Biochemical Pregnancies

H.J.A. Carp

Sheba Medical Center, Tel Hashomer

& Tel Aviv University, Israel

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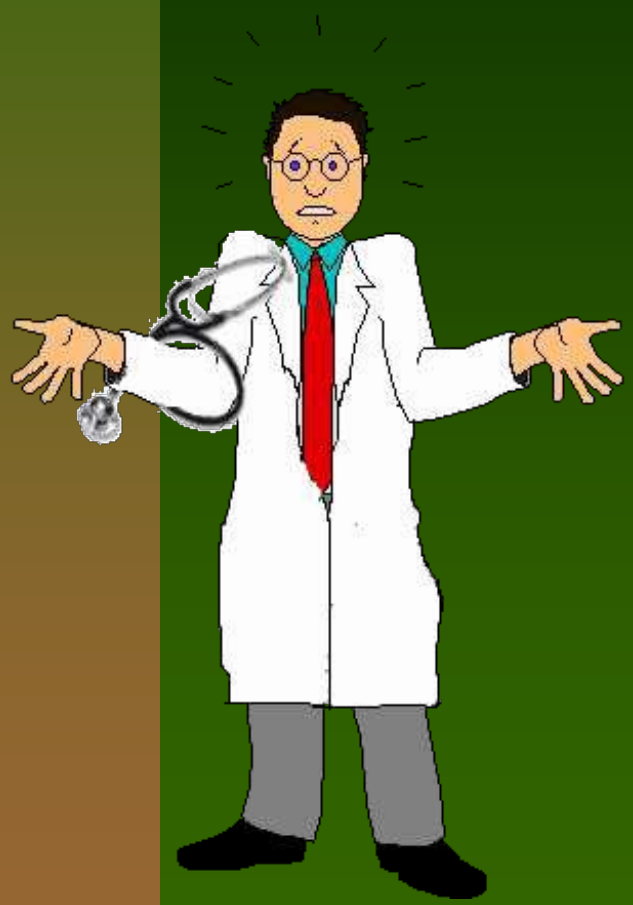


- What are biochemical pregnancies?
- What is the incidence?
- What are the implications?
- Should they be treated?
- How to treat them?

ESHRE Definition 2015

No Localisation on US

No US

Term	Description of pregnancy loss and clinical or ultrasound findings	References
Non-visualized pregnancy loss	Spontaneous pregnancy demise based on decreasing serum or urinary β-hCG levels and non-localization on ultrasound, if performed	Kolte *et al.* (2014)
Biochemical pregnancy loss	Spontaneous pregnancy demise based on decreasing serum or urinary β-hCG levels, without an ultrasound evaluation	Farquharson and Stephenson (2010)
Resolved pregnancy loss of unknown location (resolved PUL)	Pregnancy demise not visualized on transvaginal ultrasound with resolution of serum β-hCG after expectant management or after uterine evacuation without chorionic villi on histology	Barnhart *et al.* (2011)
Treated pregnancy loss of unknown location (treated PUL)	Pregnancy demise not visualized on transvaginal ultrasound with resolution of serum β-hCG after medical management	Barnhart *et al.* (2011)

Resolution after medical management

Resolution after expectant management

Definition

- No accepted definition
- Positive hCG, no clinical sign of pregnancy, no pregnancy on ultrasound (Farquharson & ESHRE 2005; Sher 2010; Schreiber et al, 2009; Annan, 2013)
- 10 - 1000 IU/l & rising level (Carp et al, 1994; De Neubourg et al, 2004)
- ≥ 2 rising values of hCG with no gestational sac 3 weeks after ET (Coulam et al, 2002)
- We suggest alternative nomenclature:-
  - Isolated elevated hCG,
  - If hCG rises, biochemical pregnancy, non visualised pregnancy or PUL.

