SERUM LEVELS OF HUMAN CHORIONIC SOMATOMAMMOTROPHIN (HCS) AND HUMAN CHORIONIC GONADOTROPHIN (HCG) FOLLOWING INTRA-AMNIOTIC INJECTION OF HYPERTONIC SALINE

by

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Introduction

Intra-amniotic hypertonic saline injection is an accepted method for terminating pregnancy in the second trimester. The study of hormonal changes following such an injection may be useful for better understanding of foeto-placental physiology, placental insufficiency or onset of labour at or before term. From this point of view it is interesting to study the levels of HCS and HCG at frequent intervals after intra-amniotic injection of hypertonic saline and to correlate these results if possible with onset of abortion.

Material and Methods

Termination of pregnancy was carried out by intra-amniotic instillation of saline. Upto 200 cc of amniotic fluid was withdrawn and replaced by 200 cc of 20% saline solution. The gestational period varied from 14 to 20 weeks. Blood samples were drawn from antecubital vein at intervals of 12-24 hours. The serum was separated and frozen at—20°C until analysis. Serum HCS and HCG were estimated by radio-immunoassay using double antibody method (Middley 1966) in 64 samples in 21 patients before and after the injection of hypertonic saline. The high concentrations of HCS and HCG in blood of pregnant women necesitated serum dilutions of 1:40 and 1:20 respectively in the assay system.

All reagents for this immunoassay were generously supplied by the National Institute of Arthritis and Metabolic Disease (NIAMD). The Second Reference Preparation of Human Menopausal Gonadotrophin (2IRP) was used as the standard for HCG. In our laboratory, the lower limit of sensitivity for the HCG assay is 0.5 mIU/ml.

Results

Table I indicates the serum HCS in these patients according to the weeks of gestation. Our results of serum HCS are in agreement with those of Saxena *et al.*, reported in 1969.

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TABLE I

Human Chorionic Somatomammotrophin (HCS) Changes After Intra-amniotic Injection of Hypertonic Saline (Results in ug/ml.)

Week of gestation	No.	Initial level (Control)	After Intra-amniotic injection							Abortion at hours
			12 hrs.	24 hrs.	36 hrs.	48 hrs.	60 hrs.	72 hrs.	84 hrs.	
14 wks.	1	1.13	—	3.15	—	—	—	—	—	26 hours
	2	1.1	2.24	—	—	—	—	—	—	24 ,,
16 wks.	1	1.5	3.48	—	2.52	—	—	—	—	48 hours
	2	1.8	—	4.0	1.42	—	1.7	—	5.5	108 ,,
	3	1.62	2.28	—	—	—	—	—	—	20 ,,
	4	1.2	0.79	—	—	—	—	—	—	24 ,,
	5	0.72	0.58	0.18	—	—	—	—	—	28 ,,
18 wks.	1	2.0	—	1.2	2.2	1.11	—	—	—	50 hours
	2	1.0	—	—	1.7	3.5	—	—	—	50 ,,
	3	4.6 (2½ hrs. after inj.)	1.24	2.25	—	—	—	—	—	36 ,,
	4	0.88	1.9	0.99	—	—	—	—	—	
	5	1.14	—	1.1	—	0.115	—	—	—	
20 wks.	1	1.35	6.85	—	6.45	—	—	—	—	41 hours
	2	1.65	1.79	1.47	—	—	—	—	—	31 ,,
	3	4.88	—	—	3.3	—	—	—	—	42 ,,
	4	5.7	6.0	—	—	—	—	6.1	—	84 ,,
	5	6.2	—	4.55	7.0	6.4	—	—	—	60 ,,
	6	1.33	2.3	0.64	—	—	—	—	—	36 ,,
	7	1.04	0.66	0.785	—	—	—	—	—	48 ,,
	8	1.05	—	2.55	—	1.10	1.0	—	—	72 ,,
	9	1.5	—	2.3	—	1.7	—	1.48	—	144 ,,

