OVARIAN ALLOTRANSPLANTATION IN HUMAN

by

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Introduction

Two unmarried patients both complained of primary amenorrhoea were considered to be cases of primary ovarian failure through cytological examination and hormonal assay. The donors were selected from cases coming for tubectomy when they gave the nearest HLA type with that of the recepients. Steroid was used as immunosuppressive in both the cases. One case had 4 cyclical menstruation and the other had 1, subsequently they passed to the phase of amenorrhoea again. Other examinations, hormonal and cytological, suggested ovarian function during the said period in the 2 cases respectively. A follow up study of 7 months of both these cases have been presented.

Case I: P.G., 30 year unmarried female, a case of primary amenorrhoea, height-4 ft 8 inches, weight-56 kg., secondary sex character-underdeveloped, breast small, hair distribution-scanty, general look-feminine, uterus-1 inch, vagina-small and narrow.

Case II: P.K., unmarried female, a case of primary amenorrhoea, height 5 ft 3 inches, weight 50 kg, secondary sex character-underdeveloped, breast very small; hair distribution—scanty; general look feminine, uterus $1\frac{1}{2}$ inches, vagina—almost normal.

Preoperatively the following investigations were done:

(i) Blood film for drum stick (St. Georges Hospital monograph 1963) (ii) Buccal smear for sex chromatin (St. Georges Hospital monograph, loc. lit) (iii) Vaginal smear for maturation index (Novak & Woodruff 1968) (iv) Urinary Gonadotrophin (Williams 1974) (v) Pregnanediol in urine (Guterman, 1944), (vi) HLA typing by fluorochromatic cytotoxic test (Elves, 1972) (New accepted HLA nomenclature was used as outlined by Duquesnoy & Fuller, 1975) (vii) Presence of lymphocytotoxic antibody in the recepient against the donor's lymphocytes by dye exclusion method (Dausset, 1973) (viii) ABO, RH group by standard slide method (ix) Urinary creatinine (Henry, 1966) (x) Endometrial Biopsy of the recepients. Chromosomal analysis was not performed.

The donors were selected from the nearest HLA type of the recepients after screening primarily by ABO & Rh grouping. The patients in the reproductive age coming for tubectomy were selected for taking out one ovary with their consent. The pre- and post-operative findings of both the cases are given in Table I & II respectively.

The operative notes in connection with these cases are as follows. Donors and recepients were opened up side by side by two team of surgeons and the ovary after it was taken out from the donor was immediately transplanted in the recepient. The total procedure was completed within 40 minutes in both the cases.

Donor for case I, a 34 year healthy young women $P_4 + 0$ came for tubectomy, selected because of HLA suitability (Table I). No antibody was present in recepient's serum against donors lymphocytes. Both the ovaries were healthy. Biopsy was taken from left ovary (Histology revealed, healthy and active ovary with graffian follicles). Right ovary with part of the tube and mesosalpinx was removed by vascular occlusion clamp, one in infundidulopelvic ligament and the other on the ovarian

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ligament along with mesosaplinx. In recepient (case I) no ovaries were visible on either side, both the tubes were long and hanging in infundibulopelvic (I.P.) ligament. The uterus was hypoplastic (1" A.V.). The I. P. ligament on the right side was clamped at a distance between the two and cut. The pedicular stump was washed with heparin (5000 I.U./ml). The donor ovary was placed in the space provided anastomosing the vessels of the I.P. ligament and the vessels of the mesosalpinx (atraumatic 00000 anacap silk suture material used.) This anatomosis was performed wearing specially made spectacle (Keeler, England) incorporating convex lens giving a magnification x 10. Since the pedicle of the donor was wider than that of the recepient's, after anastomosis of the vessels, the peritoneum and other pedicular tissue was used to cover the raw area of the stump of anastomosis. The anastomosis was further strengthened by putting in few interrupted stitches on the peritoneal surface. Before closure of the abdomen a thorough check was made for any possible leakage.

Donor for case 2, a 30 years old healthy women $P_7 + 0$ came also for tubectomy, selected because she was found to be HLA suitable for the second recepient. No antibody was found to be present in the recepient's serum against donors lymphocytes, Both the ovaries of the donor were healthy. Biopsy of the right ovary showed on histology presence of good graffian follicles. Left ovary was taken out and placed to the left side of the recepient who showed streak like ovaries in both the sides (Histology of the streak ovary of the left side—only stromal tissue, no follicles observed). The uterus was hypoplastic (1½" AV). The operative procedure was same as in case 1.

In the postoperative period for first four days Inj. Reverin (275 mg IV) twice daily and Inj. Dexamethasone (2 mg/ml), 1 ml. twice daily, then Oxytetracycline 250 mgm capsules 4 times daily for 4 more days was given. Tab Prednisolone 60 mgm daily in divided dose was given (the same dosage in both the cases was given as the body weights of the cases were close to each other) for 8 weeks, then in the course of another 10 days the steroid was tapered off. The postoperative period was uneventful and the recovery was quite satisfactory. Both the patients noticed a sense of fullness of breast from the time they had their normal menstruation one month after the operation.

