

Title

Prednisolone for repeated implantation failure associated with high natural killer cell levels:

Case Report and Risk-Benefit analysis

Short Title

Prednisolone, implantation failure and NK cell analysis

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Abstract

Women with unexplained repeated IVF failure present a considerable challenge. Some cases are believed to be due to immunological dysfunction preventing effective embryo implantation. Whilst data are still being collected doctors are faced with the dilemma of treating patients (or not) in the absence of randomised control trial evidence. This article explores a pragmatic approach in using natural killer cell analysis as a means of targeting suitable patients who might attempt treatment with additional immune suppressive therapy.

Introduction

Implantation failure manifests as either unsuccessful IVF or miscarriage. Whilst it is generally accepted that most failures are due to genetic abnormalities of the embryo, it is often difficult or impossible to prove (requiring preimplantation embryo biopsy or miscarriage products of conception for cytogenetic diagnosis). Hence a pragmatic approach has been to investigate a couple after three or more otherwise unexplained miscarriages, or three or more unsuccessful IVF cycles (Margalioth et al., 2006).

However approximately half of couples do not have any abnormality detected, and yet continue to have repeated failures. It is the reality of such unexplained cases that has led to the empirical use of immune suppressive therapy. It has been argued that this is taking advantage of desperate couples. However, there is certainly a strong theoretical case that abnormalities of the immune system could result in repeated implantation failure, and prednisolone has been used in this context for over 20 years.

This case report describes two successful pregnancies following the introduction of prednisolone therapy for a woman with previous repeated implantation failure. The rationale and dose of prednisolone was based on the diagnosis of high natural killer cell levels. A literature review is reassuring of the relatively safety of prednisolone in early pregnancy, and we encourage further studies to assess the potential benefit.

Case Report

A couple presented with a five-year history of infertility due to severe oligoasthenoteratospermia. She (LS) was 30 years old with no other significant medical history, a regular 28-day cycle and body mass index of 23. A previous laparoscopy and hysteroscopy were normal. He (GS) was aged 33 with no cause for his poor sperm count. They had already experienced three miscarriages following three ICSI cycles and five frozen blastocyst transfer cycles at another clinic. Extensive screening for repeated implantation failure included karyotype, autoantibody and thrombophilia testing. LS was found to have raised anticardiolipin antibodies and treated with low molecular weight heparin and aspirin in another ICSI cycle. This was unsuccessful, as was a further frozen embryo transfer (her 10th good quality blastocyst in total).

LS underwent a further hysteroscopy which confirmed a normal uterine cavity. She had a midluteal phase endometrial biopsy which reported a level of uterine natural killer cells of 28%, and a peripheral blood test which reported a level of NK cells of 19.1%. Both these levels were considered to be elevated based on our previous published work (King et al,

2010, Russell et al, 2011). After discussing the risks and possible benefits she agreed to take prednisolone 20mg per day from day 1 of her next ICSI cycle (her 5th) in addition to clexane and aspirin. She fell pregnant and had a normal pregnancy and healthy baby boy weighing 3.16kg. Prednisolone was stopped at 12 weeks gestation, and clexane and aspirin continued until 34 weeks gestation. The couple returned 2 years later. Following a similar protocol of prednisolone and clexane, she conceived again with a frozen embryo and had another healthy baby boy weighing 3.5kg.

Discussion

This is the first report of the use of natural killer cell testing for targeting prednisolone therapy in a woman with repeated IVF failure. It is of interest because of the long history of unexplained IVF failure in a young woman, because the use of prednisolone led to a successful pregnancy rapidly, and because she had a second successful pregnancy with similar treatment. Did prednisolone make a difference? It certainly appears like it did from the patient's perspective. However it can be argued that the pregnancies were a result of clexane and aspirin treating her anticardiolipin antibodies, or simply 'regression toward the mean'. Without randomised trials it is of course impossible to determine what brought about the success in this case. Does that make a difference? Is it reasonable to offer such therapy in these circumstances?

