 of 7, 14.2% of frozen embryo transfers resulted in a live delivery despite thin endometria. **Conclusions:** Live delivered pregnancies are possible despite thin endometria but the pregnancy rate is poor. Possibly the pregnancy rates may be better without controlled ovarian hyperstimulation.

**Key words:** Endometrial thickness; Thin endometria; Fresh and frozen transfers; Pregnancy rates.

## Introduction

Lower pregnancy rates (PRs) per embryo transfer have been demonstrated in women with thin endometrium in the late proliferative phase at the time of human chorionic gonadotropin (hCG) injection [1, 2]. One review of the literature concluded that there were no successful pregnancies following in vitro fertilization-embryo transfer (IVF-ET) when the pre-ovulatory endometrium was  $< 6$  mm (3). However subsequent to this study a successful pregnancy following IVF was reported where the maximum endometrial thickness was only 4 mm [4]. A successful delivery was also reported without IVF-ET in a natural cycle with a maximum endometrial thickness in the late proliferative phase of 4 mm [5]. The objective of the present study was to determine if these two case reports are rare or if we could find any other success in our practice in cases with very thin endometria in the proliferative phase in cycles with embryo transfer. The objective was also to determine what the likelihood of success is following embryo transfer with an endometrial thickness  $\leq 5$  mm.

## Materials and Methods

A retrospective review of all embryo transfers fresh or frozen over a 7-year time-period was carried out. Embryo transfers were identified where maximum late proliferative phase endometrium was 5 mm. Typically the policy at our IVF center is that if the endometrium is  $\leq 7$  mm the fresh or frozen embryo transfer is deferred. If however on a subsequent cycle the

maximal therapy to improve thickness has been provided then an embryo transfer will ensue.

Typically, for frozen embryo transfers maximal therapy consisting of taking 2-4 mg estradiol vaginally plus an oral graduated regimen of oral estradiol up to 8 mg in the proliferative phase could be extended as long as two to five days; also [6], less commonly vaginal sildenafil 25 mg 4x daily was been given during the proliferative phase [7].

Endometrial thickness was measured by placing calipers on the outer walls of the endometrium. Progesterone vaginal suppositories 200 mg twice daily and IM progesterone 100 mg per day was initiated in frozen ET cycles when maximum endometrial thickness was attained. Embryo transfers were performed on the fourth day of progesterone therapy. For fresh embryo transfers progesterone vaginal suppositories 200 mg twice daily were started the day after the 10,000 U hCG injection. Three-day-old embryos were used for transfers in both fresh and frozen ET cycles.

## Results

There were 35 embryo transfers performed with the late proliferative phase endometrium at a peak thickness of 5 mm. There were two clinical pregnancies (5.7% per transfer) and two live deliveries. One of the successful pregnancies occurred in a woman who had diminished egg reserve and used a minimal gonadotropin stimulation regimen and fresh embryo transfer. Her peak endometrial thickness was 5.8 mm.

Another woman had her fresh embryo transfer deferred and her first frozen ET also because of inadequate endometrial thickness using 2 mg estradiol vaginally daily from day 2 and a graduating oral estradiol regimen of 4 mg times five days, 6 mg times four days, and 8 mg

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times five days. Her peak endometrial thickness in the next cycles only reached 5 mm. Frozen ET was performed; she conceived and delivered a healthy live baby.

Another woman with low-dose gonadotropins conceived with a maximum endometrial thickness of 4 mm. She miscarried and the fetus had aneuploidy (trisomy 18).

Thus conception occurred in 2/28 (7.1%) of fresh transfers (both minimal stimulation however) and in 1/7 (14.2%) of frozen ETs.

## Conclusions

These data show that pregnancies are possible following embryo transfer despite a peak endometrial thickness in the late proliferative phase of < 6 mm.

Ten of the 28 fresh transfers were with minimal stimulation. Interestingly, the two clinical pregnancies with fresh transfer both occurred with low-dose stimulation. Thus the clinical pregnancy rate was 20% with low-dose drugs. None of the 18 women with normal stimulation conceived.

There are data supporting that in some cases the controlled ovarian hyperstimulation regimen may adversely affect embryo implantation [8, 9]. Even with low-dose gonadotropins or a graduated estrogen regimen without gonadotropins the clinical pregnancy rate in this group was lower than usual (17.6%, 3/17). The possibility exists that pregnancies would be much more rare with 4-5 mm endometrial thickness in IVF cycles with conventional controlled ovarian hyperstimulation.

Obviously one option for women with thin endometria is to use a gestational carrier. These data could suggest that one option for thin endometria in the late proliferative phase for women needing IVF-ET is either to use low-dose protocols or purposely freeze the embryos for future frozen ET [10, 11].

We did not use low-dose aspirin because we have not found it helpful in improving the endometrial thickness [12].

## References

- [1] Gonen Y., Casper R.F., Jacobson W., Blankier J.: "Endometrial thickness and growth during ovarian stimulation: a possible predictor of implantation in in vitro fertilization". *Fertil. Steril.*, 1989, 52, 446.
- [2] Check J.H., Nowroozi K., Choe J., Dietterich C.: "Influence of endometrial thickness and echo patterns on pregnancy rates during in vitro fertilization". *Fertil. Steril.*, 1991, 56, 1173.
- [3] Friedler S., Schenker J.G., Herman A., Lewin A.: "The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review". *Hum. Reprod. Update*, 1996, 2, 323.
- [4] Sundstrom P.: "Establishment of a successful pregnancy following in vitro fertilization with an endometrial thickness of no more than 4 mm". *Hum. Reprod.*, 1998, 13, 550.
- [5] Check J.H., Dietterich C., Check M.L., Katz Y.: "Successful delivery despite conception with a maximal endometrial thickness of 4 mm". *Clin. Exp. Obstet. Gynecol.*, 2003, 30, 93.
- [6] Check J.H., Graziano V., Lee G., Nazari A., Choe J.K., Dietterich C.: "Neither sildenafil nor vaginal estradiol improves endometrial thickness in women with thin endometria after taking oral estradiol in graduating dosages". *Clin. Exp. Obstet. Gynecol.*, 2004, 31, 99.
- [7] Sher G., Fisch J.D.: "Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development". *Fertil. Steril.*, 2002, 78, 1073.
- [8] Check J.H., Choe J.K., Katsoff D., Summers-Chase D., Wilson C.: "Controlled ovarian hyperstimulation adversely affect implantation following in vitro fertilization-embryo transfers". *J. Assist. Reprod. Genet.*, 1999, 16, 416.
- [9] Check J.H., Nazari P., Check M.L., Szekeres-Bartho J., Yuan W.: "Evidence that the adverse effect of controlled ovarian hyperstimulation on successful pregnancy outcome following embryo transfer may be related to premature trophoblast invasion". *Clin. Exp. Obstet. Gynecol.*, 2002, 29, 83.
- [10] Check J.H., Choe J.K., Nazari A., Summers-Chase D.: "Ovarian hyperstimulation can reduce uterine receptivity. A case report". *Clin. Exp. Obstet. Gynecol.*, 2000, 27, 89.
- [11] Check J.H., Summers-Chase D., Yuan W., Horwath D., Wilson C.: "Effect of embryo quality on pregnancy outcome following single embryo transfer in women with a diminished egg reserve". *Fertil. Steril.*, 2007, 87, 749.
- [12] Check J.H., Dietterich C., Lurie D., Nazari A., Chuong J.: "A matched study to determine whether low-dose aspirin without heparin improves pregnancy rates following frozen embryo transfer and/or affects endometrial sonographic parameters". *J. Assist. Reprod. Genet.*, 1998, 15, 579.

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# Fertilization by intracytoplasmic sperm injection with sperm with subnormal morphology using strict criteria results in lower live delivered pregnancy rates following frozen embryo transfer rather than eggs fertilized conventionally

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## Summary

**Purpose:** To determine if fertilization by intracytoplasmic sperm injection (ICSI) lowers pregnancy rates following frozen embryo transfer compared to conventional insemination similar to findings with fresh embryo transfer. **Methods:** Clinical and live delivered pregnancy and implantation rates were compared according to whether the eggs were fertilized by conventional oocyte insemination vs ICSI over a 10-year period in women whose husbands had normal semen parameters except for having normal strict morphology between 2-5%. **Results:** The clinical and live delivered pregnancy rates were 40.9% and 31.9, respectively, with ICSI vs 44.2% and 38.3% for women having conventional insemination. The difference in live delivered pregnancy rates approached statistical significance. **Conclusions:** Choosing ICSI for subnormal morphology may not only possibly lower the chance of successful pregnancy following fresh embryo transfer but possibly also following frozen embryo transfer.

**Key words:** Intracytoplasmic sperm injection; Frozen embryo transfer; Live delivered pregnancy rate; Strict morphology; Sperm.

## Introduction

Initially Van Steirtegham *et al.* suggested that fertilization with ICSI produced extremely low pregnancy rates following the transfer of frozen-thawed oocytes [1]. However, other data was not in agreement finding similar pregnancy rates following frozen ET despite fertilization with ICSI versus fresh embryo transfer pregnancy rates after fertilization by ICSI [2].

Recently some data, especially in younger women, suggested that fertilization by ICSI compared to conventional oocyte insemination resulted in lower pregnancy rates following embryo transfer [3].

To support or refute the conclusion that ICSI may lower implantation rates the present study evaluated pregnancy rates following frozen embryo transfer in couples with oocytes fertilized by ICSI vs conventional oocyte insemination.

## Materials and Methods

A retrospective review of frozen ETs over a 10-year time period was performed. There was a requirement of  $\geq 2$  embryos transferred in women aged  $\leq 36$  to be included in the study.

Clinical and delivered pregnancy rates were determined according to whether ICSI or conventional oocyte insemina-

tion was performed. The only couples selected were those where all semen parameters were normal except for strict morphology 2-5%.

The option of ICSI or conventional insemination was left up to the couple. The couples were advised that the majority of IVF centers would do ICSI for low normal morphology. They were advised however, that our data does not agree with the importance of poor morphology being associated with decreased fertility [4-6]. They were reminded that by not doing ICSI they would save money, i.e., the cost of ICSI.

The frozen thawed embryos transferred could have been derived from the intentional cryopreservation for risk of ovarian hyperstimulation syndrome or inadequate endometrial thickness, or were supernumerary ones left over in women who previously had fresh embryo transfers.

The cryopreservation method used a simplified slow-cool method avoiding a planar programmable freezer with a one-step removal of the cryoprotectant 1,2 propanediol [7]. Assisted embryo hatching was performed prior to the transfer on day 3 [5].

The clinical (ultrasound evidence of pregnancy at 8 weeks), viable (live fetus at 12 weeks) and live delivered pregnancy rates and implantation rates were then determined according to the method of fertilization.

## Results

There were 1,741 frozen embryo transfers evaluated of which 1,039 (59.6%) had ICSI performed and 702 (40.4%) had conventional oocyte insemination.

The clinical pregnancy rate per transfer was 40.9% for ICSI and for conventional insemination it was 44.2%

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( $p = 0.119$ ) with chi-square analysis. The live delivered pregnancy rate per transfer was 31.9% for ICSI and for conventional oocyte insemination it was 38.3% ( $p = 0.063$ ) with chi-square analysis.

The clinical pregnancy rates, miscarriage rates and implantations rates are shown in Table 1.

Table 1. — Pregnancy rates for conventional and ICSI insemination following transfer of frozen-thawed embryos.

	ICSI	Conventional
# transfers $\geq 2$ embryos transferred	1039	702
# pregnancies including chemical	497	359
% pregnant/transfers	47.8	51.1
# clinical pregnancies	425	310
% clinical/transfers	40.9	44.2
# chemical pregnancies only	70	41
# ectopic	2	8
# viable	359	283
% viable/transfers	34.6	40.3
# miscarriages	94	41
% miscarriage/clinical pregnancy	22.1	13.2
# deliveries	331	269
% delivered	31.9	38.3
# embryos transferred	3142	2219
Average # embryos transferred	3.0	3.2
# sacs implanted	627	463
Implantation rate	20.0%	20.9%

## Discussion

The results with frozen ET do not show as great of a difference in pregnancy rates following frozen embryo transfer according to whether fertilization had been performed by ICSI or conventional oocyte insemination as was shown with fresh embryo transfer when comparing these two types of oocyte fertilization procedures [3]. Nevertheless, there was a trend for a higher live delivered pregnancy rate, almost approaching a statistically significant difference, in favor of conventional insemination. There was a 20% higher live delivered rate by not performing ICSI.

Previously, it was found that for women with normal day 3 serum FSH undergoing IVF with normal morphology at 2-5% by strict criteria the failed fertilization rate was 1.3% with ICSI vs 1.8% with conventional oocyte insemination [3]. These data thus help support conclusions that were made with fresh embryo transfer, that if semen parameters are otherwise normal but strict normal morphology is between 2-5%, it is definitely more cost effective to fertilize the eggs with a conventional insemination technique rather than ICSI.

These data support the conclusion that either the mechanics of ICSI may cause some subtle damage that

lowers implantation potential despite creating normal appearing embryos, or that there are other more important criteria for selecting "normal" sperm than morphology that is more efficiently achieved by the zona pellucida. Perhaps there are some chemical signals that allow the zona pellucida to select better sperm so that an embryo with greater implantation potential is formed.

Even if one interprets the data that there was no significant difference in the outcomes, the data at a minimum suggest no benefit in performing the more labor intensive and more costly ICSI procedure [3, 9].

Indirectly these data support conclusions that having a low percentage of sperm with normal morphology using strict criteria may not be a very good prognosticator of male subfertility [7-9].

## References

- [1] Van Steirteghem A.C., Van der Elst J., Van den Abbeel E., Joris H., Camus M., Devroey P.: "Cryopreservation of supernumerary multicellular human embryos obtained after intracytoplasmic sperm injection". *Fertil. Steril.*, 1994, 62, 775.
- [2] Hoover L., Baker A., Check J.H., Lurie D., Summers D.: "Clinical outcome of cryopreserved human pronuclear stage embryos resulting from intracytoplasmic sperm injection". *Fertil. Steril.*, 1997, 67, 621.
- [3] Check J.H., Bollendorf A., Wilson C., Summers-Chase D., Horwath D., Yuan W.: "A retrospective comparison of pregnancy outcome following conventional oocyte insemination vs. intracytoplasmic sperm injection for isolated abnormalities in sperm morphology using strict criteria". *J. Androl.*, 2007, 28, 607.
- [4] Check J.H., Adelson H.G., Schubert B.R., Bollendorf A.: "Evaluation of sperm morphology using Kruger's strict criteria". *Arch. Androl.*, 1992, 28, 15.
- [5] Check M.L., Bollendorf A., Check J.H., Katsoff D.: "Reevaluation of the clinical importance of evaluating sperm morphology using strict criteria". *Arch. Androl.*, 2002, 48, 1.
- [6] Kiefer D., Check J.H., Katsoff D.: "The value of motile density, strict morphology, and the hypoosmotic swelling test in in vitro fertilization-embryo transfer". *Arch. Androl.*, 1996, 37, 57.
- [7] Baker A.F., Check J.H., Hourani C.L.: "Survival and pregnancy rates of pronuclear stage human embryos cryopreserved and thawed using a single step addition and removal of cryoprotectants". *Hum Reprod Update* 2, (CD-ROM), 1997.
- [8] Check J.H., Hoover L., Nazari A., O'Shaughnessy A., Summers D.: "The effect of assisted hatching on pregnancy rates after frozen embryo transfer". *Fertil. Steril.*, 1996, 65, 254.
- [9] Keegan B.R., Barton S., Sanchez X., Berkeley A., Krey L.C., Grifo J.: "Isolated teratozoospermia does not affect in vitro fertilization outcome and is not an indication for intracytoplasmic sperm injection". *Fertil. Steril.*, 2007, 88, 1583.

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# Endometriosis: a possible cause of right shoulder pain

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## Summary

Although endometriosis is a common condition it can present with a wide range of symptoms. We report a case of chronic right hypochondrial and shoulder pain which proved to be due to endometriosis.

*Key words:* Endometriosis; Sites; Presentation.

## Introduction

Endometriosis is a common condition affecting 5-10% of the general female population (ACOG 2005). The disease is mostly found in the pelvic organs and peritoneum and presents with pelvic pain, dysmenorrhoea and/or dyspareunia. We report a rare case of extrapelvic endometriosis that presented with pain in the right hypochondrium and right shoulder.

## Case Report

A 38-year-old woman was referred to the Gynaecology Clinic because of a history of secondary infertility. She had had only one pregnancy 13 years before which ended with an emergency caesarean section at 34 weeks gestation due to placental abruption. The patient had her menarche at the age of 13. Her periods were regular, always associated with dysmenorrhoea and they seemed to be getting heavier. She used the combined birth control pills for nine years following the delivery of her child and stopped using any form of contraception after that as she was trying to conceive.

There was no previous history of any medical or surgical problem. However, 18 months before her referral she began to have attacks of right shoulder and right hypochondrial pain. She was referred for medical and surgical review but the cause of the pain was never identified. On close questioning the pain was more or less of a constant dull aching nature with frequent acute exacerbations one to two days before her period. The right hypochondrial acute pain episodes used to last for the whole duration of the period while the right shoulder pain tended to get better on the second or third day. Although the patient was doing her best to live with the pain, she was barely coping and the pain started to have an impact on her life. She was also worried about the possible effect of any fertility treatment or future pregnancy on her pain.

General and abdominal examinations were unremarkable, whereas pelvic examination showed a bulky, mobile and tender uterus with no masses felt in either adnexa. The patient had had

an abdominal and pelvic ultrasound scans six months before as part of the investigations for the pain but they did not reveal any abnormality.

Initial investigations for infertility were already arranged by the general practitioner before referral and they were all normal including the semen analysis of her partner. The results of the tests were explained to the couple, and we discussed the possibility of having diagnostic laparoscopy and a tubal patency test with the patient. We also mentioned that laparoscopy might help in finding the cause of her upper abdominal pain.

Laparoscopy showed deposits of endometriosis in the pouch of Douglas, right uterosacral ligament and right ovary. Both tubes were patent and there were no pelvic adhesions. Evidence of what seemed to be extensive endometriosis was found on the peritoneum covering the under surface of the diaphragm above the liver; no other areas were affected in the abdomen. Biopsies were taken from these peritoneal lesions which later confirmed the presence of endometriosis. The operative findings and management options were discussed with the patient. Although she was initially referred with a fertility problem she decided to have a course of GnRH analogues with add-back therapy for six months to treat her pain before considering any fertility management. She was reviewed in the clinic halfway through her course and reported a marked improvement in her symptoms with the disappearance of the right shoulder pain.

## Discussion

Endometriosis can be simply defined as the presence of abnormally implanted endometrial tissue (glands and stroma) outside the uterine cavity [1]. The best estimated incidence of the condition in the general female population is between 5-10% [2]. The incidence has been shown to reach up to 30% in women being evaluated for infertility and 45% in those investigated for chronic pelvic pain [2]. The most affected sites are the pelvic organs and peritoneum, although extra pelvic sites were reported, e.g., bowel, umbilicus and lungs [3]. The extent of the disease varies from a few small spots with an otherwise normal pelvis to an extensive form with fibrosis, adhesions, distortion of anatomy with or without the formation of ovarian endometriotic cysts [4].

In this case the patient did not have any significant pelvic symptoms of endometriosis although the subfertil-

ity could have been related to the condition. The only suggestive symptoms of endometriosis, after taking a detailed history, were due to abdominal rather than pelvic involvement in the form of upper right quadrant pain referred to the right shoulder which also had a relation to the period.

The diagnosis was confirmed histologically and the condition showed marked improvement following treatment with GnRH analogues which proves that endometriosis was the underlying cause. There was a previous case report of juxtahepatic endometriosis [5] which presented only with right hypochondrial pain. Thus endometriosis can be regarded as one of the possible causes of right shoulder and right hypochondrial pain in females after exclusion of other medical and surgical causes, particularly if the history is suggestive.

## References

- [1] American College of obstetricians and gynecologists (ACOG): "Endometriosis technical bulletin". 1993, 184, 1.
- [2] American college of obstetricians and gynecologists (ACOG): "Endometriosis in adolescents committee opinion". 2005, 310, 1.
- [3] Kennedy S., Bergqvist A., Chapron C., D'Hooghs T., Dunselman G., Greb R. *et al.*: "ESHRE guideline for the diagnosis and treatment of endometriosis". *Hum. Reprod.*, 2005, 20, 2698.
- [4] Revised American Society for Reproductive Medicine classification of endometriosis. *Fertil. Steril.*, 1999, 67, 817.
- [5] Nuovo M., Bayani E., Gerold T., Leong M., Mir R.: "Endometrioid cystadenofibroma developing in juxtahepatic endometriosis: A case report". *Int. J. Surg. Pathol.*, 1998, 6, 109.

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# Troponin I and homocysteine levels in mild and severe preeclampsia

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## Summary

**Objective:** To investigate troponin I and homocysteine in pregnant women with severe and mild preeclampsia. **Methods:** 43 women with mild and 22 women with severe preeclampsia, and 34 healthy pregnant women were included in the study. Homocysteine and troponin levels of the three groups were measured at admission and compared. **Results:** Mean troponin I levels were 0.005 ng/ml, 0.0116 ng/ml and 0.007 ng/ml in healthy pregnant women and mild and severe preeclampsia, respectively. These results were similar among the three groups. Homocysteine levels were similar in the mild and severe preeclampsia groups and significantly higher than in healthy pregnant women. **Conclusions:** Troponin I levels are not significantly increased in either mild and severe preeclampsia. Homocysteine increases in preeclampsia, but the severity of preeclampsia is not correlated with homocysteine levels.

**Key words:** Troponin I; Homocysteine; Preeclampsia; Pregnancy.

## Introduction

It is well known that maternal adaptations of the cardiovascular system are needed in normal pregnancy. If pregnancy is complicated with hypertension and/or proteinuria these adaptation mechanisms may fail and result in myocardial damage [1-3].

Troponin I is a constituent of the troponin complex which regulates the interaction of actin and myosin in striated muscle. Cardiac troponin I contains an immunologically distinct N-terminus amino acid chain not expressed in skeletal isoforms. Cardiac troponin I is released into the circulation in response to myocardial injury and has been shown to be one of the most sensitive and specific markers of myocardial damage both in ischemic and nonischemic conditions [4, 5]. Population studies suggest that the 98<sup>th</sup> percentile lies at 0.03 ng/ml and that 88% of the normal population have serum cTnI of less than 0.01 ng/ml. In the setting of acute chest pain cardiac troponin I of > 0.1 ng/ml has been shown to have prognostic significance, and values above this level are taken as an indicator of significant myocardial damage [6].

Homocysteine is a sulfur containing essential amino acid derived from demethylation of dietary methionine. Plasma homocysteine levels decrease during pregnancy, probably due to changes in the renal handling of homocysteine or due to the hormonal changes associated with pregnancy [7, 8]. Plasma homocysteine concentrations are closely dependent on vitamin B (folate, vitamins B 6 and B 12, riboflavin) intake [9] and are also affected by variants of the methylenetetrahydrofolate reductase gene, particularly the thermolabile 677 C-T variant which

results in reduced activity of the enzyme [10, 11]. Recently, increased homocysteine plasma levels have been reported to occur in women with preeclampsia [12-14]. There is a discrepancy in the literature whether homocysteine levels are related to severity of preeclampsia and if troponin levels increase in all preeclampsia cases or not.

## Material and Methods

The study was composed of 99 women attending Sisli Etfal Training and Research Hospital 3<sup>rd</sup> Obstetrics and Gynecology Clinic between June 2008 and December 2008. Three groups were generated; the first group consisted of 34 normotensive pregnant women without any pregnancy complications, the second group consisted of 43 pregnant women with mild preeclampsia and the third group consisted of 22 pregnant women with severe preeclampsia. Blood samples were obtained at admission until delivery from all participants. The diagnosis of preeclampsia was based on standard criteria as outlined in the Technical Bulletin from the American College of Obstetrics and Gynecology on Hypertension in Pregnancy [15]. Women before 24 weeks of gestation and those with known pre-existing cardiac or renal disease were excluded from the study. Informed consents on the study were signed by all participants and approval was obtained from the hospital ethical committee. Serum cardiac troponin I was measured using the Beckman Access II immunoassay (Beckman Coulter, Inc., Fullerton, CA) at the Department of Clinical Biochemistry of our hospital [16]. A lower limit of detection of 0.03 ng/ml is suggested for clinical use. Maternal homocysteine concentrations were measured using high-performance liquid chromatography with electrochemical detection procedures as described previously [17]. The interassay coefficient of variation was 7.2%. All laboratory assays were performed without knowledge of case or control status.

**Statistical analysis:** Comparisons of the demographic factors, troponin and homocysteine levels of the patients in the different groups were made using analysis of variance (ANOVA). The

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post hoc Tukey test was used to find the differences. Correlation analysis was performed using Pearson's correlation test ( $r$  = correlation coefficient). SPSS version 13.0 was used for calculations;  $p < 0.05$  was considered as significant at the 95% confidence level.

## Results

Demographic characteristics of the groups are demonstrated in Table 1. Mean ages and parity of the pregnant women were similar among the three groups. Mean gestational weeks at labor were similar in the normotensive control and mild preeclampsia groups and was significantly lower in the severe preeclampsia group ( $p < 0.05$ ).

Table 1. — Characteristics of the study and control groups.

	Mild PE	Severe PE	Control	$p$
No.	43	22	34	—
Maternal age (yrs)	28.1 ± 5.6	26.5 ± 5.2	27.7 ± 6.5	NS
Parity	1.3 ± 1.6	0.6 ± 0.8	1.0 ± 1.5	NS
GA (days)	266.04 ± 16.5	235.00 ± 20.7	272.87 ± 21.2	< 0.05
Proteinuria (mg/l)	944.5 ± 98.2	8786.8 ± 2306.9	41.3 ± 13.2	< 0.001
Birthweight (g)	3114.2 ± 96.5	2020.8 ± 304	3330.7 ± 76.8	< 0.05
Apgar score	7.6 ± 0.4	6.0 ± 1.3	8.7 ± 0.2	< 0.05

PE: preeclampsia; GA: gestational age.

Mean troponin I levels were similar among the three groups. Homocysteine levels were similar in the mild and severe preeclampsia groups and significantly higher than the control group ( $p < 0.05$ ) (Table 2).

Table 2. — Mean troponin I and homocysteine levels of the groups.

	Troponin I (ng/ml)	Homocysteine (μmol/l)
Healthy pregnant women (control)	0.0050 ± 0.008	6.31 ± 2.33
Mild preeclampsia	0.0116 ± 0.043	8.43 ± 5.20
Severe preeclampsia	0.0070 ± 0.015	9.32 ± 4.45
Significance ( $p$ )	NS	< 0.001

No correlation was found between homocysteine and gestational age ( $r = 0.17$ ), between troponin I levels and gestational age ( $r = 0.15$ ), or between homocysteine and troponin I levels ( $r = 0.34$ ).

## Discussion and Conclusion

There are a limited number of studies concerning troponin I levels in preeclampsia in the literature. Several authors have reported that troponin I levels rise in both mild and severe preeclampsia [18-20]. One study even reported that it increases more in the severe form of preeclampsia [20]. However recently Joyal *et al.* reported that preeclampsia was not associated with a rise in troponin I levels [21]. This discrepancy may be due to small study groups which consist of patients with illnesses known to increase troponin I. There are many cardiac and non-cardiac reasons that can increase troponin I levels reported in the literature. All cardiac interventions and any cardiac disease causing myocardial damage may lead

to increased troponin I. Among the non-cardiac reasons, end-stage diseases, chemotherapy, renal failure, septic shock, and ultra-endurance exercises are well known [22].

We designed our study with larger groups defining preeclampsia as mild and severe forms to make this confusing data in previous studies more clear. Our results are in accord with Joyal *et al.* Further we can say that severity of preeclampsia was not related to troponin I levels. Nonetheless although statistically not significant, there was a tendency for a rise in troponin I in mild preeclampsia compared to healthy controls ( $p = 0.066$ ), but interestingly, troponin I in severe preeclampsia was very similar with healthy controls. These findings support the theory of Joyal *et al.* that an elevated troponin level can not be solely attributed to inflammatory events of preeclampsia because it is not correlated with the amount of proteinuria and nor blood pressure levels. There were no cardiac or non-cardiac reasons known to elevate troponin I in the groups in our study. Why then did some of the patients have increased troponin levels – especially the mild preeclampsia group? This could be because some of the patients may be more prone to myocardial injury when they are exposed to high blood pressure or more prone to other inflammatory processes seen in preeclampsia.

It has been clearly defined by many studies that elevated homocysteine levels are associated with preeclampsia [13, 14, 23]. Some studies even blamed homocysteine as a causative agent for preeclampsia and the severity of preeclampsia was associated with the levels of homocysteine [24]. According to our study, homocysteine levels increased in preeclampsia, but severity of disease was not related with homocysteine serum levels.

The pathophysiology of hyperhomocysteinemia in vascular disease is still under investigation. Preeclampsia produces diffuse endothelial dysfunction as evidenced by increased levels of fibronectin, thrombomodulin, endothelin and thromboxanes; there is impaired vasodilatation [25].

Endothelial dysfunction, which is thought to play a role in the pathophysiology of hyperhomocysteinemia, can be mediated by oxidative stress. This dysfunctional endothelium may explain the poor cardiovascular outcomes after years. Is this endothelial damage also responsible for alterations of troponin levels? We thought the oxidative stress and dysfunctional endothelium may be the same etiological factor increasing homocysteine and troponin levels in severe preeclamptic women in accord with some studies [18, 20].

Thus we wanted to know if there was any correlation with troponin levels and homocysteine levels in different groups. However we could not find any correlation between homocysteine and troponin I levels ( $r = 0.34$ ). Therefore we wanted to find out if this result would also be reflected in troponin levels and would they correlate? The relationship between homocysteine and coronary artery disease/cerebrovascular disease is well known. The risk of coronary artery disease is seen across a range of homocysteine levels. Some studies reported that there is

a positive association between homocysteine and ischemic heart disease; for an increase of 5  $\mu\text{mol/l}$  in homocysteine, the rate of ischemic heart disease risk increased by 84% [10, 26]. Our not finding any correlation between troponin I and homocysteine levels may be related with not finding any relation according to severity of the disease. We could not find any other study that compared these two levels so further studies are needed.

Lastly, our study was not designed to explain the etiological role of hyperhomocysteine, so we can not comment as to whether hyperhomocysteine is a causative agent or not. Whether endothelial damage is the cause or a result of preeclampsia is also unknown.

## References

- [1] Duvekot J.J., Peeters L.L.: "Maternal cardiovascular hemodynamic adaptation to pregnancy". *Obstet. Gynecol. Sum.*, 1994, 49, S1.
- [2] Lang R.M., Pridjian G., Feldman T., Neumann A., Lindheimer M., Borow K.M.: "Left ventricular mechanics in pre-eclampsia". *Am. Heart. J.*, 1997, 121, 1768.
- [3] Barton J.R., Hiatt A.K., O'Connor W.N., Nissen S.E., Greene J.W.: "Endomyocardial ultrastructural findings in pre-eclampsia". *Am. J. Obstet. Gynecol.*, 1991, 165, 389.
- [4] Triquier S., Flécheux O., Bullenger M., Castex F.: "Highly specific immunoassay for cardiac troponin I assessed in noninfarct patients with chronic renal failure or severe polytrauma". *Clin. Chem.*, 1995, 41, 1675.
- [5] Apple F.S., Falahati A., Paulson P.R., Miller E.A., Sharkey S.W.: "Improved detection of minor ischemic myocardial injury with measurement of serum cardiac troponin I". *Clin. Chem.*, 1997, 43, 2047.
- [6] Panteghini M., Bonora R., Pagani F., Buffoli F., Cuccia C.: "Rapid, highly sensitive immunoassay for determination of cardiac troponin I in patients with myocardial cell damage". *Clin. Chem.*, 1997, 43, 1464.
- [7] Murphy M.M., Scott J.M., McPartlin J.M., Fernandez-Ballart J.D.: "The pregnancy-related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study". *Am. J. Clin. Nutr.*, 2002, 76, 614.
- [8] Powers R.W., Majors A.K., Kerchner L.J., Conrad K.P.: "Renal handling of homocysteine during normal pregnancy and preeclampsia". *J. Soc. Gynecol. Investig.*, 2004, 11, 45.
- [9] de la Calle M., Usandizaga R., Sancha M., Magdaleno F., Herranz A., Cabrillo E.: "Homocysteine, folic acid and B-group vitamins in obstetrics and gynaecology". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2003, 107, 125.
- [10] Frosst P., Blom H.J., Milos R., Goyette P., Sheppard C.A., Matthews R.G. *et al.*: "A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase". *Nat. Genet.*, 1995, 10, 111.
- [11] Raijmakers M.T., Zusterzeel P.L., Steegers E.A., Peters W.H.: "Hyperhomocysteinaemia: a risk factor for pre-eclampsia?". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2001, 95, 226.
- [12] Dekker G.A., de Vries J.I., Doelitzsch P.M., Huijgens P.C., von Blomberg B.M., Jakobs C., van Geijn H.P.: "Underlying disorders associated with severe early-onset preeclampsia". *Am. J. Obstet. Gynecol.*, 1995, 173, 1042.
- [13] Rajkovic A., Catalano P.M., Malinow R.M.: "Elevated homocyst(e)ine levels with preeclampsia". *Obstet. Gynecol.*, 1997, 90, 168.
- [14] Powers R.W., Evans R.W., Ness R.B., Crombleholme W.R., Roberts J.M.: "Homocysteine and cellular fibronectin are increased in preeclampsia, not in transient hypertension of pregnancy". *Hypertens. Preg.*, 2001, 20, 69.
- [15] American College of Obstetrics and Gynecology. Hypertension in pregnancy. The College; ACOG Tech Bull., 219, 1996.
- [16] Christenson R.H., Apple F.S., Morgan D.L., Alonsozana G.L., Mascotti K., Olson M. *et al.*: "Cardiac troponin I measurement with the ACCESS immunoassay system: analytical and clinical performance characteristics". *Clin. Chem.*, 1998, 44, 52.
- [17] Malinow R.M., Sexton G., Averbuch M., Grossman, Wilson D., Upson B.: "Homocyst(e)-inemia in daily practice: Levels in coronary artery disease". *Coron. Artery Dis.*, 1990, 1, 215.
- [18] Fleming S.M., O'Gorman T., Finn J., Grimes H., Daly K., Morrison J.J.: "Cardiac troponin I in pre-eclampsia and gestational hypertension". *Br. J. Obstet. Gynaecol.*, 2000, 107, 1417.
- [19] Atalay C., Erden G., Turhan T., Yildiran G., Saracoglu F., Koca Y.: "The effect of magnesium sulfate treatment on serum cardiac troponin I levels in preeclamptic women". *Acta Obstet. Gynecol.*, 2005, 84, 617.
- [20] Yang X.F., Lu D.H., Wang Z.P., He J., Wang H.Z., Dong M.Y.: "Changes of cardiotrophin I and cystatin C in preeclampsia and clinical significance thereof". *Zhonghua Yi Xue Za Zhi.*, 2006, 86, 3190.
- [21] Joyal D., Leya F., Koh M., Besinger R., Ramana R., Kahn S. *et al.*: "Troponin I levels in patients with preeclampsia". *Am. J. Med.*, 2007, 120, 819e13-.
- [22] Ammann P., Pfisterer M., Fehr T., Rickli H.: "Raised cardiac troponins". *BMJ*, 2004, 328, 1028.
- [23] Qusada E.V., Vilaseca M.A., Laila J.M.: "Plasma total homocysteine in uncomplicated pregnancy and preeclampsia". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2003, 108, 45.
- [24] Singh U., Gupta H.P., Singh R.K., Shukla M., Singh R., Mehrotra S.S., Prasad S.: "A study of changes in homocysteine levels during normal pregnancy and preeclampsia". *J. Indian Med. Assoc.*, 2008, 106, 503.
- [25] Friedman S.A., Schiff E., Emeis J.J., Dekker G.A., Sibai B.M.: "Biochemical corroboration of endothelial involvement in severe preeclampsia". *Am. J. Obstet. Gynecol.*, 1995, 172, 202.
- [26] Funai E.F., Friedlander Y., Paltiel O., Tiram E., Xue X., Deutsch L., Harlap S.: "Long-term mortality after preeclampsia". *Epidemiology*, 2005, 16, 206.