TABLE II

Correlation Between Serum HCS Values and the Number of Abortions

Injection & Hormonal Estimation interval	Total patients	Fall in HCS No. of patients	Abortion within 12 hrs. No. of patients	Rise in HCS No. of patients	Abortion within 12 hrs. No. of patients
12	13	5	3	8	2
24	14	6	5	8	1
36	7	2	1	5	4
48	6	2	2	4	—
60	2	2	1	—	—
72	3	1	—	2	2
84	1	—	—	1	—
Total No. of patients	46	18	12	28	9

Table II shows the correlation between the HCS values and number of abortions within 12 hours. From the Table it is clear that of the 18 patients showing a fall in serum HCS levels, 12 aborted within 12 hours but of the 28 patients showing a rise in HCS, only 9 aborted within next 12 hours.

Fig. 1 represents the serum HCS levels graphically. The time of abortion is taken as 100 per cent for each patient and all individual times for blood collection from the injection are expressed as a percentage of this interval. Similarly, the initial value for each hormone is taken as 100 per cent and all changes are expressed as the percentage of the control (initial).

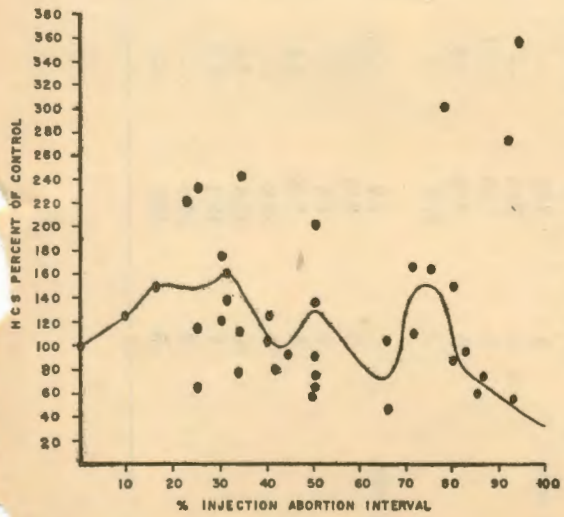

Fig. 1

From the graph it can be seen that the levels of HCS at time interval of 10-30% show a definite rise. The elevation of serum HCS is maintained upto 30-35% of the injection—abortion time interval. The HCS levels then started falling. This is followed by highly fluctuating levels of serum HCS and fails to show any correlation with the time of abortion.

Fig. 2 indicates the levels of serum HCS hormone in six individual patients of different time interval after the injection of intra-amniotic hypertonic saline.

Fig. 3 shows changes in HCS levels after intra-amniotic injection of hypertonic saline graphically.

Table III shows the serum HCG according to the weeks of gestation. Serum

TABLE III

Human Chorionic Gonadotropin (HCG) After Intra-amniotic Injection of Hypertonic Saline

Results in I.U./ml.

Weeks of gestation	No.	Initial level (control)	After Intra-amniotic injection							Abortion at hours
			12 hrs.	24 hrs.	36 hrs.	48 hrs.	60 hrs.	72 hrs.	84 hrs.	
14 wks.	1	1.82	—	1.0	—	—	—	—	—	26 hours
	2	14.4	12.0	—	—	—	—	—	—	24 ,,
16 wks.	1	2.2	1.68	—	2.0	—	—	—	—	48 hours
	2	2.3	—	2.4	3.0	—	1.4	—	2.44	108 ,,
	3	2.4	2.62	—	—	—	—	—	—	20 ,,
	4	21.6	20.8	—	—	—	—	—	—	24 ,,
	5		24.4	9.6	—	—	—	—	—	28 ,,
18 wks.	1	1.02	—	2.42	0.92	1.96	—	—	—	50 hours
	2	3.04	—	—	2.36	1.84	—	—	—	50 ,,
	3	3.06	2.24	1.9	—	—	—	—	—	36 ,,
	4	4.28	2.8	4.2	—	—	—	—	—	
	5	17.6	—	13.0	—	13.2	—	—	—	
20 wks.	1	2.6	1.52	—	2.1	—	—	—	—	41 hours
	2	2.02	0.7	2.8	—	—	—	—	—	31 ,,
	3	3.8	—	—	3.2	—	—	—	—	42 ,,
	4	2.02	1.4	—	—	—	—	1.84	—	84 ,,
	5	25.2	—	15.6	17.2	9.6	—	—	—	60 ,,
	6	15.8	7.4	6.88	—	—	—	—	—	36 ,,
	7	13.2	7.2	3.84	—	—	—	—	—	48 ,,
	8	21.6	—	13.2	—	9.06	4.2	—	—	72 ,,
	9	13.2	—	12.8	—	15.0	—	4.8	—	144 ,,

