THE USE OF STERILIZED, FREEZE-DRIED AMNION GRAFT VERSUS FRESH AMNION GRAFT AFTER HYSTEROSCOPIC LYSIS OF INTRAUTERINE ADHESIONS (RANDOMIZED COMPARATIVE STUDY)

Thesis

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ABBREVIATIONS

A.R.E. = Arab Republic of Egypt
ACOG = American College of Obstetricians and Gynecologists
AM = amniotic membrane
AMT = amniotic membrane transplantation
CO2 = Carbon dioxide
CS = Cesarean section
D&C =dilatation and curettage
DNA = Deoxyribonucleic acid
EDTA = Ethylene diamine tetra acetic acid
EM = Electron microscope
F = French
FD-AM = Freeze-Dried amniotic membrane
HIV =Human immunodeficiency virus
HLA =Human leucocyte antigen
HSG = Hysterosalpingography
ICSI = intracytoplasmic sperm injection
IUA = intrauterine adhesion
IUD = intrauterine device
IVF = in vitro fertilization
NHS = National Health Service
OD =outside diameter
PMNs = Polymorphnuclear leucocytes
R =Resectoscope
S =Scissor
TEM = Transmitted electron microscope.

Journals abbreviations

Acta Obstet Gynecol= Acta obstetricia et gynecologica Scandinavica
Ann Transplant =Annals of transplantation : quarterly of the Polish Transplantation Society
AORN =Association of periOperative Registered Nurses
Biologicals : journal of the International Association of Biological Standardization
Burns Incl Therm Inj= Burns, including thermal injury
Can J Physiol Pharmacol=Canadian journal of physiology and pharmacology
Conn Med = Connecticut medicine
Curr Opin Obstet Gynecol= Current opinion in obstetrics & gynecology
Expert Rev Med Devices=Expert review of medical devices
Hum Reprod= Human reproduction
Int J Fertil Menopausal Stud = International journal of fertility and menopausal studies
Int J Gynaecol Obstet= International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics
Invest Ophthalmol Vis Sci =Investigative ophthalmology & visual science
J Am Assoc Gynecol Laparosc= The Journal of the American Association of Gynecologic Laparoscopists
J Assam Science Society= Journal. Assam Science Society
J Biomater Sci Polym Ed=Journal of biomaterials science. Polymer edition
J Biomed Mater Res =Journal of biomedical materials research
J Clin Endocrinol Metab=The Journal of clinical endocrinology and metabolism
Reprod Biomed Online= Reproductive biomedicine online
Semin Reprod Med= Seminars in reproductive medicine
Surg Gynecol Obstet = Surgery, gynecology & obstetrics
Transplant Proc= Transplantation proceedings
Yan ke xue bao = Eye science

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Introduction

Asherman's syndrome is defined by the presence of permanent intrauterine adhesions (IUA), formed during the scarring of endometrial surface defects, and obliterating partially or completely the uterine cavity. The presence of such adhesions has been known to be associated with hypomenorrhea or amenorrhea, infertility, and obstetric complications. The most frequent causes of their formation are postpartum or postabortion overzealous dilatation and curettage. Other uterine infections or surgical trauma might also be the predisposing factor **(Asherman, 1948, Rabau and David, 1963; Forssman, 1965; Carmichael, 1970;; Schenker and Margalioth 1982; Kurman et al., 1994; and Pabuçcu et al., 1997).**

The incidence of intrauterine adhesions after one D&C was found to be 16% and most of them were mild lesions. After two and three procedures, the incidences were 14% and 32% respectively, and more than 50% were severe adhesions. It appears that there is an individual constitutional element causing certain patients to develop a severe form of (IUA) and others to be unaffected while undergoing the same traumatic procedure**.** This concept may also explain why some patients respond well to treatment, whereas others suffer from recurrent adhesions **(Polishuk and Sadovsky 1975 and Friedler et al., 1993).**

Surgical excision of the intrauterine adhesions has evolved over time. In the past, access to the uterine cavity was by direct abdominal approach using laparotomy or via the transcervical route with blind division of adhesions

using a uterine sound. This did not allow analyzing results in relation to proper visual assessment of the severity of the adhesions and the extent of uterine cavity occlusion. The use of hysteroscopy in such case since the early 1970s, particular with the newer instrumentation that allows for easier and faster dissection, has allowed rapid improvement in the diagnosis, assessment, and treatment of IUA **(March et al., 1978; Sanfilippo et al.,1982; Otubu and Olarewaju, 1989; Dicker et al., 1996; and Amer et al., 2005).**

The maintenance of the uterine cavity by some physical means along with enhancement of endometrial growth, which is often facilitated by a cyclical estrogen and progesterone treatment regiment, are important steps in the treatment of IUA. It has been reported that either an intrauterine device (IUD), or inflated pediatric Foley′s catheter balloon, can be applied to maintain the raw (endometrium-free) areas of uterine walls separated after adhesiolysis. Compared with the IUD, the use of Foley′s catheter balloon is reported to be a safer and an equal or even more effective adjunctive method of treatment **(Sugimoto, 1978; Valle and Sciarra 1988, Ozumba and Ezegwui 2002; Orhue et al., 2003, and Amer et al., 2005).**

In fact, an ideal adjunctive therapy following hysteroscopic adhesiolysis, would be the application of a biological active mechanical separator that achieves two main goals, suppression of adhesion reformation and promotion of epithelial healing. The amnion seems to be promising in this respect **(Amer et al., 2006).**

Amniotic membrane's has a unique combination of properties including the facilitation of migration of epithelial cells, the reinforcement of basal cellular adhesion and the encouragement of epithelial differentiation together with its ability to modulate stromal scarring and its anti-inflammatory and anti-bacterial activity **(Kim and Tseng 1995).**