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# Preliminary results of objective assessment of mammographic percent density

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## Summary

Breast density assessments performed by using the Breast Imaging Reporting and Data System (BI-RADS) have been completely qualitative and the American College of Radiology (ACR) fibroglandular density descriptors are mainly subjective. However, women with increased mammographic density (MD) have an increased risk of developing breast cancer. The purpose of our study was to evaluate an experimental method to quantify MD using a software utility which measures absolutely black areas as zero and absolutely white areas as 100. In grey scale areas, these values range between 0 and 100, depending on the "density" of the area. Digital screening mammograms were directly estimated with this method. We concluded that there is a significant correlation between ACR quartiles and this grey scale percentage method, although several improvements on the original idea are planned.

*Key words:* Mammographic density; Digital mammography; Breast density assessment.

## Introduction

The efficacy of mammographic screening has been established by randomized controlled trials in which absolute mortality reduction has been achieved by the ability of mammography to find ductal carcinoma in-situ and infiltrating cancers of a smaller size and earlier stage than in unscreened control groups [1-4]. Digital mammography has been shown to have at least equivalent diagnostic accuracy to screen-film mammography and it offers some potential advantages over conventional technology [5] as magnification, subtraction of parasite signals, contrast changing, reproductivity and storage.

Breast density assessments performed by using the Breast Imaging Reporting and Data System (BI-RADS) have been completely qualitative [6]. The American College of Radiology (ACR) has also developed the following set of fibroglandular density descriptors that may be used within the text of a mammogram report: "almost entirely fat" (< 25% density), "scattered fibroglandular densities" (25%-50%), "heterogeneously dense" (51%-75%), and "extremely dense" (> 75%) [7], although these estimations are mainly subjective. However, women with mammographic percent density (MPD) > 50% have an approximately three-fold increased risk of developing breast cancer [8].

The purpose of this work was to evaluate an experimental method to quantify MPD utilizing Mac OS X Software.

## Material and Methods

A prospective study on a method of calculation of breast mammography density was carried out. Our main purpose was to obtain an objective value of mammographic density for digital mammography and to "avoid" the subjectivity of the ACR classification which is not always reproducible.

The DigitalColor Meter is a Mac OS X utility that measures colors and translates the color values into those used by different color models, such as RGB (red-green-blue). In the RGB color model, the grey scale pictures have the same RGB values. In absolutely black areas all values, expressed as percentage, are zero and in absolutely white areas all values are 100. In grey scale areas the values are between 0 and 100, depending on the «density» of the area.

In digitized mammographies, pointing to the areas of mammography to be measured, and pressing Shift-Command-C, the gray scale values are copied to the clipboard. Reducing the size of mammography and expanding the examined area in a broader surface, a more representative value for the whole breast could be achieved (Figures 1 & 2 magnified).

Digital screening mammograms from 47 patients were directly estimated with the program and compared with clinical impression based on ACR quartiles, blindly estimated by the authors.

## Results

Density values in mediolateral oblique mammograms were increased in comparison with craniocaudal projections, due to the major pectoralis muscle. Therefore, to avoid the "whiteness" of the major pectoralis muscle, only craniocaudal projections were estimated.

Furthermore, the black background of mammograms (circumferentially of the breast) lowered the real density value in all mammograms. Hence, the background was changed to grey in an effort to achieve values close to those obtained by clinical estimation. This "trick" did not

Fig. 1

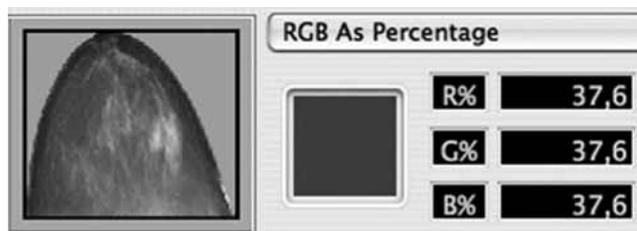


Figure 1. — “Mammographic breast density” estimated as red-green-blue percentage.

Fig. 2

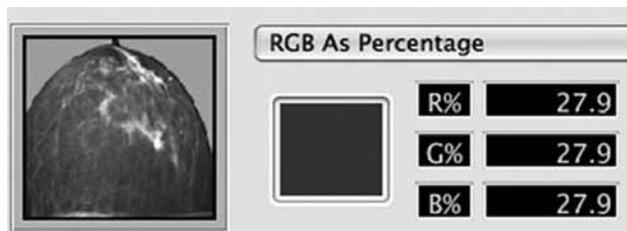


Figure 2. — “Mammographic breast density” estimated as red-green-blue percentage.

influence the quality of density estimations and density comparisons among mammograms because the breast itself remained intact and the same scale of grey was used for all mammograms. Equally, with this method, no further calculations were necessary to compare clinical estimations with the program estimations or the use of special tables, and the “automated” percentages corresponded to the clinical impression quartiles of ACR.

### Discussion

Despite the fact that a biopsy is to be undertaken for a palpable abnormality, mammography is still important to evaluate the area in question as well as to screen the remaining ipsilateral and contralateral breast tissues for clinically occult cancer.

By definition, mammographic screening involves the performance of the mediolateral oblique and craniocaudal projections. However, due to the square shape of the aperture area examined, it was not possible to avoid the major pectoralis without losing a part of breast tissue in mediolateral oblique projections.

The ACR set of fibroglandular density descriptors is a very useful method of clinical description although some degree of hesitation could arise when such a description belongs to the upper or lower limit of the previous or next category respectively, as in our example of Figure 2. In this case, our method has a degree of descriptive accuracy, although this method could be proposed more as a comparative tool among mammograms than a unique tool for a specific mammogram.

Previous studies have quantified objectively the mammographic (percent) density, correlated it with breast cancer risk and made digitized assessments of mammographic breast density in patients receiving hormonal regimens [9-11].

Taking into account that breast density may actively be related to breast cancer risk, methods of breast densitometry must be accurate, reliable, easy to learn, easy to perform, widely available, quick, cheap and repeatable.

Further validation of accuracy and possibilities of changing shapes in examined areas with similar programs are ongoing investigations at our institutions.

### Conclusions

There is a significant correlation between ACR quartiles and this grey scale percentage method, although we

plan several improvements on the original idea. Our initial ideal could prove to be an important one in mammography screening because it is based on a cheap, easy to learn and perform, relatively quick, and repeatable method.

### References

- [1] Tabar L., Fagerberg C.J., Gad A., Baldetorp L., Holmberg L.H., Grontoft O. *et al.*: “Reduction in mortality from breast cancer after mass screening with mammography. Randomised trial from the breast cancer screening working group of the Swedish National Board of Health and Welfare”. *Lancet*, 1985, *1*, 829.
- [2] Duffy S.W., Tabar L., Chen H.H., Holmqvist M., Yen M.F., Abd-salah S. *et al.*: “The impact of organized mammography service screening on breast carcinoma mortality in seven Swedish counties”. *Cancer*, 2002, *95*, 458.
- [3] Tabar L., Yen M.F., Vitak B., Chen H.H., Smith R.A., Duffy S.W.: “Mammography service screening and mortality in breast cancer patients: 20-year follow-up before and after introduction of screening”. *Lancet*, 2003, *361*, 1405.
- [4] Swedish Organised Service Screening Evaluation Group: “Reduction in breast cancer mortality from organized service screening with mammography: 1. Further confirmation with extended data”. *Cancer Epidemiol. Biomarkers Prev.*, 2006, *15*, 45.
- [5] Shiraishi A.: “Current state of digital mammography”. *Breast Cancer*, 2008, *15*, 194.
- [6] Martin K.E., Helvie M.A., Zhou C., Roubidoux M.A., Bailey J.E., Paramagul C. *et al.*: “Mammographic density measured with quantitative computer-aided method: Comparison with radiologists’ estimates and BI-RADS categories”. *Radiology*, 2006; *240*, 656.
- [7] Bondy M.L., Newman L.A.: “Assessing breast cancer risk: Evolution of the gail model”. *JNCI*, 2006, *98*, 1172.
- [8] Glide-Hurst C.K., Duric N., Litttrup P.: “A new method for quantitative analysis of mammographic density”. *Med. Phys.*, 2007, *34*, 4491.
- [9] Lundström E., Söderqvist G., Svane G., Azavedo E., Olovsson M., Skoog L. *et al.*: “Digitized assessment of mammographic breast density in patients who received low-dose intrauterine levonorgestrel in continuous combination with oral estradiol valerate: a pilot study”. *Fertil. Steril.*, 2006, *85*, 989.
- [10] McCormack V.A., Highnam R., Perry N., dos Santos Silva I.: “Comparison of a new and existing method of mammographic density measurement: intramethod reliability and associations with known risk factors”. *Cancer Epidemiol. Biomarkers Prev.*, 2007, *16*, 1148.
- [11] Shepherd J.A., Kerlikowske K.M., Smith-Bindman R., Genant H.K., Cummings S.R.: “Measurement of breast density with dual X-ray absorptiometry: feasibility”. *Radiology*, 2002, *223*, 554.

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# Correlations of fetal-maternal outcomes and first trimester 3-D placental volume/3-D power Doppler calculations

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## Summary

**Objective:** To investigate correlations between first trimester placental volume, placental vascularization indexes and the outcome of those pregnancies. The possible prediction of macrosomia and intrauterine growth restriction in the first trimester are studied. **Methods:** We prospectively examined 145 pregnant patients at 11-14 weeks of gestation using transvaginal 3D gray-scale and power Doppler ultrasound. The acquired volumes were analyzed using the VOCAL imaging program, for assessing placental volume, vascularization index (VI), flow index (FI) and vascularization flow index (VFI). The results were correlated with the pregnancy outcome. **Results:** Correlations between placental volume and the intrauterine growth restriction group of infants classified according to their anthropometric measurements were significant. As the placental volume decreases, percentage of intrauterine growth restriction increases. In the aspect of placental vascularisation indexes, VI showed a positive linear correlation with newborn weight. **Conclusion:** The 3D placental volume and blood flow calculations could be important in the prediction and easy, rapid diagnostic evaluation of fetal growth restriction presenting with placental volume and vascular tree alterations even beginning at the first trimester.

**Key words:** 3-D ultrasound; VOCAL; Placental volume; IUGR; Pregnancy outcome.

## Introduction

Three-dimensional (3-D) ultrasound (US) has been used as a tool in many placenta-based researches to evaluate the volume and power Doppler features [1]. 3-D US and virtual organ computer-aided analysis (VOCAL) software have superior ability of configuring anatomy and abnormality when compared to 2-D US [2]. Usage of 3-D power Doppler helps in diagnosing fetal and placental abnormalities and their vascularity [1]. In our study we focused on outcomes of pregnancy as IUGR and LGA. As for placental vascularization, our study revealed results of indexes of a global view of the placenta, not only a particular part of it.

3-D volumetric calculations of the placenta were made through all trimesters. The first focus of investigations was to show correlations of placental volume and fetal growth [2, 3]. The second was to predict fetal growth disproportion and pregnancy-related complications such as preeclampsia [4, 5]. The correlation of placental volume and fetal growth disproportion – especially growth restriction – was the major aim in these investigations [3-5]. Placental 3-D power Doppler indexes (vascularization index (VI), flow index (FI) and vascularization-flow index (VFI)) were used to correlate with fetal growth. Those parameters have been shown to have a positive linear correlation with first trimester fetal anthropometric calculations and the feasibility of using them in fetal delivery weight and prediction of fetal growth restriction has been noted [1-5].

The purpose of our study was to investigate the correlation between first trimester placental volume, placental

VI and infant outcomes such as weight of infants, infants classified according to their gestational week at birth, and birth weight. We also investigated the prediction of IUGR by help of placental volume and placental VI calculations as early as the first trimester.

## Materials and Methods

One hundred and forty-five pregnant volunteers in their first trimester in the period of 11-13 weeks and six days were examined after informed consent was obtained. The study had the approval of the university ethical committee. None of the patients had any gynecologic or obstetric complications in their medical history and they all had a normal course of pregnancy. Systemic and obstetric examinations were performed on the women and gestational age was confirmed by crown-rump length (CRL) with a transabdominal 3.5 MHz probe (Voluson 730 GE Expert Diamond Kretztechnik, Zipf, Austria).

After determining the placental site 3-D power Doppler volume of the placenta was obtained. Then placental volume was calculated automatically by VOCAL analysis of 3-D US. The volume acquisition lasted from 5-10 sec. To minimize errors in measurements all patients were investigated with the same setup of the system.

The placental contours were drawn manually. After defining volume of the placenta the software automatically calculated the vascular indexes: VI, FI and VFI. VI represents the percentage of the color-coded volume units (voxels) within the investigated volume. FI is the average color value of all the color voxels, and it shows the average blood flow intensity. VFI value is formed from the percentage of the color coded voxels weighted by average relative intensity of the power Doppler signal. Statistical analysis was undertaken using SPSS (Statistical Package for Social Science) for Windows 15.0;  $p < 0.05$  level was considered statistically significant.

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## Results

Demographic features of the 145 pregnant volunteers in the first trimester and newborns are listed in Table 1.

Our results show that placental volumes of the study group were significantly different when infant weights were classified according to their week of delivery as AGA, SGA, LGA, and IUGR. This significance was found when placental volume of the IUGR ( $37.0 \pm 18.4$ )  $\text{cm}^3$  group was compared with placental volume of the AGA ( $63.7 \pm 28.0$ )  $\text{cm}^3$ , SGA ( $73.1 \pm 30.4$ )  $\text{cm}^3$ , LGA ( $72.0 \pm 38.1$ )  $\text{cm}^3$  groups one by one (t: 2.88,  $p < 0.05$ ). Quantity of placental volume was also different when groups were classified as IUGR ( $37.0 \pm 18.4$ )  $\text{cm}^3$  and non-IUGR ( $64.8 \pm 28.8$ )  $\text{cm}^3$  (t: 4.001  $p < 0.005$ ). As placental volume range was 16-166  $\text{cm}^3$ , none of placental volume of the IUGR group was higher than 70  $\text{cm}^3$ . While placental volume had a correlation with birth weight, no correlation was found with birth height (t: 0.181  $p < 0.05$  vs t: 0.021  $p = 0.802$ ). Sex of the fetuses did not show any statistical difference in placental volumes of male and female newborn groups.

3-D power Doppler results and statistical analysis of patient FIs were not significantly different when infant weights were classified according to the week of delivery as AGA, SGA, LGA, and IUGR. Similar non-significant results were seen when VI and VFI were compared with infant weights classified according to their week of delivery (t: 3  $p < 0.05$  vs t: 3  $p = 0.088$ ). Statistical comparison of 3-D power Doppler indexes of the placenta with birth weight revealed a significant difference just in VI (t: 0.279  $p < 0.001$ ). No significant difference was found between birth weight and 3-D power Doppler indexes of FI and VFI of the placenta (t: 0.027  $p = 0.744$  vs t: 0.147  $p < 0.05$ ).

Table 1. — Characteristics of the 145 volunteers in the study.

Parameter	p value
Age (years, mean $\pm$ SD)	30.0 $\pm$ 4.3
Gravidy (number, mean $\pm$ SD)	1.7 $\pm$ 0.8
Parity (number, mean $\pm$ SD)	1.4 $\pm$ 0.7
Maternal weight (kg, mean $\pm$ SD)	59.8 $\pm$ 9.5
Placental volume ( $\text{cm}^3$ ; mean $\pm$ SD)	63.1 $\pm$ 28.9
Vascularization index (mean $\pm$ SD)	6.7 $\pm$ 6.9
Flow index (mean $\pm$ SD)	43.0 $\pm$ 8.6
Vascularization flow index (mean $\pm$ SD)	2.9 $\pm$ 2.9
Male newborn (number, percentage)	88; 60.7%
Female newborn (number, percentage)	57; 39%
AGA (appropriate for gestational age) (number, percentage)	120; 82.8%
LGA (large for gestational age) (number, percentage)	9; 6.2%
SGA (small for gestational age) (number, percentage)	8; 5.5%
IUGR (intrauterine growth restricted) (number, percentage)	8; 5.5%
Newborn weight (g, mean $\pm$ SD)	3381 $\pm$ 524.8
Newborn height (cm, mean $\pm$ SD)	49.9 $\pm$ 2.2
Gestational age at delivery (week, mean $\pm$ SD)	37.8 $\pm$ 1.3

## Discussion

Introduction of 3-D US and feasibility of its usage in perinatal medicine appear to have opened a new domain to investigate structures that can not be evaluated by conventional 2-D US as much as 3-D ultrasonography. In respect to volumetric calculations with 3-D US in obstetrics, numerous fetal and placental volume studies have been designed to correlate with fetal and pregnancy related outcomes [1, 2, 4, 6]. Merce *et al.* demonstrated a good reproducibility of the 3-D power Doppler parameters when applied to the study of the placental vascular tree in normal pregnancies [6]. In another study Merce *et al.* showed that 3-D Doppler indices change as pregnancy progresses and are significantly related with fetal biometry and umbilical artery Doppler velocimetry [7].

Zalud *et al.* defined normal placental and spiral artery volume in the second trimester of normal pregnancies using 3D sonography in 2007 [1]. In another study they also defined normal 3-D power Doppler vascular indexes in pregnancies between 14 and 25 weeks of singleton gestation. They found that placental and spiral artery volume blood flow increased with the advancement of gestational age [8].

Clapp *et al.* showed a correlation of placental volume calculated at 14 and 26 weeks of gestation with newborn weight in 40 patients [2]. Thame *et al.* also reported a correlation of placental volume calculated at 14, 17 and 21 weeks of gestation with newborn weight in 561 patients [3]. In our study we measured the placental volume only in the first trimester. Our results can allow us the capability and simplicity to predict newborn weight as early as the first trimester.

Hafner *et al.* have attracted attention to the connection of placental volume calculated serially at 12, 16 and 22 weeks of gestation by revealing relatively smaller placental volume in the 12<sup>th</sup> week of gestation in SGA [4]. Moreover they stated that placental growth between week 12 and 22 is too heterogeneous to justify using this method as a clinical tool, but that it could provide new information on placental physiology underlying unfavorable obstetric outcomes.

In the present study we demonstrated that placental volumes of IUGR fetuses in the period of 11-13 weeks and six days were significantly different and lower compared with placental volumes of AGA, SGA, and LGA babies. In classification of all infants in groups of IUGR and non-IUGR, statistical analysis of placental volumes in both groups consolidated these differences. In the IUGR group, placental volume value did not reach beyond 70  $\text{cm}^3$ . While placental volume decreased, the percentage of IUGR infants increased. Lower bound placental volume of all IUGR infants has approximately a 6-fold higher risk of being IUGR than upper bound placental volume of all IUGR (28% vs 4.8%). By analyzing statistically one by one each infant's birthweight and placental volume with each other, placental volume had a positive linear correlation with birth weight.

Besides volumetric calculations in 3-D US, the feature of power Doppler was another way of obtaining data from tissues three dimensionally. Placental vascularization indexes obtained by 3-D power Doppler were other parameters used in our study. VI, FI and VFI did not show any significant difference when correlated with infant weights classified according to their week of delivery as AGA, SGA, LGA, and IUGR, thus results were not meaningful. When birth weight and placental vascularization indexes were compared, only VI revealed a positive linear correlation with birth weight. Our statistical analyses show parallelism with results of studies in the literature [1, 3, 7-9]. Although these studies show that 3-D Doppler indexes change as pregnancy progresses and are significantly related with fetal biometry, in our study placental FI and VFI showed no correlation with birth weight; VI had a linear and positive relation with birth weight, thus this index can be emphasized for its reproducibility and feasibility [7].

In conclusion, the 3-D placental volumetric calculation technique is an appropriate approach for routine evaluation of the human placenta during early gestation. 3-D power Doppler sonography will especially improve investigation of early placental vascularization and its connection with fetal growth.

These results could be of great importance for the predictive and diagnostic evaluation of fetal growth restriction presenting with placental volume and vascular tree alterations as early as the first trimester.

## References

- [1] Zalud I., Shaha S.: "Placental and spiral arteries volume and gray scale value assessment by three-dimensional ultrasound in the second trimester". *J. Clin. Ultrasound*, 2007, 35, 504.
- [2] Clapp J.F. 3rd, Rizk K.H., Appleby-Wineberg S.K., Crass J.R.: "Second-trimester placental volumes predict birth weight at term". *J. Soc. Gynecol. Investig.*, 1995, 2, 19.
- [3] Thame M., Osmond C., Wilks R., Bennett F.I., Forrester T.E.: "Second-trimester placental volume and infant size at birth". *Obstet. Gynecol.*, 2001, 98, 279.
- [4] Hafner E., Metznerbauer M., Höfner D., Munkel M., Gassner R., Schuchter *et al.*: "Placental growth from the first to the second trimester of pregnancy in SGA-foetuses and pre-eclamptic pregnancies compared to normal foetuses". *Placenta*, 2003, 24, 336.
- [5] Hafner E., Philipp T., Schuchter K., Dillinger-Paller B., Philipp K., Bauer P.: "Second-trimester measurements of placental volume by three-dimensional ultrasound to predict small-for-gestational-age infants". *Ultrasound Obstet. Gynecol.*, 1998, 12, 97.
- [6] Merce L.T., Barco M.J., Bau S.: "Reproducibility of the study of placental vascularization by three-dimensional power Doppler". *J. Perinat. Med.*, 2004, 32, 228.
- [7] Merce L.T., Barco M.J., Bau S., Kupesic S., Kurjak A.: "Assessment of placental vascularization by three-dimensional power Doppler "vascular biopsy" in normal pregnancies". *Croat. Med. J.*, 2005, 46, 765.
- [8] Zalud I., Shaha S.: "Evaluation of the utero-placental circulation by three-dimensional Doppler ultrasound in the second trimester of normal pregnancy". *J. Matern. Fetal. Neonatal Med.*, 2007, 20, 299.

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# Previous cesarean section increases the risk for breech presentation at term pregnancy

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## Summary

**Purpose of investigation:** The aim of the present study was to estimate the risk for breech presentation in women with singleton pregnancies at-term who had had at least one previous cesarean section (CS) versus at least one previous vaginal delivery. **Methods:** Out of 4,269 singleton pregnancies during the study period, 2008 met the inclusion criteria. The history, the number of previous CSs, as well as maternal age, parity, birth weight, gestational age, neonatal sex and placenta previa were used to estimate the risk for breech at term. **Results:** The overall incidence of breech presentation was 3.2%, while 20% of the women had a history of at least one previous CS. The rate of breech presentation at term in singleton pregnancies after CS increased two-fold (5.3%) when compared to those with at least one previous vaginal delivery (2.6%), ( $p = 0.01$ ) [OR 2.08 (95% CI, 1.23-3.52)], while the number of the previous CSs did not correlate with breech presentation ( $p = \text{NS}$ ) [OR 0.86 (95% CI, 0.31-2.4)]. **Conclusion:** According to the present study, women with a history of at least one cesarean delivery have an increased risk for breech presentation in the subsequent singleton pregnancy at-term.

**Key words:** Breech presentation; Elective cesarean section; Abdominal delivery.

## Introduction

The rate of cesarean section (CS) has shown a stable increase over the last three decades. Apart from the obstetrical or iatrogenic indications for cesarean delivery, peroperative and anesthetic improvement, as well as advanced neonatology care contributed to the former event. The recommended indications for abdominal delivery include mainly fetal distress, labor arrest, maternal exhaustion, cephalopelvic disproportion, fetus malpresentation and maternal request [1-4]. Prophylactic (elective) CS because of previous cesarean is an additional contribution to the increased rate of abdominal delivery [2-5].

Fetal malpresentation and especially breech presentation (complete or frank) involve almost 4% of all deliveries at-term [6]. Although there is controversy related to the optimal way of delivery of fetuses in breech, elective CS for this indication has increased dramatically in both the United States and many European countries [7-9]. A recently re-published meta-analysis (2009) based on a Cochrane Database Review of 2003, assessed pregnancy outcomes after planned CS for singleton pregnancies at term [10]. The authors concluded that planned CS offered less neonatal morbidity and mortality compared to planned vaginal delivery.

An increased rate of breech presentation at term in women with previous abdominal delivery has been reported recently [11]. The primary objective of the present study was to estimate the rate of breech presentation in women with at least one previous CS compared to women with a previous vaginal delivery. Second, to recognize other possible risk factors that contribute to breech presentation in singleton pregnancies at-term.

## Material and Methods

The objective of the present study was to evaluate whether a previous CS constitutes a risk factor for breech presentation in singleton pregnancies at term, when compared to a similar group of women with at least one previous vaginal delivery. This was a retrospective study in which the obstetric records of our department were used to identify all the singleton pregnancies at term ( $> 37$  weeks) of women with a history of at least one previous delivery, with breech or cephalic presentation, between January 2004 and December 2007. Out of 4,269 singleton pregnancies, 2008 that met the inclusion criteria were enrolled in the final analysis. Stillbirths or fetuses with congenital malformations were not included in the study. Patients with previous uterine surgery (myomectomy via the abdomen or laparoscopy), uterine malformation or myomas in the present pregnancy were also excluded from the trial.

Demographic characteristics such as maternal age, the number of previous term deliveries, gestational age (weeks and days), neonatal weight, the rate of macrosomic and low birth weight (LBW) neonates were included in the present analysis. Neonates with a birth weight of more than 4,500 g were defined as macrosomic while those with a weight of less than 2,500 g as LBW.

The history of at least one previous CS or vaginal delivery was used to estimate the risk for breech presentation at-term of singleton pregnancies. Similarly, statistical analysis was performed according to the number of previous CSs (1 vs  $> 1$ ). Other factors such as parity (number of previous deliveries), maternal age ( $< 35$  vs  $\geq 35$  years), gestational age ( $\leq 40$  vs  $> 40$  weeks), neonatal sex (male vs female), placenta previa, macrosomic and LBW neonates were used to calculate the risk for breech presentation at term.

## Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science version 14.0 (SPSS Inc., Chicago, IL, USA). Continuous data expressed by mean value  $\pm$  SD (standard deviation). The Student's *t*-test or Mann-Whitney non parametric test were used to compare variables with continuous outcomes of the different groups. The chi-square and Fisher's

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exact test analysis were used for cross-tabulated comparison of the different variables that were used as risk factors for breech presentation according to our methodology. The odds ratio (OR) based on a 95% confidence interval (CI) was also estimated. All *p* values are two-sided and *p* < .05 was considered as statistical significant.

## Results

Among the 2,008 singleton term pregnancies of our cohort that met the inclusion criteria, 64 fetuses (3.2%) were identified with a breech presentation, while 1,944 (96.8%) with a cephalic presentation. In addition, 1,595 (79.4%) women had at least one previous vaginal delivery in their obstetric history, while 413 (20.6%) had at least one previous CS. Demographic characteristics of the women are presented in Table 1. The mean maternal age ( $\pm$  SD) of the women with previous vaginal delivery was  $29 \pm 5.2$  years versus  $30 \pm 5.1$  of those with previous CS (*p* < .0001). However, the rate of the women aged  $\geq 35$  or < 35 years did not significantly differ. The mean gestational week at delivery was significantly higher in women with a previous vaginal delivery ( $39 \pm 1$ ) compared to those with a previous CS ( $38 \pm 0.6$ ). Similarly, the neonatal weight was significantly higher in the group of women with a history of vaginal delivery compared to a previous CS ( $3370 \pm 460$  vs  $3176 \pm 420$ , respectively, *p* < .0001). Macrosomic births were not different between the two study groups, while LBW neonates occurred more often in women with a previous CS (5.1%) compared to those with a previous vaginal delivery (2.5%) (*p* = 0.01).

Breech presentation at term delivery in singleton pregnancies of women with a history of a previous CS (22/413) occurred more often compared to the group of women with a previous vaginal delivery (42/1,595) (5.3% vs 2.6%, respectively) which was statistically significant (*p* = 0.01) and with an OR 2.08 (95% CI, 1.23-3.52) (Table 2). In a sub-analysis of women with a history of a cesarean section, the number of previous cesarean scars (1 vs > 1 previous CS) did not significantly contribute to the breech presentation of singleton pregnancies at term (5.5% vs 4.8%, respectively) [OR 0.86 (95% CI, 0.31-2.4)] (Table 2). The risk factors that contributed to breech presentation at term in singleton pregnancies are shown in Table 3. There was a trend between the number of previous deliveries irrespective of the way (abdominal or vaginal) and breech presentation at term (> 1 previous delivery; 3.9% vs 1 previous delivery; 2.9%, *p* = 0.1). A positive association was noted between increased maternal age and breech presentation ( $\geq 35$  years; 5.4% vs < 35 years; 2.7%, *p* = 0.01). There was no association between neonatal sex or gestational week (> 40 weeks vs  $\leq 40$  weeks) and breech presentation. The incidence of macrosomic births was not different between breech and cephalic presentation (1.6% vs 0.8%, respectively). The same observation was made with regards to LBW infants (3.1% vs 3%, respectively). Finally no association was found between placenta previa and breech presentation.

Table 1. — Demographic characteristics of the women with previous vaginal and cesarean delivery.

Demographic characteristics	Previous vaginal delivery (n = 1,595)	Previous CS (n = 413)	<i>p</i> value
Maternal age (mean $\pm$ SD; years)	$29 \pm 5.2$	$30 \pm 5.1$	< .0001
$\geq 35$ years, n (%)	280 (18)	87 (21)	NS
< 35 years, n (%)	1,315 (82)	326 (79)	
Number of previous term deliveries (mean $\pm$ SD)	$2.4 \pm 0.8$	$2.3 \pm 0.6$	.01
Gestational age (mean $\pm$ SD)			
Weeks	$39 \pm 1$	$38 \pm 0.6$	< .0001
Days	$277 \pm 7.5$	$270 \pm 5$	< .0001
Neonatal weight (mean $\pm$ SD; g)	$3,370 \pm 460$	$3,176 \pm 420$	< .0001
LBW ( $\leq 2,500$ g), n (%)	40 (2.5)	21 (5.1)	.01
Macrosomic (> 4,500 g), n (%)	14 (0.9)	2 (0.5)	NS

CS = cesarean section, LBW = low birth weight, SD = standard deviation.

Table 2. — The rate of breech presentation according to the way of the previous delivery and the number of previous cesarean sections.

	Cephalic presentation (n = 1,944)	Breech presentation (n = 64)	OR (95% CI)	<i>p</i> value
Previous vaginal delivery <sup>o</sup> , n %	1,553 (97.4)	42 (2.6)	1	.01*
Previous CS <sup>o</sup> , n %	391 (94.7)	22 (5.3)	2.08 (1.23-3.52)	
only 1 previous CS	292 (94.5)	17 (5.5)	1	NS*
> 1 previous CS	99 (95.2)	5 (4.8)	0.86 (0.31-2.4)	

<sup>o</sup> at least one, \* *p* value calculated for the subgroups in breech presentation only, NS = non significant, OR = Odds ratio, CI = confidence interval, CS = cesarean section.

Table 3. — Risk factors that contribute to breech presentation at term delivery.

Risk factors	Cephalic presentation (n = 1,944)	Breech presentation (n = 64)	<i>p</i> value
Parity, n %			
1 previous delivery	1356 (97.1)	40 (2.9)	NS*
> 1 previous delivery	588 (96.1)	24 (3.9)	
Maternal age, n (%)			
< 35 years	1,597 (97.3)	44 (2.7)	.01*
$\geq 35$ years	347 (94.6)	20 (5.4)	
Gestational age at term, n (%)			
$\leq 40$ weeks	1,821 (96.7)	62 (3.3)	NS*
> 40 weeks	123 (98.4)	2 (1.6)	
Neonatal weight, n (%)			
LBW ( $\leq 2500$ g)	59 (3)	2 (3.1)	NS
Macrosomic (> 4500 g)	15 (0.8)	1 (1.6)	NS
Neonatal sex			
Male	1,031 (96.7)	35 (3.3)	NS*
Female	913 (96.9)	29 (3.1)	
Placenta previa, n (%)	4 (0.2)	0	NS

\* *p* value calculated for the subgroups in breech presentation only, LBW = low birth weight.

## Discussion

The present study showed that women with a history of CS have an increased risk of breech presentation in a subsequent singleton pregnancy at term, while the number of the previous CSs did not affect the incidence of breech presentation.

To the best of our knowledge there is only one recent publication employed with the risk of breech presenta-

tion at term delivery of a singleton pregnancy in women with a history of CS [11]. The authors observed a 4.56% rate of breech presentation after previous CS compared to 2.09% after vaginal delivery, giving a twofold relative risk (RR) 2.18 (95% CI, 1.98-2.39) and an adjusted OR of 2.12 (95% CI, 1.91-2.36) for breech presentation after previous cesarean delivery. Similarly, in our study there was a double risk for breech presentation in women with previous abdominal delivery compared to those with a previous vaginal delivery. The rate of fetuses in breech with a history of at least one cesarean section was 5.3%, while in women with at least one previous vaginal delivery 2.6% [OR 2.08 (95% CI, 1.23-3.52)]. Further analysis to estimate a possible influence of the number of previous CS (1 vs > 1) showed a similar rate of breech presentation in women with history of one previous CS (5.5%) compared to women with more than one (4.8%) ( $p = \text{NS}$ ). A similar result was shown by others [11].

Further analysis to reveal other risk factors that contribute to breech presentation at term showed a significant correlation only with maternal age ( $\geq 35$  years), which is in accordance with a previous report [11]. However, parity, gestational week, neonatal weight and placenta previa did not have any significant contribution to the breech presentation. Vendittelli *et al.* showed similar results with the present series concerning placenta previa, while using a different base analysis for gestational age (> 39 weeks <) and birth weight (> 3,000 g <) demonstrated a significant association of these with breech presentation.

The optimal way of delivery of a fetus in breech presentation appears to be a planned CS. Two previous randomized studies with a small sample size assessed the optimal way of delivery of fetuses in breech (frank and non frank) and found no significant benefits for the fetus with either method of delivery, planned CS or vaginal delivery [12, 13]. However, subsequent studies demonstrated increased risk for perinatal death and morbidity for fetuses in breech presentation during vaginal delivery, supporting that planned CS may improve perinatal outcomes [7, 14]. Thereafter, a multicenter trial (121 centers, 26 countries) randomly assigned planned CS or planned vaginal delivery in 2,088 singleton pregnancies at term with the fetus in frank or complete breech presentation. (15) In this study, perinatal outcomes (mortality and morbidity) were significantly lower in planned CS compared to planned vaginal delivery. In the same trial, the author also concluded that serious maternal complications were similar between the two delivery options. Two meta-analyses in the Cochrane Database which included three randomized studies by Collea *et al.*, Gimovsky *et al.*, and Hannah *et al.* [12, 13, 15], similarly concluded that planned CS is optimal to planned vaginal delivery as it reduces perinatal or neonatal death and serious neonatal morbidity, despite a somewhat increased rate of maternal complications [10, 16].

