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Effectivity of Heparin in Assisted Reproduction

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Abstract: Disturbances of the embryo-maternal interaction, i.e. impaired implantation, are seen in only a minor fraction of couples. These malfunctions become evident as recurrent spontaneous abortions (RSA), or repetitive implantation failure (RIF) in cases with IVF or ICSI procedures. The antiphospholidpid syndrom (APL) is the only consensus-defined syndrome associated with RSA (anticardiolipin antibodies and/or lupus anticoagulant plus clinical symptoms). Since antiphospholipid antibodies directly interfere with hemostasis (increased coagulation), heparin is an established treatment option in these cases resulting in unequivocal benefits. There is no defined antibody syndrome in RIF even if it may be assumed that it exists. Conclusive evidence for a benefit of heparin (and aspirin) in this situation is lacking as well. However, the majority of investigations including our own experience indicate that anticoagulation may be useful. Besides the extensively studied anticardiolipin antibodies, other – by far less thoroughly investigated – antiphospholid antibodies have been described. So far it is unclear if heparin may exert positive effects in women carrying these antibodies. Autoreactive immune processes may also become apparent by the emergence of further antibodies, such as antinuclear (ANA), thyreoglobulin (TGA) and thyreoperoxidase antibodies (TPO) etc. However, there is no established definition of a syndrome associated with these antibodies, TGA and TPO probably being the most relevant.

Most studies in this area including our own experience indicate that heparin may be a useful. The detection or autoantibodies per se is probably not of pathophysiological relevance if there is no ongoing pathological activation of the immune system. However, an acute autoimmune response associated with irregular antibodies may represent the pathophysiological basis of a reproductive autoimmune failure syndrome. In these cases, immune-equilibrating interventions appear to be more appropriate than heparin therapy.

Coagulation disorders, namely thrombophilia, are a frequent cause of RSA and probabably RIF as well, the most relevant being antithrombin deficiency, Factor V Leiden and prothrombin mutations. Deficiencies of protein S, protein C and factor XII and XIII are of minor importance. There is a varying degree of evidence for a benefit of heparin/aspirin in these syndromes. Heparin not only reduces the abortion rate but also lowers the risk for developmental retardation, premature birth and preeclampsia.

The effects of heparin are not restricted to anticoagulation. It is directly or indirectly (e.g. via heparan sulfate proteoglycans or heparin-binding EGF) involved in the adhesion of the blastocyst to the endometrial epithelium and the subsequent invasion. Actually, prolonged heparin treatment (14 days) resulted in an increased pregnancy rate in our patient population. Shorter courses of heparin where not effective.

Key words: assisted reproduction; IVF; Heparin; RIF(repeated implantation failure)

Introduction

Every year, around 13,000 to 14,000 children in Germany are born after in vitro fertilization using assisted reproduction techniques (ART) – an annual rate of approximately 2% of all live births. If all reproductive medical techniques are considered, 60,000 to 70,000 children – about 10% of all live births – are born after medical treatment to support conception.

Both in vitro fertilization (IVF) and intracytoplasmic sperm cell injection (ICSI) are used to overcome reproductive impediments such as tubal dysfunctions or pronounced male subfertility. However, there is no point in using IVF or ICSI in cases of dysfunctional interaction of endometrium and embryo (i.e. impaired implantation).

Taking into consideration that around 2.5 million couples in Germany are childless against their will, the relatively small population of couples with reproductive impediments due to impaired implantation [8] is of substantial significance to reproductive medicine. This population includes patients with recurrent spontaneous abortions (RSA) or chronic habitual abortions (CHA) as well as patients failing to conceive despite technically appropriate IVF or ICSI.

Why Heparin ?

