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THE POSSIBLE EFFECT OF HYDROSALPINX FLUID HUMAN EMBRYOS

Tubal factor Infertility

- IVF was initially developed as fertility treatment in a population of women without functional fallopian tubes in order to overcome mechanical obstruction.
- Tubal factor – 25-35% of female infertility
- One of the major indications for IVF-ET.
- In patients with hydrosalpinges poor IVF results have been reported compared to women with other tubal factor-related infertility.

Strandel A et al, Hum Reprod 1994; 9: 861-3

Hydrosalpinx (HS)

- Hydrosalpinx - collection of watery fluid in the uterine tube
- Heterogeneous entity with wide spectrum of pathology and distal tubal occlusion → leading to potentially different outcomes.

Shahara FI et al, Hum Reprod 1996; 11: 526-30

- Any distal tubal occlusion → can lead to HS regardless of the cause
- Main cause – Pelvic inflammatory disease (50%)
- C. Trachomatis – the most common cause of PID and is associated with tubal damage, hydrosalpinges, ectopic pregnancy, infertility.
- In 10-30% of patients undergoing IVF-ET – unilateral or bilateral HS is present.

Andersen AN et al, Hum Reprod 1996; 11:2081-4

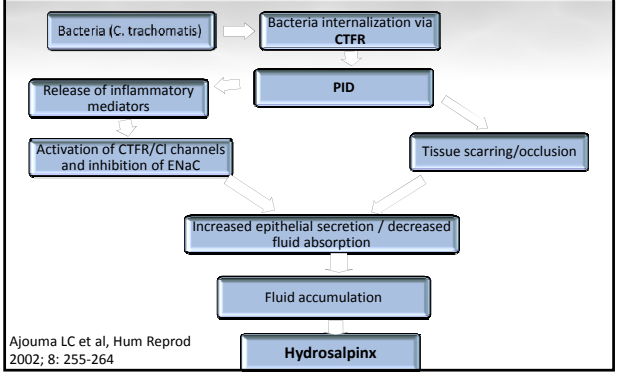
Hydrosalpinx (HS)

- **Hydrosalpinx simplex** – excessive distension and thinning of the uterine tube wall, tubal plicae are thin and widely separated.
- **Hydrosalpinx follicularis** – tube without any central cystic cavity, the lumen being broken up into compartments, plicae are fused (it covers also cases without any fluid in tubes).
- **Saktosalpinx** – dilation of the inflamed uterine tube by retained secretions (*saktos* = stuffed).

Proposed Mechanisms for HF formation after C. trachomatis infection

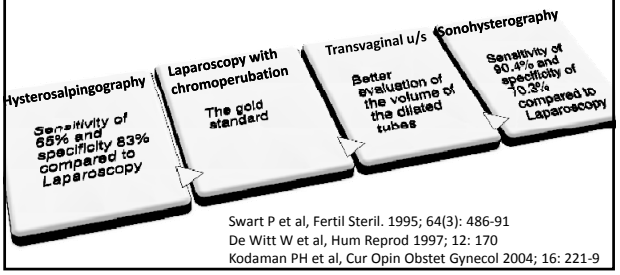
1. Destruction of oviductal epithelium → Loss of membrane polarity → ↓ expression of epithelial membrane transporters and ion channels (particularly CTFR)→ abnormal fluid secretion and reabsorption.
2. Release of inflammatory mediators → increase fluid secretion in oviduct and stimulate myometrial and myosalpingeal contractions → increase of luminal hydrostatic pressure due to distal tube occlusion → increase serosa-to-mucosa fluid flow → fluid accumulation.
3. Effect of ovarian stimulation hormones on up-regulation of CTFR → sudden increase in HF.

Ajouma LC et al, Hum Reprod 2002; 8: 255-264



Ajouma LC et al, Hum Reprod 2002; 8: 255-264

Diagnosis of Hydrosalpinx



Swart P et al, Fertil Steril. 1995; 64(3): 486-91
De Witt W et al, Hum Reprod 1997; 12: 170
Kodaman PH et al, Cur Opin Obstet Gynecol 2004; 16: 221-9

Effect of Hydrosalpinx on fertility

- Data based on several retrospective studies.
- Two meta-analyses further strengthened the association between the presence of HS and adverse IVF outcome.

➢ Presence of HS is associated with significantly lower implantation and pregnancy rates after IVF.
➢ Spontaneous abortion rates and the risk for ectopic pregnancy were increased.
➢ Embryos appear to have reduced viability.