Increased rate of CS is associated with a rise in severe obstetric morbidity. Recently, Kuklina *et al.* showed a

trend of severe complications in the United States in a period study between 1998 and 2005 [17]. The rate of at least one severe obstetric complication such as pulmonary embolism, blood transfusion, adult respiratory distress syndrome (ARDS) and renal failure increased from 0.64% at the beginning of the study period (1998) to 0.81% towards the end (2005) [17]. The authors concluded that the increased rate of CS contributed significantly to the former complications. Additionally, abnormal placentation such as placenta previa and accreta is another complication, of low rate, but with significant maternal morbidity and mortality for which a history of previous CS constitutes the main reason [18]. A previous study reported an incidence of placenta accreta of 5% in an unscarred uterus, while in women with history of four cesarean deliveries the same figure climbed to 67% [19]. Furthermore, Miller *et al.* reported an incidence of placenta accreta of 10% in women with a coexisting placenta previa and found a 2.1% incidence of placenta accreta in patients with no history of uterine scar but 38% in women with more than two CSs.

There are certain limitations in our study, with the retrospective nature and the relatively small sample size being the most important ones. However, the present series reflect the experience of a University Hospital, with more than 1,000 deliveries per year. Furthermore it was not in the scope of the present analysis to estimate the optimal way of delivery of fetuses in breech presentation.

The rising of the rate in CS is a worldwide event contributing to the increased rate of maternal morbidity and mortality as well as to serious obstetric complications, although for the benefit of the fetus the decision for abdominal delivery may be warranted [1, 2, 17]. According to the Greek experience during a 24-year period, there was an overall two-fold increase in the rate of cesarean section from 13.8% (1977-1983) to 29.9% (1994-2000). Fetal distress, breech presentation, cephalopelvic disproportion and hypertensive disorders were the main reasons for primary cesarean section, while previous CS was the most common indication with an increase in the rate from 7.7% to 10.9% in the study period [21]. A Norwegian study demonstrated that 65% of cesarean deliveries were emergency operations, mainly due to fetal distress and progress failure, while previous CS and maternal request were the principal indications for elective cesarean section [4]. The era of serious skepticism and possible redefinition of the indications for cesarean delivery has arrived. Physicians must be alert and clear in their decision to perform another abdominal delivery.

In conclusion, according to the results of the present study, women with a history of previous cesarean section have an increased risk for breech presentation at term delivery of singleton pregnancies compared to a previous vaginal delivery. The number of previous CSs is not significantly correlated with the risk of breech at term. Further studies are needed to confirm or reject the current knowledge.

## References

- [1] Porreco R.P., Thorp J.A.: "The caesarean birth epidemic: Trends, causes, and solutions". *Am. J. Obstet. Gynecol.*, 1996, 175, 368.
- [2] Penn Z., Ghaem-Maghami S.: "Indications for caesarean section". *Best Pract. Res. Clin. Obstet. Gynecol.*, 2001, 15, 1.
- [3] Stafford R.S.: "Recent trends in caesarean section use in California". *West J. Med.*, 1990, 153, 511.
- [4] Kolas T., Hofoss D., Daltveit A.K., Nilsen S.T., Henriksen T., Hager R. *et al.*: "Indications for caesarean deliveries in Norway". *Am. J. Obstet. Gynecol.*, 2003, 188, 864.
- [5] Paul R.H., Miller D.A.: "Caesarean birth: how to reduce the rate". *Am. J. Obstet. Gynecol.*, 1995, 172, 1903.
- [6] Hickok D.E., Gordon D.C., Milberg J.A., Williams M.A., Daling J.R.: "The frequency of breech presentation by gestational age at birth: A large population-based study". *Am. J. Obstet. Gynecol.*, 1980, 137, 235.
- [7] Cheng M., Hannah M.: "Breech delivery at term: a critical review of the literature". *Obstet. Gynecol.*, 1993, 82, 605.
- [8] Graves W.K.: "Breech delivery in twenty years of practice". *Am. J. Obstet. Gynecol.*, 1980, 137, 229.
- [9] Thiery M.: "Management of breech delivery". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1987, 24, 93.
- [10] Hofmeyr G.L., Hannah M.E.: "Planned caesarean section for term breech delivery (Cochrane Review) re-published". *Cochrane Database Syst. Rev.*, 2009, (1), CD000166.
- [11] Vendittelli F., Riviere O., Crenn-Hebert C., Alain Rozan M., Maria B., Jacquetin B. and the AUDIPOG sentinel network.: "Is a breech presentation at term more frequent in women with a history of caesarean delivery". *Am. J. Obstet. Gynecol.*, 2008, 198, 521.e1-6.
- [12] Collea J.V., Chein C., Quilligan E.J.: "The randomized management of term frank breech presentation: a study of 208 cases". *Am. J. Obstet. Gynecol.*, 1980, 137, 235.
- [13] Gimovsky M.L., Wallace R.L., Schiffrin B.S., Paul R.H.: "Randomized management of the nonfrank breech presentation at term: a preliminary report". *Am. J. Obstet. Gynecol.*, 1983, 146, 34.
- [14] Gifford D.S., Morton S.C., Fiske M., Kahn K.: "A meta-analysis of infant outcomes after breech delivery". *Obstet. Gynecol.*, 1995, 85, 1047.
- [15] Hannah M.E., Hannah W.J., Hewson S.A., Hodnett E.D., Saigal S., Willian A.R.: "Planned caesarean section versus planned birth for breech presentation at term: a randomized multicenter study. Term Breech Collaborative Group". *Lancet*, 2000, 356, 1375.
- [16] Hofmeyr G.L., Hannah M.E.: "Planned caesarean section for term breech delivery (Cochrane Review)". *Cochrane Database Syst Rev*, 2003, (2), CD000166.
- [17] Kuklina E.V., Meikle S.F., Jamieson D.J., Whiteman M.K., Barfield W.D., Hillis S.D. *et al.*: "Severe obstetric morbidity in the United States: 1998-2005". *Obstet. Gynecol.*, 2009, 113, 293.
- [18] Rosen T.: "Placenta accreta and caesarean scar pregnancy: overlooked costs of the rising caesarean section rate". *Clin. Perinatol.*, 2008, 35, 519.
- [19] Clark S.L., Yeh S.Y., Phelan J.P., Bruce S., Paul R.H.: "Emergency hysterectomy for obstetric hemorrhage". *Obstet. Gynecol.*, 1984, 64, 376.
- [20] Miller S.L., Chollet J.A., Goodwin T.M.: "Clinical risk factors for placenta previa-placenta accreta". *Am. J. Obstet. Gynecol.*, 1997, 177, 210.
- [21] Tampakoudis P., Assimakopoulos E., Grimbizis G., Zafrakas M., Tampakoudis G., Mandalenakis S., Bontis J.: "Caesarean section rates and indications in Greece: data from a 24-year period in a teaching hospital". *Clin. Exp. Obstet. Gynecol.*, 2004, 31, 289.

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# The effect of amniocentesis on preterm delivery rate in women with uterine myoma

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## Summary

**Objectives:** To evaluate the effect of genetic amniocentesis on the preterm delivery rate in women with uterine myoma. **Methods:** The volume of each fibroid and the relation to the placenta, myometrium and uterine corpus were recorded. Amniocentesis was performed by an experienced operator, if indicated. **Results:** During the study 14,579 pregnant women were examined and 234 had complications of uterine myomas (1.61%). Forty-three women delivered prematurely (19.46%). The results revealed that multifocal fibroids in relation to the myometrium, uterine myoma subjacent to the placenta, total myoma volume greater than 150 cm<sup>3</sup> are statistically significant independent risk factors for preterm delivery, while amniocentesis was not found to be an independent risk factor for preterm delivery. **Conclusions:** Although having uterine myoma is a fairly known cause of preterm delivery, second trimester genetic amniocentesis does not seem to have any additional adverse effect on the preterm delivery rate in women with uterine myomas.

**Key words:** Uterine myoma; Amniocentesis; Preterm delivery.

## Introduction

Women of the modern epoch tend to delay pregnancy until after their professional career or modern assisted reproductive technologies permit them to become pregnant even in advanced ages. A large demographic analysis conducted in Turkey and another study by Jacobsson *et al.* demonstrated that maternal age at first pregnancy has been increasing in the last decades [1, 2].

Uterine myoma or uterine fibroid is a frequently seen benign tumor of the female pelvis. Clinically the fibroid may be asymptomatic or may be the source of some gynecological or obstetrical problems. Preterm delivery is still the major cause of neonatal mortality and morbidity, and having myomas is reported to be a risk factor for this condition [3-6]. Uterine fibroids are more frequent after the fourth decade. Borgfelt and Andolf stated that the prevalence of myomas was 3.3% in women aged 25 to 32 years old and 7.8% for women aged 33 to 40 years old [7]. Qidwai *et al.* reported that the prevalence of uterine myoma is 1.4% and 5.6% in women younger and older than 35 years old, respectively [3].

Amniocentesis is a widely performed invasive procedure in prenatal diagnosis all over the world. Advanced maternal age is the most common indication for the amniocentesis, which is also the era when myoma prevalence increases. Positive trisomy screening tests and congenital abnormalities are the other frequent indications for amniocentesis. As the number of women who wish to have a child in advanced maternal age is increasing, the prevalence of pregnancies complicated by uterine myoma with the need for genetic amniocentesis is also rising.

Recently Salvador *et al.* concluded in a retrospective study that the gestational age at delivery was similar in women with uterine myomas who underwent amniocentesis [8].

The aim of this study was to evaluate prospectively the impact of amniocentesis on preterm delivery in pregnancies complicated by uterine myoma from the point of view of number, volume, localization and relation with the placenta.

## Methods

The study was conducted in the prenatal diagnosis unit of Istanbul University, Istanbul Faculty of Medicine between April 1, 2004 and May 31, 2008. All ultrasonographic (US) examinations and invasive procedures were performed by two expert physicians. A second trimester routine sonographic screening was performed on all pregnant women including fetal biometry, amniotic fluid volume assessment, screening for fetal structural abnormalities and placental localization.

US diagnosis of the uterine myoma was performed in the presence of a spherical or ellipsoid mass at least 1 cm in maximal diameter with different echogenicity from the surrounding myometrium. The fibroids were investigated regarding their localization in the uterus and their relation between the placenta and myometrium. The myoma was characterized as *retroplacental* if it was superposed totally or partially to the placenta and was called *away from placenta* if not in touch with the placenta. Fibroids were named *subserous*, *intramural* or *submucous* depending on their relationship to the myometrium. Finally, localization was noted as *corporal* or *lower uterine segment* in relation to the uterine corpus. Three dimensions (sagittal, axial, and transverse) of the myoma were measured in centimeters and the volume was calculated in centimeters cube (cm<sup>3</sup>).

According to previous reports, myoma localization which is less related to the obstetric complication is defined as sub-

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serous, away from placenta, corporal, smaller and single [6, 9]. These localizations and characteristics were accepted to be less related with obstetric complications.

Amniocentesis was performed (if necessary), between the 16<sup>th</sup> and 20<sup>th</sup> gestational weeks under continuous sonographic guidance using the free-hand technique with a 20-gauge needle for amniocentesis. Anesthesia, antibiotic prophylaxis or tocolytics were not used in any patient. Transplacental passage was avoided when possible and was also recorded if it occurred.

Exclusion criteria from the study included any fetal structural abnormality, chromosomal abnormality, polyhydramnios, oligohydramnios, isoimmunization, spontaneous abortion and multiple pregnancies. History of abortion in recent pregnancies, threatened abortion in the present pregnancy, maternal age, parity, gestation age at examination, gestation age at delivery and birthweight were recorded. Preterm delivery was defined as a birth occurring between the 24<sup>th</sup> and 37<sup>th</sup> week of gestation. Premature rupture of membranes was defined as the absence of contractions one hour after rupture of the membranes regardless of gestational age.

For each continuous variable, normality was checked. Comparisons were applied using the Student's *t*-test for data normally distributed. If the data was not distributed normally, an appropriate non-parametric test was chosen such as the Mann-Whitney U test. A logistic regression model was constructed to assess the independent factors on preterm delivery. We included variables with probability values < 0.1 in univariate analysis in the model as confounders. The results are presented as prevalence of odd ratios and their 95% confidence intervals. Statistical analyses of the groups were performed using the Statistical Package for the Social Sciences (SPSS) for Windows 15.0. Results are presented as mean  $\pm$  SD and median (min-max).

This study was approved by the ethics committee of Istanbul University, Istanbul Faculty of Medicine.

## Results

During the study period 14,579 pregnant women were examined and 234 had uterine myomas (1.6%). One hundred and thirty-five women had single (57.7%), 36 had two (15.38%), 27 had three (11.5%) and 36 had at least four (15.4%) uterine myomas and the mean total volume was  $392.96 \pm 812.86$ ;  $606.34 \pm 1399.51$ ;  $790.34 \pm 1494.56$  and  $1214.46 \pm 1574.40$ , respectively. Total myoma volume was < 150 cm<sup>3</sup> in 117 women (50%), 150-300 cm<sup>3</sup> in 33 women (14.10%) and > 300 cm<sup>3</sup> in 84 women (35.90%). In 151 of the women none of the fibroids were in contact with the placenta (64.53%), whereas at least one of the rest was in touch with placenta.

Five pregnancies were terminated because karyotype analysis was revealed as abnormal. Eight patients (three had amniocentesis and five did not) who had spontaneous abortions before the 24<sup>th</sup> week of gestation were also excluded from the study. One hundred and twenty-five (56.6%) of the remaining 221 had amniocentesis, whereas 96 (43.4%) did not. The most common indication for genetic amniocentesis was maternal age. Only 21 women who had amniocentesis were age 34 or younger (16.8%). During the amniocentesis placing the amniocentesis needle through the myoma or placenta was avoided. While no patient had transmyomatic amniocentesis, nine

Table 1. — Patient demographics.

	Preterm delivery (n = 43)	Term delivery (n = 178)	Significance
	Mean $\pm$ (SD) Med (min-max)	Mean $\pm$ (SD) Med (min-max)	
Maternal age	34.00 $\pm$ 4.80	35.30 $\pm$ 4.00	0.12
Gestational age at examination	34 (23-43)	36 (24-44)	0.09
Parity	19.40 $\pm$ 4.20 18 (10-30)	18.70 $\pm$ 2.80 18 (12-29)	0.25
Number of previous abortions	0.50 $\pm$ 0.80 0 (0-4)	0.60 $\pm$ 0.80 0 (0-5)	0.18
	0.90 $\pm$ 1.80 0 (0-9)	0.80 $\pm$ 1.20 0 (0-10)	

had transplacental amniocentesis. The mean gestational age at delivery for this small subgroup was  $37.22 \pm 1.92$ .

Only one patient of the 221 women presented with placental abruption at the 36<sup>th</sup> week of gestation. She did not undergo amniocentesis and two of her four uterine myomas were subjacent to the placenta. She delivered a 2,650 g healthy boy by cesarean section.

The patients were examined to determine preterm delivery. Forty-three of the 221 women delivered prematurely (19.5%). Demographic features of the women are shown in the Table 1. Maternal age, gestational age at the time of examination, parity, and previous history of abortion were all similar for the preterm and term groups.

Tables 2 and 3 show the comparisons of distribution of the fibroids from the point of view of number and volume, respectively, between the term and preterm groups. As all myomas were classified according to the relationship with the myometrium, the uterine corpus, and placenta each condition was evaluated. There were not any significant differences between the distributions

Table 2. — Comparison of preterm and term deliveries from the point of view of the number of myomas.

	Preterm delivery Mean $\pm$ (SD) Med (min-max) n	Term delivery Mean $\pm$ (SD) Med (min-max) n	p
<i>Distribution of the fibroids in relation to the uterine wall</i>			
– Submucous	1.00 $\pm$ 0.00 1.00 (1.00-1.00) 1	1.10 $\pm$ 0.40 1.00 (1-2) 8	0.3
– Subserous	1.10 $\pm$ 0.40 1 (1-2) 16	1.50 $\pm$ 0.80 1 (1-4) 66	0.2
– Intramural	1.90 $\pm$ 1.60 1 (1-9) 40	2.10 $\pm$ 2.00 1 (1-12) 133	0.2
<i>Distribution of the fibroids in relation to the uterine body</i>			
– Lower uterine segment	1.70 $\pm$ 1.40 1 (1-6) 14	1.70 $\pm$ 1.80 1 (1-12) 60	0.7
– Corporal	1.60 $\pm$ 0.90 1 (1-4) 43	2.00 $\pm$ 1.70 1 (1-10) 147	0.02
<i>Distribution of the fibroids in relation to the placenta</i>			
– Retroplacental	1.20 $\pm$ 0.50 1 (1-3) 26	1.30 $\pm$ 0.60 1 (1-3) 58	0.1
– Away from the placenta	1.80 $\pm$ 1.50 1 (1-9) 31	2.20 $\pm$ 2.00 1 (1-12) 149	0.1

Table 3. — Comparison of preterm and term deliveries in relation to myoma volume.

	Preterm delivery Mean $\pm$ (SD) Med (min-max) n	Term delivery Mean $\pm$ (SD) Med (min-max) n	p
<i>Distribution of fibroids in relation to the uterine wall</i>			
– Submucous	325.01 $\pm$ 0.00 325.10 (325.10-325.10) 1	358.95 $\pm$ 636.37 132.91 (8.58-1903.18) 8	0.3
– Subserous	588.28 $\pm$ 629.23 334.68 (32.00-2142.00) 16	1006.96 $\pm$ 1527.09 380.58 (3.54-7047.94) 66	0.5
– Intramural	480.57 $\pm$ 1071.06 180.41 (8.40-6541.04) 40	288.03 $\pm$ 539.42 97.04 (5.15-2966.40) 133	
<i>Distribution of fibroids in relation to the uterine body</i>			
– Lower uterine segment	482.33 $\pm$ 541.69 218.82 (21.50-1766.40) 14	474.30 $\pm$ 727.42 140.87 (6.29-3091.20) 60	0.5
– Corporal	489.10 $\pm$ 1039.81 217.60 (8.40-6541.04) 43	564.68 $\pm$ 1165.61 120.64 (3.54-7047.94) 147	0.5
<i>Distribution of fibroids in relation to the placenta</i>			
– Retroplacental	660.54 $\pm$ 1181.34 229.18 (12.54-5706.08) 26	575.4 $\pm$ 1114.3 135.8 (9.98-5734.2) 58	0.3
– Away from the placenta	305.0 $\pm$ 376.8 169.8 (8.40-1581.2) 31	535.9 $\pm$ 1021.7 135.1 (3.54-7047.9) 149	0.1

Table 4. — Multiple regression analysis of the preterm deliveries.

	$\beta$	Odds Ratio (OR)	CI (95.0%)	Significance
Amniocentesis	-0.44	0.64	0.26-1.58	0.33
Age > 35	0.34	1.41	0.55-3.64	0.48
Myoma localization in relation to the myometrium				
– Subserous	Ref			0.01
– Intramural	0.78	2.17	0.76-6.19	0.15
– Submucous	-0.60	0.55	0.03-9.62	0.68
– Multifocal	-1.94	0.14	0.02-0.89	0.04
Myoma localization in relation to the uterus				
– Corpus	Ref			0.93
– Lower uterine segment	-0.10	0.91	0.31-2.64	0.86
– Both	0.25	1.28	0.28-5.84	0.75
Myoma localization in relation to the placenta				
– Away from the placenta	Ref			0.03
– Retroplacental	0.99	2.70	1.09-6.71	0.03
– Both	1.33	3.79	1.06-14.95	0.05
Myoma number				
– 1	Ref			0.58
– 2	0.57	1.76	0.26-11.78	0.56
– 3	0.16	1.18	0.19-7.42	0.86
– $\geq$ 4	1.08	2.95	0.47-18.52	0.25
Myoma volume (cm <sup>3</sup> )				
– < 150	Ref			0.03
– 150-300	1.42	4.12	1.29-13.17	0.02
– > 300	1.03	2.80	1.09-7.20	0.03
Threatened abortion	0.78	2.20	0.63-7.57	0.22

$\beta$ : Beta coefficients.

for each variable, except one. Only number of myomas located in the uterine corpus was significantly lower in the preterm group compared to the term group.

The multiple regression analysis of the preterm deliveries is shown in Table 4. According to this analysis having multifocal fibroids in relation to the myometrium, having at least one uterine myoma subjacent to the placenta, and having a total myoma volume between 150

cm<sup>3</sup> and 300 cm<sup>3</sup> and > 300 cm<sup>3</sup> are risk factors for preterm delivery. Nevertheless, performing amniocentesis was not an independent risk factor for preterm delivery in women with uterine myomas (OR: 0,64). Maternal age greater than 35, the presence of threatened abortion in the present pregnancy, presence of submucous or intramural fibroids, myoma localization in relation to the uterus, and having multiple uterine myomas were the other parameters which did not increase the risk for preterm delivery.

## Discussion

Today, women have the tendency to postpone child-bearing until after their professional carriers or until new assisted reproductive technologies give them the chance of pregnancy even in advanced ages. Additionally, in the last three decades ultrasound has become an essential tool of obstetric units which allows diagnosing even asymptomatic or small myomas. Uterine fibroid is a benign tumor of the female pelvis. It may be asymptomatic or may be the cause of infertility, early pregnancy loss, premature delivery, obstructed delivery or postpartum bleeding. Its incidence rises with age. Sonographic studies report the incidence of uterine myoma throughout pregnancy as between 0.3% and 3.3% [7, 8, 10, 11]. The consequence of this situation is confronting more pregnancies complicated with uterine myomas today. In this report the incidence of uterine myomas detected sonographically during pregnancy revealed 1.61%. This finding is comparable to previous retrospective studies.

The effect of uterine myoma on pregnancy outcome has been investigated by many authors. Muram *et al.* reported that retroplacental myoma is a risk factor for several complications [11]. While Rice *et al.* [12] and Exacoustòs and Rosati [9] stated that a fibroid subjacent to the placenta is a risk factor for ablation placenta. They could not show that it increases the risk of preterm delivery. On the other hand, two other studies showed that the localization of the myoma in relation to the placenta had no sway on pregnancy outcome [6, 13]. However our study revealed that if a woman with a myoma or myomas all subjacent to the placenta or with multiple myomas with at least one subjacent to the placenta, had a significantly increased risk of preterm delivery. The OR was 2.70 and 3.79, respectively (95% CI; 1.09-6.71;  $p < 0.05$  and 95% CI; 1.06-14.95;  $p < 0.05$ , respectively).

Recent studies evaluated the size of the myoma by measuring the diameter or volume. Total myoma volume was evaluated by Exacoustòs and Rosati and they found that total myoma volume greater than 200 cm<sup>3</sup> is a risk factor for placental abruption. Nonetheless, they found that the presence of uterine myomas did not affect the preterm delivery rate [9]. Rice *et al.* also evaluated the size of uterine myomas regarding the largest diameter. They concluded that as the myoma becomes larger, the rate of complications rises concordantly [12]. In contrast, in two recent studies the authors did not find any associ-

ation between myoma size and preterm delivery [3, 13]. Our results revealed that total myoma volume between 150 and 300 cm<sup>3</sup> and > 300 cm<sup>3</sup> is associated with increased risk of prematurity. The OR was 4.12 and 2.80, respectively (95% CI; 1.29-13.17;  $p < 0.05$  and 95% CI; 1.09-7.30;  $p < 0.05$ ).

Exacoustòs and Rosati stated that women with multiple myomas have an increased risk for abortion, but they did not investigate the effect of multiple myomas on preterm delivery [9]. In another study, having multiple myomas was found to be a risk factor for malpresentation, placental retention and premature uterine contractions [6]. Conversely, in other studies the investigators could not demonstrate any relationship between the number of myomas and preterm delivery [3, 13]. Our results underlined that the number of uterine myomas did not increase the risk of preterm delivery. While the OR for having two, three or more uterine myomas is 1.76; 1.18 and 2.95 correspondingly, it did not reach significance level (95% CI; 0.26-11.78;  $p = 0.56$ , 95% CI; 0.19-7.42;  $p = 0.86$  and 95% CI; 0.47-18.52;  $p = 0.25$ ).

Like uterine myomas, the risk of chromosomal abnormality and the need of second trimester genetic amniocentesis also rise with age. In our study group 104 of the women who had amniocentesis were aged 35 or older (83.2%). This study did not demonstrate an increased preterm delivery rate in pregnancies complicated by uterine myomas when performing second trimester genetic amniocentesis (OR: 0.64; 95% CI; 0.26-1.58;  $p = 0.33$ ). Previously Salvador *et al.* evaluated the effect of amniocentesis in pregnant women with uterine myomas in a retrospective study. They composed three groups of women who had uterine myomas and underwent amniocentesis (*cases*), women who did not have uterine myomas and underwent amniocentesis (*amnio only*) and women who had uterine myomas and did not undergo amniocentesis (*myoma only*). They reported that the mean gestational age at delivery was  $37.6 \pm 5.1$  and  $37.1 \pm 5.3$  in the group of cases and myoma only respectively, which were not statistically significant [8].

While it should be avoided, in nine women an anterior placenta obliged the operator to perform the procedure transplacentally. However it is difficult to draw a conclusion from this small subgroup, but we should note that the mean gestational age was  $37.22 \pm 1.92$ . This finding is appropriate to the report of Bombard *et al.* They underlined that transplacental amniocentesis should be done by an experienced operator, far away from the cord insertion [14].

In this report we aimed to evaluate prospectively the effect of amniocentesis on the preterm delivery in women with uterine myomas. With the increasing age of the obstetric population, uterine fibroids will be more frequently detected during pregnancy. While there is a wide discrepancy in the results of the studies evaluating the effect of myoma through the pregnancy, the authors conclude that performing second trimester amniocentesis is not a risk factor for preterm delivery in women with uterine myomas.

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## References

- [1] Koc I., Ozdemir E.: "Fertility". In: Hacettepe University Institute of Population Studies, Turkey Demographic and Health Survey, 2003. Hacettepe University Institute of Population Studies, Ministry of Health General Directorate of Mother and Child Health and Family Planning, State Planning Organization and European Union. Ankara, Turkey. 2003, 4, 55. Available from <http://www.hips.hacettepe.edu.tr/tsna2003/data/English/chapter04.pdf> [Accessed February 13, 2009].
- [2] Jacobsson B., Ladfors L., Milsom I.: "Advanced maternal age and adverse perinatal outcome". *Obstet. Gynecol.*, 2004, 104, 727.
- [3] Qidwai G.I., Caughey A.B., Jacoby A.F.: "Obstetric outcomes in women with sonographically identified uterine leiomyomata". *Obstet. Gynecol.*, 2006, 107, 376.
- [4] Koike T., Minakami H., Kosuge S., Usui R., Matsubara S., Izuni A. *et al.*: "Uterine leiomyoma in pregnancy: its influence on obstetric performance". *J. Obstet. Gynecol. Res.*, 1999, 25, 309.
- [5] Rosati P., Exacoustòs C., Mancuso S.: "Longitudinal evaluation of uterine myoma growth during pregnancy: a sonographic study". *J. Ultrasound Med.*, 1992, 11, 511.
- [6] Lev-Toaff A., Coleman B.G., Arger P.H., Mintz M.C., Arenson R.L., Toaff M.E.: "Leiomyomas in pregnancy: Sonographic study". *Radiology*, 1987, 164, 375.
- [7] Borgfeldt C., Andolf E.: "Transvaginal ultrasonographic findings in the uterus and endometrium: low prevalence of leiomyoma in a random sample of women age 25-40 years". *Acta Obstet. Gynecol. Scand.*, 2000, 79, 202.
- [8] Salvador E., Bienstock J., Blakemore K.J., Pressman E.: "Leiomyomata uteri, genetic amniocentesis, and the risk of second-trimester spontaneous abortion". *Am. J. Obstet. Gynecol.*, 2002, 186, 913.
- [9] Exacoustòs C., Rosati P.: "Ultrasound diagnosis of uterine myomas and complications in pregnancy". *Obstet. Gynecol.*, 1993, 82, 97.
- [10] Katz V.L., Dotters D.J., Droegemueller W.: "Complications of uterine leiomyomas in pregnancy". *Obstet. Gynecol.*, 1989, 73, 593.
- [11] Muram D., Gillieson M., Walters J.H.: "Myomas of uterus in pregnancy: Ultrasonographic follow-up". *Am. J. Obstet. Gynecol.*, 1980, 138, 16.
- [12] Rice J.P., Kay H.H., Mahony B.S.: "The clinical significance of uterine leiomyomas in pregnancy". *Am. J. Obstet. Gynecol.*, 1989, 160, 1212.
- [13] Davis J.L., Ray-Mazumder S., Hobel C.J., Baley K., Sassoon D.: "Uterine leiomyomas in pregnancy: A prospective study". *Obstet. Gynecol.*, 1990, 75, 41.
- [14] Bombard A.T., Powers J.F., Carter S., Schwartz A., Nitowsky H.M.: "Procedure-related fetal losses in transplacental versus nontransplacental genetic amniocentesis". *Am. J. Obstet. Gynecol.*, 1995, 172, 868.

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# Prenatal diagnoses of cytomegalovirus (CMV), rubella, toxoplasmosis, varicella, parvovirus, herpes simplex and syphilis. The Lagos programme experience

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## Summary

Prenatal diagnosis of infectious diseases has been shown to be indispensable to confirm or exclude in utero infections due to cytomegalovirus, rubella, toxoplasmosis, varicella, parvovirus and herpes simplex, and a multidisciplinary approach is needed. Our report is on data obtained from 236 pregnant women at risk for the above-mentioned conditions. The specific IgM test suggested seroconversion in only 198 of these patients and 162 of them requested prenatal diagnoses by means of fetal blood sampling or amniocentesis, or both. The results are encouraging but more work is required to optimize our diagnostic approach, i.e., monoclonal antibodies and DNA probes with direct identification by means of chorionic villi sampling, which we use for prenatal diagnoses of hemoglobinopathy (DNA-genetic).

*Key words:* Prenatal diagnosis; Cytomegalovirus; Rubella; Toxoplasmosis; Varicella; Parvovirus; Herpes simplex II; Syphilis.

## Introduction

The microorganisms most frequently responsible for congenital infertility are rubella virus, cytomegalovirus, and toxoplasmosis gondii whereas hepatitis B, type II herpes virus, parvovirus, varicella and HIV1&2 cause prenatal infections based on the characteristics of transmission [1]. For cytomegalovirus (CMV) the transmission to the fetus may occur with the primary infection but may also result from reaction of a latent infection, and for rubella and toxoplasmosis the involvement of the fetus occurs only in primary maternal infections [2-5]. It should be noted that in utero infection during reinfection, unlike in cases of primary infection, rarely leads to lesions at birth, where the fetus in the case of a formally seropositive mother for CMV is considered "not to be at risk" of pathological lesions related to these infections. As a result, while the findings – prior to pregnancy to toxoplasmosis gondii and rubella virus specific antibodies – appear to suggest that fetal infections are no longer possible (or highly improbable). The findings of maternal CMV antibodies prior to pregnancy do not exclude the likelihood of reactivation with possible fetal infection, which is detectable only through isolation of the virus from the neonatal urine.

In fact, as far as CMV is concerned, prenatal diagnoses are considerably more complex than those seen in rubella and toxoplasmosis. In the case of the latter infection, the finding of specific IgM in the mother during pregnancy may safely be taken as evidence of primary infection.

In such cases, the serological investigation may be extended to include fetal blood sampling by means of fetoscopy or free needle aspiration (cordocentesis). Determination of specific IgM in fetal serum is of criti-

cal importance not only on account of the fact that maternal IgM do not cross the placenta but also because the low titre may be there for several months (up to 25 weeks) after the onset of infection [5-13]. Our present report is based on our experience of prenatal diagnoses of infectious disease at the Prenatal Diagnosis and Therapy centre, College of Medicine University of Lagos.

## Material and Method

Between December 1994 and December 2004 a total of 236 pregnant women were referred to our centre for confirmation by serological diagnoses of a possible rubella virus, cytomegalovirus, and herpes simplex infections in pregnancy (rubella, CMV, toxoplasmosis, varicella, and herpes simplex type II, parvovirus and syphilis).

Serological diagnosis in all women was carried out using the immunoenzymatic method ELISA for IgG and IgM antibodies and immunofluorescence in those with positive results. Serum titering for specific IgM was always preceded by serum absorption with IgG carrying latex particles to eliminate the rheumatic factor.

In patients in which the findings of specific IgM indicated the possibility of seroconversion occurring in pregnancy, serological investigations were extended to the fetus with fetal blood sampling, amniotic fluid sampling and sometimes fetal urine. Prior to antibody titering in fetal samples, fetal IgM was separated on columns to eliminate any possible interference in the diagnostic assay of the IgG derived mostly from the mother.

## Results

Out of the 236 patients investigated the specific IgM test suggested seroconversion in 162 cases. Seventy of these patients requested prenatal diagnoses by means of fetal blood samples or amniocentesis or both.

There were 21 positive fetal blood IgM samples out of 48 for rubella, 38 positive fetal blood IgM out of 64 for CMV, one positive fetal blood IgM out of six for herpes

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simplex type II, three positive fetal blood IgM out of six for toxoplasmosis, four positive fetal blood IgM out of 12 for varicella, and two positive fetal blood IgM out of 21 for syphilis and one fetal blood IgM out of five for parvovirus. Amniotic fluid IgM was also positive for all those with fetal blood IgM positivity samples (Table 1).

Table 1. — Prenatal diagnosis of rubella, cytomegalovirus (CMV), toxoplasmosis, varicella, herpes simplex II, syphilis and parvovirus ( $n = 236$ ).

	Rubella	CMV	Toxoplasmosis	Varicella	Herpes simplex II	Syphilis	Parvovirus
Total female patients	63	91	18	29	8	21	6
Maternal IgM (+)	48	64	6	12	6	21	5
Fetal IgM (+)	21	38	3	4	1	2	1

## Discussion

Our results of the 236 pregnant women investigated to rule out involvement of rubella virus, CMV, varicella virus, herpes simplex type II, toxoplasma gondii, syphilis and parvovirus infections revealed that only 68.64% had a recently acquired maternal infections thus confirming the reports of others [14, 15]. In this report, in cases where infections were demonstrated in pregnant women, further investigations of fetal blood samples and amniotic fluid samples were carried out. It was possible to demonstrate fetal infection in 70 (29.7%) cases and thus allowing continuation of pregnancy in the rest which led to normal condition of the neonates at delivery thus supporting our preliminary results and those of other authors [8, 10-13, 18-21].

Our results show that there is a high occurrence of congenital rubella, CMV, toxoplasmosis, Varicella zoster, Herpes simplex, syphilis and parvovirus in our environment, and antenatal screening programme should be introduced and encouraged.

Our results are encouraging but more work is required to optimize our diagnostic approach, i.e. monoclonal antibodies and DNA/RNA with direct identification by means of cordocentesis, amniocentesis and chorionic villi sampling to rule out possible placentitis involving the pathogens [7, 8].

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## References

- [1] Alford C.A. Pass R.F.: "Epidemiology of chronic congenital and perinatal infection of man". *Clin. Perinatal.*, 1981, 8, 397.
- [2] Remington J.O., McLeod R., Desmonts G.: "Toxoplasmosis". In: Remington J.S., Klein J.O. (eds.). *Infectious Diseases of the Fetus and Newborn Infants*. Philadelphia W.B., Saunders 1995, 140.

