Editorial Article

Ovulation defects despite regular menses: part III

J.H. Check, *M.D. Ph. D.*

The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, NJ (USA)

Summary

Objective: To describe subtle ovulatory defects that can contribute to infertility and/or miscarriage despite regular menses with apparent ovulation. *Methods*: By using follicular maturation studies and measurement of serum estradiol, progesterone, and LH certain imperfections in the ovulatory process can be ascertained. *Results*: Careful evaluation of follicular maturation was able to determine infertility factors, e.g., premature luteinization, luteinized unruptured follicle syndrome, and luteal phase defects. Effective treatment agents include follicular maturing drugs and gonadotropin releasing hormone antagonists in the follicular phase, human chorionic gonadotropins and leuprolide acetate at time of peak follicular maturation and progesterone in the luteal phase. *Conclusions*: Progesterone supplementation alone is more effective than follicle maturing drugs in women with luteal phase defects with mature follicles. Small doses of follicle stimulating hormone in the late follicular phase is most effective for luteal phase deficiency associated with immature follicles. Sometimes leuprolide acetate can allow egg release when hCG has failed.

Key words: Regular menses; Luteal phase defect; Premature luteinization; Luteinized unruptured follicle syndrome.

Luteinized Unruptured Follicle (Luf) Syndrome

Proteolytic enzymes may play a role in the rupture of the oocyte from the follicle. Plasminogen activator may be one of the enzymes needed to achieve release of the ovum [1], and luteinizing hormone (LH) and follicle stimulating hormone (FSH) have been shown to stimulate plasminogen activator [2]. Theoretically, human chorionic gonadotropin (hCG), because of its LH biologic activity, may help to achieve follicular rupture in patients diagnosed as having luteinized unruptured follicle (LUF) syndrome [3, 4]. Hamilton *et al.* [5] did find a lower level of mid-cycle LH in patients with LUF syndrome compared with normal ovulating women. Similarly, an increase in mid-cycle FSH levels may help to activate plasminogen and thereby effect release. Therefore, some cases of LUF may be related to insufficient FSH surge at mid-cycle, so that the use of pure FSH or human menopausal gonadotropins (hMG) as a one-time injection at the time of follicular maturation might help to achieve follicular rupture in patients with LUF syndrome.

One study has suggested that LUF syndrome may be corrected with serial injections of hMG followed by an injection of hCG when the follicle is mature [6]. Another study has suggested improved release when a single injection of hMG mixed with hCG is given at the time of follicular maturation [7]. Because of slow clearance of the FSH component of hMG, the mechanism of action may be secondary to the increase of FSH levels in the late follicular phase. However, there has been some suggestion that an excess of FSH in the late follicular phase could lead to excess glycosaminoglycan, which may, in turn, inhibits the production of hyaluronic acid needed for ovum release [8, 9]. My group found that the use of either 10,000 units of hCG or hCG in combination with hMG in a single injection at the time of follicular maturation allowed oocyte release in those with LUF syndrome in 46% (21/46) of patients [10].

A global review of the world literature on the luteinized unruptured follicle syndrome concluded that although LUF does exist as a clinical phenomenon, from a clinical standpoint it probably does not constitute a syndrome [11]. However I do not agree with the conclusion of Katz [11]. My group found that of the women taking hMG in the first cycle the egg released in 148 and 13.5% achieved a pregnancy vs only 5.4% of 56 women where we had difficulty in concluding whether the egg released or not vs 0% in 16 women where we concluded LUF [12]. Similar conclusions were reached in the second hMG cycle (pregnancy rates of 15.7%, 4.0%, and 0%, respectively [12]. These data suggest that ultrasound is in general able to make a diagnosis of non-release of oocytes [12].

Katz was willing to conclude that LUF does exist as a phenomenon thus showing that pregnancies are unlikely if the egg does not release. This is not actually in disagreement with our studies [10, 11]. However, to prove that it is a syndrome rather than a phenomenon, it is important to show that it is likely to recur. In the same study we found that if the egg released the first cycle (n = 128), 91% released the second cycle [12]. For inconclusive release (n = 53) only 30% released the second cycle [12]. For those who did not release in cycle 1 (n = 16) only 6% released the second cycle [12]. We consider LUF if by 48 hours from the initiation of the LH surge, the follicle fails to shrink by at least 5 mm. Release of the egg from the follicle is usually associated with fluid in the cul de sac.