hCG Secretion

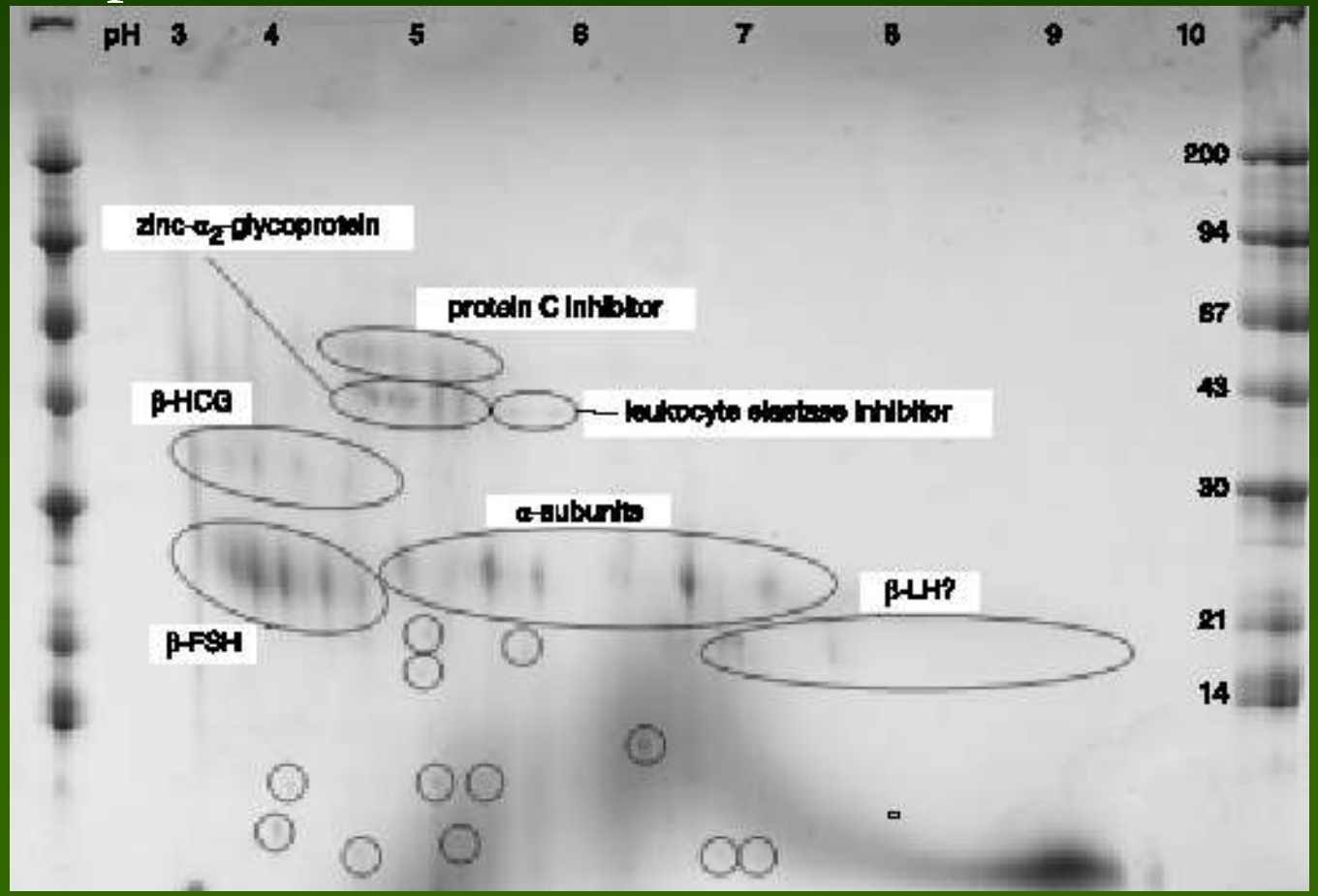
- hCG mRNA can be detected from 8 cell embryos
- hCG can be detected from 7 days after ovulation (Lopata & Hay 1989)
- Can be used clinically from 9 days after LH surge
- Positive hCG after 12 days usually taken as indicative of pregnancy

