LEUCOCYTE MIGRATION ENHANCEMENT AS AN INDICATION OF IMMUNOLOGICAL ENHANCEMENT IN DIFFERENT TRIMESTERS OF NORMAL PREGNANCY

by

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SUMMARY

The immunological status of 73 normal pregnant females in various trimesters of pregnancy, 10 non-pregnant females and 10 normal males, as depicted by leucocyte migration in migration chambers filled with M.E.M. and amniotic fluid pool antigen, was studied. Leucocyte migration enhancement and inhibition both were measured in study as well as control cases.

Introduction

In states of cellular hypersensitivity the lymphocyte migration is regularly and distinctly inhibited by the specific antigen. Since Soborg and Bendixen (1967) determined human leucocyte migration as a parameter of hypersensitivity toward an antigen, a large variety of studies have been performed utilizing the technique of leucocyte migration inhibition (LMI). While LMI is believed to represent hypersensitivity, very little information is available about the positive implication of leucocyte migration enhancement (LME), the opposing phenomenon.

Gleicher *et al* (1980) reported the enhancing effect of amniotic fluid (AF) on pregnancy leucocyte and suggested presence of an immunologic blocking factor in amniotic fluid.

The present study is concerned with evaluation of leucocyte migration in different trimesters of normal pregnancy, indicating presence of blocking substance in amniotic fluid and shows altered immunological cellular response during pregnancy.

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Material and Method

In the present study 73 pregnant females and 20 control males and non-pregnant female cases formed the material. The subjects for study were selected from the out patient and the indoor wards of U.I.S.E. Maternity Hospital, Kanpur. The various haematological, biochemical and immunological investigations were carried out in Pathology Department of G.S.V.M. Medical College, Kanpur.

In pregnant group 21 cases were investigated during 1st trimester of pregnancy, 24 in IInd and rest 28 during IIIrd trimester of normal pregnancy. All 73 patients were investigated with homologous amniotic fluid which consists of an amniotic fluid pool. Amniotic fluid (AF) was ob-

tained from 16 IInd and IIIrd trimester patients of varying parity and socio-economic status by either sterile transabdominal amniocentesis or transvaginal amniotomy. AF was immediately iced and centrifuged at 1000 g for 30 minutes at 5°C. The clear supernatent was used as antigen after dilution, 1:1 with 20% heat inactivated foetal calf serum, 100 units per ml penicillin and 1 mg per ml of streptomycin.

The leucocyte migration assays were performed according to the method of Soborg and Bendixen (1967) with some minor modifications suggested by Goldstein *et al* (1971) and Faiferman (1977). Each experiment consisted of four duplicates which were arranged to obtain the mean area of migration (MA). The migration index (MI) was calculated as:

$$MI = \frac{\text{Area of migration with medium.}}{\text{Area of migration with AF}}$$

MI above one indicates leucocyte migration enhancement (LME) below one is leucocyte migration inhibition (LMI).

Informed consent was obtained from all the patients and amniocentesis was performed for clinical indications only. Statistical evaluation of data was performed utilising analysis of variance, Fischer's t test and correlation studies.

Observations

Table I shows mean leucocyte migration with amniotic fluid (AF) in pregnancy.

Table II shows group means standard errors of difference and t values.

It is evident from Tables I and II both, that there was leucocyte migration inhibition in control male and female cases and their difference was nonsignificant ($P > 0.05$).

When mean values of migration indices in different trimesters of normal pregnancy were studied in comparison to control female and male group mean migration indices, there was significant migration enhancement in all the trimesters of pregnancy ($P < 0.001$) with maximum enhancement ($P < 0.001$) in IInd trimester.