In comparison with other biologic tissues used as reconstructive grafts, the amniotic membrane has the advantage that it is thinner and better tolerated by the patient. The amniotic membrane (AM) can be used as a substrate replacement, such that host cells can migrate into the membrane to form new and healthy tissue. The amniotic membrane is the deeper layer of the fetal membrane; it is a vascular, multilayered tissue with antiangiogenic, antiscarring and anti-inflammatory properties. Since it does not express antigens of histocompatibility, the membrane is never rejected by the receiving tissues **(Alio et al., 2005).**

Human amniotic membrane epithelial and mesenchymal cells express various antiangiogenic and antiinflammatory proteins (interleukin-1 receptor antagonist, all four tissue inhibitors of metalloproteinase (TIMPs), collagen XVIII, and interleukin-10 and Thrombospondin-1). Some of these proteins also were found in amniotic membrane stroma. These findings may explain in part the antiangiogenic and antiinflammatory effects of amniotic membrane transplantation **(Hao et al.,2000).**

One of prototypic potent cytokine systems that mediates inflammation and immune responses is the interleukin 1 (IL-1) gene family. This family is composed of three forms; two proinflammatory forms, IL-1alpha and IL-1beta, each having a precursor form, and an anti-inflammatory form:

IL-1 receptor antagonist (IL-1 RA). The anti-inflammatory effect exerted by the AM is mediated by the suppression of the expression and secretion of the IL-1 gene family**.** Amniotic membrane stromal matrix markedly suppresses lipopolysaccharide induced upregulation of both IL-1alpha and IL-1beta. These data may explain in part the effect of AM transplantation in reducing ocular surface inflammation, underscoring the unique feature of the AM as a substrate for tissue engineering **(Solomon et al., 2001).**

Amniotic membrane contains basement membrane components and various proteinase inhibitors. Furthermore, when used as a graft, the basement membrane of AM could block inflammatory insults to a damaged corneal surface. Immediate intervention for acute alkali burns with AM as a temporary patch promotes wound healing by inhibiting proteinase activity and PMNs infiltration **(Kim et al., 2000).**

Down-regulation of the transforming growth factor beta (TGF-beta) signaling system is a strategy for preventing scarring during wound healing. TGF-beta signaling system, DNA synthesis, and subsequent myofibroblast differentiation can be suppressed by an amniotic membrane matrix. This action explains in part the antiscarring results of amniotic membrane transplantation used for ocular surface reconstruction, a surgical technique applicable to other subspecialties. It may also explain in part why fetal wound healing is scarless **(Tseng and Meller, 1999).**

Human amniotic epithelial cells do not express on their surfaces HLA-A, B, C, and DR antigens, or beta 2-microglobulin. So, the acute

immune rejection does not occur after the transplantation of human amniotic epithelial cells **(Akle et al., 1981).**

The potential of the amniotic membrane's application in surgery has just realized over the last three decades**.** In fact, the use of whole human fetal membranes or amnion alone in surgery has primarily developed to aid the repair of surface epithelial defects in the skin **(Faulk et al., 1980)**, eye **(Dua et al., 1999; and Gabler and Lohmann, 2000)**, abdominal wall **(Gharib et al., 1996)** and peritoneum **(Trelford et al., 1977, and Trelford et al., 1978).**

Although the field of obstetrics and gynecology is more concerned with fetal membranes, the use of amnion has not been popular, being restricted to its use as a graft in forming an artificial vagina **(Tancer et al., 1979; Dhall, 1984; Morton and Dewhurst, 1986;Ashworth et al.,1986; and Mhaskar, 2005),** a barrier to prevent postoperative intra-abdominal adhesion formation **(Trelford et al.,1977)**, and as a biological dressing following radical vulvectomies and groin dissections **(Trelford et al., 1973).**

To our knowledge, the first study used the amnion graft after hysteroscopic lysis of intrauterine adhesions was conducted by **Amer and Abd-El-Maeboud (2006)**.The authors reported in a pilot study that; Hysteroscopic lysis of intrauterine adhesions with amnion grafting seems to be a promising procedure for decreasing recurrence of adhesions and encouraging endometrial regeneration **(Amer and Abd-El-Maeboud, 2006)**

The amnion used for the mentioned purposes has been obtained from fresh source or through a variety of preserving methods. Freezing,

Lyophilisation, preservation in glycerol and impregnated with highly concentrated glycerol. However some biological and logistic problems remain; first, this procedure does not guarantee a completely sterile amniotic membrane because of its biological origins. Second, cryopresevation of amniotic membrane requires an expensive and bulky -80ºC deep freezer **(Prabhasawat et al., 2000; Prabhasawat and Tesavibul 2001; Rama et al., 2001).**

Ideally, for clinical use, the amniotic membrane should be sterile and free of contamination. It should also be easily to obtain, transport and store for long periods without deteriorations. The sterilized, freeze – dried amniotic membrane retained most of the physical, biological and morphological characteristics of cryopreserved amniotic membrane **(Nakamura et al., 2004 and Chuck et al., 2004).**

To our knowledge, there have been no papers reporting the effectiveness of sterilized, freeze – dried amnion graft after hysteroscopic lysis of intrauterine adhesions. **Amer and Abd-El-Maeboud (2006)** study used fresh amnion graft. The authors emphasized on the requirement of randomized comparative studies to validate its benefits.

Aim of the work

The purpose of this study is to compare, in a randomized fashion, between the safety and efficacy of; sterilized, freeze-dried amnion graft versus fresh amnion graft after hysteroscopic lysis of intrauterine adhesions.