Hypothesis:
The survival of the grafts that are harvested with the FUE technique is best if the grafts are implanted in the recipient site immediately after donor harvesting.

Study Aims:
The intent of this study is to compare the out of body time duration of grafts with the hair survival and the growth rate of the grafts at 1, 3, 6 and 12 months after the transplant. It is believed that the graft survival is best when the harvested grafts are implanted immediately after extraction by the Follicular Unit Extraction (FUE) harvesting technique. This study is designed to determine:

- The optimum time period during which the grafts should be implanted back after extraction in order to have the best possible graft survival and growth rate.
- Whether simultaneous hair implantation (at the same time, within 2 minutes) has any advantage over the FUE procedure (implantation after 2-4 hours)

Importance:

In hair transplantation, the factors affecting the graft survival plays an important role in the overall results and success of the hair restoration procedure. These factors can be broadly divided into

- Donor scalp factors
- Operative factors

Donor scalp factors are defined by - percentage of terminal follicles as compared to intermediate follicle or vellus hairs, high hair per graft count, and caliber of terminal follicles.

Operative factors potentially impacting graft survival include physical damage to the grafts caused by dehydration, transection, blunt trauma done during the extraction and implantation of grafts, ischemia-reperfusion injury (IRI), storage medium & out of body time of the grafts, i.e. the transit time between graft harvesting and implantation. These are important determinants for graft survival rate.

Previous Graft Survival Studies:

Clinical studies with patients have been carried out on this subject of graft survival and out of body time. They are:
1. An in vivo study by Unger\textsuperscript{3} storing 4 mm Strip grafts in chilled NS showed the following survival rates after the following time intervals from graft harvesting to graft placement: 84\% at 2 min, 98\% at 30 min and 97\% at 60 min. Storage was in chilled 4 degrees normal saline.

2. Limmer performed an in vivo study using chilled NS with follicular unit grafts. The results were: 2 h, 95\%; 4 h, 90\%; 6 h, 86\%; 8 h, 88\%; 24 h, 79\%; 48 h, 54\%. Limmer’s "rule of thumb" is graft loss was roughly 1%/hour \textsuperscript{4}.

3. Raposio\textsuperscript{5} et al had compared the survival of grafts after five hours stored in chilled saline and saline at room temperature and found them to be similar.

4. Hwang\textsuperscript{6} et al had noted in his in vitro study that the survival of grafts stored in chilled saline at 4 degree remained was not affected much up to 6hrs.

Various studies utilizing single strip harvesting technique have demonstrated the importance of hydration, optimum temperature, and storage solution for improving the graft survival. The limitations of these studies were that they were with FUT technique. Studies focusing on follicle regrowth or survival using the FUE harvesting method, however, has not yet been studied. A review of the medical literature and Medline search confirms that no study have yet been conducted comparing the growth rates and survival of FUE grafts after one year with their transit time between extraction and implantation.

**Experimental Design:**

Patient selection for this study are males over the age of 25 with a clinical diagnosis of androgenetic alopecia (based on the history and physical examination). Patients with a suitable bald patch needed for the study are only selected. Additionally, patients will require a non-hair bearing portion of the recipient scalp region with minimal dimensions of 4cm X 1cm. In patients who will have some terminal hair then, they will be counted in the baseline reading. Patients with medical systemic, inflammatory, or any dermatological conditions negatively impacting graft survival will be excluded.

Follicular units will be harvested by the FUE technique using a 0.9 mm punch. Only naturally occurring two haired grafts (and not by dissection of the 3 or 4 hair follicular unit) that are completely intact (without any transection) and devoid of adipose tissue will be used for the study. The grafts will be stored in chilled normal saline at 4 degree Celsius until implantation.