TABLE I

Group	No. of cases	Mean M	
Male control group	10	mean range	-0.83 -0.78-0.91
Female control group	10	mean range	-0.80 -0.78-0.88
Ist trimester	21	mean range	-1.93 -1.10-2.61
IInd trimester	24	mean range	-2.83 -2.08-3.59
IIIrd trimester	28	mean range	-2.13 -1.38-2.77
Total No. of cases	93		

TABLE II

Group Mean	No. of cases	Female control (0.81)	Normal pregnant females Ist trimester (1.93)	IInd trimester (2.85)	IIIrd trimester (2.13)
Male control (0.83).	10	Diff—0.03 S.E.—0.160 t—0.188 (N.S.)	Diff—1.10 S.E.—0.137 t—8.029 ***	Diff-2.02 S.E.-0.134 t-15.075 ***	Diff-1.30 S.E.-0.132 t-9.848 **
Female control (0.80)	10	—	Diff—1.13 S.E.—0.137 t—8.248 ***	Diff-2.05 S.E.-0.134 t-15.278 ***	Diff-1.33 S.E.0.32 t-10.076 ***
Normal pregnancy Ist trim. (1.93)	21	—	—	Diff-0.92 S.E.-0.107 t-8.598 ***	Diff-0.20 S.E.0.103 t-1.942 ((N.S.)
IInd trim. (2.85)	24	—	—	—	Diff-0.72 S.E.-0.099 t-7.273 ***

N.S.—Nonsignificant ($P > 0.05$)
*** —Significant ($P < 0.001$)
Figure in ( ) in the first column signifies mean values)

Discussion

Leucocyte migration inhibition of male leucocytes was observed in presence of amniotic fluid antigen during previous studies by Gleicher *et al* (1980). Similar phenomenon was seen in the present study and there was no significant difference ($P > 0.05$) between LMI with female and male leucocytes showing that immune cellular response in non-pregnant females does not differ much from males. In both of these groups LMI indicates hypersensitivity toward embryogenic antigen present within amniotic fluid. Amniotic fluid causes significant LME with Ist, IInd and IIIrd trimesters leucocytes in comparison to the normal control male and nonpregnant female control cases ($P < 0.001$).

This enhancing effect of AF on pregnancy leucocytes suggested presence of an immunologic blocking factor in amniotic fluid which prevents recognition of feto-placental antigen by the maternal immune system. Thus *in vitro* LME may correlate *in vivo* graft enhancement.

Gleicher *et al* (1979) observed that none of other antigens except AF pool antigen exhibited migration enhancement of significant properties and that amniotic fluid is the causative factor for LME and exclude the possibility that maternal lymphocytes acquired special properties per se during regnancy.

Goldstein (1977) and Gleicher *et al* (1979) concluded that such an inducing action on subpopulation of effector cells through contact with immunologically active substance has been suggested to be mediated by circulating immune complexes/blocking antibodies of IgG class. These studies were confirmed by Masson *et al* (1977). Rocklin and co-workers

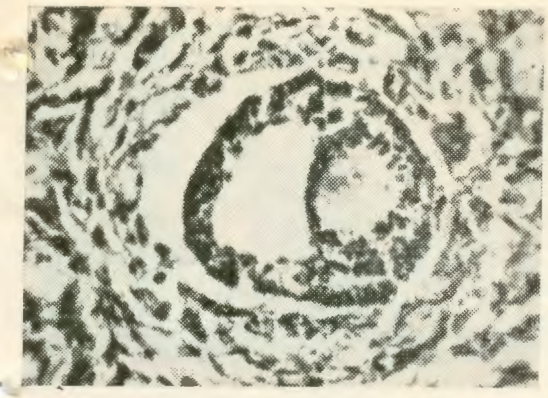

Fig. 1
Microphotograph from foetal ovary of toxaemic series (Group-B) showing advanced maturing active Graafian follicle with multi layered granulosa cells. The antrum is well developed with prominent theca interna. (H x E x 440).