- [3] Peckham C., Logan S.: "Screening for toxoplasmosis during pregnancy". *Arch. Dis. Child.*, 1993, 68, 3.
- [4] Lequien P., Thieuleux M.: "The cytomegalovirus in perinatal medicine". *Presse Med.*, 1985, 14, 2187.
- [5] Cederqvist L.L., Abdel-Latif N., Meyer J., Doctor L.: "Fetal and maternal humoral immune response to cytomegalovirus infection". *Obstet. Gynaecol.*, 1986, 67, 214.
- [6] Schluter W.W., Reef S.E., Redd S.C., Dykewicz C.A.: "Changing epidemiology of congenital rubella syndrome in the United States". *J. Infect. Dis.*, 1998 178, 636.
- [7] Rodeck C.H., Nicolaides K.H.: "The use of fetoscopy for prenatal diagnosis and treatment". *Semin. Perinatal.*, 1983, 7, 118.
- [8] Holzgreve W., Miny P.: "Chorionic villi sampling with an echogenic catheter: experiences of the first 500 cases". *J. Perinat. Med.*, 1987, 15, 244.
- [9] Holzgreve W., Miny P.: "Chorionzotendiagnostik". Weinheim, VCH Publishers, 1987.
- [10] Morgan-Capner P., Rodeck C.H., Nicolaides K.H., Craddock-Watson J.E.: "Prenatal detection of rubella-specific IgM in fetal sera". *Prenat. Diag.*, 1985, 5, 21.
- [11] Nicolaides K.H.: "Cordocentesis". *Clin. Obstet. Gynaecol.*, 1988, 31, 123.
- [12] Isada N.B., Berry S.M.: "In utero diagnosis of congenital infection". In: Gonik B (ed.) *Viral Diseases in Pregnancy*. New York, Springer-Verlag, 1994, 34.
- [13] Berry S.M.: "Cordocentesis". In: Evans M.I., Johnson M.P. Moghissi K.B. (eds.). *Invasive outpatient proceeding in reproductive medicine*, Philadelphia, Lippincott-Raven, 1997, 35.
- [14] Cesbron J.Y., Capron A., Ovlaque G., Santoro F.: "Use of a monoclonal antibody in a durable-sandwich ELISA for detection of IgM antibodies to toxoplasmosis gondii major surface protein". *J. Immun. Method.*, 1985, 83, 151.
- [15] Remington J.S., Araujo F.G., Desmonts G.: "Recognition of different toxoplasmosis antigens by IgM and IgG antibodies in mothers and their congenital infected newborn". *J. Infec. Dis.*, 1985, 152, 1020.
- [16] Kravetz J.D., Federman D.G.: "Toxoplasmosis in pregnancy". *Amer. J. Med.*, 2005, 118, 212.
- [17] Volpi A., LaTorre R.: "Malattie infettive in gravidanza: valutazione costo-beneficio di uno screening di massa". *Atti del 64° congresso SIGO*. Monduzzi Ed. 1986, T2 1503.
- [18] Ajayi G.O.: "Prenatal diagnosis and therapy". In: Okonofua F., Odunsi K. (eds.). *Comparative Obstetrics and Gynaecology for Developing Countries*. WHARC, 2003, 387.
- [19] Ajayi G.: "Prenatal diagnosis of inherited diseases and therapy". In: Agboola A. (ed.). *Textbook of Obstetrics and Gynaecology for Medical Students*, 2<sup>nd</sup> edition. Ibadan, Heinemann Educational Books, 2005, 388.
- [20] Terry G.M., Ho-terry L., Warren R.C., Rodeck C.H., Cohen A., Rees K.R.: "First trimester prenatal diagnosis of congenital rubella: a laboratory investigation". *Br. Med. J.*, 1986, 292, 930.
- [21] Ajayi G.: "Maternal infection in pregnancy". In: Agboola A. (ed.). *Textbook of Obstetrics and Gynaecology for Medical Students*, 2<sup>nd</sup> edition. Ibadan, Heinemann Educational Books, 2005, 309.

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# The relationship between cardiac adaptation to uteroplacental Doppler flow and perinatal outcome in pregnant women with diabetes

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## Summary

Diabetes is a metabolic disorder that complicates pregnancy. Early detection of patients at risk of developing complications is particularly important. Failure of normal cardiovascular adaptation that takes place in pregnancy has been associated with poor perinatal outcome in preeclamptic patients. The aim of this study was to investigate if complications were higher in diabetic patients with cardiac maladaptation. Fetal, uteroplacental Doppler and echocardiographic examinations were performed once in the second and third trimesters in diabetic and healthy pregnant patients. Physiological cardiac hypertrophy was apparent in healthy patients. This, although within normal limits, was less prominent in patients with diabetes. The majority of patients were found to have normal Doppler waveforms. The abnormal uteroplacental flow group consisted almost entirely of patients with pregestational diabetes, especially type I diabetes. Neonatal complications were most common in this group. No relationship was found between echocardiographic findings, Doppler waveforms and poor perinatal outcome.

*Key words:* Doppler; Echocardiography; Diabetes; Pregnancy.

## Introduction

The incidence of diabetes, the most common metabolic disorder in pregnancy, is 1-4% [1]. Gestational diabetes constitutes 90% of diabetes cases [2]. Diabetes during pregnancy complicates pregnancy. Even if the glucose intolerance resolves after pregnancy, these women and their offspring are at risk of developing diabetes later on [3, 4]. Therefore, pregnancy can be considered as an opportunity to identify these women and treat them before any vascular damage occurs. Early identification of diabetic pregnancies reduces maternal and fetal mortality and morbidity. The incidence of the resultant congenital anomalies, macrosomia and birth trauma decrease with good glycemic control [5].

Diabetes affects the placenta and the fetus as well as the eye, heart and kidney. Intrauterine growth restriction (IUGR) as a result of uteroplacental insufficiency and preeclampsia is more common in diabetic pregnancies [6]. With the use of Doppler flow in fetal surveillance, fetal morbidity can be reduced.

With advancing gestational age, trophoblasts invade the spiral arteries in the decidua and myometrium, and uteroplacental resistance decreases to oxygenize and nourish the baby [7]. If the trophoblastic invasion is not complete such as in preeclampsia and IUGR, uteroplacental flow decreases and is associated with poor perinatal outcome [2, 7]. In diabetic patients, particularly in those with vascular involvement, preeclampsia and IUGR are more common [8]. Assessment of diastolic function by echocardiography has proven useful in identification of patients who show cardiac adaptation and in understand-

ing the underlying mechanism in patients with abnormal uterine artery waveforms [9]. A study revealed that patients with abnormal uterine waveforms and cardiac maladaptation more frequently had pregnancy-related complications [10]. Another study using echocardiography in pregnant patients with type I diabetes detected cardiac maladaptation in these patients [11]. In this study we aimed to investigate the effect of cardiovascular adaptation in diabetic patients on uteroplacental flow and fetal outcome.

## Material and Methods

In this prospective controlled study, patients with diabetes, including gestational and pre-gestational diabetes, and healthy pregnant women whose 50 g glucose tolerance test at 24 weeks was found to be normal were recruited over a one and a half year period. The study was carried out at Hacettepe University Medical Faculty Obstetrics and Gynecology Department. Patients who smoked, had chronic diseases other than diabetes, were on medications other than insulin and vitamins, had multiple pregnancies, fetuses with known or suspected chromosomal and structural anomalies and intrauterine infection were excluded from the study. Written informed consent was obtained from all the patients and the study was approved by the local ethics committee.

In all cases gestational age was verified in the first trimester by crown-rump-length (CRL) measurements. Diabetic and healthy patients had two scans and echocardiography in the second and third trimesters. During the scan, the bilateral uterine artery, umbilical, and middle cerebral artery, and ductus venous flow were assessed. All scans were performed by one trained operator using GE Dasonics 2. The Doppler flows were obtained when the fetus was not active and did not breathe. Every fetal vessel was sampled three times; the insonation angle was below 30° and five successive waveforms were obtained from each vessel. The patient had echocardiography on the

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Table 1. — Demographic characteristics of the patients.

	Diabetes (n = 20)	Control (n = 25)	p value
Maternal age	33.2 ± 4.18	34 ± 4.24	NS
Maternal weight (kg)	71.6 ± 7.4	69.4 ± 6.9	NS
Maternal height (cm)	163 ± 7.8	161 ± 6.4	NS
Delivery week	37.2 ± 2.25	38.6 ± 1.52	NS
Birth weight	3141 ± 617	3307 ± 74	NS

NS: not significant.

Table 2. — Hemodynamic variables in the two groups.

	Diabetes (n = 20)	Control (n = 25)	p value
Pulse	91 ± 11	89 ± 13	NS
MAP (mmHg)	92.4 ± 11.7	89.9 ± 8.6	NS
CO (lt)	5.33 ± 1.42	6.31 ± 1.10	NS
SV (ml)	62.2 ± 15.9	77.4 ± 13.4	0.01
TVR (Dyn/sn/cm <sup>5</sup> )	1488 ± 669	1218 ± 326	NS

same day after a period of 20 min to avoid any possible influence of the gravid uterus on circulation. Echocardiography was done by one skilled operator with Flex Scan T57S GEVINGMED, USA. Left ventricle end-diastolic diameter, left ventricle end-systolic diameter, left ventricle end-diastolic wall thickness, end-diastolic septum thickness, fractional shortening, ejection fraction, left atrial diameter and transmitral flow pattern (E/A ratio) were assessed according to the American Society of Echocardiography criteria. Blood pressure and pulse were recorded. After delivery, cord blood was examined and birth weight and Apgar score were recorded. The babies were followed-up until discharge from the hospital. Intensive care requirement, respiratory distress syndrome, intraventricular hemorrhage and necrotizing enterocolitis cases were recorded.

#### Statistical analysis

The Student's *t*-test was used to determine distribution of the patients by age. The independent two-sample *t*-test was used to determine differences between the groups, and the dependent *t*-test for paired samples was used to evaluate the differences in variables within a group. End-diastolic wall thickness did not show any parametric distribution. The Mann-Whitney U test was used to assess intragroup differences and Wilcoxon's signed rank test was used to assess intergroup differences.

## Results

There were 20 patients in the study group and 25 patients in the control group. In the study group, 16 patients (80%) had gestational diabetes, two (10%) had type I diabetes and two (10%) had type II diabetes. Demographic characteristics were similar in both groups (Table 1).

Pulse, mean arterial pressure (MAP) and total peripheral vascular resistance (TVR) were higher in the diabetic group. Cardiac output (CO) and stroke volume (SV) were higher in the control group; however, only the difference in stroke volume was statistically significant (Table 2). Left ventricle end-diastolic (LV-EDD) and end-systolic (LV-ESD) and left atrial diameter (LAD), ejection fraction (EF), fractional shortening (FS), end-diastolic wall thickness (EDWT) and E/A ratio means are shown in Table 3.

LV-EDD, LV-ESD and EDWT increased from the second to third trimesters in both groups. Intragroup differences in LV-EDD and LV-ESD were statistically significant in both groups ( $p < 0.001$ ). There was no significant difference in LV-EDD and LV-ESD between groups. The change in EDWT was only statistically significant in the control group ( $p < 0.05$ ). EF, FS and E/A ratio decreased in the third trimester when compared to the second trimester in both groups ( $p < 0.001$ ). LAD increased in both groups. When differences in the variables between two trimesters were evaluated, only the differences in LAD ( $p < 0.001$ ) and E/A ratio ( $p < 0.005$ ) were statistically significant (Table 3). The difference in EDWT was analyzed with Wilcoxon's signed rank test and there was a significant difference between the two groups ( $p < 0.05$ ). When diastolic functions were analyzed, the E/A ratio was similar in both groups. In the subset of patients whose fetuses developed fetal distress, the E/A ratio in the second trimester was lower than the mean E/A ratio of both groups. The increased TVR decreased the E wave in these patients. However, this finding was less striking in the third trimester.

In the study group, two patients (10%) had early diastolic notch, and three had increased S/D ratios. In the control group, none of the patients had early diastolic notch and one (4%) had an increased S/D ratios in late pregnancy. Of the two patients who had early diastolic notch, one had type I diabetes and the other type II diabetes. The fetus of the type I diabetes patient developed IUGR later on. The other patient's fetus did well. Umbilical artery S/D ratios in the third trimester were 15% and 4% in the study and control groups, respectively. In the third trimester four patients had an increased S/D ratio. Three of these patients were diabetic and one was not. Two of the diabetic patients were type I diabetics. Both of the babies of the type I diabetics had IUGR, and cesarean section was required in both of them because of fetal distress. One of these fetuses had early diastolic notch as well. The cesarean rate was higher in the diabetic group. When the patients who requested elective surgery were excluded, this difference was more prominent. The main indications for cesarean in the diabetic group were fetal distress and macrosomia.

In the study group the 10 min Apgar scores for three babies were below 8. Two of these were delivered preterm and belonged to type I diabetic mothers. The preterm delivery rate was higher in the diabetic group. The indication was fetal distress in these babies and both had IUGR. They needed surfactant treatment for respiratory distress, one developed necrotizing enterocolitis and they both required long-term intensive care. One patient in the diabetic group had operative delivery because of a long second stage and maternal exhaustion. This baby was not macrosomic. In the study group two babies were macrosomic and they belonged to the mothers with gestational diabetes.

In the study group two LGA and two SGA babies had hypoglycemia. The incidence of hyperbilirubinemia was higher among the babies of the study group (Table 4).

Table 3. — The means of the study and control groups in second and third trimester (TM) and the change ( $\Delta$ ) between the two trimesters.

	Study Group (n = 20)			Control group (n = 25)		
	2 <sup>nd</sup> TM	3 <sup>rd</sup> TM	$\Delta$	2 <sup>nd</sup> TM	3 <sup>rd</sup> TM	$\Delta$
LV-EDD (cm)	4.870 ± 0.48	4.875 ± 0.42	-0.005 ± 0.185	4.92 ± 0.32	4.95 ± 0.34	-0.03 ± 0.102
LV-ESD (cm)	2.940 ± 0.42	2.985 ± 0.40	-0.045 ± 0.278	3.05 ± 0.34	3.09 ± 0.31	-0.040 ± 0.173
EF (%)	69.2 ± 5.7	69.1 ± 5.4	0.10 ± 1.50	69.5 ± 3.5	69.4 ± 3.6	0.12 ± 1.78
FS (%)	39.8 ± 5.2	39.3 ± 5.5	0.50 ± 2.72	39.6 ± 3.0	39.4 ± 2.8	0.2 ± 1.64
EDWT (cm)	0.89 ± 0.13	0.85 ± 0.89	0.05 ± 0.16	0.88 ± 0.10	0.89 ± 0.10	-0.01 ± 1.64
E/Aratio	1.35 ± 0.29	1.34 ± 0.28	0.01 ± 0.10*	1.40 ± 0.24	1.31 ± 0.19	0.09 ± 0.20*
LA(cm)	3.26 ± 0.30	3.37 ± 0.28	-0.11 ± 0.35*	3.25 ± 0.22	3.45 ± 0.25	-0.20 ± 0.24*

\*Statistically significant when the study and control group are compared.

Table 4. — Pregnancy complications and neonatal outcomes.

	Diabetes (n = 20)	Control (n = 25)
<b>Pregnancy complications</b>		
<i>Delivery</i>		
Cesarean section	10 (50%)	10 (40%)
10 minute Apgar ≤ 7	3 (15%)	1 (4%)
Fetal distress	4 (20%)	2 (8%)
Preterm delivery	4 (20%)	1 (4%)
Operative delivery	1(5%)	0 (0%)
<i>Neonatal outcome</i>		
LGA	2 (10%)	0 (0%)
SGA	2 (10%)	1 (4%)
RDS	2 (10%)	0 (0%)
IVH	0 (0%)	0 (0%)
NEC	1(5%)	0 (0%)
Hypoglycemia	4 (20%)	1 (4%)
Hyperbilirubinemia	7 (35%)	4 (16%)
Perinatal mortality	0 (0%)	0 (0%)

**Discussion**

Pregnancy is a burden on the cardiovascular system. In a healthy pregnant woman plasma volume increases starting from the first weeks of pregnancy [11]. The cardiac muscle is stretched as a result of increased preload, it hypertrophies and ventricular and atrial diameters are increased. Because of low resistance in the placental vascular bed, the TVR and afterload decrease and CO and SV increase.

Echocardiography was performed in patients whose pregnancies were complicated by IUGR and preeclampsia. It was shown that CO and intravascular space were not as large as they should have been [12]. The mechanism is not very well known yet, but inadequate intravascular volume expansion is associated with defective cardiovascular adaptation. Taegtmeier *et al.* reported that the cardiac muscle of diabetic patients shows functional, biochemical and morphologic alterations [13]. Therefore, examination of the cardiovascular system early in pregnancy helps in understanding maternal-fetal homeostasis and may prove useful in predicting complications.

In this study, with advancing gestational age, cardiac mass increased in both groups. This finding confirms other studies which found an increase in cardiac mass in pregnancy.

When the group that had developed fetal distress during labor was analyzed, the mean values of LV-EDD, LV-ESD and LAD were lower than those of both the study and control groups. However, because this sample was very small, statistical significance could not be determined, which is consistent with other studies [14]. Valensis *et al.* reported that in patients with defective placentation, left ventricular hypertrophy does not develop because of increased peripheral vascular resistance [9]. Bosio *et al.* stated that it is possible to diagnose cardiac maladaptation by echocardiography at the 12th week of pregnancy in patients with preeclampsia [15].

The sensitivity of the second trimester uterine artery Doppler assessment to detect preeclampsia and IUGR in the presence of diastolic notch and increased resistance index is reported to be 85%. Olofson *et al.* found the umbilical artery PI higher in type I diabetic patients [16]. In the fetuses with high umbilical artery PI, fetal death, IUGR, preeclampsia and chronic hypertension are more frequently seen. In this study fetal distress was 2.5 times higher in the study group. After birth, these babies develop hypoglycemia and hyperbilirinemia and more frequently require prolonged intensive care stay. Grunewald *et al.* stated that if the expected decrease in uterine artery PI does not occur, then pregnancy-related complications increase [17].

**Conclusion**

In this study the systolic and diastolic functions were similar in both groups. This can be explained in two ways. Either the groups were too small to detect any difference or most of the study group constituted gestational diabetics who had no vascular disease. Although high umbilical artery S/D ratio, notch in the uterine artery and fetal distress were more common in diabetic patients, it is hard to draw any conclusions because of the small size of the groups.

Fetuses of the diabetic patients are at risk of developing complications. Doppler examination is useful in these patients in fetal surveillance. If the expected physiologic cardiac hypertrophy does not appear, then it can be associated with poor perinatal outcome. Maternal echocardiography can identify cardiac maladaptation as early as the first trimester. Echocardiography may be used in combination with Doppler ultrasound in patients who have ele-

vated vascular resistance to increase the sensitivity. This study should be regarded as a preliminary study. New randomized controlled studies with larger sample sizes are required to reach definitive conclusions.

## References

- [1] Cunningham F.G., Gant N.F., Leveno K.J. *et al.*: "Diabetes". In: Cunningham F.G., Gant N.F., Leveno K.J. *et al.* (eds.). Williams Obstetrics, 21<sup>st</sup> edition, New York, NY. McGraw-Hill, 2001, 1359.
- [2] Ventura S.J., Martin J.A., Curtin Sc., Mathews T.J., Park M.S.: "Births: Final Data for Reports". 1998 National Vital Statistics, Vol. 48, No. 3, Hyattsville, MD, 2000.
- [3] National Diabetes Fact Sheet: General Information and National Estimates on Diabetes in the United States, 2003. Rev ed. Atlanta, Ga: Centers for Disease Control and Prevention, 2004.
- [4] Catalano P.M., Kirwan J.P., Haugel-de Mouzon S., King J.: "Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus". *J. Nutr.*, 2003, 133, 1674S.
- [5] Lucas M.J.: "Diabetes complicating pregnancy". *Obstet. Gynecol. Clin. North Am.*, 2001, 28, 513.
- [6] How H.: "Is incipient nephropaty associated with increased rate of preeclampsia in women with pregestational diabetes?". *Am. J. Obstet. Gynecol.*, 2000, 182, 79.
- [7] Cole L.A., Khanlian S.A., Kohorn E.I.: "Evolution of the human brain, chorionic gonadotropin and hemochorial implantation of the placenta: insights into origins of pregnancy failures, preeclampsia and choriocarcinoma". *J. Reprod. Med.*, 2008, 53, 549.
- [8] Sibai B.M., Caritis S., Hauth J., Lindheimer M.D., Van Dorsten J.P., MacPherson C. *et al.*: "Risks of preeclampsia and adverse neonatal outcomes among women with pregestational diabetes mellitus". *Am. J. Obstet. Gynecol.*, 2000, 182, 364.
- [9] Valansise H., Novelli G.P., Vasapollo B., Borz M., Arduini D., Galente A., Romanini C.: "Maternal cardiac systolic and diastolic function: relationship with uteroplacental resistances. A Doppler and echocardiographic longitudinal study". 2000, 15, 487.
- [10] Duvekot J.J., Cheriex E.C., Pieters F.A., Peeters L.L.: "Severely impaired fetal growth is preceded by maternal hemodynamic maladaptation very early in pregnancy". *Acta Obstet. Gynecol. Scand.*, 1995, 74, 693.
- [11] Airaksinen K.E., Ikaheimo M.J., Salmela P.I., Kirkinen P., Linnaluoto M.K., Takkunen J.T.: "Impaired cardiac adjustment to pregnancy in type 1 diabetes". *Diabetes care*, 1986, 9, 376.
- [12] Duvekot J.J., Cheriex E.C., Pieters F.A., Menheere P.P., Peeters L.H.: "Early pregnancy changes in hemodynamics and volume homeostasis are consecutive adjustments triggered by a primary fall in systemic vascular tone". *Am. J. Obstet. Gynecol.*, 1993, 169, 1382.
- [13] Taegtmeier H., McNulty P., Young M.E.: "Adaptation and maladaptation of the heart in diabetes: Part I: general concepts". *Circulation*, 2002, 105, 1727.
- [14] Poppas A., Shroff S.G., Korcarz C.E., Hibbart J.U., Berger D.S., Lindheimer M.D., Lang R.M.: "Serial assesment of the cardiovascular system in normal pregnancy: Role of arterial compliance and pulsatile arterial load". *Circulation*, 1997, 95, 2407.
- [15] Bosio P.M., McKenna P.J., Conroy R., O'Herling C.: "Maternal central hemodynamics in hypertensive disorders in pregnancy". *Obstet. Gynecol.*, 1999, 94, 978.
- [16] Olofsson P., Lingman G., Marsál K., Sjöberg N.O.: "Fetal blood flow in diabetic pregnancy". *J. Perinat. Med.*, 1987, 15, 545.
- [17] Grunewald C., Divon M., Lunell N.O.: "Doppler velocimetry in last trimester of pregnancy complicated by insulin dependent diabetes mellitus". *Acta Obstet. Gynecol. Scand.*, 1996, 75, 804

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# Do maternity hospital practices support Greek mothers' decision to breastfeed?

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## Summary

**Aim:** Previous studies have shown that the conditions in Greek maternity hospitals do not support the right of mothers and their children to breastfeed. The aim of the present report was to investigate the degree that Greek maternity hospitals have adopted the 'Ten Steps to Successful Breastfeeding'. **Methods:** The study sample comprised 140 mothers living in Athens who had recently given birth and volunteered to fill in specific questionnaires. **Results:** 40.5% of the mothers did not know what the first meal of their baby was. Regarding hospitals' practices, 68.3% of the mothers mentioned that artificial milk was brought in every meal of the neonate, while 63.6% believed that artificial milk was given to their child without their knowledge, despite the fact that they had already decided to breastfeed. Ninety percent of the mothers giving birth in public maternity hospitals and 60% delivering in private clinics mentioned that health professionals supported breastfeeding ( $p < 0.05$ ). **Conclusions:** It seems that in daily practice, Greece has not yet created an appropriate well informed and supportive environment in regard to breastfeeding.

**Key words:** Breastfeeding; Breastfeeding practice; Ten steps; Supportive environment; Decision.

## Introduction

Feeding and diet of neonates and babies are included among the issues of interest for public health, as many of the causes of child mortality stem from feeding practices. Breastfeeding decisively contributes to the good development of neonates-babies, providing them the opportunity to be protected from the influences of the external environment [1-3]. It, furthermore, contributes to securing, saving and better distribution of public health resources [4].

Despite the international declarations, recommendations and initiatives for the promotion of breastfeeding [5, 6], it is clear today that the indices describing the current situation do not comply with scientific recommendations [7]. This applies to Greece as well, since, although the percentage of mothers starting breastfeeding is generally considered satisfactory [8-11], 58.5% stop breastfeeding after the 4<sup>th</sup> month and only 17.1% continue breastfeeding for a period of 6-12 months [12].

In Greece, as well as at the international level, the main factor influencing a woman's decision to breastfeed seems to focus on the practices of health professionals as well as the relevant hospital practices [13]. Many reports have proved that women's decisions on breastfeeding are seriously influenced by midwives, pediatricians and gynecologists [14]. When no proper preparation, care and information is provided by health professionals, breastfeeding percentages are clearly reduced [15], while women wishing to breastfeed for a small period of time are especially vulnerable.

The international scientific community wishing to protect the right of mothers to information about the proper way of feeding their children ratified the Declaration on the Rights of the Child [16]. This declaration also stated that the member states of the United Nations have undertaken the obligation, among others, to create the appropriate conditions so that the mothers have access to information and training on breastfeeding [17]. The WHO and UNICEF [6] recommended the same year and for the same purpose the "Ten Steps to Successful breastfeeding" (Table 1) implementation to all hospitals.

Although in 1995 the Greek Ministry for Health sent a circular letter to all maternity hospitals in Greece regarding the implementation of the "Ten Steps", there is no Greek maternity hospital today applying all the steps [18]. Furthermore, there are very few data referring to the Greek maternity hospital practices on breastfeeding. Therefore, important bio-ethical questions are raised: Under what hospital conditions and practices are the Greek mothers asked to decide whether they are going to breastfeed or not? To what extent do these practices encourage and support the mothers' intentions to breastfeed? To what extent do they defend the mothers' right to proper information and training?

This study aims to investigate the level of adaptation and implementation of the "Ten Steps to Successful Breastfeeding" in Greek maternity hospitals and how this supports the decision of Greek mothers to breastfeed.

## Methods

In this study, mothers having given birth recently (within the last 6 years) were asked to describe their experiences and decisions about the diet of their youngest child. In order to approach

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the specific population sample, 13 child care centers were randomly visited (7 municipal and 8 private) in three random suburbs of Athens. The heads of the centers were informed about the objectives of the research and the experimental protocol. Out of the 13 child care centers, seven agreed to cooperate (5 municipal and 2 private).

The above process started in November 27, 2006 and ended in January 15, 2007. The seven child care centers that had agreed to cooperate, had at that period of time, 325 children ranging from six months to six years of age. The headmasters distributed 305 questionnaires in total, since some children (20 cases) were absent from the center during that period (e.g., due to illness) and 140 of them were filled in by the mothers. The mode of specific and final sample collection allowed applying the simple random sampling securing thus the degree of representativeness [19].

As already mentioned, the first stage was to inform the headmasters of the child care centers about the way the specific study would be conducted and it was clarified that the anonymity of the centers as well of the participating mothers would be kept. After obtaining each center's approval for the distribution of questionnaires, the mothers were informed by the headmasters as well as by an introductory letter attached to each questionnaire explaining the objectives and methodology, while ensuring anonymity. The questionnaires were initially distributed randomly to a pilot group of mothers in order to evaluate the clarity and conciseness of the questions.

The data were statistically analyzed with the use of the statistical software SPSS (Statistical Package for Social Science) 13.00. The chi square test was applied as an independent test

Table 1. — *Ten steps to successful breastfeeding.*

1. Have a written breastfeeding policy that is routinely communicated to all health care staff.
2. Train all health care staff in skills necessary to implement this policy.
3. Inform all pregnant women about the benefits and management of breastfeeding.
4. Help mothers initiate breastfeeding within half an hour of birth.
5. Show mothers how to breastfeed, and how to maintain lactation even if they should be separated from their infants.
6. Give newborn infants no food or drink other than breast milk, unless medically indicated.
7. Practise rooming-in - that is, allow mothers and infants to remain together - 24 hours a day.
8. Encourage breastfeeding on demand.
9. Give no artificial teats or pacifiers (also called dummies or soothers) to breastfeeding infants.
10. Foster the establishment of breastfeeding support groups and refer mothers to them on discharge from the hospital or clinic.

(whether two random categorical variables were statistically independent or not) using the following principles: (a) the existence of a random sample, (b) independence of observations, (c) nominal data, (d) size of the sample between  $n = 15$  and  $n = 250$  and (e) the fulfillment ensuring that 29% of the related table's cells had a maximum of expected frequency below 5 [20]. The statistical criterion T-test for two independent samples (in cases of continuous variables) was also applied with the following

Table 2. — *Data for the neonates' diet during the first hours of their lives in the maternity hospitals.*

*How much time elapsed between the time you gave birth and the neonate was brought to you for the first time in your room?\**

	After half an hour	After 1-2 hours	After 3-6 hours	After 6 to 24 hours	After 24 to 48 hour	The baby was not taken away from the mother
Public	4.2%	37.5%	25%	25%	8.3%	0%
Private	10.9%	25%	52.2%	11.9%	0%	0%
Total	9.5%	27.6%	46.5%	14.7%	1.7%	0%

Giving birth in a public/private hospital:  $p < 0.05$

*When was the first time that you breastfed after birth?\**

	The first half hour after birth	The second half hour	The first 1-6 hours	Over 6 hours	The 2 <sup>nd</sup> 24 hours	The 3 <sup>rd</sup> 24 hours	The next days
Public	0%	12.6%	33.3%	20.8%	33.3%	0%	0%
Private	1.1%	2.2%	41.3%	27.2%	22.8%	1.1%	4.3%
Total	0.9%	4.3%	39.6%	25.9%	25%	0.9%	3.4%

Giving birth in a public/private hospital:  $p = 0.260$

*What was the first meal of the neonate at the hospital?\**

	Sugar water	Artificial milk	Breast milk	Breast milk and artificial milk	I don't know
Public	20%	16%	28%	12%	24%
Private	22%	5.5%	22%	5.5%	45%
Total	21.5%	7.8%	23.3%	6.9%	40.5%

Giving birth in a public/private hospital:  $p = 0.166$

*According to the instructions given to you by the health professionals involved in breastfeeding, which of the following should you do?\**

	Breastfeeding according to a specific timetable	The neonate breastfed whenever he/she wished	Breastfeeding for a specific period	The neonate breastfed for as much as he/she wished
YES	53.4%	28.2%	48.9%	19.1%
NO	46.6%	71.8%	51.1%	80.9%
Giving birth in a public/private hospital:	$p = 0.609$	$p < 0.05$	$p < 0.05$	$p < 0.05$

\* (The sample of the mothers whose babies were taken to a Neonate Intensive Care Unit was taken out).

Table 3. — *Breastfeeding practices applied in maternity hospitals.**Which of the following practices was applied in the maternity hospital where you gave birth?*

	Artificial milk was provided every time it was asked	Artificial milk was provided in every meal	The artificial milk was given on the doctor's order
Public	38.5%	38.5%	23%
Private	11.6%	76.8%	11.6%
Total	17.9%	68.3%	13.8%

 $p < 0.05$ *Did you suspect that the personnel was giving artificial milk to your baby, without your knowledge and after you had taken the decision to breastfeed?*

	I do not believe that something like that was done	I was afraid that this was happening	I did not pay attention
Public	76.9%	19.3%	3.8%
Private	15.5%	77.4%	3.8%
Total	29.1%	63.6%	7.3%

 $p < 0.05$ *Were you allowed by the maternity hospital to give a pacifier to your baby, if you wanted?*

	Yes, I was allowed	It was not allowed	I do not know
Public	20.7%	6.9%	72.4%
Private	8.8%	5.9%	85.3%
Total	11.3%	6%	82.7%

 $p = 0.195$ *At your leaving the maternity hospital, were you given by the doctors a prescription for artificial milk?*

	Public Maternity Hospital	Private Maternity Hospital	Total sample
YES	45%	57.7%	54.8%
NO	55%	42.3%	45.2%

 $p = 0.218$ *Upon leaving the maternity hospital, were you informed about a support breastfeeding group that could help you?*

	Public Maternity Hospital	Private Maternity Hospital	Total sample
YES	13.8%	6.7%	8.8%
NO	86.2%	93.3%	91.2%

 $p = 0.216$ 

principles: (a) data of numerical or analogical type, (b) randomly chosen population sample, and (c) samples from normally distributed population [20]. The central limit theorem could not be applied since the population sample comprised mainly nominal variables and was not very large; in some cases, therefore, the non parametric Mann-Witney criterion for differences between two independent samples and Kruskal-Wallis criterion were applied.

## Results

The majority of the mothers (46.5%) stated that their baby was brought to them three to six hours after delivery. Furthermore, 39.6% of the mothers breastfed for the first time in the first one to six hours, while 40.5% stated that they did not really know what the first meal of their baby was. The mothers also stated that according to the information provided by health professionals, they had to breastfeed based on a schedule and for a specific period of time. A statistically significant difference is observed depending on the hospital where the mothers had delivered their babies ( $p < 0.0005$ ) (Table 2).

Furthermore, 68.3% of the mothers stated that they were brought artificial milk in every meal, whereas

63.6% of them believed that artificial milk was given to their baby without their knowledge and despite the fact that they had already decided to breastfeed. When leaving the hospital, over half of the mothers (54.8%) were given a prescription for artificial milk by a health professional, while 91.2% mentioned that they were not informed about the existence of support breastfeeding groups (Table 3).

Ninety percent of the mothers giving birth in public maternity hospitals and 60% of the mothers giving birth in private clinics mentioned that health professionals supported breastfeeding ( $p < 0.05$ ). Although 56.5% of the total of the mothers considered that the instructions given by doctors-midwives about breastfeeding were clear and comprehensive, 29% believed that they were given in a rush, lacking the feeling of serious planning. Furthermore, the mothers (83.5%) did not know that it is forbidden to give artificial milk to a breastfeeding neonate without a doctor's prescription (Table 4). During their stay at the hospital, 43.8% stated that they were poorly informed about breastfeeding by health professionals, while 56.1% of the mothers felt at the beginning of breastfeeding that they were little to not trained at all (Table 5).