The answer to this question revolves around the analysis of potential benefit versus potential risk. In the absence of appropriate randomised trials, a review of the best available evidence

is necessary, ongoing outcome monitoring is mandatory, and public debate leading to larger trials is recommended.

Following animal studies from the 1950s, a review of 457 women exposed to glucocorticoids (for SLE, asthma or infertility) in early pregnancy noted 2 cases of cleft palate where 0.2 should have been expected (Czeizel & Rockenbauer, 1997).

A case control study investigated 322 malformed and 503 normal control infants (Fraser & Sajoo, 1995). Although only 26 and 33 women from each group were exposed to systemic glucocorticoids during the first trimester, they reported an increased risk of cleft lip with/without cleft palate. This was also demonstrated in another study of 1184 infants with non-syndromic orofacial clefts (although only five had been exposed to corticosteroids in the first trimester) (Rodríguez-Pinilla & Luisa Martínez-Frías, 1998). A further study showed that corticosteroids were associated with orofacial clefts but not conotruncal defects, neural tube defects and limb anomalies (Carmichael & Shaw, 1999).

A meta-analysis including these trials concluded that whilst corticosteroids do not present a major teratogenic risk they convey a 3-4 fold increase in the risk of the child being born with an oral cleft (Park-Wyllie et al., 2000). This represents an increase from 0.1% to 0.3-0.4% where the baseline risk of a malformation in newborns is approximately 3%. Further reassurance was provided by a prospective study which was unable to find any teratogenic potential of systemic corticosteroids in the first trimester (Gur et al., 2004).

Glucocorticoids have also been implicated in preterm birth, gestational diabetes and hypertension, intrauterine growth restriction, and even postnatal and behavioural effects (Gur et al., 2004; Laskin et al., 1997; Michael & Papageorghiou 2008). However those studies involved women taking corticosteroids beyond the first trimester (and often at doses as high as 80mg daily). None of those complications have been reported in pregnancies treated with corticosteroids in the first trimester only.

A meta-analysis on the use of peri-implantation glucocorticoids for women without auto-antibodies undergoing assisted reproductive techniques found no benefit overall (Boomsma et al., 2007). However there may be benefit when there is evidence of immune dysfunction – such as positive antinuclear antibodies (Taniguchi, 2005), anti-DNA antibody or lupus anticoagulant (Ando et al., 1996) and antithyroid antibodies (Revelli et al., 2009).

The case report presented here made use of detailed assessment of both blood and uterine NK (uNK) cells, the assays of which have been described elsewhere (King et al., 2010; Russell et al., 2011). Elevated peripheral blood NK cell activity and uNK cell levels have been correlated with reduced IVF implantation rates (King et al., 2010; Tuckerman et al., 2010) Prednisolone may lead to improved reproductive outcomes by suppressing cytokine production and NK cytotoxicity (Thum et al., 2008). Administration of prednisolone 20mg daily has been shown to reduce uNK numbers in women with recurrent miscarriage (Quenby et al., 2005) and this approach was reported to lead to a live birth in a woman with a history of 19 recurrent unexplained miscarriages (Quenby et al., 2003). Indeed, it is that case report that provided the basis for the case presented here.

Conclusion

Prednisolone is relatively safe in early pregnancy although there is an approximate 3 times increased risk of children having an orofacial cleft. It is considered safe enough to treat various medical conditions including severe hyperemesis gravidarum, asthma, and SLE. This case report supports the careful use of prednisolone targeted for women with evidence of immunological dysfunction associated with repeated implantation failure. A randomised controlled trial is therefore required to better assess this benefit and low teratogenic risk.