The antiphospholipid syndrome (APL) is the only syndrome associated with RSA that was de-

Table 1. APA positivity in women undergoing in vitro fertilization versus controls.*

	IVF Patients		Controls	
Study	N	%	N	%
Sher et al., 1994	429	45	NA	
Gleicher, 1994	105	28	NA	
Birkenfield, 1994	139	25	NA	
Schenk, 1996	90	48	NA	
Birdsall, 1996	240	15	NA	
Kaider, 1996	42	26.2	42	4.8
Balasch, 1996	49	25	49	0
Kowalik, 1997	525	18		
Coulam, 1997	312	22.1		5
Kutteh, 1997	191	18.8	200	5.5
Denis, 1997	793	28	NA	
Eldar-Geva, 1999	173	37	NA	
Kaider, 1999	122	27.9	105	6
Chilcott, 2000	380	23.4	NA	

* N = total number of women tested for APA; % = percent positive (from Ghazeeri and Kutteh, 2002 [25])

Table 2. Pregnancy rates in women with APA after IVF

Study	APA+, H/A	APA+	APA-
Sher, 1994	82/169	4/25	47/171
Schenk, 1996	18/35		12/40
Birdsall, 1996		14/30	71/172
Denis, 1997		309/470	219/323
Kowalik, 1997		45/78	222/447
Kutteh, 1997	10/20	6/16	72/151
Eldar-Geva, 1999	11/35	17/32	
Chilcott, 2000		14/89	57/291
All pregnant	110/244 (49.1%)	403/743 (54.4%)	717/1627 (44.1%)

APA+ = presence of antiphospholipid antibodies, H/A = heparin and aspirin treatment (from: Ghazeeri and Kutteh [25])

fined in an expert consensus (International Statement on Preliminary Criteria for the Classification of the Antiphospholipid-Syndrome [70]), similarly to the clinically apparent systemic lupus erythematodes [10, 15, 20, 35, 45, 55]. Considering the pathophysiology of antiphospholipid antibodies, anticoagulant therapy using heparins is a rational approach to RSA in these patients [10, 15, 45, 55, 73], while there is no convincing evidence of a therapeutic benefit of aspirin, prednisolone or immunoglobulins in this indication [59]. Therefore, heparins can be used in cases with repetitive implantation failure following IVF or ICSI plus concomitant detection of antiphospholipid antibodies (IgG, IgM and/or lupus anticoagulant) [59].

Antiphospholipid Syndrome and ART

Antiphospholipid antibodies (APA) display a wide range of effects. Since they bind to virtually any cell membrane [68], they may as well interact with ovarian membranes, follicles, granulosa cells or spermatozoa [11, 51]. During or after the implantation process, APA may also bind to the syncytiothrophoblast or cytotrophoblast [12, 15, 29, 37]. In addition, APA may directly accelerate local coagulation, leading to extensive thrombotic occlusion of small vessels surrounding the site of implantation [9].

Ghazeeri and Kutteh [25] reviewed a number of studies on the prevalence of APA in IVF patients (Table 1) [36, 63].

Screening the IVF and ICSI patient population of our institution (4481 women undergoing a total of 7910 transfer cycles), we found an APA prevalence of only 5 percent (unpublished data). Even though this was not a systematic survey and we examined only patients with a history indicating an autoimmune process, the prevalence in our population was substantially lower than the figures observed in other studies (15-45%).

The relevant studies on heparin/aspirin treatment in patients with APA undergoing IVF procedures are listed in Table 2. Of note, the pregnancy rate is higher in patients with APA. The benefit of heparin and aspirin in this setting remains unclear, although the pregnancy rate in the treatment groups is higher than in untreated patients. Certainly, this assembly of studies does not provide adequate evidence for a reproductive benefit of heparin/aspirin in patients with antiphospholipid syndrome. However, the question is raised if heparin/aspirin may promote conception in ART independent of an antiphospholipid syndrome.

All of these studies only discriminate patients with and without APA [13], However, detection of APA by itself is not sufficient to diagnose an antiphospholipid syndrome (see above).

The proper definition of an RIF syndrome in patients with antiphospholipid antibodies to our opinion should consider:

- age < 35 years
- normal responder (≥ 8 oocytes in a long GnRH agonist protocol, following 150 I.E. FSH or HMG for 10 to 12 days)
- fertilisation rate > 60 %
- transfer of 2-3 embryos of adequate morphological quality [43, 72].