Table 4. — *The attitude of the maternity hospital health professionals as regards breastfeeding.*

*Did you feel that the medical-nursing staff of the maternity hospital:*

	had a neutral attitude	supported breastfeeding	supported an artificial mixed diet	supported artificial milk
Public	6.9%	89.6%	3.5%	0%
Private	18.1%	60%	20.9%	1%
Total	15.4%	66.9%	16.9%	0.7%

$p < 0.05$

*How did you find the instructions of the doctors-midwives about breastfeeding:*

	vague and not very concise	very clear	provided quickly and without any planning	every professional supported something different
Public	3.6%	60.7%	28.6%	7.1%
Private	4.9%	54.5%	29.7%	10.90%
Total	4.6%	56.5%	29%	9.9%

$p = 0.906$

*Did you know that it is not allowed by the maternity hospital to give artificial milk to your baby as a diet supplement while breastfeeding without a doctor's order?*

	Public	Private	Total sample
Yes	25%	14.6%	16.5%
No	75%	85.4%	83.5%

$p = 0.190$

Table 5. — *Mother's breastfeeding training, while staying at the maternity hospital.*

*When you started breastfeeding, how well trained at breastfeeding did you feel?*

	Not at all	Very little	Much	Very much
Public	14.3%	35.7%	35.7%	14.3%
Private	19.6%	38.2%	28.4%	13.8%
Total	18.2%	37.9%	29.5%	14.4%

$p = 0.898$

*When you decided to breastfeed, who guided and trained you in your first breastfeeding?*

	Mother/mother-in-law	Husband	A member of the hospital staff	Personal midwife	Personal pediatrician	Personal gynecologist	Someone else
Yes	16.7%	2.3%	60.6%	20.5%	2.3%	10.6%	4.5%
No	83.3%	97.7%	39.4%	79.5%	97.7%	89.4%	95.5%

They gave birth in a public/private hospital:

$p = 0.198$

$p = 0.054$

$p = 0.433$

$p = 0.139$

$p = 0.359$

$p = 0.992$

$p = 0.766$

*Who trained and guided you in the next cases of breastfeeding at the maternity hospital?*

	Mother/mother-in-law	Husband	A member of the hospital staff	Personal midwife	Personal pediatrician	Personal gynecologist	Someone else
Yes	15.1%	4.8%	61.9%	18.3%	0.8%	5.6%	10.3%
No	84.9%	95.2%	38.1%	81.7%	99.2%	94.4%	89.7%

They gave birth in a public/private hospital:

$p = 0.084$

$p = 0.086$

$p = 0.255$

$p = 0.996$

$p = 0.057$

$p = 0.654$

$p = 0.406$

## Discussion

According to the Declaration of the Rights of the Child, the member states of the UN [16] recognize the right of children to enjoy the best possible level of health. Furthermore, according to the main message of the World Breastfeeding Week, in 2000 [21], breastfeeding is the most important human right of a woman and a child protecting their health. The international scientific commu-

nity, therefore, in order to defend their right to proper diet and health announced the Innocenti Declaration [5], which, inter alia, is based on the application of the "Ten Steps to Successful Breastfeeding" in all maternity hospitals. No hospital in Greece officially applies all ten steps [18]. Under these circumstances, the aim of this research was to investigate the practices existing in Greek maternity hospitals regarding the information and support of mothers to breastfeeding and examine whether the

current conditions favor the mothers' autonomous and free decision-making process.

As shown by the analysis of the results, in all cases the mothers were separated from their babies immediately after delivery, while in approximately half of the cases, the baby was taken to the mother three to six hours after birth. Thus, an important instruction of the "Ten Steps" concerning rooming-in was implemented in only 15.3% of the cases, while in only 1.1% of the mothers delivering in private maternity hospitals. The majority of the mothers did not know what the first meal of their baby consisted of and, obviously, that it was not breastmilk. A similar percentage of mothers stated that they breastfed within the first six hours after birth, a percentage also shown in the study by Antoniou *et al.* [12]. The delayed initiation of breastfeeding is also shown in other Greek studies, where the percentage of women that breastfed within the first hour after birth varied from 8.5% to 3% [8, 11].

Although the WHO in the "Ten Steps" recommends free and unlimited breastfeeding, approximately half of the mothers stated that following the instructions of health professionals of the hospital, they should breastfeed according to a specific timetable and for a specific period of time. To that matter (free and unlimited breastfeeding) the mothers that delivered in public maternity hospitals were better informed than the ones in private clinics ( $p < 0.05$  and  $p < 0.005$ , respectively). As mentioned in other reports, quite often the training of the health professionals is not adequate, and consequently mothers are poorly supported [22, 18].

During their stay in the maternity hospital, a high percentage of mothers (68.3%) stated that artificial milk was brought to them at every meal, although a similar percentage (63.6%) were afraid that the maternity hospital personnel was giving artificial milk to the babies without their knowledge and despite their decision to breastfeed – practices that were more frequent in private maternity hospitals ( $p < 0.001$  and  $p < 0.0005$ , respectively). Although the free and exclusive breastfeeding during the stay in the maternity hospitals is an important factor for positive prognosis regarding the duration of breastfeeding [13] and is considered to be the most important policy of the "Ten Steps", it seemed that in Greece this is not even taken into consideration. Other reports studying these practices in Greek hospitals show that 89% of the neonates are fed with artificial milk once or twice during the first days of their lives [11], while only 19.1% are exclusively breastfed in the maternity hospitals [8].

The mothers having given birth in public hospitals, mentioned at a more frequent rate (89.6%) than the ones having given birth in private hospitals (60%), that the health professionals support breastfeeding in their daily practice. The mothers having given birth in private maternity hospitals stated that health professionals supported a mixed diet or had a neutral attitude towards breastfeeding ( $p < 0.05$ ). Although approximately half of the mothers found the instructions of the health professionals about breastfeeding extremely clear, 29% of them said that the

instructions were given in a hurry, lacking serious planning, while 37.9% of the women found themselves poorly trained for breastfeeding. According to Theofilogiannakou *et al.*'s study [11], only 34% of Greek mothers get informed about the advantages of breastfeeding by their doctors.

Although Greece has ratified the Agreement for the Rights of the Child, has recognized the need for information and support of the mothers in issues of health and diet, and has also adopted the "Ten Steps" [23] of the "Innocenti Declaration", the present study showed that none of the recommended by the world organizations "Ten Steps" was fully applied.

It seemed, therefore, that breastfeeding practices applied in Greek maternity hospitals do not create the appropriate environment to provide sufficient information and support to the mothers. Consequently, these practices do not favor the mothers' free decision making process. Further investigation will provide the necessary information to the National Health System enabling control and promotion of breastfeeding in Greece.

## References

- [1] Howie P.W.: "Protective effect of breastfeeding against infection in the first and second six months of life". *Adv. Exp. Med. Biol.*, 2002, 503, 141.
- [2] Scariati P.D., Grummer-Strawn L.M., Fein S.B.: "A longitudinal analysis of infant morbidity and the extent of breastfeeding in the United States". *Pediatrics*, 1997, 99.
- [3] Morley R., Lucas A.: "Nutrition and cognitive development (review)". *Br. Med. Bull.*, 1997, 53, 123.
- [4] Ball T., Bennet D.: "The economic impact of breastfeeding". *Pediatr. Clin. North. Am.*, 2001, 48, 253.
- [5] UNICEF/WHO: "Innocenti Declaration". Florence, 1990.
- [6] WHO/UNICEF: "Protecting, promoting and supporting breastfeeding: the special role of maternity services". WHO, Geneva, 1989.
- [7] American Academy of Pediatrics Work Group on Breastfeeding: "Breastfeeding and the use of human milk". *Pediatrics*, 1997, 100, 1035.
- [8] Pechlivani F., Vassilakou T., Sarafidou J., Zachou T., Anastasiou C.A., Sidossis L.S.: "Prevalence and determinants of exclusive breastfeeding during hospital stay in the area of Athens, Greece". *Acta Paediatr.*, 2005, 94, 928.
- [9] Ladomenou F., Kafatos A., Galanakis E.: "Risk factors related to intention to breastfeed, early weaning and suboptimal duration of breastfeeding". *Acta Paediatr.*, 2007, 96, 1441.
- [10] Bakoula C., Nicolaidou P., Veltsista A., Prezerakou A., Moustaki M., Kavadias G. *et al.*: "Does exclusive breastfeeding increase after hospital discharge; A Greek study". *J. Hum. Lact.*, 2007, 23, 165.
- [11] Theofilogiannakou M., Skouroliakou M., Gounaris A., Panagiotakos D., Markantonis S.: "Breast-feeding in Athens, Greece: factors associated with its initiation and duration". *J. Pediatr. Gastroenterol. Nutr.*, 2006, 43, 379.
- [12] Antoniou E., Daglas M., Iatrakis G., Kourounis G., Greatsas G.: "Factors associated with initiation and duration of breastfeeding in Greece". *Clin. Exp. Obstet. Gynecol.*, 2005, 32, 37.
- [13] Daglas M., Antoniou E., Pitselis G., Iatrakis G., Kourounis G., Creatsas G.: "Factors influencing the initiation and progress of breastfeeding in Greece". *Clin. Exp. Obstet. Gynecol.*, 2005, 32, 189.
- [14] Swanson V., Power K.G.: "Initiation and continuation of breastfeeding: theory of planned behaviour". *J. Adv. Nurs.*, 2005, 50, 272.
- [15] DiGirolamo A.M., Grummer-Strawn L.M., Fein S.B.: "Do perceived attitudes of physicians and hospital staff affect breastfeeding decisions?". *Birth.*, 2003, 30, 94.

- [16] United Nations General Assembly. Convention on the Rights of the Child. New York, 1989.
- [17] UNICEF Press Release: Breastfeeding and Family Diet, with Affection and Health. Providing other kinds of food while breastfeeding continues, Breastfeeding Week, 1-7 November, 2005.
- [18] Cattaneo A., Yngve A., Koletzko B., Guzman L.R.: "Protection, promotion and support of breastfeeding in Europe: current situation". *Public Health Nutrition*, 2004, 8, 39.
- [19] Kyriazi Nota, Sociological Research: Critical Review of the Methods and Techniques, 4<sup>th</sup> edition, Athens, 2002, Ellinika Gramata.
- [20] Dafermos Vassilis, Social Statistics with SPSS, Ziti Publications, Thessaloniki, 2005.
- [21] UNICEF Press Release: Breastfeeding is your right! Breastfeeding Week, 1-7 November, 2000.
- [22] Taveras E.M., Li R., Grummer-Strawn L., Richardson M., Marshall R., Rego V.H. *et al.*: "Opinions and practices of clinicians associated with continuation of exclusive breastfeeding". *Pediatrics*, 2004, 113, 283.
- [23] Document of the Ministry of Health and Welfare, Primary Health Care Directorate, Breastfeeding - irremovable right of every neonate, A.P. 9/10/1995.

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# Effects of selective and non-selective cyclooxygenase (COX) inhibitors on postoperative adhesion formation in a rat uterine horn model

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## Summary

**Objective:** To investigate the effects of cyclooxygenase (COX) inhibitors including celecoxib, indomethacin, and nimesulide on postoperative adhesion formation. **Material and Methods:** Forty-eight female Wistar-Albino rats were randomly divided into four groups: control (saline solution), celecoxib, indomethacin, and nimesulide groups. The uterine horns of rats were traumatized with unipolar electrocautery. Drugs of each group and saline in the control group were instilled on traumatized areas of horns as intraperitoneally. After three weeks, the extent and severity of adhesions with a standardized scoring system were evaluated. **Results:** The extent and severity of postoperative adhesions were significantly reduced in nimesulide group compared with the control group. The extent but not severity of adhesions in rats given indomethacin was significantly reduced. Celecoxib showed no significant reduction in the extent and severity of adhesions. **Conclusion:** Nimesulide is more effective than the other COX inhibitors in the prevention of postoperative adhesions in rats.

**Key words:** Adhesion; Cyclooxygenase; Nimesulide; Celecoxib; Indomethacin.

## Introduction

Postoperative pelvic adhesions cause various medical problems including infertility, chronic pelvic pain, bowel obstruction, and increase in health expenses [1, 2]. Despite modern surgical techniques, adhesion formation and reformation are still an unavoidable event in reproductive pelvic surgery. Although many adjuvants have been tested in animal models and clinical trials, intraperitoneal fluid instillates and barrier methods are used in clinical practice [3-6]. However, effective application is limited by technical difficulties, including the need for hemostasis and removal of excess peritoneal fluid [7, 8].

Adhesions are the results of the inflammatory response to tissue trauma, infection, hemorrhage, or foreign materials in the peritoneal space. This inflammatory response is due largely to the local release of eicosanoids, including prostaglandins and leukotrienes, caused by tissue trauma [9]. Non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit prostaglandin production, have been shown to decrease adhesion formation [10, 11]. Most of the available NSAIDs may inhibit both cyclooxygenase-2 (COX-2) and cyclooxygenase 1 (COX-1), showing no selectivity for either of the COX isozymes, whereas several NSAIDs express selectivity for COX-1 over COX-2. In most previous reports, the effects of non-selective and selective COX inhibitors have been investigated on postoperative adhesion formation, however,

there are few studies comparing the effects of these drugs [10-14]. In this study, we compared the effects of COX inhibitors, celecoxib (a highly selective COX-2 inhibitor), indomethacin (a nonselective COX inhibitor) and nimesulide (a partially selective COX-2 inhibitor) on postoperative adhesion formation in a rat uterine horn model.

## Materials and Methods

Forty-eight female Wistar albino rats at the age of 10-12 weeks, weighing 200-220 g were used. They were housed five animals to a cage, with the appropriate diet and water ad libitum. All rats were observed for several days to ascertain health before operations. All procedures were approved by and performed under the guidelines of the Animal Care and Use Committee of Cumhuriyet University.

Each rat was anesthetized with ketamine hydrochloride (40 mg/kg intravenously). Before the surgery, the abdomen was shaved and prepared with a povidone iodine solution. Using a sterile technique, a 3 cm midline vertical incision was made and both uterine horns were exposed, and then a 2 cm segment of each uterine horn was traumatized at ten spots on the antimesenteric surface using unipolar cautery. Care was taken to avoid gross bleeding from injured sites. Handling of other tissues was minimized. Rats were randomly assigned into four groups each consisting of 12 rats. Treatment groups were as follows: (i) control, saline solution only; (ii) celecoxib, (iii) indomethacin and (iv) nimesulide groups. Before the final throw of the abdominal closure, saline solution and drugs were instilled immediately after injury onto uterine horns. Dosages of drugs were determined as 0.5 mg/ml according to our previous study [13]. All treatments were given in 2 ml volumes. The incision

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was closed in a single layer, excluding the peritoneum, with a running 4-0 monofilament delayed absorbable suture. The total operative time was less than 10 min. Rats were allowed to recover for three weeks. Celecoxib was obtained from the Pharmacia Corp, Chesterfield, MO and indomethacin and nimesulide were obtained from Sigma, St Louis, MO, USA.

On postoperative day 21 animals were sacrificed by cervical dislocation. The previous abdominal incisions were visually inspected for integrity. A transverse subcostal incision was made above the cephalad extent of the midline laparotomy site, and the abdominal cavity was inspected for the presence of adhesions. The extent and severity of adhesions in the operation site for each uterine horn were evaluated according to Linsky *et al.*'s criteria [15] and recorded by an investigator blinded to the treatment groups. The extent of adhesions was evaluated as follows: 0, no adhesion; 1, 25% of traumatized area; 2, 50% of traumatized area; 3, total involvement. The severity of adhesions was measured as follows: 0, no resistance to separation; 0.5, some resistance (moderate force required); 1, sharp dissection needed.

Data are expressed as mean  $\pm$  SD. Analysis of the adhesion extent and severity scores was done by one-way ANOVA with a Tukey post-hoc test. Significance was assumed when the *p* value was less than 0.05.

## Results

There was no mortality in the study groups. Forty-eight rats recovered without incident after operation and resumed preoperative physical activity and feeding patterns postoperatively. All animals appeared healthy and were evaluated. There were no signs of impaired wound healing or bleeding complications.

The mean  $\pm$  SD extent score of adhesions in the control, celecoxib, indomethacin, and nimesulide groups was recorded as  $1.58 \pm 0.99$ ,  $0.83 \pm 0.93$ ,  $0.50 \pm 0.52$ , and  $0.33 \pm 0.65$ , respectively. As shown in Figure 1, the extent of adhesion scores were significantly lower in indomethacin and nimesulide groups than those of the control group. Although the celecoxib group appeared to have a lower extent of adhesions than those of the control group, the difference was not significant. No differences were found in the extent of adhesions between the celecoxib, indomethacin and nimesulide groups.

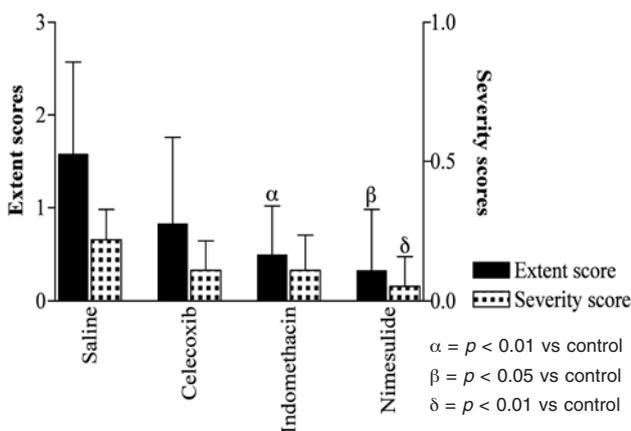


Figure 1. — The extent and severity of adhesions in the control, celecoxib, indomethacin and nimesulide group.

The mean  $\pm$  SD severity score of adhesions in the study groups was as follows: severity in the control, celecoxib, indomethacin, and nimesulide groups was  $0.66 \pm 0.32$  in control group,  $0.33 \pm 0.32$  in celecoxib group,  $0.33 \pm 0.38$  in the indomethacin group, and  $0.16 \pm 0.32$  in the nimesulide group. The severity scores of adhesions was significantly lower in the nimesulide group than those of the control group. Although the severity score of adhesions was lower in the celecoxib and indomethacin groups than that of the control group, there was no statistically significant difference (Figure 1). No significant differences were found between the celecoxib, indomethacin, and nimesulide groups.

## Discussion

Adhesion formation follows the sequence of tissue inflammation, fibrin deposition, and collagen formation. Inflammation occurring as an initial response to peritoneal injury is an integral part of postsurgical repair and leads to extravasation of serum and cellular elements. [16]. The site of peritoneal injury is covered predominantly by polymorphonuclear cells entangled in fibrin strands, which are soon outnumbered by macrophages. When normal fibrinolysis occurs, islands of mesothelial cells proliferate throughout the injury site and completely cover the defect within four to five days. If normal fibrinolysis is inhibited by several factors, macrophages persist and fibroblasts proliferate at this site. Within five days, the fibrin network between adherent structures is replaced by fibrous adhesions of bundles of collagen and fibroblasts. Inflammatory mediators such as prostaglandins (PGF2 $\alpha$  and PGE2) might play an important role in this process of adhesion formation [17]. It has been shown that anti-inflammatory drugs that suppress prostaglandin synthesis were able to prevent adhesion formation following surgical trauma to peritoneum [10, 11, 13, 18]. The suppressive effect of anti-inflammatory drugs on prostaglandin synthesis is mediated by inhibition of cyclooxygenases. Cyclooxygenases (COXs) catalyze the conversion of arachidonic acid to prostaglandin H<sub>2</sub>, which serves as the common precursor for the synthesis of prostaglandins, prostacyclins, and thromboxanes. COXs exist in three isoforms (COX-1, COX-2 and COX-3), which exhibit similar catalytic properties but differ in terms of regulation of expression [19, 20]. The existing nonsteroidal antiinflammatory drugs (NSAIDs) differ in their relative specificities for COX-2 and COX-1. NSAIDs used traditionally, including indomethacin, ibuprofen, and flurbiprofen inhibit the activities of COX-1 and COX-2 non-selectively. In contrast, recently developed NSAIDs such as nimesulide, and celecoxib are designed to inhibit COX-2 selectively.

There is evidence that both traditional NSAIDs and selective inhibitors of COX-2 may prevent adhesion formation postsurgically in animal models [12-14]. DeLeon and Greene have been reported that non-selective COX inhibitors including indomethacin and ibuprofen were effective in reducing postoperative adhesions [14, 21].

Firstly, we have previously demonstrated that nimesulide, selective COX-2 inhibitor, reduced the formation of postoperative adhesion in rat uterine horn model [13]. Greene *et al.* investigated the effects of both selective (celecoxib, rofecoxib) and non-selective COX-2 inhibitors (aspirin, indomethacin, ibuprofen) on the formation of postsurgical adhesions [14]. They found that celecoxib produced a maximal reduction in adhesion formation compared with rofecoxib and the nonselective COX-2 inhibitors. They did not investigate the effect of nimesulide in their study. In the present study, we compared the effects of nimesulide, indomethacin and celecoxib on adhesion formation. Although treatment with nimesulide and indomethacin did lead to a significant reduction in adhesion formation, celecoxib did not reduce as well as nimesulide and indomethacin. Nimesulide significantly reduced both the extent and severity of adhesions whereas indomethacin significantly reduced only the extent of adhesions. Some possible explanations may be suggested based on different results of these studies. First, there are methodologic differences between studies. In the study of Greene *et al.*, drugs were administered orally to male mice, while drugs were applied intraperitoneally to female rats in our study. Second, nimesulide might prevent adhesion formation through different mechanisms accompanying COX-2 inhibition. In addition, it has been suggested that nimesulide is different from both non-selective COX and selective COX-2 inhibitors [22].

The data of the present study and above-mentioned studies are consistent with the hypothesis that both COX-1 and COX-2 mediate postoperative adhesion formation. Additionally, these results suggest that other factors except COXs may also be responsible for adhesion formation. During peritoneal repair, the cellular events appear to be coordinated at least in part by cytokines that function as chemoattractants and immunostimulants. Interleukin-6 (IL-6), transforming growth factor- $\alpha$ , epidermal growth factor, transforming growth factor- $\beta$  and interleukin-1 $\alpha$  have been found to be adhesionogenic, whereas antibodies to IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-1 (IL-1) reduce postoperative adhesion formation [21, 23-26]. The anti-inflammatory properties of nimesulide may also contribute to its inhibitory effect on TNF- $\alpha$  production [27]. Interleukin-6 is a cytokine that is produced by macrophages as well as by activated fibroblasts. Saba *et al.* have determined that IL-6 had a major role in peritoneal adhesion formation and using IL-6 neutralizing antibodies preoperatively, did lead to a reduction of adhesion formation without a significant effect on wound healing. [23]. Nimesulide at therapeutic concentrations is a potent inhibitor of IL-6 production [28]. Although celecoxib has an inhibitory effect on IL-6, this effect is not higher than that of nimesulide. In the study of Bianchi *et al.* it was found that the effects of nimesulide on synovial fluid concentrations of interleukin (IL)-6 and IL-8 were more marked than for celecoxib [29]. Nimesulide also decreases histamine release from tissue mast cells and

inhibits the production of platelet-activating factor by human basophils [30]. These findings may explain why the efficacy of nimesulide was better than celecoxib on adhesion formation. In conclusion, nimesulide was more effective in the prevention of postsurgical adhesions in the rat uterine horn than the other COX inhibitors. The mechanism of action of nimesulide seems to be multifactorial and not limited to the inhibition of COX-2. Further studies should be performed to substantiate these initial observations in human and animal trials.

## References

- [1] Diamond M.P., Schwartz I.B.: "Prevention adhesion development". In: Sutton C. and Diamond M.P. (eds.). *Endoscopic Surgery for Gynecologists*, 2<sup>nd</sup> edition, Philadelphia, Bailliere Tindall, 1998, 398.
- [2] Kössi J., Salminen P., Rantala A., Laato M.: "Population-based study of the surgical workload and economic impact of bowel obstruction caused by postoperative adhesions". *Br. J. Surg.*, 2003, 90, 1441.
- [3] Farquhar C., Vandekerckhove P., Watson A., Vail A., Wiseman D.: "Barrier agents for preventing adhesions after surgery for subfertility". *Cochrane Database Syst. Rev.*, 2000, CD000475.
- [4] Tsuji S., Takahashi K., Yomo H. *et al.*: "Effectiveness of antiadhesion barriers in preventing adhesion after myomectomy in patients with uterine leiomyoma". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2005, 123, 244.
- [5] Mais V., Bracco G.L., Litta P., Gargiulo T., Melis G.B.: "Reduction of postoperative adhesions with an auto-crosslinked hyaluronan gel in gynaecological laparoscopic surgery: a blinded, controlled, randomized, multicentre study". *Hum. Reprod.*, 2006, 21, 1248.
- [6] Brown C.B., Luciano A.A., Martin D., Peers E., Scrimgeour A., diZerega G.S., Adept Adhesion Reduction Study Group: "Adept (icodextrin 4% solution) reduces adhesions after laparoscopic surgery for adhesiolysis: a doubleblind, randomized, controlled study". *Fertil. Steril.*, 2007, 88, 1413.
- [7] DeCherney A.H., diZerega G.S.: "Clinical problem of intraperitoneal postsurgical adhesion formation following general surgery and the use of adhesion prevention barriers". *Surg. Clin. North Am.*, 1997, 77, 671.
- [8] Larsson B.: "Efficacy of Interceed in adhesion prevention in gynecologic surgery: a review of 13 clinical studies". *J. Reprod. Med.*, 1996, 41, 27.
- [9] Samuelsson B., Granstrom E., Green K., Hamberg M., Hammarstrom S.: "Prostaglandins". *Annu. Rev. Biochem.*, 1975, 44, 669.
- [10] Rodgers K.E., Girgis W., St Amand K., Campeau J.D., diZerega G.S.: "Reduction of adhesion formation by intraperitoneal administration of various anti-inflammatory agents". *J. Invest. Surg.*, 1998, 11, 327.
- [11] Cofer K.F., Himebaugh K.S., Gauvin J.M., Hurd W.W.: "Inhibition of adhesion reformation in the rabbit model by meclizemate: an inhibitor of both prostaglandin and leukotriene production". *Fertil. Steril.*, 1994, 62, 1262.
- [12] Jarrett J.C. 2<sup>nd</sup>, Dawood M.Y.: "Adhesion formation and uterine tube healing in the rabbit: a controlled study of the effect of ibuprofen and flurbiprofen". *Am. J. Obstet. Gynecol.*, 1986, 155, 1186.
- [13] Guvenal T., Cetin A., Ozdemir H., Yanar O., Kaya T.: "Prevention of postoperative adhesion formation in rat uterine horn model by nimesulide: a selective COX-2 inhibitor". *Hum. Reprod.*, 2001, 16, 1732.
- [14] Greene A.K., Alwayn I.P., Nose V., Flynn E., Sampson D., Zurakowski D. *et al.*: "Prevention of intra-abdominal adhesions using the antiangiogenic COX-2 inhibitor celecoxib". *Ann. Surg.*, 2005, 242, 140.
- [15] Linsky C.B., Diamond M.P., Cunningham T., Constantine B., DeCherney A.H., diZerega G.S.: "Adhesion reduction in the rabbit uterine horn model using an absorbable barrier, TC-7". *J. Reprod. Med.*, 1987, 32, 17.

- [16] Raftery A.: "Cellular events in peritoneal repair". In: diZerega G.S. *et al.* (eds): "Pelvic surgery: adhesion formation and prevention". New York, Springer-Verlag, 1997.
- [17] Golan A., Stolik O., Wexler S., Langer R., Ber A., David M.P.: "Prostaglandins-a role in adhesion formation. An experimental study". *Acta Obstet. Gynecol. Scand.*, 1990, 69, 339.
- [18] Siegler A.M., Kontopoulos V., Wang C.F.: "Prevention of postoperative adhesions in rabbits with ibuprofen, a nonsteroidal anti-inflammatory agent". *Fertil. Steril.*, 1980, 34, 46.
- [19] Jouzeau J.Y., Terlain B., Abid A., Nedelec E., Netter P.: "Cyclooxygenase isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs". *Drugs*, 1997, 53, 563.
- [20] Garavito R.M., DeWitt D.L.: "The cyclooxygenase isoforms: structural insights into the conversion of arachidonic acid to prostaglandins". *Biochim Biophys Acta*, 1999, 23, 1441, 278.
- [21] De Leon F.D., Toledo A.A., Sanfilippo J.S., Yussman M.A.: "The prevention of adhesion formation by nonsteroidal antiinflammatory drugs: an animal study comparing ibuprofen and indomethacin". *Fertil. Steril.*, 1984, 41, 639.
- [22] Suleyman H., Cadirci E., Albayrak A., Halici Z.: "Nimesulide is a selective COX-2 inhibitory, atypical non-steroidal anti-inflammatory drug" (review). *Curr. Med. Chem.*, 2008, 15, 278.
- [23] Saba A.A., Kaidi A.A., Godziachvili V., Dombi G.W., Dawe E.J., Libcke J.H., Silva Y.J.: "Effects of interleukin-6 and its neutralizing antibodies on peritoneal adhesion formation and wound healing". *Am. Surg.*, 1996, 62, 569.
- [24] Williams R.S., Rossi A.M., Chegini N., Schultz G.: "Effect of transforming growth factor beta on postoperative adhesion formation and intact peritoneum". *J. Surg. Res.*, 1992, 52, 65.
- [25] Hershlag A., Otterness I.G., Bliven M.L., Diamond M.P., Polan M.L.: "The effect of interleukin-1 on adhesion formation in the rat". *Am. J. Obstet. Gynecol.*, 1991, 165, 771.
- [26] Kaidi A.A., Nazzal M., Gurchumelidze T., Ali M.A., Dawe E.J., Silva Y.J.: "Preoperative administration of antibodies against tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) and their impact on peritoneal adhesion formation". *Am. Surg.*, 1995, 61, 569.
- [27] Dogan M.D., Ataoglu H., Akarsu E.S.: "Nimesulide and diclofenac inhibit lipopolysaccharide-induced hypothermia and tumour necrosis factor-alpha elevation in rats". *Fundam Clin. Pharmacol.*, 2002, 16, 303.
- [28] Henrotin Y.E., Labasse A.H., Simonis P.E., Zheng S.X., Deby G.P., Famaey J.P. *et al.*: "Effects of nimesulide and sodium diclofenac on interleukin-6, interleukin-8, proteoglycans and prostaglandin E2 production by human articular chondrocytes in vitro". *Clin. Exp. Rheumatol.*, 1999, 17, 151.
- [29] Bianchi M., Brogginini M., Balzarini P., Franchi S., Sacerdote P.: "Effects of nimesulide on pain and on synovial fluid concentrations of substance P, interleukin-6 and interleukin-8 in patients with knee osteoarthritis: comparison with celecoxib". *Int. J. Clin. Pract.*, 2007, 61, 1270.
- [30] Bevilacqua M., Magni E.: "Recent contributions to knowledge of the mechanism of action of nimesulide". *Drugs*, 1993; 46 (suppl. 1), 40.

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# Dinoprostone vaginal insert versus intravenous oxytocin to reduce postpartum blood loss following vaginal or cesarean delivery

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## Summary

**Objective:** To compare the impact of a dinoprostone vaginal insert and intravenous oxytocin in reducing blood loss of women undergoing vaginal or cesarean delivery. **Methods:** This study was conducted among term singleton pregnancies delivered vaginally or by elective cesarean section. In the vaginally delivered cases, active management of the third stage of labor was conducted. During cesarean delivery, 20 IU of intravenous oxytocin was administered. Women, who either delivered via the vaginal or abdominal route, were then randomly allocated to receive 10 mg vaginal dinoprostone insert for 12 hours (group I, n: 100) or intravenous oxytocin (group II, n: 100), respectively. **Results:** Mean blood loss and need for additional uterotonic and postpartum hemoglobin and hematocrit levels at 24 and 36 hours after delivery did not differ between the two groups. Women allocated to the dinoprostone vaginal insert arm experienced more nausea and vomiting. **Conclusion:** Dinoprostone vaginal insert was as effective as intravenous oxytocin in the prevention of postpartum blood loss.

**Key words:** Postpartum blood loss; Dinoprostone vaginal insert; Vaginal delivery; Cesarean delivery.

## Introduction

Postpartum hemorrhage remains in the top five causes of maternal deaths in both developed and developing countries [1]. The period following the birth of baby and first hours postpartum are crucial in the prevention, diagnosis and management of postpartum hemorrhage (PPH). The injection of the most commonly used uterotonic drug, oxytocin, has proven to be very effective in reducing the incidence of PPH [1, 2]. Misoprostol, a new PGE1 analogue, has been suggested as an alternative for routine management of the third stage of labor [3]. Moreover, it has been mainly used by different routes of administration for the prevention of PPH, results of which have showed promising results, indicating similar efficacy in comparison to oxytocic agents [4-7]. Given its low cost, ease of administration, and stability misoprostol use was a good option for the prevention of PPH [8, 9].

Dinoprostone (PGE2 analogue) vaginal insert has been recently implemented as an alternative agent for labor induction with high efficacy in achieving cervical ripening and successful labor induction [10-14]. Controlled-release dinoprostone, delivered over 24 h from a vaginal insert, results in cervical ripening within 12 h in most women. It is marginally more effective than immediate release formulations and has a comparable efficacy to misoprostol [15].

However, there is paucity in the literature regarding the use of the controlled-release dinoprostone vaginal insert in the prevention of postpartum blood loss. Within this

context, this study represents the first attempt in the literature to investigate the efficacy of dinoprostone in the prevention of PPH.

## Materials and Methods

Approval for this study was obtained from the Institutional Ethical Board of the University Hospital. All the patients were informed and consented to take part into the study. This prospective, randomized study enrolled 200 term singleton pregnancies undergoing spontaneous vaginal (n: 56) and elective cesarean delivery (n: 144) from December 2007 to May 2008. Exclusion criteria were known sensitivity to prostaglandins, excessive postpartum hemorrhage with hemodynamic instability that necessitated blood transfusion, assisted vaginal delivery, use of epidural anesthesia and cases with labor induction. All the women enrolled to this study met the inclusion and exclusion criteria. Intrapartum blood loss was not taken into consideration in any case.

In the vaginal delivery, active management of the third stage of labor was implemented, consisting of early cord clamping, 10 IU intravenous oxytocin infusion (Synpitan fort®, 5 IU ampoule, Deva, Istanbul) following the delivery of the anterior shoulder of the baby and controlled cord traction. In cases undergoing cesarean section, 20 IU intravenous oxytocin infusion was given after the delivery of the placenta.

Following the vaginal or cesarean delivery, 100 women (group I) were randomly allocated to controlled-release PGE2 vaginal insert with a constant delivery of 0.3 mg/hr (Propess®, Vitalis Sağlık Ürünleri Danışmanlık ve Tic Ltd., Turkey) for 12 hours following the insertion instead of oxytocin. An equal number of women (group II) were assigned to intravenous oxytocin infusion in balanced solution (10 IU oxytocin for vaginal as described above for active management of the third stage of labor and 20 IU oxytocin for cesarean delivery, infused 24 hours postpartum, respectively). Randomization was done independ-

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ently through the hospital pharmacy by random allocation. All deliveries were attended by an experienced obstetrician and a senior resident.

Patient demographic characteristics such as age, number of gravidity, parity, living children, mode of delivery, gestational age at birth and neonatal birthweight were determined in both groups. Estimated amount of postpartum blood loss was assessed via a gravimetric method by counting the blood-filled pads within 24 hours of postpartum. Dry weight of the pads was assessed prior to delivery and found to be equivalent to 30 ml. Main outcome measures were the amount of bleeding, need for additional oxytocins, hemoglobin and hematocrit level changes during the postpartum period and drug-related side-effects such as nausea, vomiting, shivering, pyrexia and diarrhea and postpartum vaginal or endometrial infections in groups I and II, respectively.

Statistical analysis was performed using SPSS 10.0 (SPSS 10.0, Chicago IL, USA). Results are presented as the mean  $\pm$  SEM. Test of normality was performed by the one-way Kolmogorov-Smirnov test. Patient demographic characteristics and main outcomes were analyzed by the Student's t-test and the chi-square test, and Fisher's exact test or Wilcoxon log-rank test, where applicable. A two-sided  $p$  value of  $< 0.05$  was set to be statistically significant.