Revised manuscript accepted for publication December 7, 2006

The use of hCG or hCG mixed with hMG is not the only treatment for LUF. As mentioned in the previous discussion of premature luteinization in anovulatory cycles where follicle maturing drugs are used, GnRH agonists can be used for their short-term stimulatory effect [13, 14]. Leuprolide acetate has also been used in some "flare-up" IVF protocols to try to recruit a greater response to gonadotropins [15]. We performed a study to evaluate the efficacy of leuprolide acetate given in a dosage of 1 mg every 12 hours x 3 to release oocytes in hMG-treated women. For the first hMG cycles, LUF was present in 44.3% of those taking 10,000 units hCG vs 21.7% of those treated with the GnRH agonist [16]. Interestingly in cycle 2, none of the four women failing to release an oocyte in cycle 1 released an oocyte in cycle 2 when given hCG again but 15 of 18 women (83.3%) released when given leuprolide acetate [16].

When women fail to release oocytes they should be questioned as to whether they are taking any prostaglandin inhibitor, e.g., aspirin. If all medical measures fail the only option left is to manually retrieve the oocytes and then fertilize them in vitro.

Premature luteinization

Premature luteinization in which LH surges result in a rise in progesterone before follicular maturation is achieved, is a recognized problem in women who have been treated with hMG to induce multiple follicular growth [17]. Moreover, premature luteinization has been appreciated as a cause of pregnancy failure in anovulatory women who were given hMG in an attempt to stimulate single follicular maturation [18, 19].

Using a definition of a woman reaching a serum progesterone level greater than 1.5 ng/ml following an LH surge prior to the serum estradiol reaching 200 pg/ml and the follicle average diameter failing to attain 18 mm, we found even in natural cycles without follicle maturing drugs a frequency of premature luteinization of 13% (52/400) [20].

Treatment options are similar to those already discussed in the section on anovulation with normal estrogen when this complication occurs using follicle maturing drugs. A GnRH agonist can be used, e.g., leuprolide acetate for ten days amd beginning the mid-luteal phase a short course of GnRH agonist is used from day 2-4 (use of GnRH agonists in this manner seems to suppress the subsequent LH surge) or gonadotropins and a GnRH antagonist could be used, e.g., cetrorelix or ganirelix at some point in the follicular phase. A cheaper option would be to add ethinyl estradiol 20-40 μg concomitant to the use of gonadotropins. Higher doses of estrogen seem to exert a negative effect on LH release from the pituitary whereas lower doses of estrogen enhance LH release.

Luteal phase defects

One question debated by infertility specialists is whether there is an entity where women have fairly regular menses, release an oocyte from a follicle, make progesterone during the second half of the menstrual cycle but still have infertility related to insufficient secretion of progesterone. It is well known that high pregnancy rates are found following transfer of fertilized donor eggs in women artificially treated with estrogen for two weeks and then progesterone in addition prior to and after embryo transfer but the pregnancies would not be achieved without progesterone. A pregnancy would be easily terminated by taking a progesterone receptor antagonist, e.g., mifepristone. Thus, it seems very logical that some women may be infertile related to a progesterone deficiency. The corpus luteum is responsible for the secretion of progesterone as well as estradiol during the second half of the cycle, known as the luteal phase, and thus a progesterone deficiency from post ovulation until menses is known as a luteal phase deficiency or defect. Though as mentioned there are many infertility specialists that do not consider this entity as a cause of persistent infertility, the first manuscript published suggesting the luteal phase defect to be a cause of persistent infertility was in 1949 [21].