No studies have been performed in patients with definite RIF after IVF or ICSI [64]. The issue is further complicated by the fact that studies on the reproductive pathophysiology of APA so far have been only performed on antibodies targeting cardiolipin. The clinical relevance of antibodies against other phospholipids such as phosphatidylserin, phosphatidylinositol, phosphatidyl-glycerol, phosphatidylethanoleamine etc. remains unclear [2, 46, 49, 57, 60]. The same holds true for antibodies against β2 glycoprotein, a transmembrane protein associated with the HLA complex [3]. There is no consensus whatsoever on the pathophysiological role of antibodies directed against these molecules in general and on their implication in implantatio failure [4, 5, 14, 18, 24, 31, 52, 53, 56, 66]. Therefore, it comes as no surprise that standardized assays are available for anti-cardiolipin antibodies only [30, 43].

After an initial phase of a more liberal approach to heparin/aspirin therapy in patients positive for autoantibodies, we recently returned to a more restrictive policy. Detection of antiphospholipid antibodies and/or lupus anticoagulans is no longer sufficient, and an indication for heparin/aspirin therapy requires the diagnosis of a RIF syndrome as defined above. In these cases, we prescribe a low-molecular heparin plus aspirin (50-100 mg/d) starting with the embryo transfer. If a stable pregnancy is initiated, therapy is continued until week 12, in high-risk patients (e. g. twins) the heparin/aspirin regimen is extended to week 24. The results are encouraging and did not deteriorate due to our more restrictive policy. However, antiphospholipid syndromes accoring to our definition (see above) are comparatively rare in ART patients.

Other Autoantibodies

Other autoantibodies such as antinuclear antibodies (ANA), thyreoglobulin antibodies (TGA), thyreoperoxidase antibodies (TPO), and antisperm antibodies (ASA) have been investigated in patients with RSA and those participating in IVF programs. Study results on these issues are listed in Table 3 and 4.

Clinical RSA or RIF syndromes have nor been described for these antibodies [23]. Actually, it is unlikely that ANA or ASA may impede embryo implantation. Conversely, antithyreoglobulin antibodies (ATA) may well be of pathophysiological relevance, considering the close histological similarities of thyreoid and syncytiothrophoblast tissue [48].

Accordingly, the use of heparin/aspirin in patients positive for these autoantibodies is not sufficiently documented [25, 26]. However, the results

Table 3. ANA frequency in patients undergoing IVF versus controls.

Study	IVF Patients ANA+ (%)	Controls ANA+ (%)	P value
Geva, 1994	1/21 (4.7)	NA	NA
Birkenfield, 1994	18/56 (32.1)	0/14 (0)	< 0.02
Geva, 1995	11/50 (22)	2/80 (2.5)	< 0.05
Cubillos, 1997	16/43 (37.2)	2/35 (5.7)	< 0.05
Kaider, 1999	52/122 (42.6)	2/112 (1.8)	< 0.05
Lucena, 1999	27/100 (27)	11/62	0.19

NA = not available (from: Ghazeeri and Kutteh, 2002 [25])

Table 4. Antithyroid antibodies in women undergoing embyo implantation after IVF

Outcome	Total (%)	TG/TPO+ (%)	TG/TPO- (%)	P value
Biochemical	39 (4.5)	5 (3.5)	34 (4.7)	0.66
Clinical loss	57 (6.5)	9 (6.3)	48 (6.8)	1.00
Delivered	474 (54.3)	78 (54.5)	396 (54.2)	1.00
Not pregnant 303 (34.7)	51 (35.7)	252 (34.5)	0.48	
Total	873	143	730	-

(from: Ghazeeri and Kutteh, 2002 [25])

Table 5. Effect of sperm-bound ASA on IVF fertilization.