## Results

Demographic characteristics such as age, number of gravidity, parity, abortions, gestational age at delivery, and mode of delivery did not differ between group I and group II, respectively (Table 1). Hemoglobin (g/dl) and hematocrit (%) levels before and 24 and 36 hours after delivery were not significantly changed in the two groups (Figure 1). Number of pads and estimated blood loss (ml) did not differ between the two groups, even the cases that were sub-analyzed in terms of mode of delivery (Table 2). Women allocated to dinoprostone vaginal insert arm experienced more nausea (9% vs 2%;  $p < 0.05$ ) vomiting (5% vs 0%). Moreover, diarrhea (n: 1), pyrexia (n: 1) and shivering (n:1) were only seen in group I cases. Days of hospitalization ( $5.5 \pm 3.2$  days vs  $5.2 \pm 3.3$  days) did not differ between group I and II, respectively. There were 12 cases in the dinoprostone group who felt uncomfortable during the 12 hours of insertion. There were no cases of vaginal or endometrial infection in the two groups during postpartum follow-ups.

Table 1. — Demographic characteristics of women in group I (controlled-release dinoprostone vaginal insert) and group II (intravenous oxytocin infusion), respectively.

Clinical parameters	Group I (Dinoprostone) (n = 100)		Group II (Oxytocin) (n = 100)	
	VD (n: 28)	CS (n: 72)	VD (n: 28)	CS (n: 72)
Age (years)	28.8 $\pm$ 5.1	29.7 $\pm$ 5.6	29.7 $\pm$ 5.9	29.9 $\pm$ 6.0
Gravidity (n)	2.1 $\pm$ 1.1	2.1 $\pm$ 1.2	2.1 $\pm$ 1.1	2.1 $\pm$ 1.1
Parity (n)	0.8 $\pm$ 0.7	0.8 $\pm$ 0.8	0.7 $\pm$ 0.8	0.7 $\pm$ 0.9
Abortion (n)	0.3 $\pm$ 0.7	0.3 $\pm$ 0.8	0.4 $\pm$ 0.8	0.4 $\pm$ 0.7
Living children (n)	0.7 $\pm$ 0.7	0.7 $\pm$ 0.8	0.6 $\pm$ 0.8	0.6 $\pm$ 0.7
Gestational age (weeks)	37.6 $\pm$ 2.4	37.8 $\pm$ 2.6	37.3 $\pm$ 2.9	37.4 $\pm$ 3.1
Birth weight (g)	2990 $\pm$ 660	2882 $\pm$ 652	2914 $\pm$ 770	2920 $\pm$ 780

VD: vaginal delivery, CS: cesarean section;  $p$  value was non significant for all comparisons.

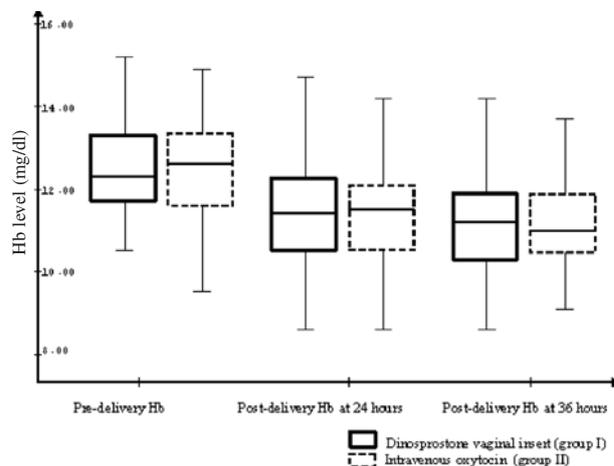


Figure 1. — Pre- and post-delivery (at 24 hours and 36 hours postpartum) hemoglobin (Hb) levels within 95% confidence intervals in groups I and II, respectively.

Table 2. — Pre- and post-delivery hemoglobin (Hb, g/dl) and hematocrit (Hct %) levels, number of pads, amount of estimated blood loss (ml) and need for additional oxytocin injection in groups I and II, respectively. Data are presented as mean  $\pm$  SEM.

Clinical parameters	Group I (Dinoprostone) (n = 100)		Group II (Oxytocin) (n = 100)	
	VD (n: 28)	CS (n: 72)	VD (n: 28)	CS (n: 72)
Pre-delivery Hb	12.4 $\pm$ 1.3	12.4 $\pm$ 1.2	12.3 $\pm$ 1.2	12.2 $\pm$ 1.2
Pre-delivery Hct	36.2 $\pm$ 1.2	35.1 $\pm$ 3.2	35.2 $\pm$ 2.3	36.3 $\pm$ 2.4
Post-delivery Hb*	11.5 $\pm$ 1.5	11.2 $\pm$ 1.4	11.2 $\pm$ 1.3	11.1 $\pm$ 1.2
Post-delivery Hb * *	11.3 $\pm$ 1.3	11.2 $\pm$ 1.2	11.1 $\pm$ 1.1	11.0 $\pm$ 1.1
Post-delivery Hct*	35.1 $\pm$ 2.7	34.2 $\pm$ 1.1	33.1 $\pm$ 1.2	34.9 $\pm$ 1.7
Post-delivery Hct**	34.4 $\pm$ 1.3	33.8 $\pm$ 1.3	33.8 $\pm$ 2.1	33.9 $\pm$ 1.3
Estimated blood loss (ml)	170 $\pm$ 54	173 $\pm$ 45	169 $\pm$ 37	171 $\pm$ 34
Number of pads	5.8 $\pm$ 1.5	5.8 $\pm$ 1.4	5.6 $\pm$ 1.2	5.7 $\pm$ 1.1
Need of additional oxytocin	—	2	—	—

\*24 hours postpartum, \*\* 36 hours postpartum, VD: vaginal delivery, CS: cesarean section;  $p$  value was non significant for all comparisons.

## Discussion

Based on the results of the current study, the 10 mg dinoprostone vaginal insert was as effective as intravenous oxytocin in terms of postpartum blood loss in both vaginal and cesarean deliveries. Several studies and meta-analyses have suggested that prostaglandin analogues, especially PGE1, were effective in reducing postpartum hemorrhage but were associated with more side-effects [16, 17].

The dinoprostone vaginal insert has not yet been used prophylactically to reduce postpartum blood loss in women with different modes of delivery [3, 5, 18, 19]. Hence, our study constitutes the first one in the literature that enables physicians to discuss PGE2 analogue use in this setting. In contrast to PGE2 analogues, PGE1 analogues (misoprostol) have a range of potential benefits including ease of use with different route of administra-

tion (rectal, buccal), low cost and stability at room temperature, the latter of the two were found to be drawbacks of the PGE2 analogues [11, 20-22]. Dinoprostone vaginal insert application per se is much more expensive ( $92.9 \pm 2.3\$$  vs  $5.8 \pm 2.1\$$ ,  $p < 0.05$ ) and needs to be stored in cold temperatures (between  $-10$  and  $-20^\circ\text{C}$ ) based on our experience. Although there is no apparent study in the literature comparing the cost of dinoprostone in the setting of postpartum blood loss prevention, in the context of labor induction, Ramsey *et al.* [23] stated that misoprostol is more cost-effective than comparable commercial dinoprostone prostaglandin preparations.

In general, despite its comparable effectiveness to oxytocin, PGE1 or PGE2 analogues should be considered as a useful option in settings where women receive no uterotonic agents [3]. The dinoprostone vaginal insert was as effective as intravenous oxytocin for the prevention of PPH. However, this approach has a high cost and is not easy to store. Hence, in most parts of Turkey, other PG analogues like PGE1 preparations seem to be a good option for postpartum blood loss prevention in settings where injectable oxytocic agents are not available [25]. However, none of the prostaglandins of different routes of administration and dosage are comparable to conventional injectable uterotonics like oxytocin or methylergonovine maleate [24].

To conclude, the dinoprostone vaginal insert was shown to be effective in the prevention of postpartum blood loss. However based on the current study, this approach is not cost-effective in terms of postpartum blood loss as compared to PGE1 preparations. This basic knowledge should be considered in the decision-making process regarding which drug to use for the prophylaxis of PPH.

## References

- [1] McDonalds: "Management of third stage of labor". *J. Midwifery Womens Health*, 2007, 52, 254.
- [2] Ramanathan G., Arulkumaran S.: "Postpartum hemorrhage". *J. Obstet. Gynaecol. Can.*, 2006, 28, 967.
- [3] Gulmezoglu A.M., Forna F., Villar J., Hofmeyr G.J.: *Cochrane Database Syst. Rev.*, 2007, 18, CD000494.
- [4] Baskett T.F., Persad V.L., Clough H.J., Young D.C.: "Misoprostol versus oxytocin for the reduction of postpartum blood loss". *Int. J. Gynaecol. Obstet.*, 2007, 97, 2.
- [5] Blum J., Alfrevic Z., Walraven G., Weeks A., Winikoff B.: "Treatment of postpartum hemorrhage with misoprostol". *Int. J. Gynaecol. Obstet.*, 2007, 99 (suppl. 2), S202.
- [6] Ozkaya O., Sezik M., Kaya H., Desdicoglu R., Dittrich R.: "Placebo-controlled randomized comparison of vaginal with misoprostol in the prevention of postpartum hemorrhage". *J. Obstet. Gynaecol. Res.*, 2005, 31, 389.
- [7] Hamm J., Russell Z., Botha T., Carlan S.J., Richichi K.: "Buccal misoprostol to prevent hemorrhage at cesarean delivery: a randomized study". *Am. J. Obstet. Gynecol.*, 2005, 192, 1404.
- [8] Derman R.J., Kodkany B.S., Goudar S.S., Geller S.E., Naik V.A., Bellad M.B *et al.*: "Oral misoprostol in preventing postpartum haemorrhage in resource-poor communities: a randomised controlled trial". *Lancet*, 2006, 368, 1248.
- [9] Langenbach C.: "Misoprostol in preventing postpartum hemorrhage: a meta-analysis". *Int. J. Gynaecol. Obstet.*, 2006, 92, 10.
- [10] Strobelt N., Meregalli V., Ratti M., Mariani S., Zani G., Morana S.: "Randomized study on removable PGE2 vaginal insert versus PGE2 cervical gel for cervical priming and labor induction in low-Bishop-score pregnancy". *Acta Obstet. Gynecol. Scand.*, 2006, 85, 302.
- [11] Tanir H.M., Sener T., Yildiz C., Kaya M., Kurt I.: "A prospective randomized trial of labor induction with vaginal controlled-release dinoprostone inserts with or without oxytocin and misoprostol+oxytocin". *Clin. Exp. Obstet. Gynecol.*, 2008, 35, 65.
- [12] Wing D.A.: "Misoprostol Vaginal Insert Consortium. Misoprostol vaginal insert compared with dinoprostone vaginal insert: a randomized controlled trial". *Obstet. Gynecol.*, 2008, 112, 801.
- [13] Facchinetti F., Venturini P., Verocchi G., Volpe A.: "Comparison of two preparations of dinoprostone for pre-induction of labour in nulliparous women with very unfavourable cervical condition: a randomised clinical trial". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2005, 119, 189.
- [14] Marconi A.M., Bozzetti P., Morabito A., Pardi G.: "Comparing two dinoprostone agents for cervical ripening and induction of labor: a randomized trial". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2008, 38, 135.
- [15] Rath W.: "A clinical evaluation of controlled-release dinoprostone for cervical ripening-a review of current evidence in hospital and outpatient settings". *J. Perinat Med.*, 2005, 33, 491.
- [16] Mousa H.A., Alfrevic Z.: "Treatment for primary postpartum haemorrhage" (review). *Cochrane Database Syst. Rev.*, 2007 (1), CD003249.
- [17] Ng P.S., Lai C.Y., Sahota D.S., Yuen P.M.: "A double-blind randomized controlled trial of oral misoprostol". *Gynecol. Obstet. Invest.*, 2007, 63, 55.
- [18] Baruah M., Cohn G.M.: "Efficacy of rectal misoprostol as second-line therapy for the treatment of primary postpartum hemorrhage". *J. Reprod. Med.*, 2008, 53, 203.
- [19] Vimala N., Mittal S., Kumar S.: "Sublingual misoprostol versus oxytocin infusion to reduce blood loss at cesarean section". *Int. J. Gynaecol. Obstet.*, 2006, 92, 106.
- [20] Parsons S.M., Walley R.L., Crane J.M., Matthews K., Hutchens D.: "Rectal misoprostol versus oxytocin in the management of the third stage of labour". *J. Obstet. Gynaecol. Can.*, 2007, 29, 711.
- [21] Meckstroth K.R., Whitaker A.K., Bertisch S., Goldberg A.B., Darney P.D.: "Misoprostol administered by epithelial routes: Drug absorption and uterine response". *Obstet. Gynecol.*, 2006, 108 (3 Pt 1), 582.
- [22] Anderson J.M., Etches D.: "Prevention and management of postpartum hemorrhage". *Am. Fam. Physician*, 2007, 75, 875.
- [23] Ramsey P.S., Harris D.Y., Ogburn P.L. Jr, Heise R.H., Magtibay P.M., Ramin K.D.: "Comparative efficacy and cost of the prostaglandin analogs dinoprostone and misoprostol as labor preinduction agents". *Am. J. Obstet. Gynecol.*, 2003, 188, 560.
- [24] Wei S., Fraser W.D.: "Misoprostol and intramuscular prostaglandins do not prevent postpartum haemorrhage more than injectable uterotonics" (review). *Evid Based Med.*, 2008, 13, 82.
- [25] Prager M., Eneroth-Grimfors E., Edlund M., Marions L.: "A randomised controlled trial of intravaginal dinoprostone, intravaginal misoprostol and transcervical balloon catheter for labour induction". *BJOG*, 2008, 115, 1443.

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# Psychological factors of hyperemesis gravidarum by using the SCL-90-R questionnaire

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## Summary

**Background:** Hyperemesis gravidarum is known as a complex disease with interaction of biological, psychological and sociocultural factors. Our study was an attempt to understand the psychological effects on hyperemesis gravidarum by using an objective scale. **Methods:** Thirty-four pregnant women with hyperemesis gravidarum who were hospitalized in the Obstetrics and Gynecology Department of Dr. Lutfi Kirdar Kartal Education and Research Hospital in Istanbul, Turkey comprised the patient group and asymptomatic pregnant women who came for routine antenatal visits to our clinic were enrolled in this study as the control group between March 1, 2007 and October 15, 2008. Women in both groups filled in the Symptom Check List (SCL-90-R) questionnaire. The data collected from both groups were analyzed by using the Student's t-test (SPSS 13.00). Frequencies of high SCL scores between groups were analyzed by chi-square tests. **Results:** The patients with hyperemesis gravidarum had higher distress scores than those in the control group. The mean value of global severity index (GSI) was 1.03 in the patient group and 0.64 in the control group. The difference was statistically significant ( $p < 0.005$ ). The most significant difference between the two groups was in somatization subscales ( $p < 0.0001$ ). **Conclusion:** Hyperemesis gravidarum is a complex disorder with psychological aspects. Considering this fact can help us deal with the disorder.

**Key words:** Hyperemesis gravidarum; SCL-90-R; Psychology.

## Introduction

Nausea and vomiting in pregnancy are very common; 50-90% of women in early stages of pregnancy are affected by the condition called morning sickness or hyperemesis gravidarum [1, 2].

Hyperemesis gravidarum (HG) is a condition that can accompany persistent nausea and vomiting. It causes dehydration, electrolyte imbalance, ketosis and nutritional deficiencies in pregnant woman. It affects about 0.5-1% of pregnant women [3]. HG can be associated with substantial morbidity including organ damage or retarded fetal growth without proper medical management [4]. Although it is a rare condition, severe hyperemesis is refractory to treatment with fluid and electrolyte replacement and antiemetics. It is associated with multiple hospitalizations, social isolation and psychological morbidity. Women may request termination of pregnancy for intractable, serious hyperemesis or their obstetricians may recommend it [5, 6].

Both physiological and psychological etiologies have been proposed for the condition. High levels of estrogen, human chorionic gonadotropin, increasing levels of androgen hormones and transient maternal hyperthyroidism have been considered for HG [7, 8]. Gastrointestinal etiologies were also investigated in the literature [9, 10].

Moreover, psychological factors like lack of emotional support, depression, and personality disorders have been considered for HG in the literature [11, 12]. Although, there are some studies on the association between hyperemesis gravidarum and psychological factors in the literature, an objective relationship has not been established [13-15].

The Symptom Check List 90 Revised (SCL-90-R) was designed to assess patient self reporting by Derogatis *et al.* [16]. It is a widely used and well searched instrument for investigation of psychological distress and psychopathology. Dag validated the Turkish version of the SCL-90-R in 1991 [17]. Researchers have used the questionnaire to evaluate the Turkish population in the literature [18-20].

An objective relationship between HG and psychological condition has not been well shown in the literature. The SCL-90-R is a good questionnaire to evaluate the psychological condition of patients objectively [21].

We assumed that some of the subscales of the questionnaire express the condition of mental health that could be effective in understanding the psychological condition of hyperemesis. We compared the Global Severity Index (GSI) and somatization (som), obsessive-compulsive (obs), depression (dep), and an additional scale, anxiety (anx), subscales of patients and the control group in this study.

## Materials and Methods

The research was conducted prospectively between March 1, 2007 and October 15, 2008 at the Department of Obstetrics and

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Gynecology, Dr. Lutfi Kirdar Kartal Education and Research Hospital in Istanbul, Turkey.

The patients were diagnosed with HG based on the following criteria: intractable nausea and vomiting, dehydration, loss of more than 5% of body weight, electrolyte imbalance and ketone bodies in urine samples.

Patients who wanted to participate responded to the SCL-90-R questionnaire. The same number of asymptomatic pregnant women who came for first trimester routine antenatal exams to our outpatient clinic were enrolled in the study as the control group.

Women with a history of psychiatric disorders, endocrine or gastrointestinal disease and multiple pregnancies were excluded from the study. All patients had basic biochemical and urine tests. Patients who had abnormal liver enzyme levels, thyroid function tests or urinary infections were also excluded from the study. The study was explained and written informed consent was given by all patients.

SCL-90-R consists of 90 questions concerning a patient's distress symptoms in the previous seven days [22]. Each item is rated based on the Likert score, a five-point scale (0-4) from "not at all to extremely" [23].

In clinical practice the SCL-90-R is used to reflect the general symptom level or GSI of individuals. The sum of scores rated is divided into the total number of questions (90). The result gives the GSI of the patient. A score more than one is significant for psychological evaluation in the questionnaire.

The SCL-90-R has nine primary symptom dimensions: 90 items in the questionnaire include somatization, obsessive-compulsive disorder, interpersonal sensitivity, depression, anxiety, anger-hostility, phobic anxiety, paranoid ideation and psychotic-related questions. They are placed randomly in the questionnaire.

The sum of scores rated by patients for questions related to each symptom dimension is calculated and divided into the total number of those questions. The results are final scores for each item. A score more than one is considered significant in the SCL-90-R.

GSI scores and subscales of the patients and control group were evaluated.

Patients filled in the SCL-90-R questionnaire in a comfortable, quiet room designed for the study. The participants read and answered the questionnaire alone before being hospitalized. The GSI and scores of somatization, obsessive-compulsive, depression, and anxiety subscales were calculated properly according to the questionnaire. Patients with high SCL-90-R scores were consulted at the psychiatric department of the hospital as well.

Data of the study were analyzed with SPSS 13.00. We used the Student's t-test to compare mean values of groups and the chi-square test to compare frequencies. A *p* value less than 0.05 was considered statistically significant.

**Results**

Thirty-four patients with hyperemesis gravidarum selected according to our research criteria completed the SCL-90-R questionnaire. The mean age was 26 ± 3.7 years (range 18-33 years). The mean age of the 34 patients in the control group was 25 ± 4.8 years (range 18-34 years) with no significant differences. Patient characteristics are shown in Table 1. Women in the control group were approximately 9.6 ± 3.0 gestational weeks

and patients with HG 9.0 ± 2.4 gestational weeks with no significant differences.

SCL-90R questionnaire results are shown in Table 2. The mean GSI score of patients was 1.03 ± 0.57 and the control group's mean GSI score was 0.64 ± 0.48. The difference was statistically significant (*p* < 0.004). The difference between the two groups was also statistically significant in mean somatization subscale scores (*p* < 0.0001). The highest scores of subscales were in the somatization subscale in the patient group. An additional subscale on eating disorders was not significantly different between the two groups.

High GSI scores (score more than 1) were calculated in 19 women with HG (55.5%) and six patients in the control group (17.6%). The difference between percentages was statistically significant (*p* < 0.01). Frequency of scores higher than one are shown in Table 3. Frequencies of high scores in all subscales were higher in the HG group than in the controls. However, only the anxiety subscale had more patients with a score higher than one in the HG group (*p* < 0.05) (Table 3).

Table 1. — Patient characteristics.

	Groups*	Mean	Std. Deviation	Std. Error (mean)	<i>p</i> values
age	1	26.1176	3.70720	.63578	
	2	25.0000	4.77367	.81868	ns
gravida	1	2.3529	1.29994	.22294	
	2	1.9118	1.11104	.19054	ns
para	1	1.1176	1.09447	.18770	
	2	.6471	.77391	.13272	ns
weeks	1	9.3529	3.22756	.55352	
	2	9.4412	1.79547	.30792	ns

\*1: Patients with HG; \*2: Control group; ns: Not significant

Table 2. — SCL-90-R results.

	Groups*	Mean	Std. Deviation	Std. Error (mean)	<i>p</i> values
GSI	1	1.0350	.57590	.09877	
	2	.6471	.48109	.08251	< .005
Somatization	1	1.5114	.71589	.12277	
	2	.9075	.57545	.09869	ns
Obsessive-compulsive disorder	1	1.0882	.71423	.12249	
	2	.7422	.64194	.11009	< .05
Depression	1	1.3765	.82743	.14190	
	2	.9644	.72656	.12460	<.05
Anxiety	1	1.1340	.79032	.13554	
	2	.6863	.60083	.10304	
Additional scale	1	1.1114	.90142	.15459	<.05
	2	.8641	.55594	.09534	

\*1: Patients with HG; \*2: Control group; ns: Not significant.

Table 3. — Frequencies of high scores.

Groups*	Total	GSI	Patients with score > 1			
			Som	Ob/Comp	Dep	Anx
1	34	19.00	26.00	17.00	22.00	18.00
2	34	6.00	16.00	11.00	13.00	8.00
	$\chi_2$	9.15	3.7	1.5	3.76	5
	<i>p</i> values	<0.01	ns	ns	ns	< 0.05

\*1: Patients with HG; \*2: Control group; ns: Not significant.

## Discussion

The SCL-90-R is a self-reporting outcome measure in psychiatric research and primary care settings [23]. It is an objective, measurable and proper method to understand psychological conditions of patients.

GSI and subscales of the questionnaire; somatization, obsessive-compulsive, depression and anxiety and additional subscales of both groups were evaluated according to the SCL-90R questionnaire.

The mean GSI score of patients with HG was higher compared to the control group (Table 2). Additionally, more patients with a GSI score higher than one were in the HG group (Table 3).

The HG group had higher means of subscales except for the additional scale which included questions on eating disorders (Table 2). The somatization score was significantly higher in the HG group than in control group. Table 2 shows the relationship between hyperemesis gravidarum and psychological conditions of patients. Somatization was the most important psychological symptom related to HG in our study group but did not seem to be related to eating disorders.

Studies in the literature have found that somatization can cause some gastrointestinal disorders like irritable bowel syndrome [24, 25]. Our study findings support that somatization disorders were mostly related to HG patients.

When we compared frequencies of high subscale scores, only the anxiety subscale was more frequent in the HG group (Table 3). Increased anxiety symptoms could be the result of the condition as well as the reason for it.

In the literature, studies showed that gastrointestinal disorders are less refractory to treatment when anxiety level falls [26].

Hyperemesis gravidarum is defined as severe nausea and vomiting and consequently a pregnant woman is unable to maintain a good nutritional status. Although more than 50% of women have morning sickness as a result of changes in hormone levels, the cause of hyperemesis is still uncertain [1]. As mentioned above, there are several factors that explain the etiology of the condition: gastrointestinal disorders, elevated hormone levels, and vitamin deficiencies are reported in the literature [27].

We designed this study in an attempt to understand the psychological part of this condition. Uncertain relationships between subscales of the SCL-90-R and unrealistic evaluations of patients are disadvantages of the questionnaire.

The diagnostic efficiency of the SCL-90-R has been well tested [28, 29, 30]. More than 90% of the patients were correctly classified psychologically by using the SCL-90R questionnaire according to Pederson and Karterud's study in 2004 [31]. They found that the traditional SCL-90-R has a high predictive power with respect to any symptom disorder according to DSM-IV. However, the differential predictive power has been reported as less satisfactory [32, 33].

Although GSI and somatization scores of the questionnaire were significantly higher in the HG group, it is difficult to conclude that psychological disorders of pregnant women were the only reason for HG in this study. Patients with hyperemesis are not in a good state of health. Effects of poor health on psychological status of patients are also not clear.

On the other hand, we know that most patients in clinical practice present complex co-occurring symptom disorders [32], and the diagnostic picture has boundaries between many disorders, e.g., depression may be accompanied with anxiety disorder. In the literature it was found that patients with pain-related complaints had high scores on the somatization subscale [33]. Patients with depression reported more emotional distress as a result of that study.

Our study was not aimed at a full psychiatric evaluation of patients. Fell *et al.* found that the relative risk of psychiatric illness is 4.5 among pregnant women with a history of hyperemesis when compared with healthy women [34]. The purpose of our study was to understand the efficacy of the psychological condition on hyperemesis gravidarum. We found that the SCL-90-R is capable of selecting patients who need psychiatric help and that psychological aspects are important in the approach to the disorder.

In conclusion, the SCL-90-R questionnaire is a practical and effective test to screen patients psychologically, especially women who are hospitalized for hyperemesis gravidarum. Severe and intractable cases with high questionnaire scores should especially be evaluated.

## References

- [1] Holmgren C., Aagaard-Tillery K.M., Silver R.M., Porter T.F., Varner M.: "Hyperemesis in pregnancy: an evaluation of treatment strategies with maternal and neonatal outcomes". *Am. J. Obstet. Gynecol.*, 2008, 198, 56.
- [2] Gross S., Librach C., Cecuttia A.: "Maternal weight loss associated with hyperemesis gravidarum: a predictor of fetal outcome". *Am. J. Obstet. Gynecol.*, 1989, 160, 906.
- [3] Bergin P.S., Harvey P.: "Wernicke's encephalopathy and central pontine myelinolysis associated with hyperemesis gravidarum". *Br. Med. J.*, 1992, 305, 517.
- [4] Shah A., Mathew V., Shah P.: "Severe hyperemesis gravidarum leading to hepatorenal failure, a rare and challenging case". *J. Obstet. Gynaecol.*, 2008, 28, 1023.
- [5] Poursharif B., Korst L.M., Macgibbon K.W., Fejzo M.S., Romero R., Goodwin T.M.: "Elective pregnancy termination in a large cohort of women with hyperemesis gravidarum". *Contraception*, 2007, 76, 451.
- [6] Ziaei S., Hosseiny F.S., Faghihzadeh S.: "The efficacy low dose of prednisolone in the treatment of hyperemesis gravidarum". *Acta Obstet. Gynecol. Scand.*, 2004, 83, 272.
- [7] Trogstad L.I.S., Stoltenberg C., Magnus P., Skjaerven R., Irgens L.M.: "Recurrence risk in hyperemesis gravidarum". *BJOG*, 2005, 112, 1641.
- [8] Tan J.Y.L., Loh K.C., Yeo G.S.H., Chee Y.C.: "Transient hyperthyroidism of hyperemesis gravidarum". *BJOG*, 2002, 109, 683.
- [9] Cevrioglu A.S., Altidis M., Yilmazer M., Fenkci I.V., Ellidokuz E., Kose S.: "Efficient and non-invasive method for Helicobacter pylori in gravida with hyperemesis gravidarum: Helicobacter pylori stool antigen test". *J. Obstet. Gynaecol. Res.*, 2004, 30, 136.
- [10] Nelson-Piercy C.: "Hyperemesis gravidarum". *Curr. Obstet. Gynecol.*, 1997, 7, 98.

- [11] Meighan M., Woo A.F.: "The impact of hyperemesis gravidarum on maternal role assumption". *JOGNN*, 2005, 34, 172.
- [12] Karpel L., deGmeline C.: "Psychological approach to hyperemesis gravidarum". *J. Gynecol. Obstet. Biol. Reprod.*, 2004, 33, 623.
- [13] Swallow B.L., Lindow S.W., Masson E.A., Hay D.M.: "Psychological health in early pregnancy: relationship with nausea and vomiting". *J. Obstet. Gynaecol.*, 2004, 24, 28.
- [14] Iatrakis G.M., Sakellaropoulos G.G., Kourkoubas A.H., Kabounias S.E.: "Vomiting and nausea in the first 12 weeks of pregnancy". *Psychoter. Psychosom.*, 1998, 49, 22.
- [15] Poursharif B., Korst L.M., Fejzo M.S., MacGibbon K.W., Romero R., Goodwin T.M.: "The psychosocial burden of hyperemesis gravidarum". *J. Perinatol.*, 2008, 28, 176.
- [16] Derogatis L.R., Lippman R.S., Covi L.: "SCL90. An outpatient psychiatric rating scale preliminary report". *Psychopharmacol. Bull.*, 1973, 9, 13.
- [17] Dag I.: "Belirti Tarama Listesi (SCL 90-R) nin universite ogrencileri icin guvenirligi ve gecerliligi". *Turk. Psikiatri Dergisi*, 1991, 2, 5.
- [18] Sayar K.: "Predictors of somatic symptoms in depressive disorder". *Gen. Hosp. Psychiatry*, 2003, 25, 108.
- [19] Celikel F., Cumurcu B., Koc M., Etikan I., Yucel B.: "Psychologic correlates of eating attitudes in Turkish female college students". *Compr. Psychiatry*, 2008, 49, 188.
- [20] Nickel M., Cangoez B., Bachler E., Muehlbacher M., Lojewski N., Mueller-Rabe N. *et al.*: "Bioenergetic exercises inpatient treatment of Turkish immigrants with chronic somatoform disorders: A randomized, controlled study". *J. Psychosom. Res.*, 2006, 61, 507.
- [21] Derogatis L.R.: "SCL90-R: Symptom Checklist-90-R: administration, scoring and procedures manual". Minneapolis, MN, National Computer systems, 1994.
- [22] Vassend O., Skrondal A.: "The problem of structural indeterminacy in multidimensional symptom report instruments. The case of SCL-90-R". *Behaviour Research Therapy*, 1999, 37, 685.
- [23] Schmitz N., Hartkamp N., Brinshwitz C., Michalek S., Tress W.: "Comparison of the standard and the computerized versions of the Symptom Check List (SCL-90-R): a randomized trial". *Acta Psychiatr. Scand.*, 2000, 102, 147.
- [24] Locke G.R., Weaver A.L., Melton J.L., Talley N.J.: "Psychosocial factors are linked to functional gastrointestinal disorders: a population based nested case control study". *Am. J. Gastroenterol.*, 2004, 40, 350.
- [25] Spiegel B.M.R., Kanwal F., Naliboff B., Mayer E.: "The impact of somatization on the use of gastrointestinal health-care resources in patients with irritable bowel syndrome". *Am. J. Gastroenterol.*, 2005, 100, 2262.
- [26] Lee O.Y., Fitzgerald L.Z., Naliboff B., Schmulson M., Liu C., Fullerton S. *et al.*: "The impact of advertisement and clinic populations in symptoms and perception of irritable bowel syndrome". *Aliment. Pharmacol. Ther.*, 1999, 13, 1631.
- [27] Piercy C.N., Fayers P., Swiet M.: "Randomized, double blind, placebo controlled trial of corticosteroids for the treatment of hyperemesis gravidarum". *BJOG*, 2001, 108, 9.
- [28] Gottschalk L.A., Stein M.K., Shapiro D.H.: "The application of computerized content analysis of speech to the diagnostic process in a psychiatric outpatient clinic". *J. Clin. Psychol.*, 1997, 53, 427.
- [29] Schmitz N., Kruse J., Tress W.: "Improving screening for mental disorders in the primary care setting by combining the GHQ-12 and SCL-90R subscales". *Compr. Psychiatry*, 2001, 42, 166.
- [30] Collo B., Suzanne L.: "Culture and validity of the Symptom Check List 90 Revised and profile of mood in New Zeland student sample". *Am. Psychol. Ass.*, 2003, 9, 185.
- [31] Pederson G., Karterud S.: "Is SCL-90R helpful for the clinician in assessing DSM-IV symptom disorders?". *Acta Pshychiatr. Scand.*, 2004, 110, 215.
- [32] Dver I.R., Phillips K.A., Warshaw M.G., Dolan R.T., Shea M.T., Stout R.L. *et al.*: "Patterns of personality pathology in patients with generalized anxiety disorder, panic disorder with and without agorophobia and social phobia". *J. Pers. Disord.*, 2001, 15, 60.
- [33] Cirrlirle F., Guelm J.D.: "Pain complaints in depressed inpatients". *Psychopathology*, 2000, 33, 307.
- [34] Fell D.B., Dodds L., Joseph K.S., Allen V.M., Butler B.: "Risk factors for hyperemesis gravidarum requiring hospital admission during pregnancy". *Obstet. Gynecol.*, 2006, 107, 277.

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# Spontaneous uterine rupture in a nulligravida female presenting with unexplained recurrent hematometra

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## Summary

Spontaneous rupture of an unscarred uterus in reproductive-age women is exceedingly rare, especially in the context of dysfunctional bleeding and a patent cervical canal. A 25-year-old nulligravida female, who reported recent onset of metromenorrhagia and anemia, was initially admitted for surgical management of unexplained hematometra requiring dilation and curettage. The patient remained with intermittent vaginal bleeding for the following six months on continuous progestin therapy. She then re-presented with enlarged hematometra and uterine rupture, which was surgically repaired. Despite exhaustive conservative treatment to preserve fertility, hysterectomy was eventually required due to recurrent uterine rupture. Idiopathic recurrent hematometra can result from the rare combination of uncontrolled dysfunctional bleeding and absence of outflow obstruction.

*Key words:* Uterine rupture; Unexplained hematometra; Hysterectomy.

## Introduction

Hematometra refers to retrograde accumulation of the menstrual blood in the uterine cavity usually as result of lower genital tract obstruction, most commonly at the level of the cervical canal and involving iatrogenic, infectious or malignant etiologies [1]. It is rare for a hematometra to be present in the absence of an identifiable cause. In a pre-puberal girl, unexplained hematometra has been reported in an initially obstructed and over-distended hemiuterus despite its surgical correction [2]. However, persistence of hematometra in the absence of outflow obstruction is exceedingly rare in a young nulliparous female. The following is a description and discussion of an unusual case of uterine rupture in a young female with unexplained recurrent hematometra.