Determining that there was an insufficient amount of progesterone has been based predominantly over the years by showing an out-of-phase endometrial biopsy. The original description of the histological changes that occur with each progressive day from ovulation to menses was by Noyes *et al.* [22]. There is some controversy over the methodology used to determine endometrial changes in "normal" women since the studies were performed on infertility patients who were assumed to have normal ovulation because their menstrual cycles were regular and they had blocked fallopian tubes as a contributing cause (but was it the only cause of their infertility?). Controversy also exists as to whether to do the biopsy in the mid or late luteal phase [23-28]. Also debate exists as to whether abnormal should be considered $\leq$ or $>$ 2 days out-of-phase, i.e., having an earlier date than what it should be based on e.g., time of ovulation [29-32].

We had previously found that almost all women who achieved a successful pregnancy attained an average 18-24 mm diameter dominant follicle associated with a serum E2 level $\geq$ 200 pg/ml [33]. We subsequently considered this as the standard for a mature dominant follicle. We evaluated a group of 100 women with $>$ 1 year of infertility with regular menses who had an endometrial biopsy performed in the late luteal phase that dated more than two days early [34]. Using the definition of a mature follicle, 58% appeared to make a mature follicle. Interestingly 77% of the women randomly assigned to progesterone vaginal suppository treatment in the luteal phase conceived within six months (n = 31) with a miscarriage rate of 4.1% vs only 11.1% conceiving with either clomiphene citrate or hMG (n = 27) and 67% miscarried [34]. Interestingly, taking 25 women who failed to successfully conceive with follicle maturing drugs and then treating them with progesterone vaginal suppositories 64% conceived with a miscarriage rate of 6.2% [34].

In that same study the women who released oocytes from immature follicles did not fare nearly so well with progesterone vaginal suppositories alone (25% pregnancy rate in 6 months with no miscarriages). A higher percentage (70%) conceived with follicle maturing drugs but the miscarriage rate was high (57%) so only 30% had a live birth [34]. The best result was with the combination of follicle maturing drugs in the follicular phase and progesterone support in the luteal phase (70% pregnancy rate but a miscarriage rate of only 7.1% [34].

Based on these data my philosophy has been to determine in women with luteal phase defects whether they attain a mature follicle or not. If they do make a mature follicle then we treat only with progesterone in the luteal phase. If they do not attain a mature follicle they are given follicle maturing drugs plus progesterone. Frequently so as to not create multiple follicles we may allow the dominant follicle to split off from the other follicles and then just boost it along with a small dosage of gonadotropin, e.g., 75 IU for two to three days. This prevents the problem of recruitment of multiple follicles as could happen when the drugs are started earlier in the menstrual cycle.

It could be suggested that instead of determining whether the women make a mature follicle or not, why not just put all women with luteal phase defects on both follicle maturing drugs and progesterone. The reason that we do not double cover is that it behooves physicians to find the most effective therapy with the least side-effects or complications. As mentioned the risk of multiple births should be avoided and this risk is more likely with the use of clomiphene citrate or gonadotropins. Clomiphene frequently causes an adverse cervical mucus necessitating intrauterine insemination which costs the couple time and money. Clomiphene is associated with various symptoms of menopause as a side-effect and can cause depression. With multiple follicles there is a greater chance of some failing to have an egg release leading to an increased risk of subsequent ovarian cysts. There is always the risk with these drugs of the complication of ovarian hyperstimulation syndrome which can lead to ovarian torsion and loss of the ovary, massive edema, predilection to deep vein thrombosis and acute tubular necrosis and hyperkalmia from decreased renal blood supply.

However, there is one more reason why I prefer not to use follicle maturing drugs if not necessary and that is that they can lead to an abnormal uterine environment leading to an inhibition of embryo implantation [33, 35, 36]. When a luteal phase defect is associated with decreased egg reserve as manifested by an increase day 3 serum FSH or a decreased inhibin B level, the adverse effect of follicle maturing drugs is even worse and the effect may be on the embryo itself [37, 38].