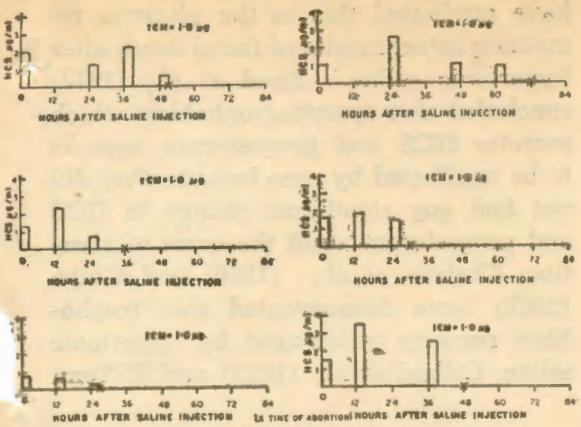

Fig. 2

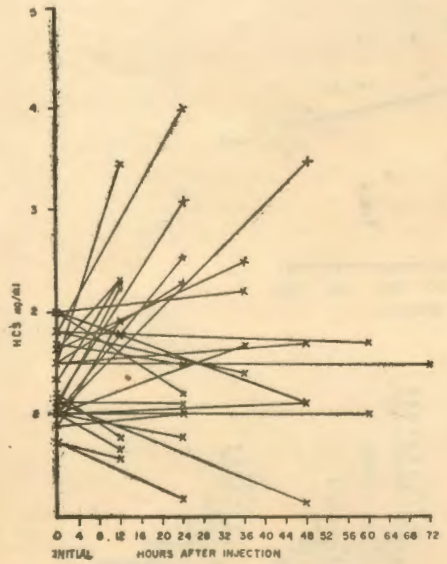

Fig. 3

HCG in these patients varies from 1.02 o 25.2 I.U./ml.

Table IV also shows correlation bet- een HCG values and number of abor- ns within 12 hours.

From this Table it is clear that when the fall is 25%, 46% of patients abort in the next 12 hours. When the fall is 50% only 35% of patients abort in the ext 12 hours.

TABLE IV

Correlation Between Serum HCG Values and the Number of Abortions

Time Interval Inj. & Hormonal Estimation Interval	No. of patients	Percent of HCG Fall						Percent of HCG Rise					
		25%		50%		75%		25%		50%		75%	
		No. of patients	No. of abortions on 12 hrs.	No. of patients	No. of abortions on 12 hrs.	No. of patients	No. of abortions on 12 hrs.	No. of patients	No. of abortions on 12 hrs.	No. of patients	No. of abortions on 12 hrs.	No. of patients	No. of abortions on 12 hrs.
12	11	5	1	4	–	–	–	1 (9%)	1	–	–	–	–
24	12	1	–	5	3	1	–	–	–	1	–	–	–
36	7	5	4	–	–	–	–	1	–	–	–	–	–
48	6	1	–	3	2	–	–	1 (13%)	–	–	–	1	1
60	2	–	–	1	–	1	1	–	–	–	–	–	–
72	2	1	1	–	–	–	–	–	–	–	–	–	–
84	1	–	–	–	–	–	–	–	–	–	–	–	–
Total		13	6	14	5	2	1	3	1	1	–	1	1

Only one patient showed a sustained rise in serum HCG after hypertonic saline injection, the other two patients showed a transient rise at different study intervals. This means that there is no definite correlation between the amount of fall in serum HCG and the injection—abortion interval.