Discussion

Allotransplantation in human was planned in cases where the different parameters set up for this study would indicate virtual absence of ovary. In this series in one case (case 1) there was absence of both the ovaries and in the other rudimentary ovaries were present where histology revealed presence of only stromal tissue. Donor/Recepient homologous blood group (ABO & Rh) was not only taken into account, a near HLA match of the donor/recepient was also done by screening numerous possible donors in either case. Linder (1961) in his experimental work stated that ovarian allografts between some strains of mice differing at weak histocomptibility loci often survived. Our attempt of allotransplantation of ovary in human was also done after a series of similar animal experiments in rabbits and dogs, results of which will be published shortly. Vascular anastomosis was attempted using special spectacle which gave 10 times magnification and this facilitated the operative procedure. In this procedure the vascular stapling machine if available would have been helpful (Androsov, 1975). Steroid only was used as immunosuppressives. Azathioprine due to its toxicity (Kirpstovaky 1976) was not given to either of the patients.

The preoperative vaginal cytology (Table I) in both the cases showed lack of oestrogenic influences, the so called androgenic reaction was there. Vaginal smear study is considered to be a good parameter for endocrine graft survival (Gittes, 1972) and that was adequately proved in this study (expecting the result in the 4th month of case 1 which gave some what akin to the preoperative state for which no explanation could be provided) as shown in Table II. The oestro-

TABLE I
Pre-operative Laboratory Findings of Recepients

Blood Group & Rh	HLA type (also of the donors)	Sex chromatin in buccal smear	Drum stick in blood film	Maturation index of vaginal smear	Gonado-trophin in urine	Preg-nanediol in Urine	Urinary Creatinine excretion	Endometrial Biopsy
ABO = O RhD = Positive (The donor was also of the same Group)	HLA — A2 A10 B8 Weak for HLA — B13 Donor: HLA — A2 A3 B8 Weak for HLA — B27	Nil	Nil	Parabasal cells/ intermediate cells-Superficial cells (P:I:S)/ 31/35/34	12 units 24 hours	Weakly Positive	0.92 gm/ 24 hrs.	Scanty glands in early profiferative phase. Stromal tissue in abund-ance
ABO = O RhD = Positive (The donor was also of the same groups	HLA — A3 — A9 — B7 — B8 Donor: HLA — A3 — A9 — B5 Weak for BW 17	2%	6%	P.I.S.: 40/35/25	8 units 24 hours	Negative	0.50 Gm/ 24 hours.	Small bit of tissue showing slightly tortuous glands without evidence of secretion. No abnormality in stromal tissue.

TABLE II

Postoperative Laboratory Findings of the Recepients

	2 weeks					1st month					2nd month					3rd month				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Case 1	ND	9.32	WP	0.56	—	19:50:31	9.2	P	0.56	Y	15:58:27	8.0	SP	0.60	Y	NC	8.55	P	0.89	Y
Case 2	ND	7.2	N	0.50	—	10:60:30	7.0	WP	0.75	Y	NC	10.7	N	0.85	Z	NC	8.20	N	0.90	Z

	4th month					5th month					6th month					7th month				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Case 1	35:50:15	7.0	P	0.50	Y	NC	5.60	WP	0.89	Z	NC	9.60	WP	0.55	Z	NC	10.70	N	0.90	Z
Case 2	ND	8.0	N	0.80	Z	NC	7.85	N	0.52	Z	NC	8.70	N	0.50	Z	NC	9.71	N	0.50	Z

Vaginal smear (done 1 week before the expected date of menstruation)

A: Maturation index = Parabasal cells: Intermediate cells: Superficial cells.

B: Gonadotrophils of 24 hrs. urine, units per 24 hrs.

C: Urinary Pregnandiol:

NC: Same as in preoperative state

N: Negative.

WP: Weakly positive.

P: Positive.

SP: Strongly positive.

D: Urinary Creatinine = Gm/24 hrs.

E: Menstruation

Z = No.

ND: Not done

genic influence was manifested in vaginal cytology as long as the patients had menstruation and again the vaginal cytology study gave results as preoperative state after the cessation of menstrual cycle (on 5th month in 1st case and on 2nd month in 2nd case).

Urinary gonadotrophin showed gross pituitary activity in the preoperative state but the gonadotrophin level went down in both the cases (Table II) indicating possible ovarian activity. Results of gonadotrophin bioassay however was more satisfactory in case I than in case 2 which again corroborated with the overall result in the two cases. Following cessation of menstruation the urinary gonadotrophin again showed an upward trend. The urinary pregnanediol estimation results also behaved in an identical way, reaction became negative after the menstruation stopped in these cases. Urinary creatinine estimation was performed to assess the rejection of the transplanted tissue. From the result it is assumed that this is not a very sensitive parameter for such assessment in ovarian transplantation cases. Regarding the physical change, both the patients felt a sense of fullness of breast before and during the menstruation.

However, from this study it is evident that the allotransplanted ovaries functioned for 4 months and 1 month in the first and the second recepients respectively. This is considered to be a variable success keeping in mind that even if vascular anastomosis is not done the ovarian tissue is likely to survive for some time. Castellino and Sturgis (1958) in their studies in monkeys showed that following castration if the animals' ovaries were reimplanted in the region of broad ligament and retroperitoneally for about 2 months there was spontaneous vaginal bleeding. After the castration there was atrophic vaginal smear which became recornified in 6 weeks. Similar result was also obtained by ovarian tissue in semipermeable millipore filter membrane in a pocket in the abdomen following castration. Here also the tissue survived for 4 months. Necessarily it may be claimed that in the first case of this series vascularity possibly was established, though use of laparoscope could have helped in this assessment. In the second case this tissue may have survived, naturally, for one month.

Summary

Ovarian Allotransplantations were attempted in two human cases with variable success. Attempt of this nature was possibly the first of its kind in this country. The results of the allo-transplantations have been presented.

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