Why is progesterone so important? One possibility is that the main effect of progesterone is to inhibit natural killer cell rejection of the fetal semi-allograft by the induction of a protein known as the progesterone-induced blocking factor [39-41]. There is evidence that the allogeneic stimulus of the fetus induces de novo progesterone receptors on CD8+ T cells (gamma/delta T cells) [42, 43]. These are relatively weak receptors and only when exposed to a very high concentration of progesterone (possibly only such a level is generated at the maternal-fetal interface) do these gamma/delta T cells with progesterone receptor express the PIBF protein. The PIBF protein, in turn inhibits natural killer cell cytolysis [44]. It is not clear what are the main fetal antigens that trigger the progesterone receptor induction [45]. Thus either a lack of progesterone or the inability to stimulate progesterone receptors on gamma/delta T cells may lead to a luteal phase defect. There are data that the more likely deficiency is insufficient progesterone [40]. If despite adequate progesterone supplementation there is inadequate PIBF expression then the possibility exists that there was inadequate progesterone receptor induction on the gamma/delta T cells. Possibly the fetus is not sufficiently foreign to the mother or the mother has a weakened response to a normal fetal stimulus [45, 46]. In these cases there may be a need for a better allogenic stimulus, e.g., paternal or pooled lymphocytes [47-50].

So supposing a couple has more than one year of infertility, the semen analysis and post-coital test are normal, the wife makes a mature follicle and releases the egg, and her endometrial biopsy is in-phase and tubal studies are normal? What is the next step? Immediately go to IVF-ET? Empirically treat with follicle maturing drugs? My preference based on these discussions would be to simply treat the woman with luteal phase vaginal progesterone (vaginal progesterone may create better endometrial concentrations compared to oral and even parenteral related to lymphatic transfer from the vagina to the endometrium). This is based on the fact that I question the significance of an in-phase endometrial biopsy when the most important thing to measure would be whether adequate PIBF expression has been achieved. Though this testing is not available now commercially this will happen in the future by the Elisa method now that the PIBF protein has been purified by recombinant DNA technology and a monoclonal antibody can be made.

References

[1] Beers W.H., Strickland S., Reich E.: "Ovarian plasminogen activator: Relationship to ovulation and hormonal regulation". *Cell.* 1975, *6*, 387.

[2] Strickland S., Beers W.H.: "Studies on the role of plasminogen activator in ovulation". *J. Biol. Chem.*, 1976, *251*, 5694.

[3] Marik J., Hulka J.: "Luteinized unruptured follicle syndrome: A subtle cause of infertility". *Fertil. Steril.*, 1978, *29*, 270.

[4] Koninckx P.R., Heyns W.J., Corvelyn P.A., Brosens I.A.: "Delayed onset of luteinization as a cause of infertility". *Fertil. Steril.*, 1978, *29*, 266.

[5] Hamilton C.J.C.M., Wetzels L.C.G., Evers J.L.H. *et al.*: "Follicle growth curves and hormonal patterns in patients with the luteinized unruptured follicle syndrome". *Fertil. Steril.*, 1985, *43*, 541.

[6] Temmerman M., Devroey P., Naaktgeboren N. *et al.*: "Incidence, recurrence and treatment of the luteinized unruptured follicle syndrome". *Acta Eur. Fertil.*, 1984, *15*, 179.

[7] Check J.H., Chase J.S., Adelson H.G., Dietterich C.: "New approaches to the diagnosis and therapy of the luteinized unruptured follicle syndrome". *Int. J. Fertil.*, 1986, *30*, 29.

[8] Schweitzer M., Jackson J.C., Ryan R.: "The porcine ovarian follicle. VII. FSH stimulation in vitro (3H)-glucose-serum incorporation in mucopolysaccharides". *Biol. Reprod.*, 1981, *24*, 332.

[9] Coulam C.B., Bustillo M., Shculman J.D.: "Empty follicle syndrome". *Fertil. Steril.*, 1986, *46*, 1153.

[10] Check J.H., Dietterich C., Nowroozi K., Wu C.H.: "Comparison of various therapies for the luteinized unruptured follicle syndrome". *In. J. Fertil.*, 1992, *37*, 33.

[11] Katz E.: "The luteinized unruptured follicle and other ovulatory dysfunctions". *Fertil. Steril.*, 1988, *50*, 839.