Study	Positive Criteria (Method)	Oocyte Fertilization Rate	
		Positive ASA	Control
Clarke, 1985	≥ 80% G and A (IBT)	18/66 (27)	47/65(72)
Mandebaum, 1987	≥ 20% G or A (IBT)	23/33 (70)	224/350 (64)c
De Almeida, 1989	≥ 70% G or A (IBT)	6/43 (14)	31/52 (60)
Kato, 1990	≥ 30% G and A (IBT)	9/50 (18)	39/54 (72)
Witkin, 1992	≥ 65% G or A (IBT)	6/43 (14)	137/34 (56)a
Janssen, 1992	≥ 50% G and A (IBT)	142/295 (48)	201/344 (58)
Rajah, 1993	≥ 34% G or A (IBT)	53/105 (51)	93/128 (73)
Lähteenäki, 1993	≥ 40% G (MAR)	68/283 (24)	30/72 (42)
Acosta, 1994	≥ 36% G or A (IBT)	N/A (42)	N/A (73)
Sukcharoen, 1995	≥ 20% G or A (IBT)	124/165 (75)	974/1412 (69)c
Ford, 1996	≥ 25% G or A (IBT)	209/544 (38)	380/555 (68)
Vasquez-Levin, 1997	≥ 20% G (MAR)	46/104 (44)	65/77 (84)
Clarke, 1997	≥ 80% G or A (IBT)	31/156 (20)	428/685 (62)b
Culligan, 1998	≥ 15% G or A (IBT)	82/123 (67)	497/792 (63)a, c
Kutteh, 1999	≥ 50% G or A (IBT)	112/145 (77)	186/252 (74)c
Check, 2000	≥ 80% G or A (IBT)	11/20 (55)	NA

IBT = immunobead test; MAR = mixed agglutination reaction; N/A = not assayed; a = estimated from data presented in paper; b = controls were the same couples treated in a second cycle with ICSI; c = no adverse effect of ASA on fertilization rate (from: Ghazeeri and Kutteh, 2002 [25]).

published so far support an association of ATA with RSA and/or RIF. Several studies report on a benefit of heparin/aspirin in ATA-positive patients (see [25] for review).

In our institution, we currently tend to a more restrictive use of heparin/aspirin, because the detection of a single irregular antibody does not necessarily indicate an ongoing pathological situation requiring treatment. In many cases, the antibodies listed above merely represent "serological scarring" (see below).

Is There such a Thing as the "Reproductive Autoimmune Failure Syndrome (RAFS)" ?

The RAFS was first describes by Gleicher [27]. Since he published his description of the putative syndrome in 1989, virtually no studies dealing with RAFS or supporting its existence have appeared. However, quite a number of authors investigated specific autoantibodies and their potential role in RSA and RIF (see above).

Conflicting data on the role of in ANA, ASA und ATA in RIF raise the question if the detection of autoantibodies by itself is of pathophysiological relevance [28, 33, 34]. The most likely candidates for a role in reproductional failure are APA and ATA. While APA and ATA may well enhance coagulation causing local clotting of small vessels, this is very unlikely for other antibodies.

Currently, the crucial question appears to be if irregular antibodies indeed cause an acute pathological activation of the immune system. After this issue has been largely neglected in most pub-

lished studies, some authors recently began to look more closely at this essential aspect [21, 58]. Besides activation or proliferation of natural killer cells and endometrial large granular leucocytes, they also investigated the general balance of the immune system. Obviously, a general activation of a Th1 and/or Th2 response (i.e. a cellular immune response) causes quite unfavourable conditions for the implanting embryo and the subsequent pregnancy (see Clark [7] and Raghupathy [54] for reviews).

While a pathological activation of the immune system does not necessarily require anticoagulant therapy, the use of heparin may well be indicated in antibody syndromes affecting blood vessels and coagulation such as APA and most likely ATA [32].

RSA, RIF and Hypercoagulation

Syndromes associated with hypercoagulation tend to cause RSA and presumably RIF. This is well documented for antithrombin deficiency, factor V Leiden (APC resistance) and the prothrombin 20210 mutation. Other thrombophilias, such as protein C or S and FXII or FXIII deficiencies, may be involved in RSA and RIF as well [47, 61]. These thrombophilias are found at an astonishingly high prevalence of 5-7% in the general population [69].

The benefit of heparin/aspirin in these thrombophilic syndromes may vary in magnitude although it is generally well documented [40, 42, 74]. Heparin substantially reduces the rate of spontanous abortion, fetal growth retardation, preterm birth, preeclampsia as well as RIF. The abovementioned studies did not rule out the presence of thrombophilias in their patient populations. Considering their high prevalence, at least 5-7% of all participants may have been affected. Since this subpopulation is likely to benefit from heparin therapy, this is a plausible explanation for the increased conception rates in heparin-treated patients.