Strandel A et al, Fertil Steril 1999; 71: 282-86
Zeyneloglu et al, Hum Reprod 1998; 70: 492-499
Camus et al, Hum Reprod 1999; 14: 1243-49
Strandel A et al, Hum Reprod 2001; 16: 2403-10

Theories for the detrimental effect of Hydrosalpinx on IVF-ET outcome

1. Impairment of ovarian function, follicular development and oocyte quality
2. Endometrial damage
3. Purely dilutional effect of HSF on essential nutrients and substrates
4. Direct cytotoxic effect on gametes and embryos
5. Inflammatory response with production of proiflammatory cytokines
6. Mechanical washout of embryos
7. Effect on endometrial receptivity and inhibition of embryo implantation

1. Impairment of ovarian function, follicular development and oocyte quality

- Increase in embryo degeneration, growth arrest and abnormal development of untrasferred embryos with a concurrent decrease in normal blastulation and cryopreservation of blastocysts for patients with HS compared to patients with other tubal pathology.
- Demonstration of the hypothesis for the potential negative effect of HS on ovarian function and oocyte quality is the lack of improvement of the above mentioned factors despite surgical treatment of HS.

Freeman MR et al, Hum Reprod 1998; 13: 983-6

2. Endometrial damage

- Hypothesis for simultaneous damage to the endometrium at the time of the original tubal infection → resulting in permanent diminished capacity for implantation.

Strandel A et al, Hum Reprod 1994; 9: 861-3

- Subsequent studies do not support this hypothesis implying that the diminished potential of the endometrium for implantation is transient, such as pre-IVF surgical correction of HS lead to restoration of endometrial integrin expression and subsequently to increased implantation and birth rates.

Meyer WR et al., Hum Reprod 1997; 12: 1393-8
Bildirici I et al., Hum Reprod 2001; 2422-26
Johnson NP et al., Cochrane Database Syst Rev 2004
Strandel A et al, Hum Reprod 2001; 2403-10

3. Purely dilutional effect of HSF on essential nutrients and substrates

- The inhibitory effect of HF on embryonic development may be due to the absence of essential nutritional factors.
- Embryos cultured in 50% HF/50% culture medium showed development comparable to the controls.
- Addition of energy sources (lactate or pyruvate) to 100% HF largely correct this deficiency, however, the inhibitory effect could not be completely overcome → indicating that either other factors essential for the development are also missing from HF or this may be due to embryotoxicity of HF.

Murray CA et al, Hum Reprod 1997; 12: 2504-7

4. Direct cytotoxic effect on mouse embryos

- HF toxic effect to mouse embryos has been demonstrated even at low concentrations of HF, whereas three studies showed such effect only in undiluted HF.
- Another study in mice models failed to demonstrate impaired embryo development potential even in presence of 100% human HF suggesting that non-species-specific embryogenetic factors might be present in human hydrosalpinx fluid.

Saito S et al, J Obstet Gynecol Res; 2000; 26: 455-9

- Two studies on human embryos were conducted → without confirming any obvious toxic effect on embryo development.

Summary of studies on human blastocyst development in HF

Author	No of HF	No of embryos	Measured outcome	Culture system	% of HF	Blastocyst development (%)	P	Conclusion
Granot 1998	4 pooled	3PN 34	8-16 cell stage	Not stated	1 50 100	100 100 100	 NS NS	No embryotoxic effect on human embryos
Strandel 1998	12 separate from 8 patients	183 52	Blastocyst development	Under mineral oil Open vs mineral oil	0 50 100 50 50	35.3 33.3 13.9 57.7 57.5	 NS 0.026 NS	Deficiency of nutritients No lipophilic embryotoxic factor

AIM

- Investigation of the effect of human hydrosalpinx fluid (HF) on the development and blastulation of mouse embryos
- Role of growth factors (EGF and IGF-I) in culture medium with and without HF

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9

Material and Methods

2100 two-cell stage mouse embryos recovered from oviduct 34-40h after hCG administration	HF from 14 women with uni- or bilateral hydrosalpinx was aspirated during laparoscopy	Culture medium – Ham F-10 without hypoxanthine + BSA (9:1)	Various HF concentrations were analyzed (5%, 20% and 30%)

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9

Blastulation Index (BI)

Percentage of blastocyst development rate of the test	Percentage of blastocyst development rate of the control

BI < 0.5 → embryotoxic
BI >1.0 embryotrophic
BI between o.5 and 1.0 – inhibitory or adverse effect on embryonic development

EGF and IGF-I concentrations

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9

Results

	Control	5% HF	20% HF	30% HF	P Value
Blastocysts (%)	54.2	58.6	47.4	44.9	<0.5
Blastulation Index		1.11±0.4	0.97±0.5	0.98±0.9	0.842
EGF (pg/ml)	11.2±1.9	2.13±0.6	2.47±1.0	4.18±0.4	<0.001
IGF-I (pg/ml)	1.32±0.9	1.19±0.9	0.13±0.04	0.22±0.15	0.5