[12] Check J.H., Adelson H.G., Dietterich C., Stern J.: "Pelvic sonography can predict ovum release in gonadotrophin-treated patients as determined by pregnancy rate". *Hum. Reprod.*, 1990, *5*, 234.

[13] Check J.H., Wu C.H., Adelson H.G.: "Case report: Opposite response to the addition of leuprolide acetate to human menopausal gonadotropin therapy in two perimenopausal women". *Int. J. Fertil.*, 1990, *35*, 343.

[14] Check J.H., Wu C.H., Check M.L.: "The effect of leuprolide acetate in aiding induction of ovulation in hypergonadotropic hypogonadism: a case report". *Fertil. Steril.*, 1988, *49*, 542.

[15] Garcia N., Campo S., Panetta V., Venneri M., Siccardi P., Dargenio R., DeTomasi F.: "Induction of ovulation with purified urinary follicle-stimulating hormone in patients with polycystic ovarian syndrome". *Am. J. Obstet. Gynecol.*, 1985, *151*, 635.
[16] Check J.H., Nazari A., Barnea E.R., Weiss W., Vetter B.H.: "The efficacy of short-term gonadotrophin-releasing hormone agonists versus human chorionic gonadotrophin to enable oocyte release in gonadotrophin stimulated cycles". *Hum. Reprod.*, 1993, *8*, 568.
[17] Fleming R., Coutts J.R.: "Induction of multiple follicular growth in normally menstruating women with endogenous gonadotropin suppression". *Fertil. Steril.*, 1986, *45*, 226.
[18] Zimmerman R., Buhnet H.W., Weise H.C., Leidenberger F.R.: "Preliminary report about a modified gonadotropin (human menopausal gonadotropin/human chorionic gonadotropin). Treatment in infertile patients with premature luteinization". *Fertil. Steril.*, 1984, *41*, 714.
[19] Fleming R., Haxton M.J., Hamilton M.P., McCure G.S., Black M.P., MacNaughton M.C., Coutts J.R.: "Successful treatment of infertile women with oligomenorrhea using a combination of LHRH agonist and exogenous gonadotropins". *Br. J. Obstet. Gynaecol.*, 1985, *92*, 369.
[20] Check J.H., Chase J.S., Nowroozi K., Dietterich C.J.: "Premature luteinization: treatment and incidence in natural cycles". *Hum. Reprod.*, 1991, *6*, 190.
[21] Jones GES: "Some newer aspects of the management of infertility". *J. Am. Med. Assoc.*, 1949, *141*, 1123.
[22] Noyes R.W., Hertig A.T., Rock J.: "Dating the endometrial biopsy". *Fertil. Steril.*, 1950, *1*, 3.
[23] Jones G.S.: "The luteal phase". *Fertil. Steril.*, 1976, *27*, 35.
[24] Soules M.R., Wiebe R.H., Aksel S., Hammond C.B.: "The diagnosis and therapy of luteal phase deficiency". *Fertil. Steril.*, 1977, *28*, 1033.
[25] Wentz A.C.: "Endometrial biopsy in the evaluation of infertility". *Fertil. Steril.*, 1980, *33*, 121.
[26] Noyes R.W.: "The underdeveloped secretory endometrium". *Am. J. Obstet. Gynecol.*, 1959, *77*, 929.
[27] Shepard M.K., Senturia Y.D.: "Comparison of serum progesterone and endometrial biopsy for confirmation of ovulation and evaluation of luteal function". *Fertil. Steril.*, 1977, *28*, 541.
[28] Guatray J.P., De Brux J., Tajchner G., Robel P., Mouren M.: "Clinical investigation of the menstrual cycle. III. Clinical, endometrial and endocrine aspects of luteal defect". *Fertil. Steril.*, 1981, *35*, 296.
[29] Annos T., Thompson I.E., Taymor M.: "Luteal phase deficiency and infertility. Difficulties encountered in diagnosis and treatment". *Obstet. Gynecol.*, 1980, *55*, 705.
[30] Shangold M., Berkeley A., Gray J.: "Both midluteal serum progesterone levels and late luteal endometrial histology should be assessed in all infertile women". *Fertil. Steril.*, 1983, *40*, 627.