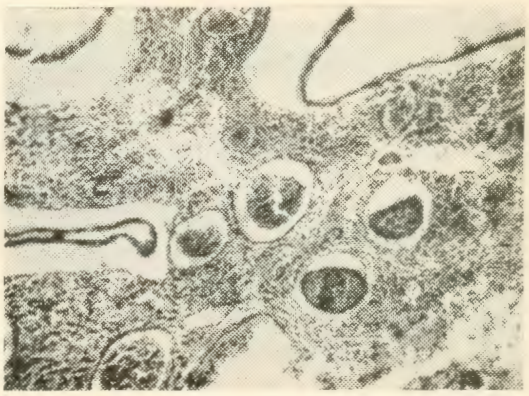

Fig. 2
Microphotograph from foetal ovary of toxaemic series (Group-C) showing Graafian follicles in different stages of maturation with a few follicles showing cystic change. (H x E x 110).

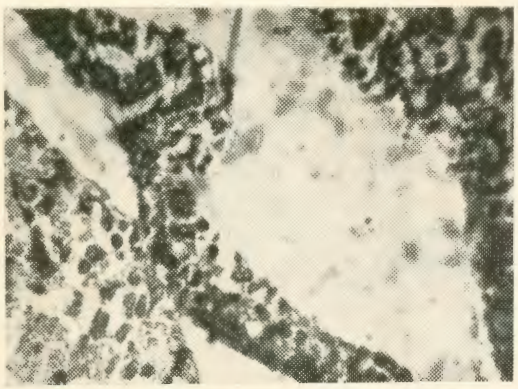

Fig. 3
Microphotograph from foetal ovary of toxaemic series (Group-D) showing wall of a ruptured cystic follicle with evidence of theca luteinisation and haemorrhage. (H x E x 440).

Maternal Toxaemia and Foetal Activity—II Testis—Ghosh et al pp. 427-430

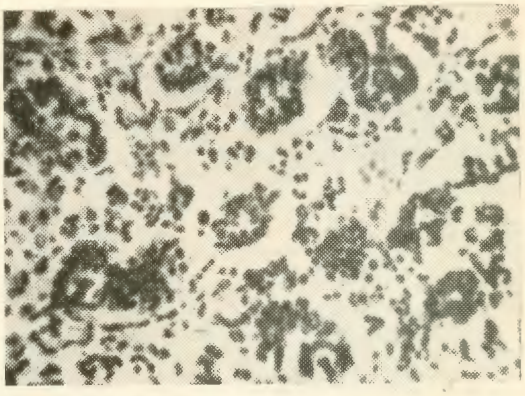

Fig. 1
Microphotograph from foetal testis of toxaemic group showing prominent interstitial cells dispersed diffusely and in small clumps. Seminiferous cords are well developed. (H x E x 440).

Ultrasonographic Studies in Cases with Bleeding—Kumari & Seetha pp. 431-434

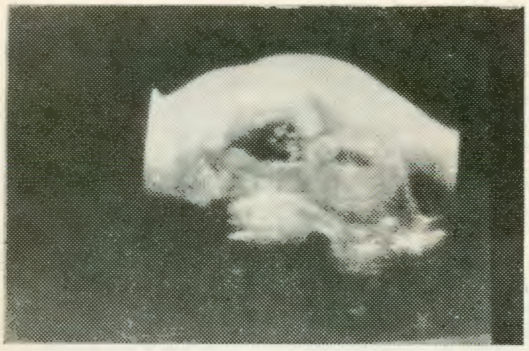

Fig. 1
Complete placenta praevia.

Fig. 2
Partial Placenta Praevia.

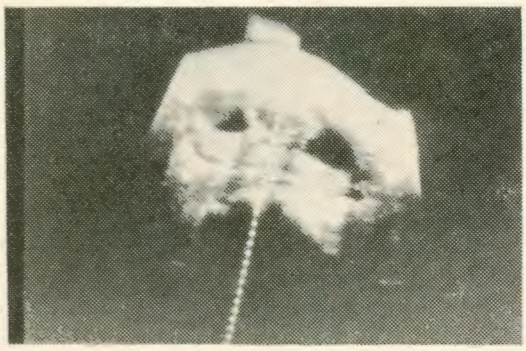

Fig. 3
Low lying placenta.