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9

	5% HF	20% HF	30% HF	P Value
Embryotoxic, n (%)	0 (0)	2 (14.3)	3 (21.4)	0.204
Impaired blastocyst formation, n (%)	7 (50)	7 (50)	6 (42.9)	0.9
Embryotrophic, n (%)	7 (50)	5 (35.7)	5 (35.7)	0.6

	Control	Embryotrophic	Embryotoxic	P Value
EGF (pg/ml)	11.2±1.9	1.15±0.4	2.25±0.7	<0.001
IGF-I (pg/ml)	1.30±0.9	0.22±0.4	0.60±0.4	0.43

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9

Development of two-cell stage mouse embryos was not affected at low (<30%) HF concentrations.

Statistically significant difference in EGF concentration between culture medium without HF and media containing various concentrations of HF.

Trend for lower levels of IGF-I in culture media containing HF compared with the control.

Even apparently normal blastulation is affected by any concentration of HF because of low embryonic EGF.

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9

5. Inflammatory response with production of proiflammatory cytokines

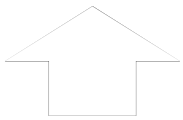
- The chemical content of HF does not seem to be remarkably different compared with tubal fluid from healthy fallopian tubes apart from hemoglobin and protein levels in cases with haematosalpix.
- Negative bacterial cultures from HF.
- Slightly elevated concentration of endotoxin - as a sign of previous infection.
- Individual variations in the HF content with regard to concentrations of electrolytes, glucose, proteins, endotoxins, cytokines, osmolarity and pH may explain the variations in HF effects on gametes, fertilization, embryo development and pregnancy.

Ng EHY et al., Hum Reprod 2000; 15: 772-7
Granot I, Hum Reprod 1998; 13: 1620-24
Strandel A et al, Hum Reprod 1998; 13; 2921-25

- **Hypothesis**: Balance of cytokines and GFs in HF – responsible for poor IVF outcome in patients with HS.

Embryotrophic

Embryotoxic



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Are cytokines and growth factors responsible for the detrimental effects of hydrosalpingeal fluid on pregnancy rates after in vitro fertilization-embryo transfer?

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Crozer-Chester Medical Center, Upland, Pennsylvania; The New York Hospital-Cornell Medical Center, New York, New York; and The University of North Carolina, Chapel Hill, North Carolina

HF from 10 infertile women with HS were examined for the presence of:

Interferon – γ + TGF-β2 + TNF-α + EGF

Interferon –γ	TGF-β2	TNF-α	EGF
• Not detected	• Not detected	• Detected in 5of 10 HF	• Detected in 7 of 10

Alteration in substances secreted from the tubal epithelium that reflux into the uterine cavity may explain the negative effects of HF on pregnancy rates after IVF-ET

The absence of EGF from half of HF samples may reflect a lack of production by damaged tubal epithelium → decrease in an embryotrophic factor → decrease in pregnancy rates

Journal of Assisted Reproduction and Genetics, Vol. 21, No. 7, July 2004 (© 2004)

The Presence of Cytokines and Growth Factors in Hydrosalpingeal Fluid[1]

Annika Strandell,[2,3] Jane Thorburn,[2] and Ann Wallin[2]

HF from 18 tubes on 17 patients vs controls form serum and folicular fluid from 15 patients without HS undergoing IVF were examined for the presence of:

IL-8 | IL-12 | IL-1α | EGF | GM-CSF | LIF | TNFα | IFNγ | TGFβ2

	Hydrosalpinx fluid (n=18) Number detected (%)	**Follicular fluid (n=15)** Number detected (%)	**Serum (n=15)** Number detected (%)
IL-8	18 (100)	1 (7)	15 (100)
IL-12	15 (83)	1 (7)	2 (13)
IL-1a	14 (78)	1 (7)	1 (7)
IFNγ	5 (28)	0	1(7)
TNFα	10 (56)	0	0
TGFβ2	14 (78)	0	15 (100)
LIF	9 (50)	14 (93)	1 (7)
EGF	18 (100)	15 (100)	15 (100)
GM-CSF	12 (67)	0	0

- All the chosen cytokines – present in HF (involvement in inflammatory reactions or expression in the female reproductive tract).
- Only LIF and EGF commonly present in follicular fluid.
- EGF also commonly present in serum, as was IL-8 and TGFβ2.
- **IL-8, IL-12, IL-1α and TNFα – primary inflammatory cytokines** → more commonly in HF suggesting a retained negative effect of a tubal infection.
- **TGFβ2**- also found in endometrium, developing oocytes and embryos in mouse studies.