## Case Report

A 25-year-old nulligravida female presented to the emergency department with a three-month history of intermittent heavy vaginal bleeding, new onset of dizziness, and fever. The patient reported a previously regular 28-day interval menses since menarche at age 13 and denied any prior history of sexual activity. Her past medical history was significant for migraines with aura. A transvaginal ultrasound (TVS) depicted a distended blood-filled uterine cavity measuring 8.4 x 6.8 x 7.6 cm, consistent with the diagnosis of hematometra. Intravenous antibiotics were initiated for presumed endometritis, and she underwent an uncomplicated dilation and curettage (D&C) where approximately 200 cc of dark blood was drained from the uterine cavity. Interestingly, the cervical canal was dilated quite easily and a vaginal exam revealed blood from the cervical canal prior to the procedure. Blood transfusion was given for persistent anemia. The final pathology of the specimen revealed

only a late secretory endometrium. Since the patient's history of migraines with aura contraindicated the use of estrogen therapy, depot medroxyprogesterone acetate was recommended as treatment for her dysfunctional uterine bleeding (DUB). During the following six months, the patient remained with controlled but intermittent vaginal bleeding. A follow-up pelvic examination one month later revealed a normal size uterus. Two weeks after the third dose of depot medroxyprogesterone acetate, the patient was readmitted to the hospital with lower abdominal pain and uterine bleeding. A pelvic computed tomography (CT) scan revealed a 7.7 x 7.5 x 5 cm blood-filled uterine cavity with a markedly thin uterine wall. A pelvic magnetic resonance imaging (MRI) scan confirmed a 4 cm uterine wall rupture with blood in the peritoneal cavity (Figure 1). The patient underwent an exploratory laparotomy to repair the uterine defect and drainage of intrauterine blood clots. A bleeding diatheses work-up was completely within normal limits. Leuprolide acetate was started in an attempt to cease her menstrual function. A few days following hospital discharge, the patient was readmitted to the hospital with recurring hematometra, an enlarged uterus approximating 16 weeks in size, and an imminent uterine rupture as depicted by MRI. The options of hypogastric artery ligation or selective uterine arterial embolization were discussed, but they were deferred due to the patient's desire for future fertility and for conservative management. Diagnostic laparoscopy revealed an intact uterus without free fluid in the abdomen. Under laparoscopic guidance, the cervix was easily dilated followed by evacuation of 300 cc of intrauterine blood clots. Again no apparent mechanical outflow obstruction was found. A normal pelvic arteriography excluded the possibility of vascular malformation as the cause of the uncontrolled bleeding. After considerable discussion of the benefits of estrogen administration and the risk of stroke in a young woman with severe and uncontrolled DUB and history of migraine with aura, the decision was made to proceed with intravenous equine conjugated estrogen administration. After 24 hours of intravenous estrogen therapy, no improvement in uterine bleeding was noted, as reflected by a progressively enlarging uterus and by a significant reduction in hematocrit. Images from a repeat MRI

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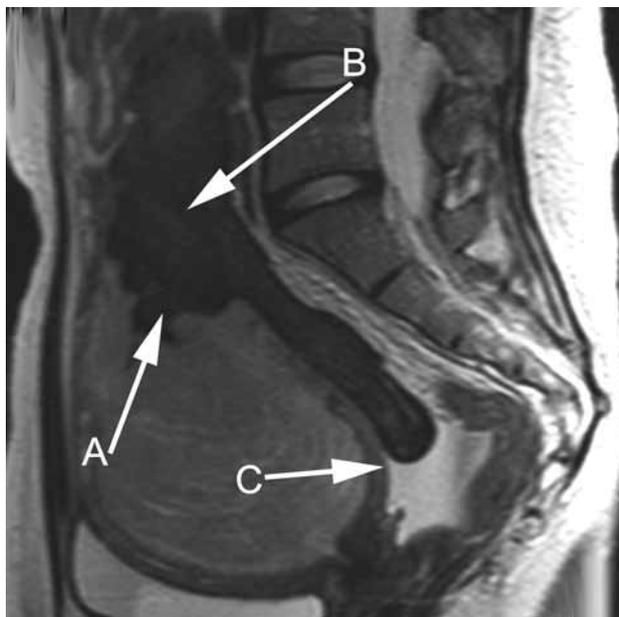


Figure 1. — T2 weighted magnetic resonance imaging depicted a markedly distended uterus filled with blood. Arrow A indicates the site of uterine wall rupture in the fundal area. Arrow B indicates blood clots extravasating into the abdominal cavity, and arrow C indicates the markedly thinned myometrium.

scan were consistent with recurrent uterine rupture. Furthermore, developing fever and increasing lower abdominal tenderness were consistent with endometritis. The patient received a blood transfusion followed by a supracervical hysterectomy. The final pathology revealed a 4.5 x 1.5 cm uterine wall defect with extensive myometrial edema and hemorrhage. Chronic endometritis and adenomyosis were also present.

## Discussion

The etiology of the unexplained hematometra formation in the patient described remains a mystery, especially given the previous history of normal menses since menarche and recent onset of self-perceived metromenorrhagia. It is plausible that the suspected endometritis, discovered during an earlier admission, might have resulted in cervical obstruction or sub-occlusion since endometritis has been suggested as a possible etiology for cervical obstruction and acute hematometra formation in a recent report [1]. However, this would not have justified the subsequent recurrent events. Other speculative but possible etiologies include: obstruction of the endocervical canal with debris, blood clots or endometrial tissue resulting in outflow obstruction and retention of menstrual blood within the uterine cavity. Similar to the patient we presented here, uncontrolled uterine bleeding, secondary either to dysfunctional uterine bleeding or intrauterine arterial malformation, have been reported to result in large hematometra in postmenopausal women and young women, respectively [3, 4]. The question remains whether in the setting of severe uterine bleeding, the

hematometra can still be formed despite a patent cervical canal. Unfortunately, a definite conclusion can not be drawn solely based on isolated case reports.

The most intriguing aspect of this case was the rapid reaccumulation of the blood in the uterine cavity despite the drainage of hematometra and advanced mechanical cervical dilation. Since no identifiable causes of outflow obstruction could be recognized, we suggested that the over-distended uterine cavity might have resulted in permanent functional impairment leading to hypocontractility of the myometrium and inability to expulse retained blood in the uterine cavity. The analogy can be compared to a deflated balloon unable to return to the initial maximum contractile state. The patient's history of persistent vaginal bleeding and the observation of an easy cervical dilation corroborated the absence of outflow obstruction. Donnez *et al.* have recently reported a case of didelphic uterus with an obstructed hemivagina resulting in ipsilateral hematocolpos and hematometra. Despite the resection of the vaginal septum, hematometra recurred in the hemiuterus in the absence of mechanical obstruction. The same author has suggested a probable loss of contractility of the myometrium and absence of recovery of normal uterine cavity after over distension as the cause of recurrent hematometra. If the hypothesis is proven to be correct, due to the potential damage of the myometrium with an over-distended uterine cavity, early detection and treatment of a large hematometra might be a logical approach to prevent its recurrence. Further studies are needed to confirm the speculation.

Uterine rupture in a non-gravid and unscarred uterus is exceedingly rare. Spontaneous uterine rupture has been reported in young nulligravida women presenting with arterial malformations [3]. Previous reports have acknowledged an association between uterine rupture and adenomyosis, with the inference that characteristic cyclic changes from these "invaded endometrial glands and stroma" may result in chronic inflammation, hemorrhage, and tissue necrosis of the native myometrial layer. This may lead to compromised tissue integrity and consequent myometrial tearing [5]. Although adenomyosis was found in the uterine specimen of the patient presented here, there is currently not enough evidence to establish a direct causal association between adenomyosis and uterine rupture. Theoretically, the intermittent uterine wall distension might have predisposed the infiltration of endometrial cells deep into the myometrium, thus accounting for the adenomyosis lesion found within the myometrium in the case described. While the plausible explanation for the uterine rupture might be obvious – excessive intraluminal pressure exceeding the distensible capacity to weaken and thin the myometrium leading to its rupture – the underlying mechanism leading to large hematometra formation remains elusive in the absence of evident lower genital tract obstruction.

Estrogen therapy is the mainstay in the treatment of life-threatening or uncontrolled DUB in women of reproductive age. Given the patient's history of migraines with aura, estrogen therapy was medically contraindicated due

to the presumed increased risk of stroke. Evidence from a meta-analysis of six case-control studies suggested that patients with migraines who use estrogen therapy were two to four times more likely to have an ischemic stroke as compared to non-user migraine patients; the risk is even greater when migraines are accompanied by aura [6]. While there is consensus to avoid estrogen therapy in women of reproductive age with migraines, the controversy remains as to whether or not estrogen therapy, when medically indicated, could be safely used in selective young healthy females having migraines with aura [7]. Although speculative, it is possible that early intervention with intravenous estrogen could have ameliorated the patient's uncontrolled DUB and prevented the progressively enlarging hematometra formation leading to uterine rupture. Traditionally, progestin has been successfully used as an alternate to estrogen for the control of DUB when estrogen is medically contraindicated. Intermittent vaginal bleeding might occur while on progesterone therapy due to endometrial atrophy, but rarely does uncontrolled and persistent bleeding requiring multiple blood transfusions occur, as is presented here.

The physiopathology behind the uncontrolled bleeding of our patient is unclear. The patient reported here failed to respond to pharmacological suppression of the endometrium and the uterine bleeding continued despite the use of estrogen, progesterone or GnRH agonist. A similar experience was reported by Donnez *et al.*, to prevent hematometra recurrence in one of the hemiuteri before the definitive surgical removal [2]. Shreikant *et al.* reported a similar unsuccessful experience to control the endometrial bleeding in a postmenopausal woman presenting with recurrent hematometra using a high dose of medroxyprogesterone acetate [4]. Whether a different pharmacological regimen would be more effective in preventing the recurrence of unexplained hematometra is currently unknown. In our patient, hysterectomy was eventually indicated due to the uncontrolled bleeding and ascending pelvic infection. The optimal treatment for hematometra in the absence of mechanical obstruction remains an area for further exploration.

## Conclusion

In summary, this case illustrated persistent hematometra in a young nulligravida woman in the absence of any evident mechanical obstruction of the lower genital tract. The challenging aspect of the management was the ineffectiveness of a conservative approach with subsequent development of recurrent hematometra in a young patient who desired fertility preservation. The uncontrolled and recurrent nature of her uterine pathology led to uterine rupture and eventually necessitated hysterectomy as the definitive treatment. The optimal treatment for the unexplained hematometra deserves further investigation.

## References

- [1] Sherer D.M., Gorelick C., Gupta A., Dalloul M., Sokolovski M., Zinn H.L. *et al.*: "Acquired hematometra and hematomtrachelos in an adolescent with dysfunctional uterine bleeding". *J. Ultrasound Med.*, 2006, 25, 1599.
- [2] Donnez O., Jadoul P., Squifflet J., Donnez J.: "Difelphic uterus and obstructed hemivagina: recurrent hematometra in spite of appropriate classic surgical treatment". *Gynecol. Obstet. Invest.*, 2007, 63, 98.
- [3] Chan K.S., Tan C.K., Mak C.W., Chia C.C., Kuo C.Y., Yu W.L.: "Computed tomography features of spontaneously perforated pyometra: a case report". *Acta Radiol.*, 2006, 47, 226.
- [4] Bollapragada S.S., Sikhar S., Rae D.W., Walker R.E.: "Unexplained postmenopausal hematometra". *Acta. Obstet. Gynecol. Scand.*, 2006, 85, 121.
- [5] Ding D.C., Chu T.Y., Liu J.Y.: "Menstruation-induced uterine rupture". *Int. J. Gynaecol. Obstet.*, 2000, 69, 171.
- [6] Curtis K.M., Mohllajee A.P., Peterson H.B.: "Use of combined oral contraceptives among women with migraine and nonmigrainous headaches: a systematic review". *Contraception*, 2006, 73, 189.
- [7] Tzourio C., Kittner S.J., Bousser M.G., Alperovitch A.: "Migraine and stroke in young women". *Cephalalgia*, 2000, 20, 190.

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# Emergent intrauterine resuscitation in a fetus with transient congenital anemia - case report

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## Summary

A 24-week gestation complicated by fetal hydrops was prepared for intrauterine transfusion to treat possible fetal anemia. During the therapeutic transfusion procedure, blood samples could be not taken from the umbilical vein, and severe fetal bradycardia developed. Given the severity of the condition and the fetal position, transfusion was performed through the most accessible part of the fetal heart, which was the right atrium. Just after the transfusion procedure, fetal cardiac tamponade developed, and the fetal heart collapsed. Subsequently, urgent fetal pericardiac tamponade decompression was performed.

*Key words:* Cordocentesis; Intrauterine transfusion; Cardiac tamponade; Pericardiocentesis.

## Introduction

Intrauterine intravenous transfusion can be performed in the treatment of fetal hydrops complicated with fetal anemia. The procedure has complications like fetal loss, vascular damage, fetal bradycardia, and preterm labor [1]. When it is not possible to perform the transfusion through the umbilical vein, intracardiac transfusion can be chosen as an alternative route which is more dangerous. The intracardiac transfusion procedure has severe complications like fetal loss, bradycardia, asystole and pericardiac tamponade [2].

We report a case of intrauterine transfusion complicated with fetal bradycardia, which was overcome with intracardiac transfusion, but complicated with pericardiac tamponade and treated with urgent pericardiac tamponade decompression incidentally.

## Case Report

A 34-year-old woman was referred to our clinic in the 24<sup>th</sup> week of her gestation due to a diagnosis of fetal hydrops. In the detailed scan, fetal abdominal ascites, pleural effusion, a MCA-PSV level of 88.7 cm/s (> 2 MoM), a dirotic pattern in the umbilical artery blood flow and a pulsatile flow in the umbilical vein were detected. The indirect Coombs test was negative, and the patient was found to be immunized for toxoplasmosis, rubella and cytomegalovirus. Cordocentesis was performed. During the procedure, a small quantity of dark red fetal blood was removed from the umbilical vein. Hematological and serological parameters were: hemoglobin (Hb) 1.7 g/dl, hematocrit (Hct) 5.14%, a negative result for Parvovirus B19 IgM and a positive result for IgG. Due to severe fetal anemia, we decided to perform intrauterine intravascular transfusion the following day. A Type O, Rh (-), freshly packed, cytomegalovirus antibody-negative, irradiated erythrocyte suspension with Hb of 22.6 g/dl and 62.8% Hct was prepared. Pancuronium bromide was used to paralyze the fetus. Using a 22-gauge spinal needle,

we twice attempted to aspirate blood in order to measure the initial Hb level. Despite the correct positioning of the tip of the needle in the umbilical vein of the transplacental cord, no sample could be taken. Meanwhile, acute fetal bradycardia lasting more than 60 seconds and vasospasm were detected, so we decided to execute an emergent intrauterine intracardiac transfusion instead of accessing the hepatic portion of the umbilical vein. We immediately entered the amniotic cavity with a new 22-gauge spinal needle and directed it to the fetal heart. Given the position of the fetus, it was impossible to puncture either of the ventricles, so we penetrated the right atrium as the most accessible heart region and took a blood sample (fetal Hb level was not measured and Hct level was 2.9%), and we then transfused 150 ml of erythrocyte suspension into the right atrium. After the transfusion, the fetal heart rate recovered to normal. After removing the needle, an echo lucent area developed around the fetal heart. The heart started flickering, and heart dimensions decreased. Pericardiac tamponade was therefore strongly suspected, and in response, the pericardiac area was again penetrated with the spinal needle, and 6 ml of blood was promptly taken. Thereafter, we observed that the heart became dilated, the dimensions returned to normal and fetal cardiac activity improved within a few seconds. After the transfusion, fetal Hb and Hct levels and MCA-PSV were all normal. Routine follow-up was performed. Two weeks after the transfusion oligohydramnios was detected. Thereafter, the amniotic fluid index (AFI) was increased to 18 cm on the third week of transfusion and increased echogenity was detected, possibly due to intraamniotic bleeding. Six weeks after the transfusion AFI was 12 cm.

At the 32<sup>nd</sup> gestational week, two months after intrauterine transfusion, pleural effusion and increased MCA-PSV (66.4 cm/s) were diagnosed and a second transfusion through the intravascular route was successfully performed. At the 35<sup>th</sup> week of pregnancy, due to premature rupture of membranes and a previous cesarean scar, a cesarean section was performed. At the end of the first postpartum month, the baby developed anemia, and a third transfusion was performed postnatally. The pediatric hematology unit made a diagnosis of transient intrauterine hematopoiesis repression, possibly due to Parvovirus B19 infection. After two years of follow-up, the child was healthy and had normal developmental scores.

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## Discussion

We have reported an unusual case in which an intrauterine intravascular transfusion performed to treat severe anemia was adjusted to an intracardiac transfusion procedure upon complication by fetal cardiac tamponade, and which we managed with urgent pericardiocentesis. Several points warrant discussion. First, regarding the repeated cordocentesis on the running day, Mari *et al.* [3] reported a false-positive rate of 12% for MCA-PSV. Therefore, we suspected a possible false-positive MCA-PSV and decided to perform a two-step approach for diagnosis and transfusion. In our opinion with this approach, we obviate preparing blood due to a false indication.

Another point related to repeated cordocentesis is the risk of fetal loss in a hydropic fetal condition. However, this risk is actually not that high when the procedure is performed correctly. Previously, van Kamp *et al.* [1] reported perinatal loss rates per procedure in the absence and presence of fetal hydrops as 1.4% and 2.5%, respectively. Nonetheless, the failure of the second cordocentesis procedure could be attributed to severe fetal anemia and low intravascular pressure as well as local vasospasm that might have developed due to the procedure.

Another event of this case was that the intrauterine intracardiac transfusion was performed via the right atrium. The safety of ventricular punctures, especially the apical part of the left ventricle, has previously been reported [4, 5]. This should be the site of choice both anatomically and physiologically because it is safe and distant from the great arteries and the transfused blood will easily traverse to the fetal brain and fetal coronaries, as well as general circulation and the placenta. Although when transfusion via the umbilical vein fails, the intrahepatic route should be considered as a secondary option for access. However in our case, due to the speed of events, gravity and urgency of the life-threatening condition, we entered into the closest and most accessible part of the fetal heart that the fetal position permitted. Westgren *et al.* [2] reported two cases of right atrium puncture during intracardiac transfusion. One of their cases was complicated with hemopericardium, while the other case was complicated with severe fetal bradycardia. In our opinion, this case and Westgren *et al.*'s experience suggest that great caution should be taken with puncture of the right atrium, which might end with hemopericardium collection. The most common complications of intracardiac transfusion are severe bradycardia and asystole, which might occur even when the transfusion is performed

through the left ventricle. Another serious complication following intracardiac transfusion is fetal death [6, 7].

The fluctuation in the AFI after the transfusion could be related with the oliguric and polyuric resolution phases of acute renal failure of the fetus due to severe anemia. An important clinical observation is that fetal physiologic reactions may mimic the same physiological renal response as in adults in the case of severe fetal hypotension. This observation can be re-evaluated in experimental animal models to make a strict conclusion.

In summary, this case shows that in cases of fetal hydrops and severe fetal anemia, when intravascular transfusion is complicated, fetal intracardiac transfusion could be life-saving. As a caution, when intracardiac transfusion is selected, the ventricles should be the main or ideal targets of the needle. If the right atrium is chosen instead, the risk of cardiac tamponade development should be taken into account, and the operator must be prepared for urgent pericardiocentesis.

## References:

- [1] van Kamp I.L., Klumper F.J., Oepkes D., Meerman R.H., Scherjon S.A., Vandenbussche F.P., Kanhai H.H.: "Complications of intrauterine intravascular transfusion for fetal anemia due to maternal red-cell alloimmunization". *Am. J. Obstet. Gynecol.*, 2005, 192, 171.
- [2] Westgren M., Selbing A., Stangenberg M.: "Fetal intracardiac transfusions in patients with severe rhesus isoimmunisation". *Br. Med. J.*, 1988, 296, 885.
- [3] Mari G., Deter R.L., Carpenter R.L., Rahman F., Zimmerman R., Moise K.J. Jr. *et al.*: "Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses". *N. Engl. J. Med.*, 2000, 342, 9.
- [4] Rodeck C.H., Roberts L.J.: "Successful treatment of fetal cardiac arrest by left ventricular exchange transfusion". *Fetal Diagn. Ther.*, 1994, 9, 213.
- [5] von Kaisenberg C.S., Grebe S., Schleider S., Kuhling-von Kaisenberg H., Venhoff L., Meinhold-Heerlein I.: "Successful intrauterine intracardiac transfusion in monozygotic twins affected by parvovirus B-19". *Fetal Diagn. Ther.*, 2007, 22, 420.
- [6] Elliott J.P., Foley M.R., Finberg H.J.: "In utero fetal cardiac resuscitation: a case report". *Fetal Diagn. Ther.*, 1994, 9, 226.
- [7] Westgren M., Jabbar F., Larsen J.F., Rahman F., Selbing A., Stangenberg M.: "Introduction of a programme for intravascular transfusions at severe rhesus isoimmunization". *J. Perinatal Med.*, 1988, 16, 417.

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# Chondrosarcoma in the left hemipelvis imitating a pelvic ovarian mass in pregnancy: a case report

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## Summary

*Purpose of investigation:* With the advent of routine sonography in pregnancy there has been an increase in the incidence of adnexal/pelvic masses. The differential diagnosis is most commonly ovarian. The complexity of diagnosing a pelvic mass in pregnancy is discussed. Chondrosarcomas most commonly occur in the pelvis and are rare in pregnancy. The clinical presentation and management in pregnancy are discussed. *Presentation:* We report on a case of pelvic chondrosarcoma in pregnancy imitating a pelvic ovarian mass on imaging. *Conclusion:* Clinicians should be aware of this diagnosis among the differentials of a pelvic mass presenting in pregnancy to enable timely and appropriate treatment.

*Key words:* Chondrosarcoma; Pregnancy.

## Introduction

Primary bone and soft-tissue tumors occur rarely in pregnancy. Though chondrosarcoma most commonly involves the pelvis, its occurrence in pregnancy is rare. The treatment of pelvic chondrosarcoma presents a challenging problem in musculoskeletal oncology. This may be hampered by a coexistent pregnancy.

We report the case of a woman who in the 20<sup>th</sup> week of pregnancy presented with a complaint of pain and numbness in the left lower limb approximately ten weeks after an apparently innocuous mass had been detected on dating (US) scan.

## Case Report

A woman presented at ten weeks' gestation in a second ongoing pregnancy. At her dating ultrasound (US) scan in the region of the left ovary an 8 cm complex cystic mass was demonstrated. Tumour marker CA-125 was slightly raised at 36 (normal range < 35). At her 20-week detailed scan, the cyst was reassessed with no observed changes. At this point she complained of pain and numbness in the left lower limb. She was managed conservatively with further US scans showing no changes. A vaginal delivery was planned and she had a ventouse delivery at 40 weeks of gestation with an uneventful postnatal period.

At six weeks postpartum follow-up the patient complained of increasing left hypochondrial pain and numbness of her thigh. On physical examination a firm irregular mass was noted attached to the left iliac crest in the left hypochondrium. Transvaginal ultrasound (TVS) showed a normal postpartum uterus, normal ovaries and no free fluid. An urgent staging computed tomography (CT) of the abdomen and pelvis showed a large soft tissue mass in the left iliac fossa in close relation to the left iliac wing with focal areas of calcification within the mass. An exophytic lesion was seen leading into the soft tissue mass. There was no evidence of invasion into adjacent abdominal and pelvic structures, retroperitoneal or pelvic lymphadenopathy.

Chondrosarcoma was entertained as a diagnosis. Following a multidisciplinary discussion the patient was urgently referred to the regional oncology orthopaedic centre.

At the centre arrangements were made to have full staging studies. A magnetic resonance imaging (MRI) scan showed a large chondroid tumour within the left hemipelvis arising from a pre-existing osteochondroma. CT chest scan showed a small nodule in the right lobe. A bone scan did not show any bony metastases. Histology of the tumour biopsy showed a low-grade chondrosarcoma. The patient subsequently underwent excision of the pelvic chondrosarcoma, which was confirmed as grade 1 chondrosarcoma. She made a satisfactory postoperative recovery. In view of the nodule noted on CT chest scan a multidisciplinary decision was made to repeat the scan in three months.

## Discussion

Pelvic masses in pregnancy have an overall incidence of four percent [1]. Most of these however, are ovarian in origin [1]. The advent of routine sonography in early pregnancy has led to an increase in the incidence of pelvic masses. The diagnosis is often made on clinical examination or following an incidental finding during a routine ultrasound. However the clinical examination of a pelvic mass in pregnancy may be hampered by a co-existing gravid uterus.

Chondrosarcoma, a soft tissue tumour, most commonly occurs in the pelvis. It rarely occurs in pregnancy. Gestational age at diagnosis has been shown to range from 11 weeks to two months postpartum. Chondrosarcomas have been known to grow in size during pregnancy [2]. Diagnosis may be delayed in pregnancy as initial non specific complaints such as pain, discomfort or numbness are often presumed to be symptoms of pregnancy [2].

Diagnosis is primarily made by imaging. This is compounded by the wide spectrum of characteristics that may present. MRI shows the extent of intraosseous and soft tissue involvement preoperatively [3]. CT is especially recommended for imaging in the pelvis to enable discer-

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nation of the pattern of bone destruction and presence of matrix mineralisation [3]. Radiotherapy may be required following incomplete resection, and chemotherapy may be effective in mesenchymal chondrosarcoma [3].

Treatment aims to achieve complete resection of the tumour with a wide en bloc excision the preferred surgical treatment. This includes hemipelvectomy and less invasive limb salvage procedures with less associated morbidity [4]. Surgery may be safely performed in pregnancy while deferring potentially toxic therapies to the puerperium [5]. Recurrence of pelvic chondrosarcoma may be difficult to manage, and the best outcome is associated with clear margins at the time of initial tumour resection [6]. Radical surgery in recurrences may be associated with a 50% long-term survival [6]. Spontaneous vaginal delivery has been described in women following hemipelvectomy [7].

Outcome and survival is influenced by tumour stage and the surgical margin achieved. Factors associated with poor outcome in high-grade tumours include pelvic location, local recurrence, tumour size greater than 100 cm<sup>2</sup>, aneuploidy, histological grade of 3 and a dedifferentiated type of tumour [8]. Adequate surgical excision is a determinant for a favourable outcome in low-grade chondrosarcoma [8].

Though the occurrence of chondrosarcoma is rare in pregnancy, clinicians should be aware of this possibility when a pelvic mass is diagnosed antenatally. The association with non responsive pain to analgesics or numbness in the pelvis may be due to tumour in the pelvic bone. Optimal outcome would involve appropriate investiga-

tions and an early multidisciplinary approach to treatment with involvement of an obstetrician and orthopaedic oncologist.

## References

- [1] Spencer C.P., Robarts P.J.: "Management of adnexal masses in pregnancy". *Obstet. Gynaecol.*, 2006, 8, 14.
- [2] Komiya S., Zenmyo M., Inoue A.: "Bone tumours in the pelvis presenting growth during pregnancy". *Arch. Orthop. Trauma Surg.*, 1999, 119, 22.
- [3] Gelderblom H., Hogendoorn P.C., Dijkstra S.D., Rijswijk C.S., Krol A.D., Taminiou A.H. *et al.*: "The clinical approach towards chondrosarcoma". *Oncologist.*, 2008, 3, 320.
- [4] Pring M.E., Weber K.L., Unni K.K., Sim F.H.: "Chondrosarcoma of the pelvis. A review of sixty-four cases". *J. Bone Joint Surg. Am.*, 2001, 83, 1630.
- [5] Maxwell C., Barzilay B., Shah V., Wunder J.S., Bell R., Farine D.: "Maternal and neonatal outcomes in pregnancies complicated by bone and soft-tissue tumors". *Obstet. Gynecol.*, 2004, 104, 344.
- [6] Weber K.L., Pring M.E., Sim F.H.: "Treatment and outcome of recurrent pelvic chondrosarcoma". *Clin. Orthop. Relat. Res.*, 2002, 397, 19.
- [7] Heetkamp A., Fijians H.W., Papatsonis D.N.: "Spontaneous delivery after hemipelvectomy because of chondrosarcoma: a case report and review of the literature". *Am. J. Perinatol.*, 2008, 25, 255.
- [8] Lee F.Y., Mankin H.J., Fondren G., Gebhardt M.C., Springfield D.S., Rosenberg A.E. *et al.*: "Chondrosarcoma of bone: an assessment of outcome". *J. Bone Joint Surg. Am.*, 1999, 81, 326.

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# D-dimer levels as a predicting factor for DIC following single twin death: a case report and review of the literature

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## Summary

**Background:** Intrauterine death of one twin in the second or third trimester occurs in about 1% of all twin pregnancies. The retention in utero of the dead fetus may be associated with maternal disseminated intravascular coagulation. **Case:** We present the case of a diamniotic monochorionic pregnancy with intrauterine death of the first twin at 24 weeks. The pregnancy reached 33 weeks. In our case although all coagulation factors were within normal limits, D-dimer levels were significantly high, without any evidence though of any clotting problems to the mother. **Conclusion:** The role of D-dimers is practically unknown in multiple pregnancies. It seems that the interpretation of elevated D-dimer levels is still of limited value for prediction or prognosis of thromboembolic complications of multiple pregnancies.

**Key words:** D-dimers; Twin pregnancy; DIC; Intrauterine death.

## Introduction

Intrauterine death of one twin in the second or third trimester occurs in about 1% of all twin pregnancies [1]. In monochorionic twins the most common cause for this complication is the twin to twin transfusion syndrome (TTS). Intrauterine death of a fetus in a monochorionic pregnancy may be associated with adverse outcome for the co-twin. The risk of death or neurological handicap in such cases is at least 30% to the surviving twin due to hypotension, vascular occlusion due to thrombi or hemorrhage resulting from coagulation disorders [1]. The interval between the occurrence of intrauterine death and organ damage varies and cerebral complications can even predate fetal demise. The death of one twin in utero will usually stimulate uterine activity and most pregnancies will deliver within three weeks, usually resulting in prematurity and compounding problems in the surviving co-twin [2]. Retention in utero of a dead fetus may lead to maternal disseminated intravascular coagulation (DIC). The thromboplastin-like material released from the necrotic fetal tissue may activate the maternal coagulation system, resulting in hemostatic failure.

Prior to 34 weeks of gestation most centers would advocate administration of antenatal steroids, tocolysis and conservative management with continuous fetal monitoring until 34 weeks. The evidence is not clear regarding the best mode of delivery and if it affects the outcome of the surviving twin [3, 4].

We present the case of a diamniotic monochorionic pregnancy with intrauterine death of the first twin at 24 months plus three weeks. Pregnancy reached 33 weeks and the patient delivered normally. Clotting parameters and especially D-dimer levels were monitored in order to predict DIC of the mother.

## Case Report

A 34-year-old para 1 woman with a diamniotic monochorionic twin pregnancy was referred at 24 weeks and three days to the emergency clinic of our department with the diagnosis of intrauterine death of one twin. She had no past medical history. Her first pregnancy was uncomplicated and she delivered vaginally at 39 weeks. According to her medical records the detailed ultrasound (US) scan at 23 weeks showed severe twin-to-twin transfusion syndrome (TTS), with the donor twin weighing 220 g with absent end-diastolic flow, and the recipient twin weighing 380 g with normal Doppler studies. On examination the intrauterine death of the one twin (twin B) was confirmed and the remaining twin (twin A) showed normal cardiac activity, cephalic presentation, normal amniotic fluid and growth (490 g) and normal Doppler studies of umbilical artery and ductus venosus. A more detailed scan revealed enlargement of the heart without any structural defects, occupying half of the chest cavity, which was attributed to the TTS. The blood work-up results upon admission were reassuring apart from D-dimer levels (Hb: 10.6 g/dl, WBC: 9.8 10<sup>3</sup>/μl, PLT: 331 10<sup>3</sup>/μl, INR: 0.9, PT: 9.7 sec, aPTT: 28 sec, fibrinogen: 451 and D-dimer: 7702 μg/l). The patient was subsequently admitted to the high dependency unit and was started on enoxheparine (40 mg s.c. daily). Thorough information was given to the parents about the possible risks for the remaining fetus and the maternal wellbeing due to the risk of DIC. Clotting status and full blood count were checked every three days. No significant changes were observed during hospitalization apart from D-dimer levels gradually falling to plateau at 2440-2550 μg/l at 28 weeks of gestation (Table 1). Betamethazone (24 mg IM divided in two doses) was administered within 24 hours from admission. Detailed US scans were performed weekly to assess fetal status and wellbeing. Fetal growth, cardiac activity, fetal movements, amniotic fluid and Doppler studies were normal. The pregnancy reached 33+0 weeks before spontaneous contractions started during the night and despite intravenous tocolysis (ritrodine) administration, the patient delivered vaginally within three hours a live male infant weighing 1,810 g with Apgar scores of 6 at 1 min and 8 at five min. The infant was admitted to the neonatal intensive care unit for observation. The mother's post-

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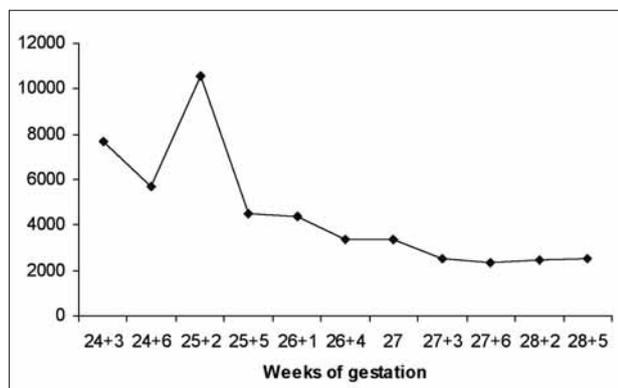


Figure 1. — D-dimer levels.

partum hospitalization was uneventful and the infant was discharged after 12 days. The infant showed no obvious clinical signs of neurological problems, although head circumference was smaller than the 3<sup>rd</sup> centille. US scan of the brain was not satisfactory due to the small anterior fontanelle. Subsequent brain magnetic resonance imaging (MRI) showed calcifications and enlargement of the right and left ventricles. Follow-up appointments were arranged by the neonatologists for further evaluation of the infant.

## Discussion

Intrauterine fetal death leads to a gradual depletion of maternal coagulation factors. Thromboplastic substances are released into maternal circulation and may trigger DIC, which occurs in about one-third of patients who retain a dead fetus in utero for longer than four to five weeks [5].

Increased D-dimer levels indicate increased fibrinolysis following fibrin formation. D-dimer assays are used to measure fibrin degradation products and they have been a useful marker for the early diagnosis of DIC and thromboembolism [6]. D-dimer is formed by plasmin-mediated proteolysis of cross-linked fibrin. D-dimer levels have been found to increase significantly in normal pregnant women in comparison to non pregnant women [7-9]. This can be attributed to the physiological hemostatic balance displacement towards hypercoagulability. In multiple gestations this imbalance in hemostasis is even more exaggerated [10]. The physiological alterations associated with multiple gestations, initiated by placental and fetal production of proteins and steroids, can be another reason for the increased levels of D-dimer occurring. Bar *et al.* showed that D-dimer levels in 49 women with normal twin pregnancies were significantly higher than in women with singleton pregnancies [11]. Morikawa *et al.* studied 48 normal singleton and twin pregnancies and concluded that there was a progressive increase in prenatal D-dimer levels, which is consistently higher in women with twin pregnan-

cies [10]. Enhanced coagulation-fibrinolysis activity that normally occurs during late gestation is more exaggerated in women with twin pregnancies [12, 13].

In the present case, although all coagulation factors were within normal limits, D-dimer levels were significantly increased, without occurrence of clotting problems (DIC) in the mother. The clinical significance and etiology of increased D-dimer levels in normal singleton pregnancies is unclear and practically unknown in multiple pregnancies. It seems that the interpretation of elevated D-dimer levels is still of limited value for prediction or prognosis of thromboembolic complications of multiple pregnancies.