False Positive

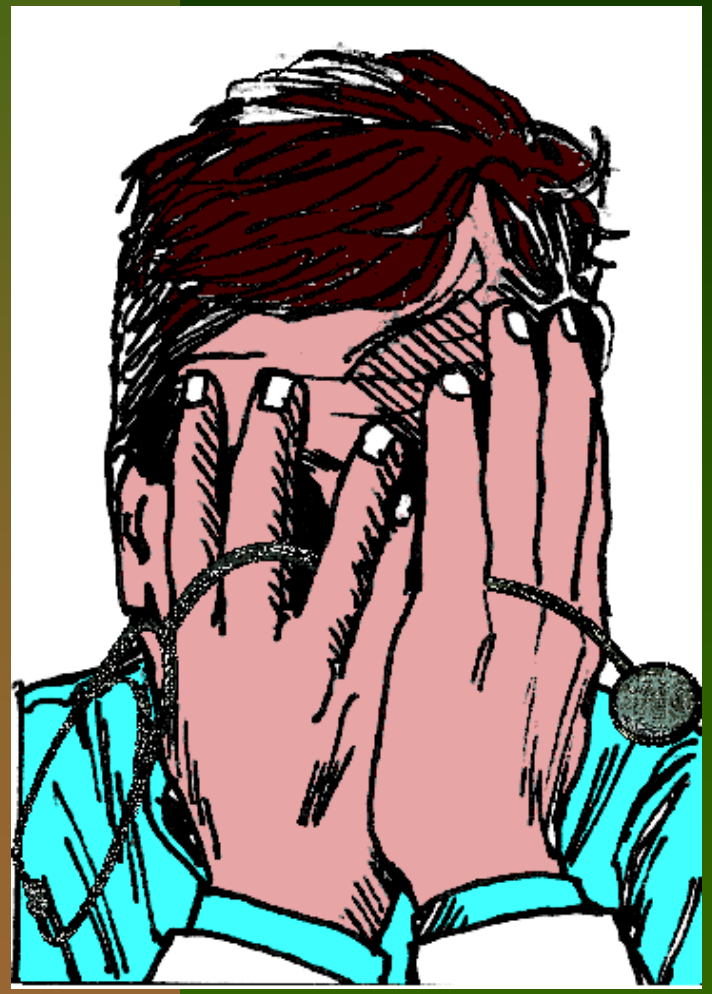
- Tests so sensitive that phantom, endometrial or pituitary hCG can be detected.
- Tests use animal antibodies raised to hCG. If anti-animal antibodies present after exposure to animals, results may be confounded.
- Intra & interlaboratory variation
- If hCG used for ovulation induction, it may still be present after 12 days
- These are raised isolated hCG's, not biochemical pregnancies

Contaminants in Gonadotrophins

- In hMG (Van de Weier et al, 2003)
- In Corifollitropin α (Kol, 2018)



Pitfalls in Diagnosis



- If no hCG has been administered, how reliable is laboratory?
- If hCG has been administered, one test is no evidence.
- In order to diagnose pregnancy, look for rising hCG levels.
- BUT, rising level must be sufficient to exclude intra and interlaboratory variation

Recurrent Biochemical Pregnancies

- Age 40
- 1. 2001 Undefined “Biochemical pregnancy”
- 2. 2001 Twins 1 Sacrococcygeal tumor IUFD 18w
- 2 Microcephalus 32w
- 3. 2003 Missed abortion 10w FHL
- 4. 2005 LSCS ♂ 3045
- 5. 2006 Biochemical pregnancy βhCG = 600
- 6. 2007 Biochemical pregnancy βhCG = 1200
- 7. 2008 Biochemical pregnancy βhCG = 800
- 8. 2009 Biochemical pregnancy βhCG = 866

- Standard investigation showed no aPL, thrombophilia, uterine anomaly, hormonal imbalance or parental chromosomal aberration

Incidence of Biochemical Pregnancies

- In fertile patients – 13-22% (Wilcox et al, 1988, Ellish et al, 1996, Zinaman et al, 1996, Coulam et al, 1997, Hakim et al, 1995)
- Isolated elevated hCG - 4% of Liu et al's series (1988)
- In infertile population incidence (14% - 18%) **not higher** after IVF (Salumets et al, 2006; Zeadna et al, 2015)
- Incidence **higher** after IVF to infertile population, 30% to 50% (Coulam et al, 1995:6) 70% vs 21% (RR, 2.6; 95% CI, 1.8 to 3.8) (Hakim et al, 1995)
- Increased maternal age at IVF/ICSI was the only parameter elevating the biochemical pregnancy rate in Salumets et al's (2006) series pf 1242 FET cycles.

Embryo Causes

- Delayed implantation may be cause or result of pregnancy loss, as hCG may be insufficient to allow implantation
- Only 8 of 36 biochemical pregnancies produced > 40% hCG-H on the day of implantation. All (100%) of normal term pregnancies produced greater than 40% hCG-H. (Saski et al, 2008)
- If implantation delayed, slow rise in hCG - abnormal embryonic development may have occurred after implantation due to chromosomal or other embryonic factors (Liu & Rosenwaks, 1991)

Maternal Causes

- Endometrial thickness. Biochemical pregnancies - in 7/32 (21.9%) if endometrium < 9 mm, on day of hCG when undergoing ovulation induction. 0/49 when endometrium ≥ 9 mm. (Dickey et al, 1993).
- hCG secretion by invading trophoblast negatively modulated by endothelin-1 (ET-1), PG F2α found in endometrium (Lenton et al, 1998)