- Cytokines with a proliferate effect and known to be produced in the human reproductive tract (**LIF, EGF, GM-CSF**) – commonly found in HF → growth promoting properties of HF.
- LIF and GM-CSF present in at least half of HF.
- Impairment of implantation in HS patients with lack of certain growth factors.
- Discrepancy in the results concerning IFN-γ and TGF-β2 between this study and the findings of previous study.

- The discrepancy may result from the small sample size.
- May be important the time lapse form the original infection.
- Heterogeneity of hyndrosalpinges even within an individual with bilateral HS → HF express a variety of properties ranging from embryotoxicity to growth promotion.

Human Reproduction Vol.17, No.1 pp. 128–133, 2002

The significance of cytokines, chemical composition, and murine embryo development in hydrosalpinx fluid for predicting the IVF outcome in women with hydrosalpinx

Chin-Der Chen, Jehn-Hsiahn Yang, Ko-Chen Lin, Kuang-Han Chao, Hong-Nerng Ho and Yu-Shih Yang[1]

The HFs were analyzed for the presence of:

EGF + INFγ + LIF + TNF-α

Various cytokine concentrations in HF in women who achieving and not achieving pregnancy.

- A trend for higher concentration of EGF and lower concentrations of LIF and INFγ in the pregnancy group compared to the non-pregnancy group.

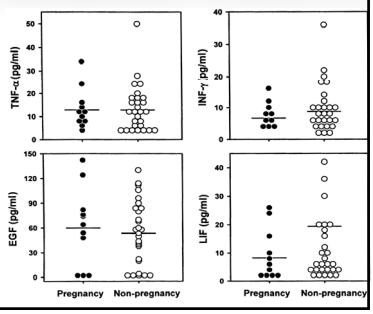

Table III. Comparison of diagnostic performance of different modalities for predicting the IVF outcome in patients with hydrosalpinx.

Parameter	Area under ROC curve	Asymptotic significance[a]	Best cutoff	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Positive likelihood ratio
Age (per additional year)	0.705	0.049	32.5	72.7	53.4	40.0	84.2	1.57
No. of embryos transferred	0.500	1.000	2.5	72.7	25.0	27.6	70.0	0.97
No. of good quality embryos transferred	0.589	0.391	1.5	72.7	39.3	32.0	78.6	1.19
Murine blastocyst formation rate								
10% hydrosalpinx fluid (%)	0.709	0.053	40.0	63.6	64.3	41.2	81.8	1.78
50% hydrosalpinx fluid (%)	0.807	0.005	53.3	63.6	85.7	63.6	85.7	4.45
100% hydrosalpinx fluid (%)	0.794	0.007	11.1	72.7	67.8	47.1	86.4	2.26
LIF (pg/ml)	0.611	0.305	8.1	72.7	42.9	33.3	80.0	1.27
EGF (pg/ml)	0.444	0.608	75.2	72.7	32.1	29.6	75.0	1.07
TNF-α (pg/ml)	0.435	0.550	12.3	72.7	42.9	33.3	80.0	1.27
INF-γ (pg/ml)	0.559	0.099	7.0	72.7	50.0	36.4	82.3	1.45
pH	0.498	0.922	7.7	44.4	86.3	57.1	79.1	3.24
Osmolality (mOsmol/kg)	0.458	0.717	261	63.6	35.7	28.0	71.4	0.99
Glucose (mg/dl)	0.570	0.356	13	72.7	46.4	34.8	81.3	1.36
Total protein (g/dl)	0.672	0.141	0.1	54.5	71.4	42.9	80.0	1.91
Lactate (mmol/l)	0.635	0.248	2.4	81.8	42.9	36.0	85.7	1.43
Logistic regression model[b]	0.860	0.001	39.8%	63.6	89.3	70.0	86.2	8.35

[a]Null hypothesis: true area = 0.5.
[b]Variables include a 50% hydrosalpinx fluid mouse assay blastocyst formation rate of ≥53.3%, patient age, and no. of good quality embryos transferred.
LIF = leukaemia inhibitory factor; EGF = epidermal growth factor; TNF-α = tumor necrosis factor-α; INF-γ = interferon-γ; ROC = receiver operative characteristic.