[31] Murthy Y.S., Arronet G.H., Parekh M.C.: "Luteal phase inadequacy: its significance in infertility". *Obstet. Gynecol.*, 1970, *36*, 758.
[32] Cooke I.D., Morgan C.A., Parry T.E.: "Correlation of endometrial biopsy and plasma progesterone levels in infertile women". *J. Obstet. Gynecol. Br. Commonw*, 1972, *76*, 647.
[33] Check J.H.: "Progesterone therapy versus follicle maturing drugs – possible opposite effects on embryo implantation". *Clin. Exp. Obstet. Gynecol.*, 2002, *29*, 5.
[34] Check J.H., Nowroozi K., Wu C.H., Adelson H.G., Lauer C.: "Ovulation-inducing drugs versus progesterone therapy for infertility in patients with luteal phase defects". *Int. J. Fertil.*, 1988, *33*, 252.
[35] Check J.H., Check M.L.: "A case report demonstrating that follicle maturing drugs may create an adverse uterine environment even when not used for controlled ovarian hyperstimulation". *Clin. Exp. Obstet. Gynecol.*, 2001, *28*, 217.
[36] Check J.H., Check M.L.: "Evidence that failure to conceive despite apparent correction of ovulatory defects by follicle-maturing drugs may be related to premature trophoblast invasion". *Med. Hypoth.*, 2002, *59*, 385.
[37] Roberts J., Spandorfer F., Souls S.J., Kashyap S., Rosenwaks Z.: "Taking a basal follicle-stimulating hormone history is essential before initiating in vitro fertilization". *Fertil. Steril.*, 2005, *83*, 37.
[38] Check M.L., Check J.H., Wilson C., Choe J.K., Krotec J.: "Outcome of in vitro fertilization-embryo transfer according to age in poor responders with elevated baseline serum follicle stimulation hormone using minimal or no gonadotropin stimulation". *Clin. Exp. Obstet. Gynecol.*, 2004, *31*, 183.
[39] Check J.H., Szekeres-Bartho J., O'Shaughnessy A.: "Progesterone induced blocking factor seen in pregnancy lymphocytes soon after implantation". *Am. J. Reprod. Immunol.*, 1996, *35*, 277.
[40] 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.
[41] Szekeres-Bartho J., Kilar F., Falkay G., Csernus V., Torok A., Pacsa A.S.: "Progesterone-treated lymphocytes of healthy pregnant women release a factor inhibiting cytotoxicity and prostaglandin synthesis". *Am. J. Reprod. Immunol. Microbiol.*, 1985, *9*, 15.
[42] Szekeres-Bartho J., Szekeres Gy., Debre P., Autran B., Chaouat G.: "Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody". *Cell. Immunol.*, 1990, *125*, 273.
[43] Chiu L., Nishimura M., Ishii Y., Wieda M., Maeshima M., Takedani Y., Shibata Y., Todokora 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.
[44] Szekeres-Bartho J., Barakonyi A., Polgar B., Par G., Faust Z., Palkovics T. *et al.*: "The role of gamma/delta T cells in progesterone-mediated immunomodulation during pregnancy: a review". *Am. J. Reprod. Immunol.*, 1999, *42*, 44.
[45] 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.
[46] 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.
[47] Check J.H., Arwitz M., Gross J., Peymer M., Szekeres-Bartho J.: "Lymphocyte immunotherapy (LI) increases serum levels of progesterone induced blocking factor (PIBF)". *Am. J. Reprod. Immunol.*, 1997, *37*, 17.
[48] Check J.H., Targuini 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 aborters". *Gynecol. Obstet. Invest.*, 1995, *39*, 257.
[49] 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.
[50] 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 review". *Clin. Exp. Obstet. Gynecol.*, 2005, *32*, 85.

Address reprint requests to:
J.H. CHECK, M.D., Ph.D.
7447 Old York Road
Melrose Park, PA 19027