Paternal Causes

- No information on biochemical pregnancies, only RPL & RIF
- Abnormal packaging of sperm chromatin leads to DNA fragmentation. (Schagdarsurengin et al, 2012)
- High DNA damage as demonstrated by increased DFI or sperm SCSA for Nick-End Labeling (TUNEL), Halo test or sperm aneuploidy tests
- Meta-analysis of 11 studies on sperm DNA damage & MA after IVF indicated that sperm DNA damage is predictive of pregnancy loss after ART. (Zini et al, 2008)

Immunologically mediated
(Coulam et al, 2003)

- Blood samples from 122 women with implantation failure (negative pregnancy test) compared to 20 women with biochemical pregnancies
- Biochemical pregnancies - higher prevalence of aPL (80% vs 28%, $P < 0.0001$).
- Prevalence of ANA, elevated NK cells not different between the two groups.

Implications of Biochemical Pregnancies (1)

- Biochemical pregnancies (BP) lead to patients leaving IVF programs (Pearson et al, 2009)
- BP ‘s positive predictor of future IVF pregnancies (Templeton et al, 1996; Wright et al, 2002; Bates, 2002; Macklon et al, 2002; De Neubourg, 2004, Haas et al, 2012)
- But negative predictor for ART outcomes. BP cases have higher BP and SA rates (Yang et al, 2015)
- Each non-visualized pregnancy loss - RR of live birth = 0.90 (95% CI 0.83; 0.97), equivalent to the RR conferred by each additional clinical miscarriage (Kolte, et al, 2014).
- If exclusively recurrent biochemical pregnancies, risk of EUP - 27% (Christiansen 2011)

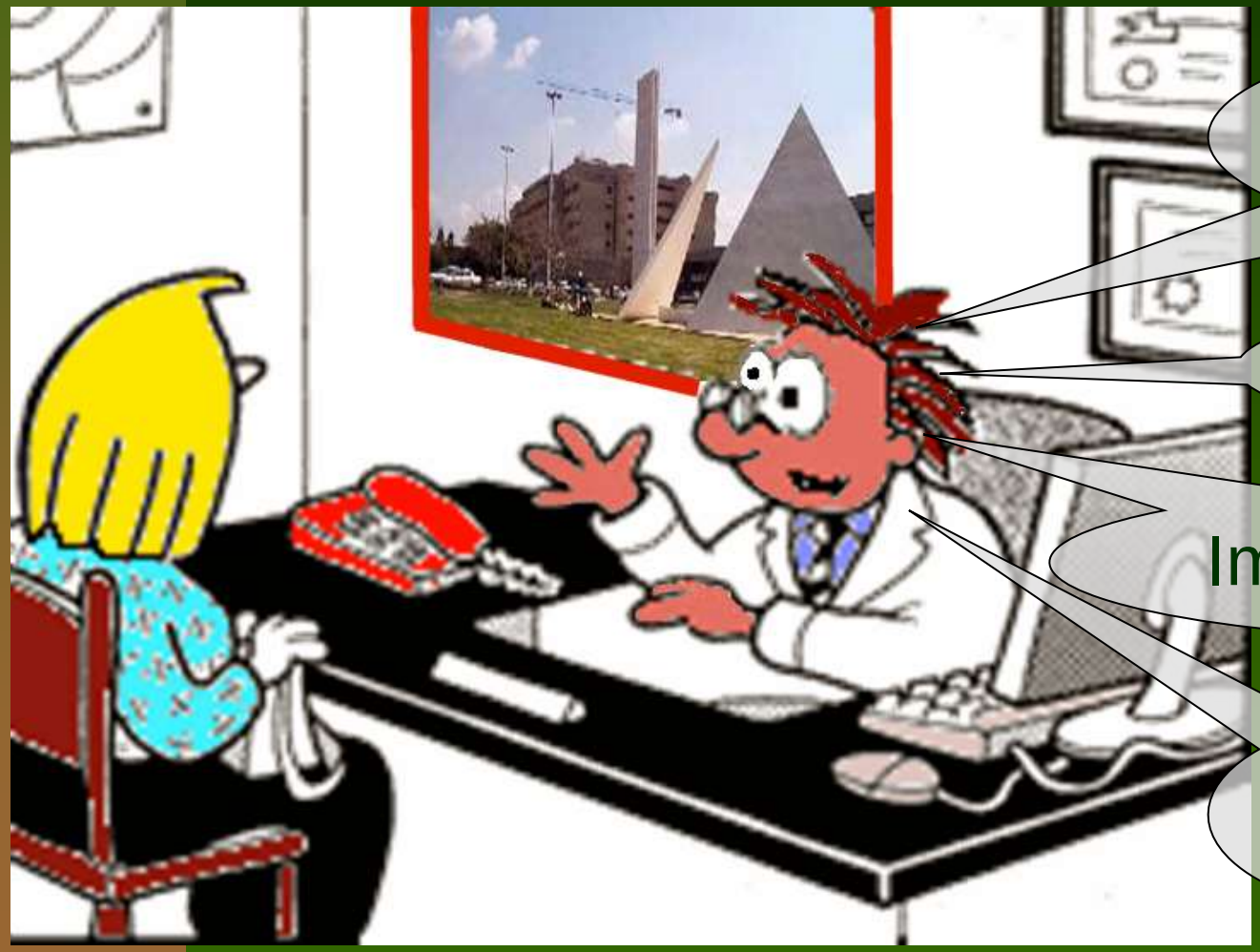
Implications of Biochemical Pregnancies (2)