Fig. 4 shows the alterations in serum HCG in all patients during injection-abortion interval. As shown in the figure, the serum HCG showed a steady decline after injection of hypertonic saline and had fallen to 56% of the initial level before the onset of abortion.

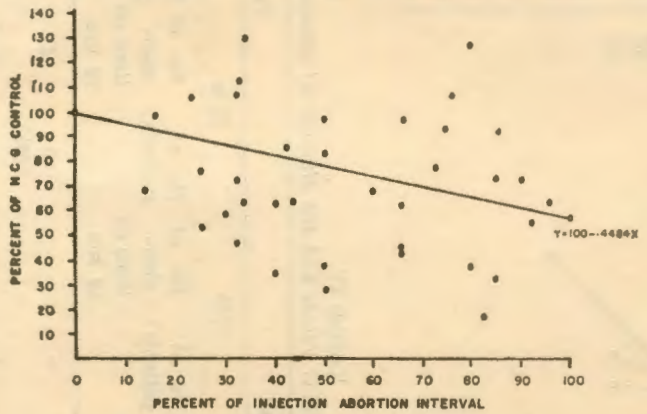

Fig. 4

Fig. 5 illustrates the relationship between the HCG levels and time after injection in six patients.

Fig. 6 shows changes in HCG levels after intra-amniotic injection of hypertonic saline graphically.

Discussion

Estimations of human chorionic somatomammotrophin (HCS) levels are considered as a sensitive indicator of the endocrine function of the placenta (Spona *et al.*, 1971). Saxena *et al*, (1971) have failed to show any change in HCS following intra-amniotic saline injection. They have attributed this to the placenta remaining intact inspite of foetal death after hypertonic saline. Raud *et al.*, (1972) concluded that syncytiotrophoblast which secretes HCS and progesterone appears to be unaffected by urea because they did not find any significant change in HCS and progesterone until the onset of abortion. Christie *et al.*, (1966) and Csapo (1967) have demonstrated that trophoblast remains undamaged by hypertonic saline. Gillard *et al.*, (1973) and El-Tomi

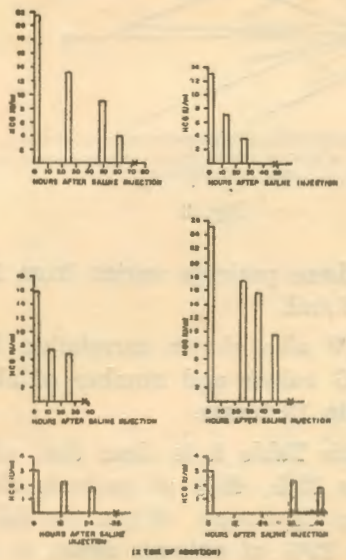

Fig. 5

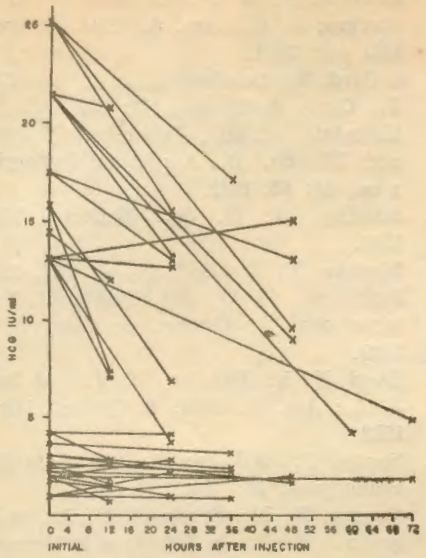

Fig. 6

(1971) have failed to show any correlation between the levels of HCS and the onset of labour. However, they have found a fall in HCS concentration at the time of labour. Raud *et al.*, (1972) and Pulkkinen and Kivikovski (1969) attribute decrease of HCS and progesterone at the time of onset of abortion as a result of increased intrauterine pressure and contractions.

