

**Adverse Effect of Human Hydrosalpingeal Fluid on the Development of Mouse Embryo (II)**

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**= Abstract =**

In our previous study, we observed that hydrosalpingeal fluid (HSF) adversely effect mouswe embryo development and hatching. The aim of this study was to evaluate the effect of HSF as assessed by the blastocyst development rate (BDR) and by cell counting in vitro. HSF was collected from ninie patients undergoing salpingoneostomy to correct hydrosalpinx. Two-cell embryos were obtained from superovulated ICR mice. T6 medium and T6+0.4% bovine serum albumin were used as control media. T6 medium containing 10% or 50% HSF and 100% HSF from each patient were used as test media. Nine to 15 embryos were cultured in microdrops prepared from each of these media To assess the total cell number within each blastocyst, the blastocysts were fixed and stained with Hoechst 33342 to facilitate cell counting. The mean BDR in two control media were 88.7% and 85.3%. The mean BDR in media containing 10%, 50%, 100% HSF were 90.0%, 89.4% and 75.2%\*, respectively (\*: p<0.05). The overall mean cell count (+ SEM) in control media were 86.9 + 3.2 and 91.0 + 3.8. The BDR was affected adversely only by 100% HSF and not in media containing 10% or 50% HSF. Mean cell counts were decreased significantly only in blastocysts cultured 100% HSF (63.3 + 4.6; p<0.01) but not in blastocysts cultured in 10% or 50% HSF (90.8 + 4.2 and 81.9 + 6.1, respectively). Thus, it is concluded that HSF has no embyotoxic effect but has a mildly negatively effect on embryonic growth and development.

(Anderson et al., 1994; Kassabji et al., 1994; Vandromme et al., 1995; Flemming et al., 1996).

(Shelton et al., 1996; ,1997).  
가

( , 1997),

가

1.

(Hydrosalpingeal fluid, HSF)

, 300rpm 10 ICR strain , 7 IU pregnanct

-20°C

mare serum gonadotropin (PMSG, Sigma) 48 7 IU human  
 gonadotropin (hCG, Sigma) , hCG 46  
 (copulation plug) flushing 2

2.

0.22um filter (Millipore) 3  
 Group 가 , T6 10% 50%  
 100% Group 가 T6 medium T6 medium 0.4% bovine serum  
 albumin (BSA; Gibco) 2 Group 가 T6 medium T6 medium 5가  
 (Sigma) (culture dish) microdrop , mineral oil  
 5% CO<sub>2</sub>가 가 37°C incubator  
 6 2- 9- 15  
 72

3.

(Blastocyst development rate;BDR)  
 가 2-  
 glutaraldehyde Ebert (1985) 1%  
 Sigma) slide Bisbenzimidazole solution (10ug/ml; Hoechst 33342,  
 fluorescent microscope (Optiphot-2, Nikon)

4.

chi-square test  
 Duncan's multiple range test  
 가  
 p < 0.05

Osmolarity 277-297 mOsm , pH 7.9-8.4  
 incubation 7.37-7.51 Table 1  
 9  
 (p=0.34). T6, T6+BSA, 10% HSF, 50% HSF, 100% HSF  
 (n=97, 102, 100, 104, 105) 88.7%, 85.3%, 90.0%, 89.4%, 75.2%\* (\*; p<0.05,  
 1). 100% HSF

Table 2  
 가 T6, T6+BSA, 10% HSF, 50%  
 HSF, 100% HSF 81, 86, 88, 81, 79 (+SEM)  
 86.9+3.2, 91.0+3.8, 90.8+4.2, 81.9+6.1, 63.3+4.6\* (\*; p<0.01, 2).  
 100% 가

가  
 Mukherjee et al.(1997) 10% 100%  
 가 5% (0%) , Sachdev et  
 (toxicity) 가  
 al.(1997) 1% 가 61%가 100%  
 , Murray et al.(1997) 100% ( , 1997)  
 75% 가  
 Sawin et al.(1997) human trophoblast Schats et al.(1997)



Katz, E., Akman, M.A., Damewood, M.D. and Garcia, J.E. (1996) Deleterious effect of the presence of hydrosalpinx on implantation and pregnancy rates with in-vitro fertilization. *Fertil. Steril.*, 66, 122-125.

Koong, M.K., Jun, J.H., Byun, H.K. *et al.* (1996) Adverse effect of hydrosalpingeal fluid on development of mouse embryo in vitro. (Abstr.) *In: 9th. World Congress on Human Reproduction and 5th. World Conference on Fallopian Tube in Health and Disease, Philadelphia, PA, USA, May 28 June 1, 1996.* Abstr. No. FR 24.

Koong, M.K., Song I.O., Son I. P. *et al.* (1997) Effect of hydrosalpinx and its surgical correction on pregnancy and implantation rates following IVF and embryo transfer. (Abstr.) *In: 13th. Annual Meeting of European Society for Human Reproduction and Embryology, Edinburgh, United Kingdom, June 22 25, 1997.* Abstr. No. p-111

Mansour, R.T., Aboulghar, M.A., Serour, G.I. and Riad, R. (1991) Fluid accumulation of the uterine cavity before embryo transfer: A possible hindrance for implantation. *J. In Vitro. Fertil. Embryo Transfer*, 8, 157-159.

Meyer, W.R. and Beyler, S.A. (1995) Deleterious effects of hydrosalpinges on in vitro fertilization and endometrial integrin expression. *Assisted Reprod. Rev.*, 5, 201-203.