A Study of Long Term Effect of Lippes Loop on Endometrium—Sanyal et al pp. 582-585

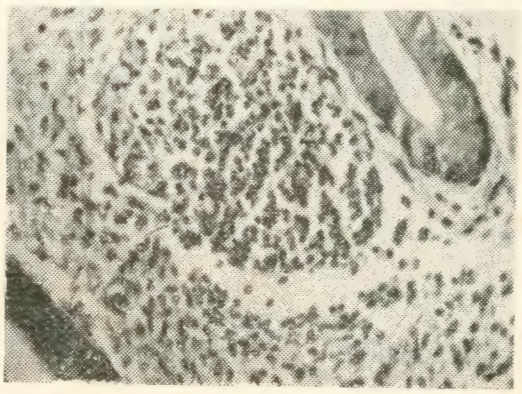

Fig. 1
Section shows endometrial glands in the proliferative phase, the stromal cells are normally stellate, oedema of stroma and focal lymphocytic cell collection within normal limit. (H x E x 320).

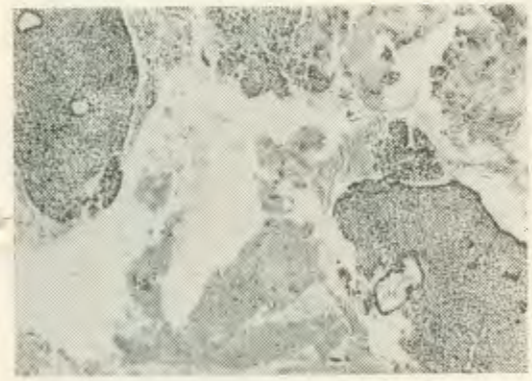

Fig. 2
Section shows endometrium having mixed glands i.e. one gland in the proliferative phase and the other gland in the other bit in secretory phase. (H x E x 80).

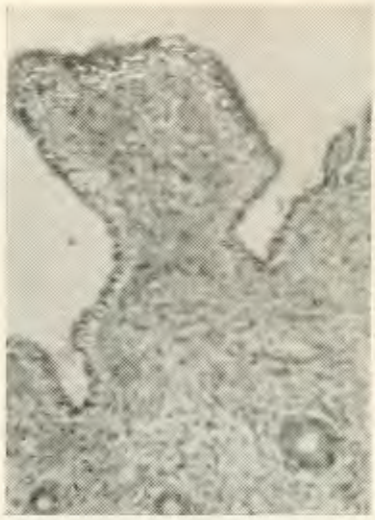

Fig. 3
Section shows micropolyp formation from the surface epithelium composed mostly of stromal cells, lined by surface epithelium. (H x E x 170).

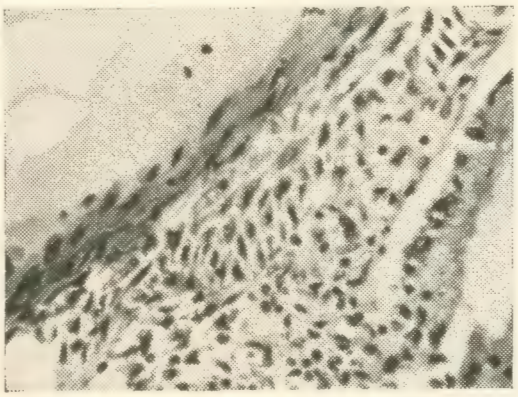

Fig. 4
Section shows squamous metaplasia of surface epithelium of endometrium. (H x E x 320).

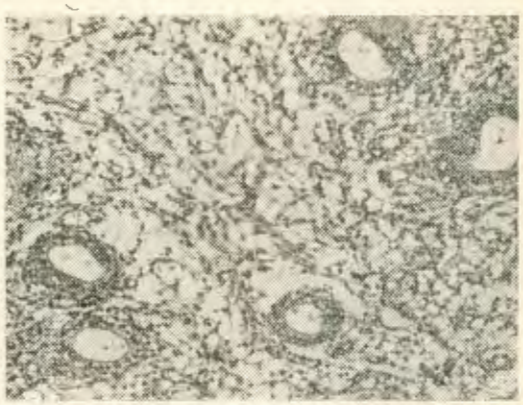

Fig. 5
Section shows focal foamy histiocytic cell collection in the stroma of endometrium. (H x E x 220).