In our series we did not find any significant correlation between the levels of HCS, the post-injection fall of HCS and the onset of abortion. However, 66% of patients who showed a fall in serum HCS compared to the initial level aborted in the next 12 hours. As against this, only 33% of patients who showed rise in serum HCS aborted in the next 12 hours.

Holmdahl *et al.*, (1971) have reported transient rise in peripheral plasma progesterone immediately after intra-amniotic injection of saline in the majority of cases (13 out of 19). They attribute it to sudden release of progesterone from the placenta in the peripheral circulation. A possible explanation they have given for this phenomenon is the abrupt change in the intra-amniotic osmolarity which theoretically might cause compression of placental tissues due to dehydration. The same mechanism may be responsible for the rise of HCS in our cases. However, they do not consider that this transient rise was a consistent phenomenon. They found a significant decrease in peripheral plasma progesterone after the infusion of hypertonic saline into amniotic cavity, but this could not be correlated to the onset of clinical labour (also quoted by Manabe 1970).

A progressive damage of HCG producing elements in the placenta occurs after hypertonic saline injection (Raud *et al.*, 1972). The present study showed a mean fall in serum HCG of 56% before abortion occurred after intra-amniotic hypertonic saline injection. The decrease in serum HCG indicated that blockage of HCG secretion must occur shortly after saline injection. Raud *et al* (1972) attribute this steady decrease in serum HCG throughout the injection-abortion interval to the cytotrophoblastic disruption.

Summary

(1) Radio-immunoassay of HCS and HCG was performed in 21 patients before and after intra-amniotic injection of saline.

(2) There was a steady decline in serum HCG after intra-amniotic injection of hypertonic saline.

(3) A rise in serum HCS was noted in 13 patients after intra-amniotic injection of hypertonic saline.

(4) There was no definite correlation between the fall in the serum HCS and HCG and the injection—abortion interval.

Thus, the study fails to show any endo-

crine theory for the induction of abortion in these cases of medical termination of pregnancy.

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References

1. Christie, J., Anderson, A. B. M., Turnbull, A. C. and Beck, J. S.: J. Obst. & Gynec. Brit. Cwlth., 73: 394, 1966.
2. Csapo, A.: Year Book of Obst. & Gynec. edited by Greenhill J. P. Year Book Medical Publishers, Chicago p. 146, 1967.
3. El-Tomi, A. E. F., Crystale, C. D. and Stevens, V. C.: Am. J. Obst. & Gynec., 108: 649, 1971.
4. Gillard, M., Letchworth, A. T. and Chord, T.: Obst. & Gynec., 41: 774, 1973.
5. Holmdahl, T. H., Johansson, E. D. B. and Nilsson, B. A.: Acta Endocrinologica., 66: 82, 1971.
6. Middley, A. R. Jr.: Endocr., 79: 10, 1966.
7. Manabe, Y. J.: Endocr., 46: 127, 1970.
8. Pulkkinen, M. O. and Kivikovski, A.: International J. Gynec. & Obst. 7: 279, 1969.
9. Raud, H. R., Balsdon, M. J. and Collins, J. A.: Am. J. Obst. & Gynec., 113: 887, 1972.
10. Spona, J. and Janish, H.: Acta Endocrinologica., 68: 401. 1971.
11. Saxena, B. N., Emerson, K. Jr. and Selenkow, H. A.: In Vitamins & Hormones. Advances in Research and Applications. Edited by—Harris, R. S., Munson, P. L., Diczfalusy, E., Glover, J. 29: Academic Press, New York and London, P. 127, 1971.
12. Tyack, A. J., Parsons, R. J., Miller, D. R., Pennington, G. and Hall, R.: The J. Obst. & Gynec. Brit. Cwlth., 80: 548-552, 1973.