- ASRM & ACOG do not recognise biochemical pregnancies as miscarriages as BP may be isolated raised hCG, or EUP.
- However, ESHRE does recognise BP as pregnancy

Should We Treat Biochemical Pregnancies?

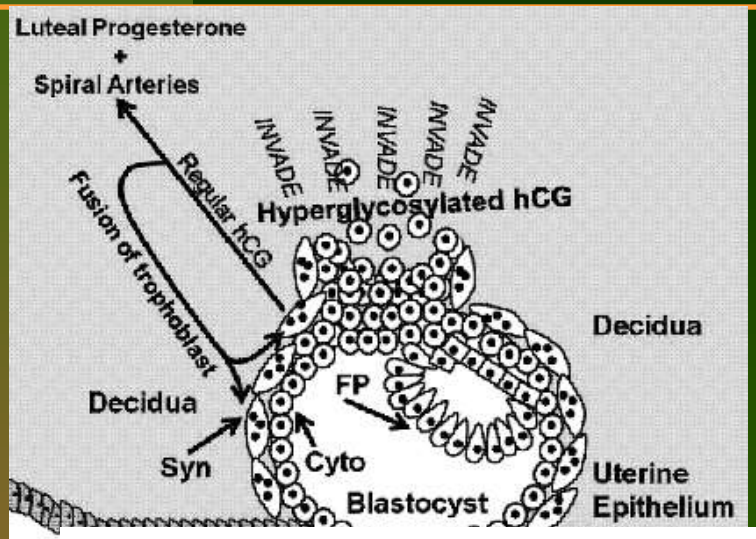
- May be non viable or persistent (PUL), MTX may be required.
- After recurrent biochemical pregnancies, few figures available from literature
- If 1 BP – little need to treat.
- If 2 BP – little need to treat. ESHRE says Treat
- If >3. Treat as RPL
- Results of 40 subsequent pregnancies available with >2 biochemical pregnancies, no treatment, from our database
  - 21 subsequent live births (53%),
  - 8xMA, 10 x biochemical pregnancies, 1xEUP.

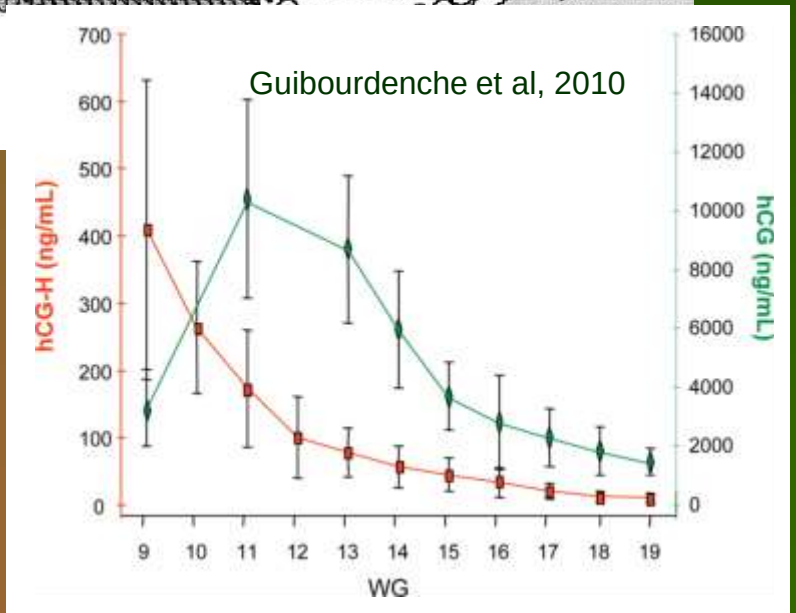
Possible Lines of Management



- Hormone supplementation
- Anticoagulants
- Immunopotentiation
- P.G.S.