In our institution, we routinely screen all patients for thrombophilias. If confirmatory testing yields positive results, we generally prescribe heparin/aspirin, since thrombophilias may have deleterious effects throughout gestation. This notion is illustrated by the fact, that three of our patients developed deep vein thrombosis, in one case followed by pulmonary embolism, even though they received prophylaxis with low-molecular weight heparins (see [47, 61]).

Further Effects of Heparin

Heparin, as a natural substance, exerts several effects besides anticoagulation that have been elucidated only recently. Some of them may well be relevant in reproduction. Some factors interacting with heparin, such as heparansulfate proteoglycan (HSPG), heparin-binding EGF-like growth factor (a member of the EGF family of proteins), and HIP (heparin interacting protein) [1, 38, 39], may have a role in reproductional funtions such as blastocyst adhesion to the endometrial epithelium, subsequent blastocyst invasion and growth stimulation of certain syncytiothrophoblast cells. Thus, an extensive body of evidence supports a physiological role of heparin in some essential steps of the reproductional process.

A retrospective study (1998-2002) investigating the effects of a short (five day) course of heparin in our institution did not provide evidence of any significant benefit with regard to these effects. However, a 14-day heparin treatment indeed was, albeit retrospectively, associated with higher conception rates. Therefore, heparin may positively affect reproductional success rates due to mechanisms independent of its effects in patients with autoantibodies and hypercoagulation.

In recent years, reproduction medicine tends to focus more and more on endometrial receptivity, which is not only implicated in RIF but in the age-associated decrease in the rate of successful implantations as well – an effect that is independent of oocyte and embryonic genetics. Endometrial receptivity is greatly reduced in nulliparous women over 40 years of age. Conversely, the receptivity and therefore the reproductional success rates positively correlate with the number of previous pregnancies leading to childbirth. A gradual decline in HPSG or HB-EGF-like growth factor levels or their response to activating stimuli may well be involved in these effects. Actually, another retrospective analysis (1998-2002) of patients treated in our institution revealed that women over age 38 did benefit from heparin treatment in terms of increased pregnancy rates while this effect was not seen in younger women.

Summary

Currently, there is no published consensus on the effects of heparin on pregnancy rates in women with autoantibody syndromes undergoing ART. Positive heparin effects are highly likely in patients with antiphospholipid syndromes due to the hypercoagulation induced by these antibodies. There is a broad consensus on the benefit of heparin in women with RSA associated with an antiphospholipid syndrome (or systemic lupus erythematodes). Current investigations tend to focus on the undelying pathological immune activation [41, 50, 67] rather than the detection of specific irregular antibodies.

Heparin may not be the treatment of choice in cases with predominant immune activation rather than hypercoagulation. Therefore, retrospective studies investigated the use of glucocorticoids or LNCC [17, 25, 62, 65, 71]. While Clark [7] reviews some of the investigations performed in this field, he completely ignores the studies by Kutteh and Ghazeeri [25, 75, 26, 43-45]. This may relate to the fact that these authors [75] as well as the Royal College of Obstetricians and Gynaecologists [57a] did not find convincing evidence for a therapeutic effect of these interventions.

Finally, we should not ignore the psychosomatic situation of our patients. Many couples presenting at a tertiary care reproductive center have unsuccessfully undergone all kinds of interventions beforehand and may have read more on reproductional techniques and immunology of reproduction than their attending physicians. It may not be easy to handle the high expectations of these couples, and it may be justified to consider therapeutic options outside the range of strictly evidence-based therapies. In some of these desperate cases, the prescription of drugs like heparin may even be successful due to an unexpected placebo effect (More due to an "eminence based" than an evidence based effect).

There is a broad consensus on the benefit of heparin in patients with disorders associated with hypercoagulation, such as a documented APC resistance or a prothrombin mutation. In the light of their high prevalence of 5-7% in the general population, screening for these thrombophilias certainly merits closer consideration.

In patients with thrombophilia, heparin should be administered in therapeutic dosages, since venous thrombotic events have repeatedly been observed in patients treated with the usual prophylactic regimens.

Further positive effects of heparin on a number of steps in the reproductional process (e.g. blastocyst adhesion) have been described and documented on the physiological level. The results of retrospective studies performed at our institution appear to support a clinical benefit. Larger prospective studies are warranted to investigate this interesting aspect of heparins.

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