## References

- [1] Sebire N., Nikolaides K.: "Multiple pregnancy. The 11-13+6 weeks scan". *Fetal Medicine Foundation*, 2004, 4, 95.
- [2] Cameron A., Macara L., Brennan J., Milton P.: "Twin pregnancy. Fetal medicine for the MRCOG and Beyond". *RCOG press* 2002, 9, 123.
- [3] Ong S.S.C., Zamora J., Khan K.S., Kilby M.D.: "Prognosis for the co-twin following single-twin death: a systematic review". *BJOG*, 2006, 113, 992.
- [4] Clearly-Goldman J., D'Alton M.: "Management of single fetal demise in a multiple gestation". *Obstet. Gynecol. Surv.*, 2004, 59, 285.
- [5] Letsky E.: "Coagulation defects in pregnancy and the puerperium. Turnbull's Obstetrics". *Third edition Churchill Livingstone*, 2002, 20, 311.
- [6] Hellgren M.: "Hemostasis during normal pregnancy and puerperium". *Semin. Throm. Hemost.*, 2003, 29, 125.
- [7] Duchinski T., Miedzinska P., Szczepanski M.: "Hemostatic variables in patients with intrauterine fetal death". *Int. J. Gynecol. Obstet.*, 1993, 42, 3.
- [8] Francalanci I., Comeglio P., Liotta A.A., Cellai A.P., Fedi S., Parretti E. *et al.*: "D-dimer concentrations during normal pregnancy as measured by ELISA". *Thromb. Res.*, 1995, 78, 399.
- [9] Nolan T.E., Smith R.P., Devoe L.D.: "Maternal plasma D-dimer levels in normal and complicated pregnancies". *Obstet. Gynecol.*, 1993, 81, 235.
- [10] Morikawa M., Takashi Y., Turuga N., Cho K., Yamada H., Sakuragi N. *et al.*: "Coagulation-fibrinolysis is more enhanced in twin than in singleton pregnancies". *J. Perinat. Med.*, 2006, 34, 392.
- [11] Bar J., Blickstein D., Hod M., Bar-Hava I., Ben-Rafael Z., Rahmay-Babai J. *et al.*: "Increased D-dimer levels in twin gestation". *Thrombosis Research*, 2000, 485.
- [12] Epiney M., Boehlen F., Boulvain M., Reber G., Antonelli E., Morales M. *et al.*: "D-dimer levels during delivery and the postpartum". *J. Thromb. Haemost.*, 2005, 3, 268.
- [13] Petersen I.R., Nyholm H.C.: "Multiple pregnancies with single intrauterine demise. Description of twenty-eight pregnancies". *Acta Obstet. Gynecol. Scand.*, 1999, 78, 202.

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# Heterotopic pregnancy: case report

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## Summary

Heterotopic pregnancy is the simultaneous development of an intrauterine pregnancy and ectopic pregnancy. It is a potentially fatal condition and rarely occurs in natural conception cycles. A high incidence of heterotopic pregnancy is reported in pregnancies following an assisted reproduction technique (ART) with embryo transfer in utero. We report the case of heterotopic pregnancy via ART in a 42-year-old primigravida. She presented with pelvic pain and intraabdominal fluid collection. She was treated with laparoscopic surgery. At present the intrauterine pregnancy is in normal evolution.

*Key words:* Heterotopic pregnancy; Ectopic pregnancy; Laparoscopy; Pelvic pain; Hemoperitoneum.

## Introduction

When a diagnosis of ectopic pregnancy is made, the coexistence of an intrauterine pregnancy and ectopic pregnancy should never be excluded if the pregnant patient has undergone ART and has painful symptomatology and hemoperitoneum.

## Case Report

A 42-year-old primigravida was admitted the Obstetrics Emergency Room of the University General Hospital "G. Martino", Department of Obstetrics and Gynecology, Messina. The patient was in the 7<sup>th</sup> week of gestation via assisted reproductive technology (ART). She presented at our hospital due to pelvic pain after an ultrasound (US) scan had been performed by her gynecologist.

The US showed an intrauterine gestational sac containing a sole embryo with a subchorionic hematoma; cardiac activity was noted. In the left adnexa there was a complex and very suspicious image due to a non evolutive ectopic pregnancy. Ten years before the patient underwent laparoscopic surgery for a left ovarian endometriotic cyst.

After hospital admission the patient was subjected to blood tests: red blood cell count was 3,740,000 mmc; hemoglobin 11.3 g%; and  $\beta$ hCG 12,778 mIU/ml. The patient's general condition was good; she was normotensive with normal temperature and cardiac activity. Examination revealed lower abdominal tenderness, mainly in the lower left quadrant without muscular rigidity. Pelvic examination revealed an anteverted, enlarged, and floating uterus. A tender painful adnexal mass was palpable on the left fossa during bimanual examination, and cervical lateral movement was painful. No vaginal bleeding nor contractions were present.

The blood test was repeated 12 hours after hospitalization: red blood cells: 2,780,000 mmc, hemoglobin 8.4 g (- 2 g%).

US scan showed an enlarged uterus corresponding to an initial pregnancy with an intrauterine gestational sac containing a sole embryo; cardiac activity was noted. The gestational sac was surrounded by a 3 x 1.8 cm subchorionic hematoma. The Retzius space was filled with blood. The pouch of Douglas was

completely filled, partly with fluid and partly with coagulated blood. In the left adnexa a 2.4 x 2.4 cm complex formation was found and an emergency laparoscopy was performed followed by therapy with progestins.

During laparoscopic optics a large quite quantity of partly coagulated blood in the pouch of Douglas (approximately 600 ml) and a modest amount of fluid and blood in the Retzius space were seen. The uterus was enlarged more than double and movable. The ectopic pregnancy was situated in the ampullary part of the integral left tube. The hemoperitoneum was cleared away and a left salpingo-oophorectomy was performed followed by washing with saline solution. The tube was taken out in a laparoscopic endo-bag. Abdominal US (postoperative day 2) revealed a sole 12 mm embryo and cardiac activity was noted. The hematoma found previously at the admission hospital had enlarged (3.6 x 2.5 mm).

A second abdominal US done on postoperative day 6 revealed a fetal crown-rump length (CRL) increase corresponding to the amenorrhea period, and there was normal fetal cardiac activity.

The hematoma was slightly reduced. A further US done on postoperative day 9 revealed a fetal CRL increase from 18 mm to 21.2 mm. Regular fetal cardiac activity and fetal movement were noted. The hematoma was 13 x 20 mm.

The patient underwent one last US which revealed regular fetal cardiac activity, CRL of 25 mm, and the hematoma reduced to 26 x 7 mm.

The patient was discharged and put on progestin therapy (hydroxyprogesterone caproate), intra-muscle ampoules (one every three days), folic acid, martial therapy and reconstituent solutions. She is followed regularly at the obstetrics clinic.

At present, the intrauterine pregnancy is in normal evolution and the progestin therapy has been suspended.

## Discussion

Heterotopic pregnancy is a rare type of ectopic pregnancy and occurs in various forms. The incidence of heterotopic pregnancy is estimated at 1:30,000 [1]. However, in the last 20 years there was been an almost four-fold increase in the incidence of ectopic pregnancy in the general population and a corresponding increase in the incidence of heterotopic pregnancy [2].

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Fig. 1

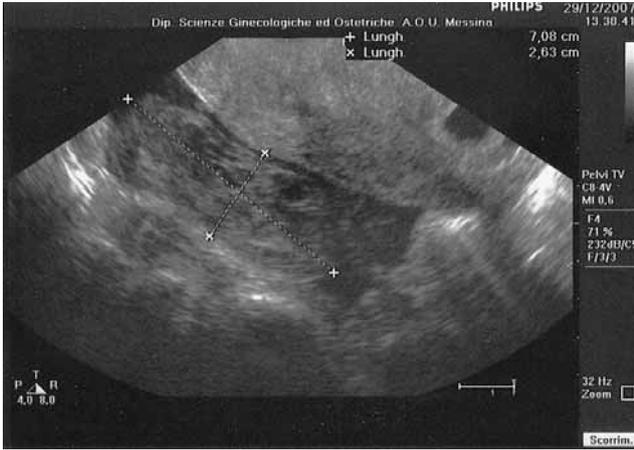


Fig. 2

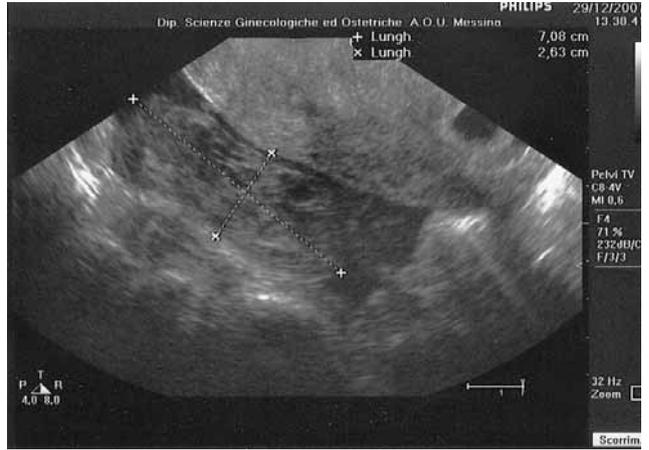


Fig. 3



Fig. 4



Fig. 5



Fig. 6



Fig. 7

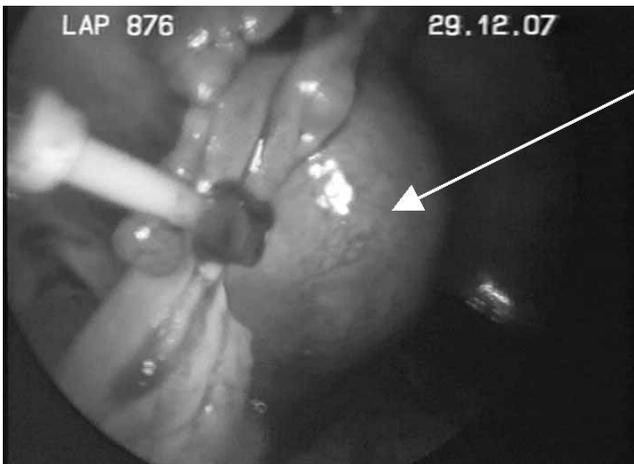


Figure 1. — Pelvic coagulated blood.

Figure 2. — Ectopic pregnancy.

Figure 3. — Ectopic pregnancy and hemoperitoneum.

Figure 4. — Subchorionic hematoma with intrauterine gestational sac.

Figure 5. — Intrauterine pregnancy.

Figure 6. — Hemoperitoneum.

Figure 7. — Left tubaric pregnancy.

This increase has been attributed to:

- increase in the incidence of pelvic inflammatory disease
- prevalent use of IUDS
- increase in tubal surgery, microsurgery
- pharmacologic ovulation stimulation
- ART
- endometriosis.

Each of these risk factors increases the risk for ectopic pregnancy from 2-7 times above the general population, with pelvic inflammatory disease having the most significant effect [3].

Heterotopic pregnancies are usually diagnosed between the 5<sup>th</sup> and 34<sup>th</sup> week of gestation: 70% of heterotopic pregnancies were diagnosed between the 5<sup>th</sup> and 8<sup>th</sup> week of gestation, 20% between the 9<sup>th</sup> and 10<sup>th</sup> week and 10% after the 11<sup>th</sup> week [4].

Our case was diagnosed at the 7<sup>th</sup> week of amenorrhea. Early diagnosis of a heterotopic pregnancy is often difficult because clinical symptoms are lacking. Usually, signs of an extrauterine pregnancy predominate. Reece *et al.* [1] defined four common presenting signs and symptoms for heterotopic pregnancy: abdominal pain, adnexal mass, peritoneal irritation and enlarged uterus.

Abdominal pain is reported in 83% of heterotopic pregnancies and hypovolemic shock with abdominal tenderness in 13%; half of the patients do not complain of vaginal bleeding [5-11]. In our patient vaginal bleeding was not present but she had pelvic pain and diffuse abdominal tenderness resulting from intraperitoneal bleeding without tubal breakage.

Transvaginal US is a safe aid in the diagnosis of heterotopic pregnancy, however sometimes the differential diagnosis between a tubaric pregnancy and hemorrhagic corpus luteum cyst is difficult [12-14].

Certainly US visualization of cardiac activity in both intrauterine and extrauterine gestations removes any doubts. Moreover, fetal cardiac motion can have a different time of onset [15-17]. In fact Reece *et al.* [1] described a case in which intrauterine heart motion was observed six days after the onset of extrauterine fetal heart activity. Serial samples of serum  $\beta$ hCG can be misleading in the diagnosis of ectopic pregnancy, whereas the presence of blood in the pelvic cavity and a survey of peripartum hemorrhage are certainly more revealing [18]. We do not believe that culdocentesis is helpful in the differential diagnosis because a US scan can easily aid in identifying the presence of peritoneal hemorrhage. Beyond all doubt the gold standard treatment both for ectopic and heterotopic pregnancy is laparoscopic surgery with minimal manipulation of the uterus [19-22].

In our case, we preferred laparoscopy, even if the hemoperitoneum was present, as the patient has innumerable benefits with laparoscopy, and also because our center has remarkable experience in laparoscopic surgery [23-28].

Our patient was submitted to a left laparoscopic salpingo-oophorectomy. Linear salpingo-oophorectomy was not done to avoid the possible persistence of tubaric trophoblastic tissue as it could have interfered with the serial

sampling of serum  $\beta$ hCG for the intrauterine pregnancy considering the patient's age and normal morphology of the contralateral tube [29-33]. The postoperative course has been regular without changes in vital parameters. The patient was discharged on postoperative day 10 and put on progestin therapy. Since then US scans have pointed out a remarkably decreased dysjunction area. In the intrauterine gestational sac, regular fetal cardiac activity and a gradual progressive and constant increase in fetal CRL are indices of normal evolution of the intrauterine pregnancy. At present, the patient is being followed by our department and her pregnancy is in normal evolution.

In conclusion, this was a rare case of heterotopic pregnancy occurring in a patient who conceived after ART (FIVET). The remote possibility of an intrauterine pregnancy should be suspected when confronted with a patient's chart characterized by pelvic pain and intraperitoneal fluid blood collection.

## References

- [1] Reece E.A., Petrie R.H., Sirmans M.F., Finster M., Todd W.D.: "Combind intrauterine and extrauterine gestations: a review". *Am. J. Obstet. Gynecol.*, 1983, 146, 323.
- [2] De Voe R.W., Pratt J.H.: "Simultaneous intrauterine and extrauterine pregnancy". *Am. J. Obstet. Gynecol.*, 1948, 56, 1119.
- [3] Westrom L.: "Incidence, prevalence and trends of acute pelvic inflammatory disease and its consequence in industrialized countries". *Am. J. Obstet. Gynecol.*, 1980, 138, 880.
- [4] Tal J., Hoddad S., Gordon N., Timor-Tritsch I.: "Heterotopic pregnancy after ovulation induction and assisted reproductive technologies: a literature review from 1971 to 1993". *Fertil. Steril.*, 1996, 66, 1.
- [5] Louis-Sylvestre C., Morice P., Chapron C., Duibuisson J.B.: "The role of laparoscopy in the diagnosis and management of heterotopic pregnancies". *Hum. Reprod.*, 1997, 12, 1100.
- [6] Lemus J.F.: "Ectopic pregnancy: an update". *Curr. Opin. Obstet. Gynecol.*, 2000, 12, 369.
- [7] Abusheikba N., Salba O., Brinssden P.: "Extrauterine pregnancy following assisted conception treatment". *Hum. Reprod. Update*, 2000, 5, 80.
- [8] Diallo D., Aubard Y., Piver P., Baudet J.H.: "Grossesse heterotopique: a propos de 5 cas et revue de la literature". *J. Gynecol., Obstet. Biol. Reprod.*, 2000, 29, 131.
- [9] Gruber I., Lahodny J., Illmensee K., Losch A.: "Heterotopic pregnancy: Report of three cases". *Wien Klin. Wochenschr.*, 2002, 114, 229.
- [10] Oliveira F.G., Abdelmassih V., Abdelmassih Oliveira S., Abdelmassih R., Nagy Z.P.: "Heterotopic triplet pregnancy: report and video of a case of a ruptured tubal implantation with living embryo concurrent with intrauterine twin gestation". *Reprod. Biomed. Online*, 2002, 5, 313.
- [11] Terzioglu N., Corse R., Streitmatter A., Feige A.: "Intact heterotopic pregnancy located simultaneously in the fallopian tube and the uterus". *Artemis.*, 2003, 4, 49.
- [12] Hirsch E., Cohen L., Hecht B.L.: "Heterotopic pregnancy with discordant ultrasonic appearance of fetal cardiac activity". *Obstet. Gynecol.*, 1992, 79, 824.
- [13] Robertson E.E., Smith W., Moye M.A., Brinssden P.R., Hansen J.N., Lewis P.M. *et al.*: "Reduction of ectopic pregnancy by injection under ultrasound control". *Lancet*, 1987, 1, 974.
- [14] Condous G., Okaro E., Khalid A., Lu C., Van Huffel S., Timmerman D., Bourne T.: "The accuracy of transvaginal ultrasonography for the diagnosis of ectopic pregnancy prior to surgery". *Hum. Reprod.*, 2005, 5, 1404.
- [15] Reynolds J.D., Booth J.V., De la Fuente S., Punhahitananda S., McMahon R.L., Hopkins M.B., Eubanks W.S.: "A review of laparoscopy for non-obstetric-related surgery during pregnancy". *Curr. Surg.*, 2003, 60, 164.

- [16] De la Fuente S.G., Pinheiro J., Gupta M., Eubanks W.S., Reynolds J.D.: "Early postnatal behaviour deficits after maternal carbon dioxide pneumoperitoneum during pregnancy". *Surg. Endosc.*, 2003, 17, 1823.
- [17] Daniel A., Dumesic M.D., Mark A., Damario M.D., Donna R., Session M.D.: "Interstitial heterotopic pregnancy in a woman conceiving by in vitro fertilization after bilateral salpingectomy". *Mayo Clin. Proc.*, 2001, 76, 90.
- [18] Varras M., Akrivis C., Hadjopoulos G., Antonius N.: "Heterotopic pregnancy in a natural conception cycle presentation with tubal rupture. A case report and review of the literature". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2003, 106, 79.
- [19] Habana A., Dokras A., Giraldo J.L., Jones E.E.: "Cornual heterotopic pregnancy: contemporary management option". *Am. J. Obstet. Gynecol.*, 2000, 182, 1264.
- [20] Pschera H., Gatterer A.: "Laparoscopic management of heterotopic pregnancy: A review". *J. Obstet. Gynaecol. Res.*, 2000, 3, 157.
- [21] Pasic R.P., Hammons G., Gardner J.S., Hainer M.: "Laparoscopic treatment of corneal heterotopic pregnancy". *J. Am. Assoc. Gynecol. Laparosc.*, 2002, 9, 372.
- [22] Sills E.S., Perloe M., Kaplan C.R., Sweitzer C.L., Morton P.C., Tucker M.J.: "Uncomplicated pregnancy and normal singleton delivery after surgical excision of heterotopic (corneal) pregnancy following in vitro fertilization/embryo transfer". *Arch. Gynecol. Obstet.*, 2002, 266, 181.
- [23] Chang Y., Lee J.N., Yang Ch., Hsu S.C., Tsai E.M.: "An unexpected quadruplet heterotopic pregnancy after bilateral salpingectomy and replacement of three embryos". *Fertil. Steril.*, 2003, 80, 218.
- [24] Hoopman M., Wilhelm L., Possover M., Nawroth F.: "Case report: heterotopic triplet pregnancy with bilateral tubal and intrauterine pregnancy after IVF". *Reprod. Biomed. Online*, 2003, 6, 345.
- [25] Soriano D., Shrim A., Seidman D.S., Goldenberg M., Mashiach S., Oelsner G.: "Diagnosis and treatment of heterotopic pregnancy compared with ectopic pregnancy". *J. Am. Assoc. Gynecol. Laparosc.*, 2002, 9, 353.
- [26] Oyawoye S., Chander B., Pavlovic B., Hunter J., Gadir A.A.: "Heterotopic pregnancy: successful management with aspiration of corneal/interstitial gestational sac and instillation of small dose of methotrexate". *Fetal. Diagn. Ther.*, 2003, 18, 1.
- [27] Siegel J.C., Jack R.E.: "Heterotopic pregnancy after two prior ectopic pregnancy: A case report". *J. Reprod. Med.*, 2006, 51, 729.
- [28] Abojji A.P., Fawole A.A., Adeniyi T.O.: "Heterotopic pregnancy: A case report". *Niger. J. Med.*, 2001, 10, 37.
- [29] Kelly A.J., Sowter M.C., Trinder J.: "The management of tubal pregnancy". *Clinical Guidelines. NHS Executive*, 2004.
- [30] Daponte A., Spyridakis M., Ioannou M., Vanakara P., Tzovaras G., Hatzitheofilou K., Messinis I.E.: "Live birth after laparotomy for concurrent heterotopic pregnancy and appendicitis in a 6 weeks IVF pregnancy". *Arch. Gynecol. Obstet.*, 2007, 275, 397.
- [31] BenAmi I., Panshi M., Ushakov F., Vaknin Z., Herman A., Raziell A.: "Recurrent heterotopic pregnancy after bilateral salpingectomy in an IVF patient: Case report". *J. Assist. Reprod. Genet.*, 2006, 23, 333.
- [32] Cholkeri-Singh A., Labarge A.: "Spontaneous heterotopic triplets: A case report". *Fertil. Steril.*, 2007.
- [33] Schroepfel T.J., Kothari S.N.: "Heterotopic pregnancy: A rare cause of hemoperitoneum and acute abdomen". *Arch. Gynecol. Obstet.*, 2006, 274, 138.

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# Idiopathic infantile arterial calcification: prenatal diagnosis and postnatal presentation

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## Summary

Idiopathic infantile arterial calcification (IIAC) is a rare disease of unknown etiology, which is characterized by arterial calcification. A 29-year-old primigravida at 33 weeks' gestation was referred for further evaluation for polyhydramnios. An ultrasonographic examination revealed an intrauterine growth restricted fetus, pericardial effusion, increased renal cortical echogenicity with sparing of corticomedullary differentiation, and diffuse arterial calcifications involving the aorta, pulmonary artery, common iliac arteries, renal arteries, and common carotid arteries. At 35 weeks of gestation a cesarean section was performed because of fetal distress. A 1,900 g male infant was delivered. Postnatal examination confirmed the diagnosis of IIAC with dysmorphic features (clinodactily and low-set ears) and normal constitutional karyotype. The baby died when he was four months old in the newborn care unit. During routine obstetric ultrasonography, the combination of polyhydramnios and intrauterine growth restriction may necessitate examination of the major vessels for presumptive a diagnosis of IIAC.

*Key words:* Artery; Calcification; Fetus; Prenatal Diagnosis.

## Introduction

Idiopathic infantile arterial calcification (IIAC) is a rare disease characterized by arterial calcification and intimal proliferation of large and medium-sized arteries, with more than 160 cases described in the literature [1]. Most of these cases were diagnosed by postnatal radiologic demonstration or at autopsy. However, there are rare reports of prenatal diagnoses and postnatal follow-up and to our best knowledge no case of IIAC with dysmorphic features has been reported yet.

We describe a case of prenatal IIAC diagnosed by ultrasound with the findings of polyhydramnios, intrauterine growth restriction and major vessel calcifications and also dysmorphic features which were diagnosed postnatally.

## Case Report

A 29-year-old primigravida at 33 weeks of gestation was referred to our School of Medicine from another hospital because of polyhydramnios. An ultrasonographic examination was performed using a Siemens ultrasound scanner with a 3-5 MHz multifrequency convex transducer (Antares, Germany).

The biparietal diameter measured 32.1 weeks, the femur length measured 27.5 weeks, and abdominal circumference measured 29.5 weeks, on ultrasound (US) examination. Fetal weight was estimated to be 1,400 g (3-5 percentile of birth-weight for gestational age) on US examination. A biophysical profile was normal apart from a high (24 cm) amniotic fluid index. A small pericardial effusion, atrioventricular septal defect, increased renal cortical echogenicity with sparing of corticomedullary differentiation, and diffuse arterial calcifications involving the aorta, pulmonary artery, common iliac arter-

ies, renal arteries, and common carotid arteries were noted (Figure 1a/b). We evaluated these findings as appropriate for IIAC and recommended close follow-up examinations for hydrops fetalis. Two doses of 12 mg of betamethasone were introduced intramuscularly for induction of fetal lung maturity. Maternal screening for structural abnormalities, metabolic disorders, anemia, and infections were unremarkable.

At 35 weeks of gestation, a cesarean section was performed under spinal anesthesia because of fetal distress. A 1,900 g male infant was delivered. The Apgar scores after 1 and 5 min were 3 and 5, respectively. The baby was intubated and ventilated because of the respiratory distress. On physical examination, low-set ears, bilateral clinodactily of the 2<sup>nd</sup> fingers, and a cardiac souffle were detected. Blood biochemistry showed normal values for calcium, phosphate, alkaline phosphate, and 25-hydroxyvitamin D. Renal function and hematologic parameters were normal. TORCH, VDRL, and HIV screens were negative.

A chest radiograph showed hilar enlargement and suspicious radio-opacities in the mediastinum. On echocardiography, calcifications of the aorta and the pulmonary arteries, mild tricuspid insufficiency, secundum type atrioventricular septal defect, and severe pulmonary hypertension were noted.

An abdominal US revealed marked calcification in the abdominal aorta and iliac arteries. Increased renal parenchymal echogenicity with sparing of corticomedullary differentiation was also detected.

Computed tomography (CT) of the chest, abdomen, and pelvis was performed using a Siemens Somatom Sensation (Erlangen, Germany) to facilitate a thorough evaluation and achieve a precise diagnosis. Multiplanar reformatted images were also obtained. There was diffuse calcification of the pulmonary arteries, ascending aorta, aortic arch, descending thoracic aorta, abdominal aorta, and common iliac arteries (Figures 2a/b). Cranial magnetic resonance imaging and skeletal survey were normal. Normal constitutional karyotype was found and no specific genetic abnormalities were noted. Although intensive newborn care support was given, the baby died when he was four months old because of sepsis.

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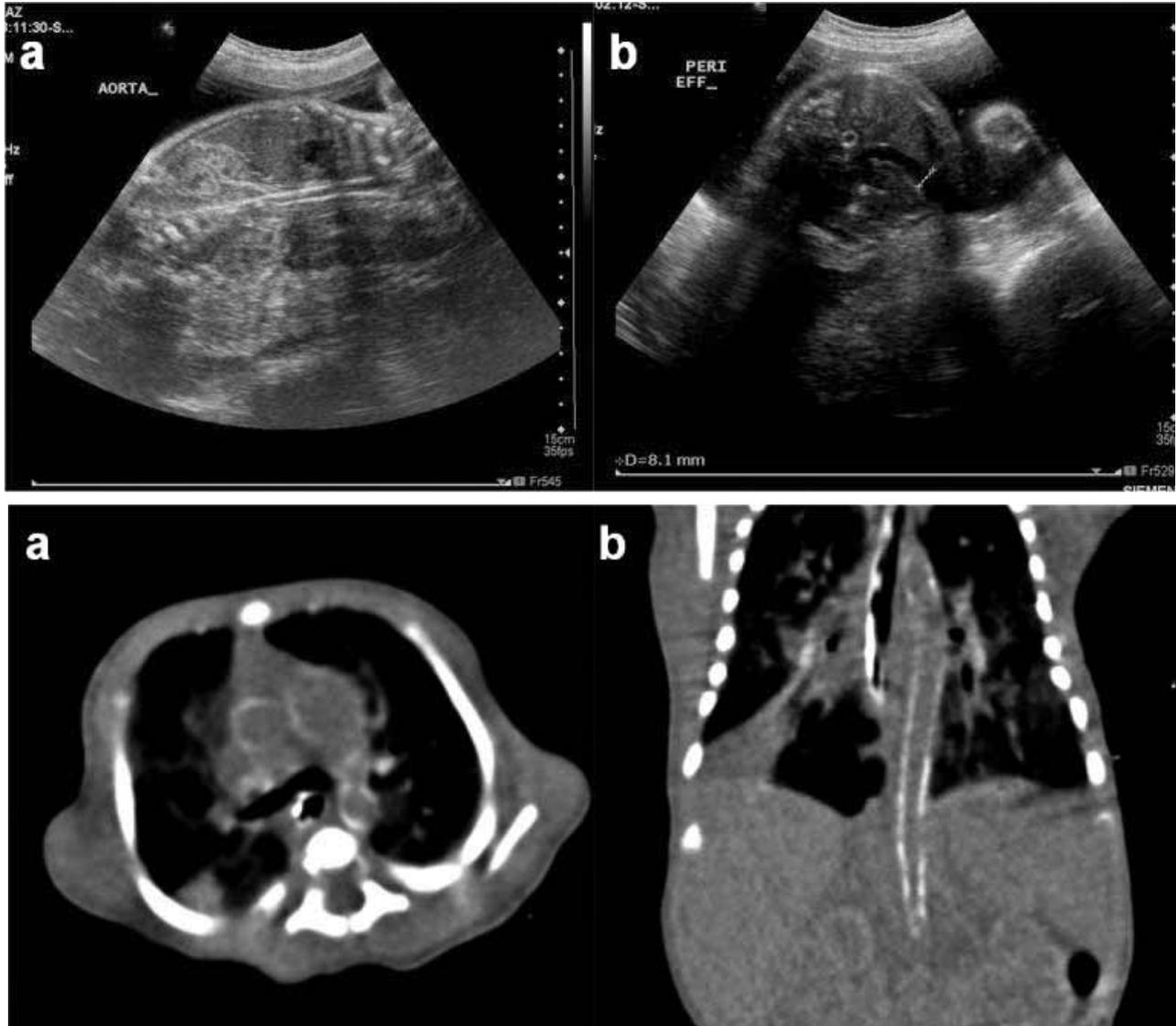


Figure 1. — a) Prenatal sonogram showing the hyperechogenic renal artery and abdominal aorta, interpreted as calcifications. Increased renal echogenicity is also seen, b) Pericardial fluid and descending aorta calcification can be seen.

Figure 2. — a) Axial CT image of the thorax showing circumferential calcification of the ascending and descending aorta, and the pulmonary trunk, b) Coronal reformation shows aortic calcification.

## Discussion

IIAC, characterized by medial calcification and intimal proliferation of large and medium-sized arteries, was first described in 1901 [2, 3]. The etiology of this disease is not known. The inheritance pattern is thought to be autosomal recessive. Rutsch *et al.* [4] and Numakura *et al.* [2] reported that mutations within the ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene (chromosome 6q22-q23) gave rise to inactivation of ENPP1 and were associated with the disorder [5]. ENPP1 is an essential physiologic inhibitor of calcification [6].

Generalized arterial calcification can occur from hyperparathyroidism secondary to chronic renal disease, antepartum hypervitaminosis D of the gravida, a disorder of calcium-phosphorus metabolism, or arteritis due to an

intrauterine infection [7]. These disorders should all be excluded prior to establishing a diagnosis of IIAC. In the case presented here there were no abnormalities linked to these disorders.

The co-occurrence of IIAC with dysmorphic features has not been reported in the literature. We demonstrated low-set ears and clinodactyly of the 2<sup>nd</sup> fingers in our case. These findings may be shown to represent the characteristics of a novel syndrome, pending reports of new cases with similar findings.

The gold standard for diagnosis of IIAC in living patients is arterial biopsy, while in postmortem cases the diagnosis is made at the time of autopsy. Radiologic modalities, such as CT and US, aid in the diagnosis without using invasive procedures [8]. The widespread

use of US facilitates the prenatal diagnosis of IIAC. IIAC may present as hydrops fetalis, polyhydramnios, and arterial calcification on prenatal US [7]. Additional secondary findings include hepatomegaly, cardiomegaly, and increased renal echogenicity [9].

Although most patients die within the first six months of life, generally because of cardiac failure, there have been rare reports of long-term survival in the literature [4, 8]. Some have reported spontaneous resolution of the calcifications [10, 11], and others have identified successful medical therapy with bisphosphonates [8, 12, 13]. Van der Sluis *et al.* [8] described long-term follow-up with etidronate therapy for up to 25 years.

In conclusion, prenatal diagnosis of IIAC may provide the opportunity to refer such cases to tertiary centers for close supervision by an obstetrician, neonatologist, radiologist and pediatric cardiologist. US is an easily accessible diagnostic tool which aids in the prenatal diagnosis and postnatal evaluation of this disorder, while CT easily reveals calcifications in the larger and smaller arteries, without the need for invasive procedures, like arterial biopsy or autopsy. During routine obstetric US, the combination of polyhydramnios and intrauterine growth restriction may necessitate examination of the major vessels for a presumptive diagnosis of IIAC.

## References

- [1] Azancot A., Diehl R., Dorgeret S., Sebag G., Baumann C., Vuillard E. *et al.*: "Isolated pericardial effusion in the human fetus: a report of three cases". *Prenat. Diagn.*, 2003, 23, 193.
- [2] Numakura C., Yamada M., Ariyasu D., Maesaka A., Kobayashi H., Nishimura G. *et al.*: "Genetic and enzymatic analysis for two Japanese patients with idiopathic infantile arterial calcification". *J. Bone Miner. Metab.*, 2006, 24, 48.
- [3] Bryant J.H., White W.A.: "Case of calcification of the arteries and obliterative endarteritis associated with hydronephrosis in a child aged six months". *Guys Hosp. Rep.*, 1901, 55, 17.
- [4] Rutsch F., Ruf N., Vaingankar S., Toliat M.R., Suk A., Höhne W. *et al.*: "Mutations in ENPP1 are associated with 'idiopathic' infantile arterial calcification". *Nat. Genet.*, 2003, 34, 379.
- [5] Tran K.H., Boechat M.I.: "Idiopathic infantile arterial calcification: imaging evaluation and the usefulness of MR angiography". *Pediatr. Radiol.*, 2006, 36, 247.
- [6] Rutsch F., Vaingankar S., Johnson K., Goldfine I., Maddux B., Schauerte P. *et al.*: "PC-1 nucleoside triphosphate pyrophosphohydrolase deficiency in idiopathic infantile arterial calcification". *Am. J. Pathol.*, 2001, 158, 543.
- [7] Saigal G., Azouz E.M.: "The spectrum of radiologic findings in idiopathic arterial calcification of infancy: pictorial essay". *Can Assoc. Radiol. J.*, 2004, 55, 102.
- [8] Van der Sluis I.M., Boot A.M., Vernooij M., Meradji M., Kroon A.A.: "Idiopathic infantile arterial calcification: clinical presentation, therapy and long-term follow-up". *Eur. J. Pediatr.*, 2006, 165, 590.
- [9] Whitehall J., Smith M., Altamirano L.: "Idiopathic infantile arterial calcification: sonographic findings". *J. Clin. Ultrasound*, 2003, 31, 497.
- [10] Sholler G.F., Yu J.S., Bale P.M., Hawker R.E., Celermajer J.M., Kozlowski K.: "Generalized arterial calcification of infancy: three case reports, including spontaneous regression with long-term survival". *J. Pediatr.*, 1984, 105, 257.
- [11] Rosenbaum D.M., Blumhagen J.D.: "Sonographic recognition of idiopathic arterial calcification of infancy". *AJR Am. J. Roentgenol.*, 1986, 146, 249.
- [12] Thiaville A., Smets A., Clercx A., Perlmutter N.: "Idiopathic infantile arterial calcification: a surviving patient with renal artery stenosis". *Pediatr. Radiol.*, 1994, 24, 506.
- [13] Vera J., Lucaya J., Garcia Conesa J.A., Aso C., Balaguer A.: "Idiopathic infantile arterial calcification: unusual features". *Pediatr. Radiol.*, 1990, 20, 585.

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