hCG and Recurrent Biochemical Pregnancies

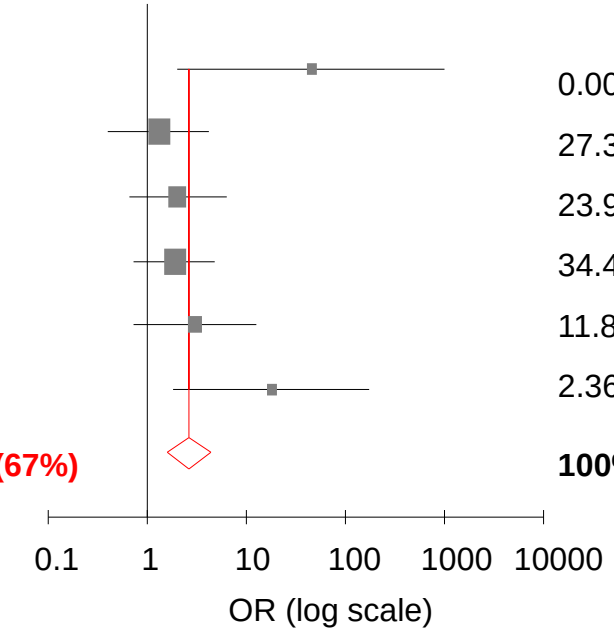




- Shallow or ineffective invasion leads to pregnancy failures (Juniaux et al, 2006).
- HCG-H - 90% of total hCG in first 2–3 weeks when invasive trophoblast activity is high (Cole, 2009; Evans, 2015).
- Can pregnancy failures be prevented by hCG-H at time of implantation?
- Quest Diagnostics has patent on hCG-H assay (B152).
- Generally, commercially available hCG used instead

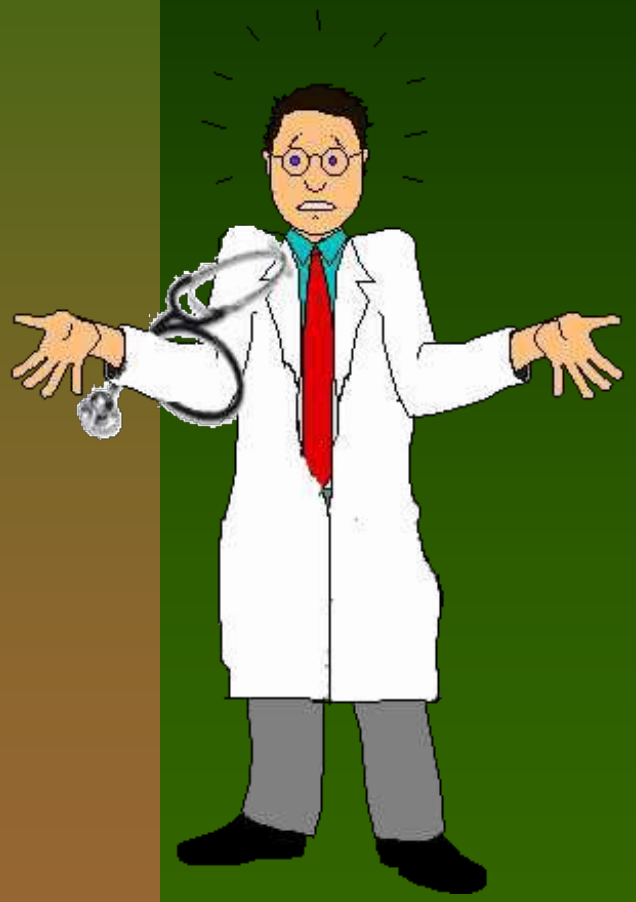
hCG in RPL: Updated Metaanalysis

Study ID	Year	Exposed	Control	Weight	OR with 95% CI
Harrison	1985	10/10	3/10	0.00%	45 (2.0114 to 1006.7466)
Harrison	1992	30/36	31/39	27.36%	1.2903 (0.3999 to 4.1636)
Quenby & Farquharson	1994	35/41	29/39	23.99%	2.0115 (0.6528 to 6.1985)
El Zibdeh	2005	41/50	34/48	34.45%	1.8758 (0.7234 to 4.8641)
Sadler & Bailie	1978	19/23	11/18	11.84%	3.0227 (0.7194 to 12.7002)
Svigos	1982	12/13	6/15	2.36%	18 (1.829 to 177.1499)
META-ANALYSIS:		**147/173 (85%)**	**114/169 (67%)**	**100%**	**2.6512 (1.583 to 4.4402)**