References

- Ando T, Suganuma N, Furuhashi M, Asada Y, Kondo I, Tomoda Y. 1996. Successful glucocorticoid treatment for patients with abnormal autoimmunity on in vitro fertilization and embryo transfer. *Journal of Assisted Reproduction and Genetics* 13: 776-781.
- Boomsma CM, Keay SD, Macklon NS. 2007. Peri-implantation glucocorticoid administration for assisted reproductive technology cycles. *Cochrane Database Syst Rev* 1, CD005996.
- Carmichael SL, Shaw GM. 1999. Maternal corticosteroid use and risk of selected congenital anomalies. *American Journal of Medical Genetics* 86: 242-244.
- Czeizel AE, Rockenbauer M. 1997. Population-based case-control study of teratogenic potential of corticosteroids. *Teratology* 56: 335-340.
- Fraser FC, Sajoo A. 1995. Teratogenic potential of corticosteroids in humans. *Teratology* 51: 45-46.
- Gur C, Diav-Citrin O, Shechtman S, Arnon J, Ornoy A. 2004. Pregnancy outcome after first trimester exposure to corticosteroids: a prospective controlled study. *Reproductive Toxicology* 18: 93-101.
- Kemeter P, Feichtinger W. 1986. Prednisolone improves the pregnancy rate of IVF. A prospective randomized study. *Fertilitat* 2: 71-76.
- King K, Smith S, Chapman M, Sacks G. 2010. Detailed analysis of peripheral blood natural killer (NK) cells in women with recurrent miscarriage. *Human Reproduction* 25: 52-58.
- Laskin CA, et al. 1997. Prednisone and Aspirin in Women with Autoantibodies and Unexplained Recurrent Fetal Loss. *New England Journal of Medicine* 337: 148-154.

Margalioth EJ, Ben-Chetrit A, Gal M, Eldar-Geva T. 2006. Investigation and treatment of repeated implantation failure following IVF-ET. *Human Reproduction* 21: 3036-3043.

Michael AE, Papageorgiou AT. 2008. Potential significance of physiological and pharmacological glucocorticoids in early pregnancy. *Human Reproduction Update* 14: 497-517.

Park-Wyllie L, et al. 2000. Birth defects after maternal exposure to corticosteroids: Prospective cohort study and meta-analysis of epidemiological studies. *Teratology* 62: 385-392.

Quenby S, Farquharson R, Young M, Vince G. 2003. Successful pregnancy outcome following 19 consecutive miscarriages: case report. *Hum Reprod* 18: 2562 - 2564.

Quenby S, Kalumbi C, Bates M, Farquharson R, Vince G. 2005. Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertility and Sterility* 84: 980-984.

Revelli A, Casano S, Piane LD, Grassi G, Gennarelli G, Guidetti D, Massobrio M. 2009. A retrospective study on IVF outcome in euthyroid patients with anti-thyroid antibodies: effects of levothyroxine, acetyl-salicylic acid and prednisolone adjuvant treatments. *Reproductive biology and endocrinology : RB&E* 7: 137.

Rodríguez-Pinilla E, Luisa Martínez-Frías M. 1998. Corticosteroids during pregnancy and oral clefts: A case-control study. *Teratology* 58: 2-5.

Russell P, Anderson L, Lieberman D, Tremellen K, Yilmaz H, Cheerala B, Sacks G. 2011. The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure: I: Techniques. *Journal of Reproductive Immunology* 91: 90-102.

Taniguchi F. 2005. Results of prednisolone given to improve the outcome of in vitro fertilization: embryo transfer in women with antinuclear antibodies. *J Reprod Med* 50: 383-388.

Thum MY, Bhaskaran S, Abdalla HI, Ford B, Sumar N, Bansal A. 2008. Prednisolone Suppresses NK Cell Cytotoxicity In Vitro in Women With a History of Infertility and Elevated NK Cell Cytotoxicity. *American Journal of Reproductive Immunology* 59: 259-265.

Tuckerman E, Mariee N, Prakash A, Li TC, Laird S. 2010. Uterine natural killer cells in peri-implantation endometrium from women with repeated implantation failure after IVF. *Journal of Reproductive Immunology* 87: 60-66.