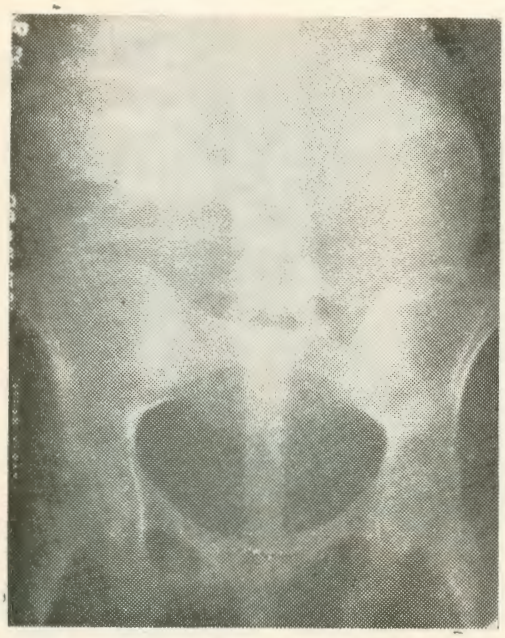

Fig. 1
Skiagram of the abdomen.

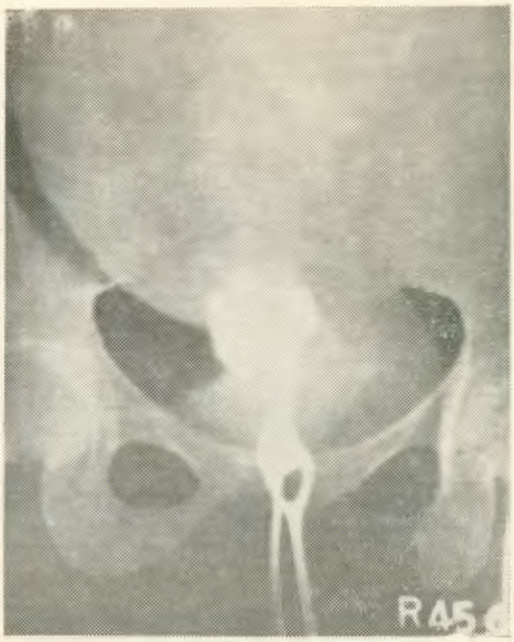

Fig. 2
Hysterogram preoperative.

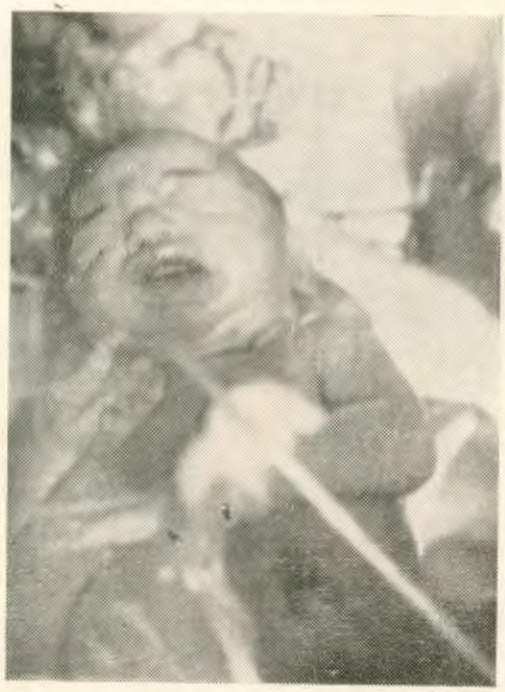

Fig. 3
The child just salvaged from the pseudosac.