- Benefit 18%
- OR for live birth = 2.65 (1.58-4.44)
- ≥ 2 miscarriages, No. miscarriages not quoted
- All trials used u-hCG

hCG Supplementation in Recurrent Biochemical Pregnancies (Authors Series)



≥2 Biochemical	3 Biochemical	≥4 Biochemical
50/75 (67%)	20/28 (71%)	7/10 (70%)
21/40 (53%)	10/17 (59%)	1/3 (33%)

- Not significantly different to 21/40 (53%) in control group
- Power analysis shows that 410 patients required to show statistical significance
- **Does this merit treatment? Yes**
- But, we need larger series or collaborative figures to reach statistical significance
- Big Data needed. SART does not list BP's

IVIg in Recurrent Biochemical Pregnancies

- Only used in our series in patients with >5 RPL & ≥ 5 biochemical pregnancies
- 9/19 pregnancies (47%) terminated in subsequent live births
- Patient No.1. Age 33, 10 biochemical pregnancies, gravida 0
- Pregnancies from IVF & spontaneous pregnancies
- Advised ovum donation.
- No cause found for pregnancy loss, 46XX, 46XY, no aPL, no thrombophilia, normal hysteroscopy. Normal FSH, LH, HPRL. Prolonged cycles.
- Treated by IVIg, prior to pregnancy 11, and as soon as pregnancy diagnosed
- Pregnancy 11, hMG. ♂3870g. Pregnancy 12, hMG. ♂3500g. Pregnancy 13, spontaneous ♂3450g.

Future Directions

- Higher numbers of BP's should be treated as RPL
- Primary diagnosis: embryonic, maternal or paternal
- Is parental karyotyping sufficient? High definition, low definition or exome sequencing
- NIPT at early stages
- In RPL, endometrium may lose selectivity. Is it over selective in BP?

Thank You for Listening

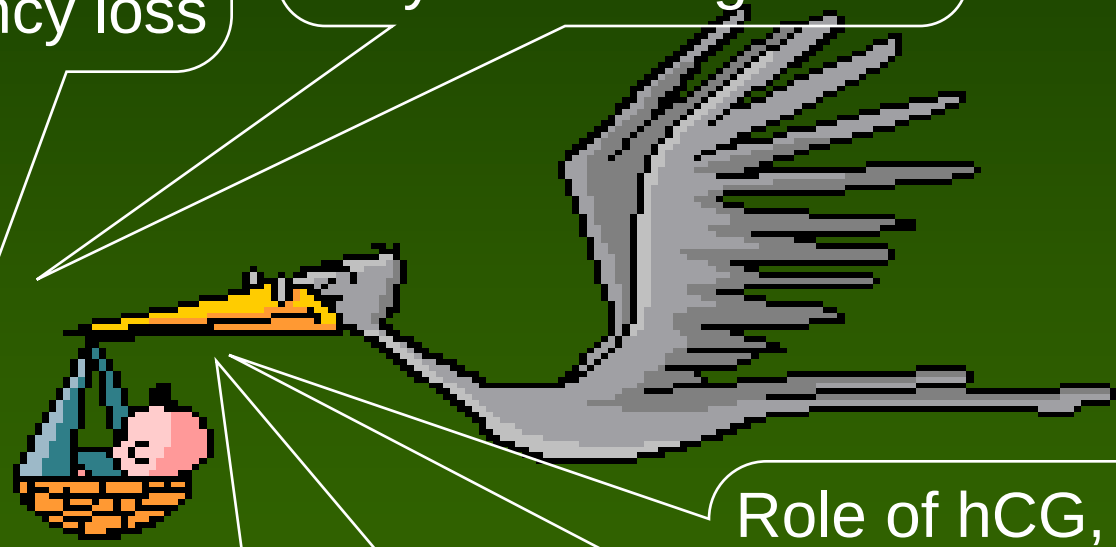
hCG is major determinant of CL function, implantation & immunomodulation. Low levels of hCG may explain pregnancy loss