Chen C et al., Hum Reprod 2002; 17: 128-33

- Only the blastocyst development rates in 50 and 100% HF, and patient age showed asymptomatic significance.
- The chemical composition and cytokine concentrations of HF were not predictive of subsequent IVF outcome.
- A **mouse embryo assay** in 50% HF may potentially serve as a predictor of subsequent IVF outcome in women with HS (sensitivity – 64%, specificity – 86%, positive likelihood ratio – 4.5)

Chen C et al., Hum Reprod 2002; 17: 128-33

6. Mechanical washout of embryos

- Fluid collection in the uterine cavity was associated with a lower pregnancy rate of IVF treatment.

Levi AJ et al., Hum Reprod 2001; 16: 2610-5

- Simple mechanical hindrance for embryo implantation due to leakage of HF in the uterine cavity.

Mansour RT et al., J Vitro Fertil Embryo Transfer 1991; 8: 157-9

Bloeche M et al., Hum Reprod 1997; 12: 703-5

- Embryonic apposition to the endometrial surface is compromised when a fluid interface exists.

Shahara FI, Hum Reprod 1999; 14: 577-8

7. Effect on endometrial receptivity and inhibition of embryo implantation

- Recipients with HS receiving donor oocytes or cryopreserved embryos also experience lower implantation rates and higher abortion rates compared with normal control individuals. Cohen M et al., Hum Reprod 1999; 14: 1087-9
  Akman M et al., Hum Reprod 1996; 11: 1013-4
- Presence of HS was associated with inflammatory endometrial response in vivo that consist of statistically significant elevations of leucocytes and IL-2.

Copperman AB et al., Fertil Steril 2006; 86: 972-6

- A decrease in HOX-10 expression in the endometrium of patients with HS. Daftary G et al., Fertil Steril 2002; 78: 577-80

7. Effect on endometrial receptivity and inhibition of embryo implantation

- A study on murine embryos showed that the number of implantation sites or number of fetuses was not affected by the presence of HF. Rawe VJ et al., Fertil Steril 1997; 68: 668-70
- The use of endometrial co-culture enhanced the blastocyst, hatching and outgrowth formation in studies on mouse embryos. Spandorfer SD et al., Fertil Steril 1999; 71: 619-26
- A negative impact of HF was only evident in a minimum concentration 75% of HF in the presence of endometrial co-culture compared with 50% without co-culture.

7. Effect on endometrial receptivity and inhibition of embryo implantation

- Integrin $\alpha_v\beta_3$, a marker of endometrial receptivity, was down-regulated during the implantation period in patients with HS.
- 70% of patients demonstrated an increased integrin expression after surgical correction of their HS.

Meyer WR et al., Hum Reprod 1997; 12: 1393-8

- The proportion and the density of pinopodes in the peri-implantation endometrium were not affected by the presence of HS, while LIF, integrin β3 and MUC1 were significantly reduced in patients with HS.

Li L et al., Eur J Odstet Gynecol Reprod Biol 2010; 151: 171-5

Conclusions

- The negative impact of HS on IVF-ET could be supported by the consistency of data from retrospective studies an meta-analyses.
- A pleiad of theories exists to justify the deleterious impact of HS on IVF outcome.
- The theories of the presence of embryotoxic factor in HS was initially supported by mouse studies, but was not confirmed subsequently in human embryo studies.

Conclusions

- Individual variations in the context and the heterogeneity of HS may be the reason for the different effects on embryo development and consequently for the discrepancies in the results among studies.
- The potential impact of peri-ovulatory cytokine profile in HF as a predictor of subsequent IVF outcome awaits further clarification.

Conclusions

- Cytokines and growth factors are normal components of tubal fluid and are important for embryo development and implantation.
- Distortion of the balance between the concentrations of different embryotoxic and embryotrophic cytokines can negatively affect reproductive system with consequent impairment on implantation rate.
- Differences in the presence of cytokines among different HF samples.

Loutradis D et al., Gynecol Endocrinol 2005; 20: 26-9
Barmat L et al., Fertil Steril 1999; 72: 1110-12
Strandell A et al., J Assist Reprod Gen; 21: 241-246

Conclusions

- Low concentration levels or even the absence of cytokines and growth factors and especially of EGF in culture medium with HF may serve as an assay that could predict IVF outcome.
- Mouse embryo assays of HF may potentially serve as a predictor of subsequent IVF outcome in women with HS and the technique of aspiration of HF for analysis may be useful in the selection of appropriate treatment option for patients with HS undergoing IVF.

Conclusions

- HF exerts a deleterious effect on the endometrium altering the receptivity or simply by affecting embryonic apposition to the endometrial surface.
- All of these theories may be involved and a combination of the above-mentioned factor is responsible for the low implantation rate in women with HF.
- There is certainly a need for future studies to explore and elucidate the exact mechanisms through which HS exerts its negative effects.