Utero-Cutaneous Fistula—Goyal & Shukla pp. 596-597

Fig. 1
Showing the site of the fistula.

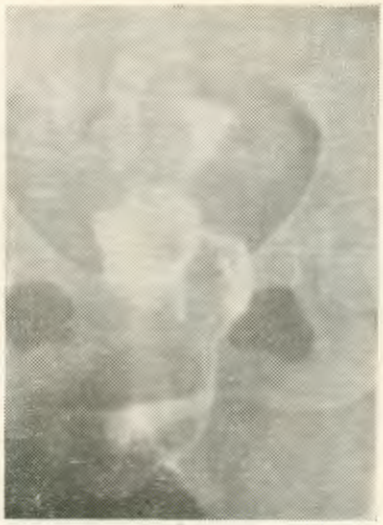

Fig. 2
Hysterosalpingograph showing the connection of fistula and the uterus.

Rare Complication of Menstrual Regulation—Maru & Bandi p. 598

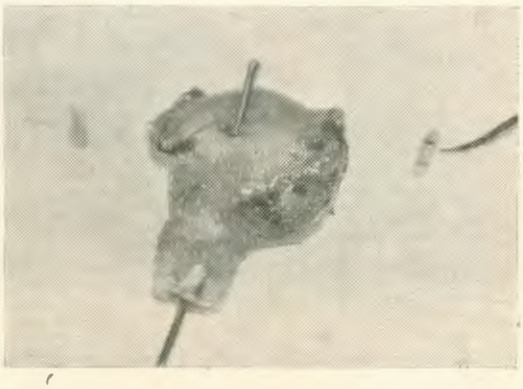

Fig. 1
The site of perforation in the uterus.

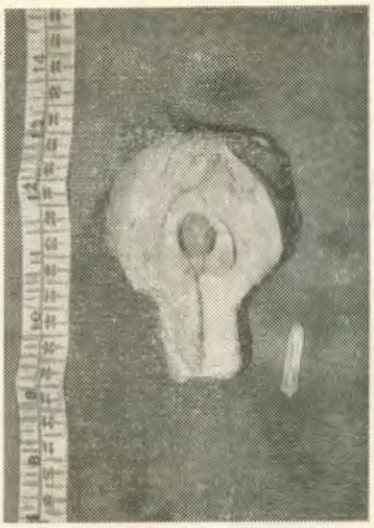

Fig. 2
The broken tip of cannula and uterus with gestation sac.

Fig. 1
Showing cut open uterus and cut open tumour attached to the fallopian tube. Ovary is clearly seen separated from tumour.

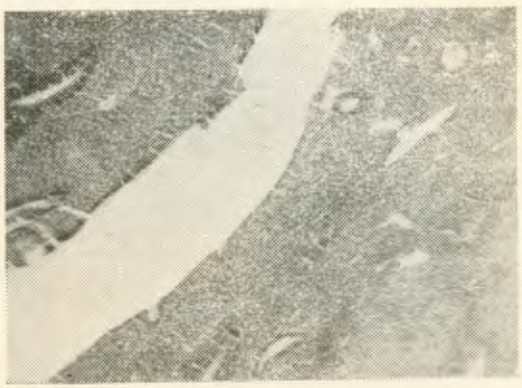

Fig. 2
Section from the uterine wall showing endometrial glands with stroma. The glands in the centre show marked dilatation.

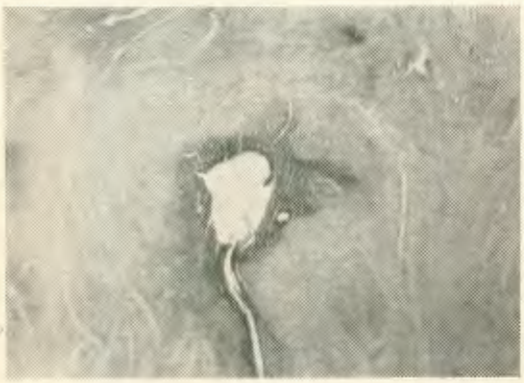

Fig. 3
Section from the tumour showing leiomyomatous picture with endometrial dilated glands and stroma in the centre.