Recurrrent biochemical pregnancies may be early miscarriages

False positive results

Inter & intra-laboratory variation

No definition of biochemical pregnancy



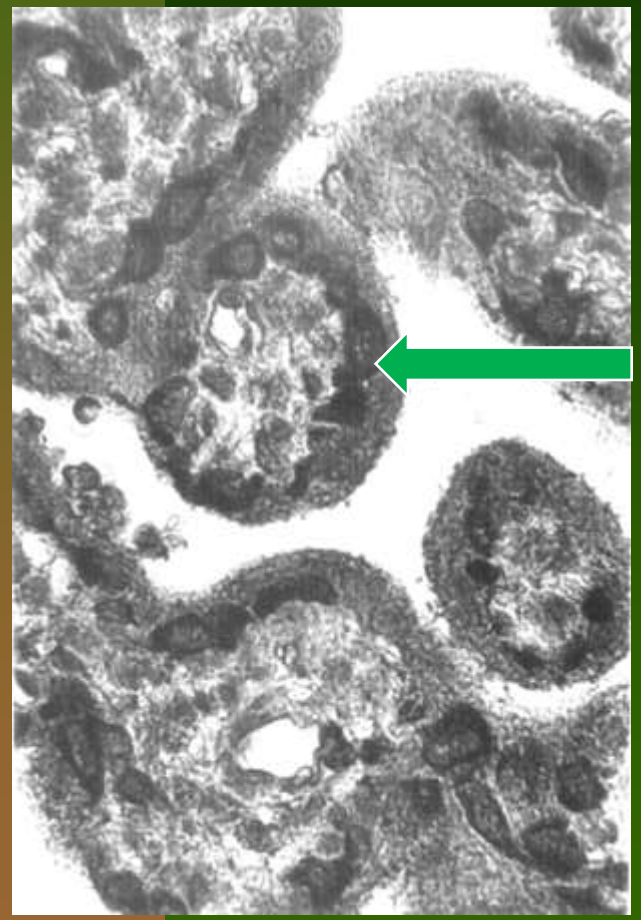
Suggested definition 10-1000iu/l & rising levels

Role of hCG, anticoagulants, IVIg etc. require clarification.

Heparins in Infertility

- Heparins affect adhesion, via heparan sulfate proteoglycans or heparin-binding of blastocyst to endometrium & subsequent invasion (Fiedler & Würfel, 2004)
- Qublan et al (2008) - enoxaparin significantly increased implantation & pregnancy rates over placebo after ≥ 3 IVF failures (20.9% vs. 6.1% p<0.001)
- Sher et al, (1998) 687 aPL+ women (1050 IVF cycles). 46% births with Heparin/Aspirin, 17% births without H/A
- Berker et al, (2011) LMWH had no beneficial effect on pregnancy outcomes in patients with ≥2 implantation failures, outcomes better after ≥3 implantation failures, but not statistically significant.
- ASRM practice bulletin (1999) did not recommend heparins in aPL seropositive patients with infertility

aPL: Trophoblast Effects



- aPL inhibit trophoblast differentiation "in vitro" (Quenby et al, 2005)
- Binding of aPL to β2GP1, → breakdown of phospholipid adhesion molecules in trophoblast, → trophoblast failure & prothrombotic effects (Lyden et al, 1992).
- aPL may affect trophoblast invasiveness, implantation, placentation, significantly reduces hCG release & early embryonic development (Shurtz-Swirsky et al, 1993; di Simone et al, 2000).
- aPL associated with reduced LIF (Kralickova et al, 2007) (LIF essential for implantation)

Non Anticoagulant Actions of Heparin / Enoxaparin

- Heparin inhibits gene expression and production of TNF-α (Baram, 1997; Pevni et al, 2005) & increases serum TNF-BP-I, (Lantz et al, 1991)
- Both heparin & LMWH limit anti-inflammatory response including neutrophil extravasation & decreasing vein wall permeability (Downing et al, 1998)
- In vitro, heparin restores ability of trophoblast to secrete hCG, which is inhibited by aPL (Schurtz-Svirsky et al, 2003)
- Heparin inhibits apoptotic nuclease responsible for DNA fragmentation in cells undergoing apoptosis (Widlak & Garrard. 2006)

Biochemical Pregnancies & Anticoagulants

- No English language literature
- Sarto et al, (2001). 35 women selected for enoxaparin from 105 women with thrombophilia & ≥1 preclinical pregnancy loss,
- With antithrombotic therapy, 30/35 (85%) ended in live birth vs 16/105 (15%) of pregnancies prior to treatment ($p < 0.001$).
- This is before & after model. Suffers from selection bias