Miyazaki, T., Kuji, N., Sugakara, M. *et al.* (1991) An analysis of factors associated with ectopic pregnancy following in vitro fertilization and embryo transfer. *Nippon Sanka Fujinka Gakkai Zasshi*, 3, 1496-1500.

Murray, C.A., Clarke, H.J. and Tulandi, T. (1996) Effects of human hydrosalpinx fluid on mouse embryo development. (Abstr.) *In: the 52nd. Annual Meeting of the American Society of Reproductive Medicine, Boston, MA, USA, Nov. 2-6, 1996.* Abstr. No. P-050.

Murray, C.A., Clarke, H.J. Tulandi, T. and Tan, S.L. (1997) Inhibitory effect of human hydrosalpingeal fluid on mouse preimplantation embryonic development is significantly reduced by the addition of lactate. *Hum. Reprod.*, 12, 2504-2507.

Mukherjee, T., Cook, C.A., Copperman, A.B. *et al.* (1996) Hydrosalpinx fluid has embryotoxic effects on murine embryogenesis: a case for prophylactic salpingectomy. *Fertil. Steril.*, 66, 851-853.

Poe-Zeigler, R., Shelton, K., Toner, J.P. *et al.* (1995) Salpingectomy(ies) improves the pregnancy rate after IVF in patients with unilateral or bilateral hydrosalpinx. *J. Assist Reprod Genet.*, 12, S65.

Rawe, V.J., Compton, M.G., Liu, J. *et al.* (1997) Effect of human hydrosalpinx fluid on murine embryo development and implantation. *Fertil. Steril.*, 68, 668-670.

Sachdev, R., Kemmann, E., Bohrer, M. and El-Danasouri, I. (1997) Detrimental effect of hydrosalpinx fluid on the development and blastulation of mice embryos in vitro. *Fertil. Steril.*, 68, 531-533.

Sharara, F.I. and McClamrock, H.D. (1997) Endometrial fluid collection in women with hydrosalpinx after human chorionic gonadotrophin administration: a report of two cases and implications for management. *Hum. Reprod.*, 12, 2816-2819.

Schats R., Lens, J.W. and Wit, de W. (1997) Survival of spermatozoa in hydrosalpinx fluid are not impaired. (Abstr.) *In: 13th. Annual Meeting of the European Society of Human Reproduction and Embryology, Edinburgh, Scotland, UK.*, June 22-25, 1997, Abstr. No. O-226.

Shelton, K.E., Butler, L., Toner, J.P., Oehniger, S. and Muasher, S.J. (1996) Salpingectomy improves the pregnancy rate in in-vitro fertilization patients with hydrosalpinx. *Hum. Reprod.*, 11, 523-525.

Strandell, A., Waldenstrom, U., Nilsson, L. and Hamberger, L. (1994) Hydrosalpinx reduces in-vitro fertilization/embryo transfer pregnancy rates. *Hum. Reprod.*, 9, 861-863.

Sawin, S.W., Wang, C.L., Mola, J.R.L. *et al.* (1997) Hydrosalpinx fluid enhances human trophoblast viability and function in vitro: implications for embryonic implantation in assisted reproduction. *Fertil. Steril.*, 68, 65-71.

Vandromme, J., Chasse, E., Lejeune, B. *et al.* (1995) Hydrosalpinges in in-vitro fertilization: an unfavorable prognostic feature. *Hum. Reprod.*, 10, 576-579.

Vasquez, G., Boeckx, W. and Brosens, I. (1995) Prospective study of tubal mucosal lesions and fertility in hydrosalpinges. *Hum. Reprod.*, 10, 1075-1078.

Zouves, C., Eranus, M. and Gomel, V. (1991) Tubal ectopic pregnancy after in vitro fertilization and embryo transfer: a role for proximal occlusion or salpingectomy after failed distal tubal surgery? *Fertil. Steril.*, 56, 691-695.

Table I. Comparison of blastocyst development rates (%) using media with and without HSF and HSF alone by patient from which HSF was obtained.

Patient	T6 100% HSF	T6+0.4% BSA	T6+10% HSF	T6+50% HSF
1	100(10)	82(11)	91(11)	90(10)
2	100(10)	90(10)	100(10)	73(11)
3	67(12)	92(12)	92(13)	77(13)
4	83(12)	83(12)	91(11)	73(11)
5	100(14)	80(15)	93(14)	73(15)
6	100( 9)	91(11)	80(10)	80(10)
7	80(10)	90(10)	100(10)	73(11)
8	70(10)	70(10)	80(10)	67(12)
9	100(10)	91(11)	82(11)	75(12)

Data in parentheses indicate the number of mouse embryos used.

Table 2. Mean cell counts for blastocysts cultured in T6, T6+0.4% BSA, T6+10% HSF, T6+50% HSF or 100% HSF alone, relative to each patient. The mean cell counts at each media were expressed as the average cell counts for all of the blastocysts cultured in a single microdrop.

Figure 1. Mean blastocyst development rate (BDR) for mouse embryos cultured in T6, T6+0.4% BSA, T6+10% HSF, T6+50% HSF or in 100% HSF alone. Results are expressed as mean  $\pm$  SEM. The statistical differences are denoted by asterisks (\*: $P < 0.05$  compared with all other groups).

Figure 2. Overall mean cell counts for blastocysts using the nine HSF patient samples for each medium preparation. The results are expressed as mean  $\pm$  SEM. The statistical differences are denoted by asterisks (\*: $P < 0.01$  compared with two control groups).