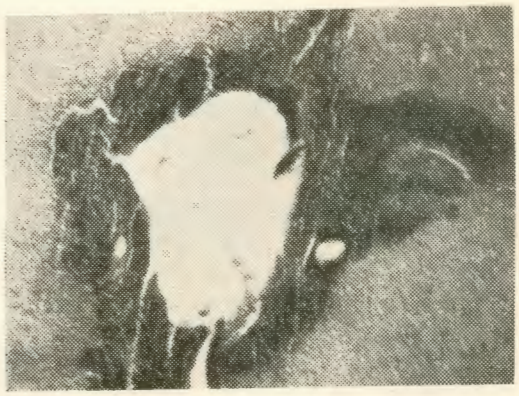

Fig. 4
Same as above but with higher magnification endometrial stroma is more clearly seen.

Fig. 1
Scar marks on either side of medial aspect of thighs represent the sites from where gracilis muscles were taken off for sling operation (Photograph taken 6 weeks after delivery).

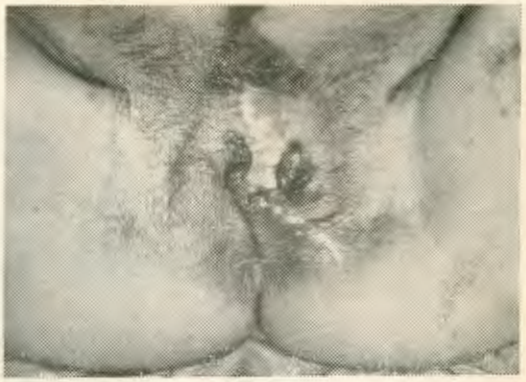

Fig. 2
Photograph shows replacement of mons veneris by an irregular scar (sire of primary operation). There is absence of clitoris. The anterior ends of labia majora are widely separated. Scar of left mediolateral episiotomy is seen (Photograph taken 6 weeks after delivery).

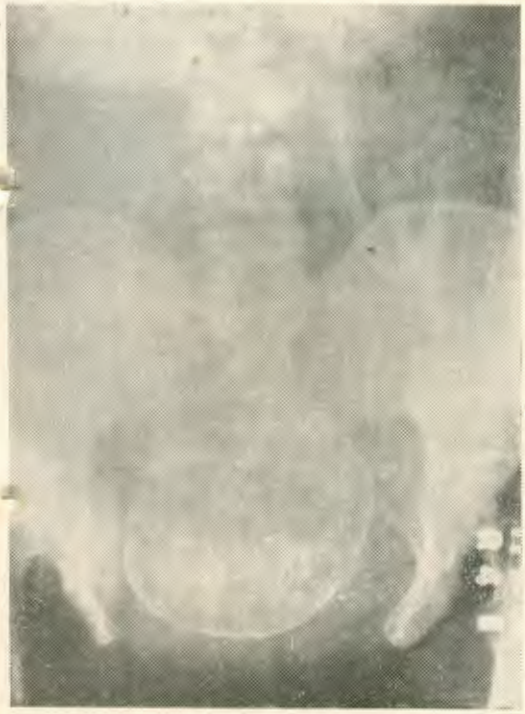

Fig. 3
P-A view of pelvis which shows wide separation of pelvic bones anteriorly. The junction between the separated bony ends being filled with fibromuscular tissue.

Dizygotic Twin-One Molar Pregnancy—Sapre et al p. 588

Fig. 1
Molar placenta and five month foetus with normal placenta.

Delivery Through Central Rupture of Perineum—Jha & Sahay p. 594

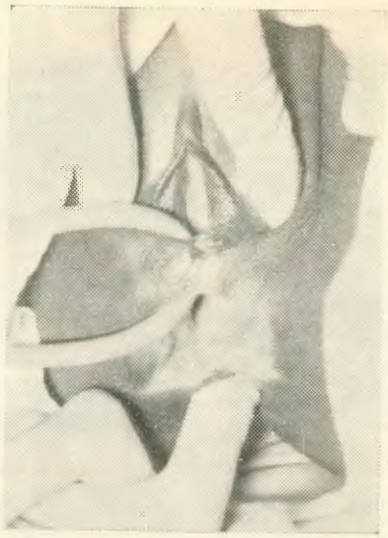

Fig. 1
A photograph showing the central rupture of perineum.

Case Report of Uterointestinal Fistula—Sharma et al p. 595

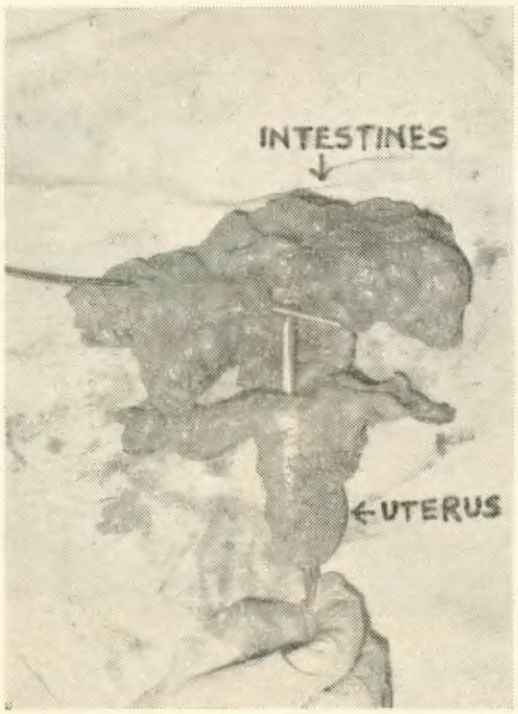

Fig. 1
Uterus with adnexa along with excised segment of the sigmoid colon.

(1976) reported presence of blocking immunoglobulin of IgG class in normal pregnancy sera and amniotic fluid. Both have been linked to nonrejection of homograft *in vivo* and LME *in vitro*. Activity of this immunoactive factor increases with increasing period of gestation. Gleicher and co-workers (1980) did not find any LME in Ist trimester of pregnancy but in our present study we found significant LME with Ist trimester leucocytes ($P < 0.001$) also when compared with control males and females showing presence of same blocking factor in amniotic fluid.

LME in IIIrd trimester was less than LME in IInd trimester, difference being statistically significant ($P < 0.001$). This could be explained on the basis of the fact that the immunological response is depressed during pregnancy which is maximum in IInd trimester. After IInd trimester the risk of foetal allograft rejecion is reduce and now the immunological phenomenon starts reverting back to normal.

References

1. Faiferman, I., Gleicher, N. and Cohen, C. J.: J. Natl. Cancer, Inst. 59: 1593, 1977.
2. Gleicher, N., Cohen, C. J. and Kerenyi, T. D.: Am. J. Obstet. Gynec. 134: 897, 1979.
3. Gleicher, N., Deppe, G. and Cohen, C. J.: Obstet. Gynec. 54: 335, 1979.
4. Gleicher, N., Beers, P., Kerenyi, T. D., Cohen, C. J. and Gusberg, S. B.: Am. J. Obstet. Gynec. 136: 1, 1980.
5. Goldstein, M. S., Shore, B. and Gusberg, S. B.: Am. J. Obstet. Gynec. 111: 751, 1971.
6. Masson, P. L. P., Delire, M. and Cambriaso, C. L.: Nature. 266: 542, 1977.
7. Oldstein, M. B. A., Tishom, A. and Morelta, L.: Nature, 269: 333, 1977.
8. Rocklin, R. E., Kitzmiller, J. L. and Carpenter, C. B.: N. Engl. J. Med. 295: 1209, 1976.
9. Soborg, M. and Bendixen, G.: Acta. Medica. Scand. 181: 2, 1967.