In the recipient area, 4 non-contiguous test sites are identified (in the mid scalp/ vertex area), each of 1cm X 1cm area. The test area should be devoid of any terminal hair, which if present is counted using Dermalite epiluminescence microscopy device and the necessary adjustments are made during the final count. There should be atleast 1cm area between each of the text boxes inorder to differentiate in regular and macro photography. The slits in the recipient area are made with 0.9mm blade in the
parallel orientation with the depth adjusted according to the average follicle length. Thirty (30) slits are made in each test site and the grafts are implanted 2 minutes, 30 minutes, 2 hours and 4 hours after extraction in each of these areas. The location of each test area with the time period after which the grafts are implanted in each are noted. In all these areas the grafts are placed at a density of 30 grafts/sq.cm.

Post procedure instructions are given to all the patients and they are advised to return at 1 month, 3 months, 6 months and 1 year after the surgery. During each follow up visit, the following evaluation and data will be collected (Hair Mass Index and photographs) and recorded as per the details given below.

**Data Collection:**

*Instrumentation*

*Observations will be recorded at 1, 3, 6, 9 and 12 months using the following:*

1. Hair Check device *(Divi Int’l Co., Miami, FL, USA)*

*Methodology*

1. Measurements with HairCheck device.

At the above mentioned time intervals an experienced blinded doctor or technician will use the Hair Check device *(Divi Int’l Co., Miami, FL, USA)* as per the user instructions to measure the hair mass index (HMI) of the study area. Three measurements of each test area will be taken and average for each area is recorded and the ratio of hair mass between the 4 groups is calculated. This ratio will provide the “percentage of hair mass maintained”. For an accurate measurement using the Hair Check device the values obtained for the study area should be at least 40 units. If the hair mass of the grafts is less than 40 units, the other protocol mentioned below will be followed.

At the end of one year, if the study area will contain less than 40 hairs, the following protocol devised by Dr. James Harris will be utilized:

1. Obtain a silk thread (3-0 or 4-0) and create a bundle of approximately 30-40 strands measuring approximately 3 cm in length.
2. Measure the bundle with the HairCheck device three times and record the average of the three measurements.
3. Measure the hair in each of the two study areas along with the silk bundle three times and record the average of the three measurements.
4. Subtract the average measurement of the test area from the average measurement of the silk bundle to obtain the HMI for that area. Perform this calculation for all the test areas.)
2. Standardized, non-magnified photographs will also be taken to compare the “global” appearance of each test site. If there is any existing hair present in between these test areas, permanent ink will be used. The macro photos will also be taken using Canon 6D with high resolution. The hair in each of the 4 study areas will be photographed.

The counting process will be repeated three times per test area and the average of the three values will be the “terminal hair count” per test area. The person performing the count will be blinded to the out of body time of the grafts contained in each test region.

Blinded observers will evaluate the “global” photographs to assess gross graft growth according to a predetermined scale. The process will be repeated for all test areas in all patients and the results recorded.

Manual count: The hair in each test region will be subjected to a “manual” count whereby the individual terminal hairs will be counted by a staff member blinded to the type of graft contained in the test area. The process will be repeated three times per test area and the average of the three values will be the “terminal hair counts” per test area.

The hair in the study areas will then be cut to a length of approximately 2-3 mm. All photography will be forwarded to the PI, who will have the hair counts done by an experienced hair restoration technician. The hair counts will be tracked by study ID number only, and whether they were baseline (BL) or final study (FS) photos.

**Data Analysis:**

The data will be collected and entered into Excel sheets. The percentages of grown vs transplanted hair will be calculated to determine the survival rate of the hair grafts for each of the transition time between extraction and implantation: (a) the surviving hair counts in each test area will be tallied and divided by the number of hairs implanted to give a “hair survival rate” for each study areas,

The values of hair mass in study areas, obtained by the method described above, will also be compared between all the four test sites. In addition to this, the HMI/hair value will be calculated by dividing HMI with the hair count in the area as per the ISHRS study of Strip v/s FUE).

Baseline data will first be analyzed using a two-tailed independent t-test to determine if a statistically significant mean difference exists, in terms of hair count, between the 4 test sites. If no statistically significant mean difference in baseline data is found to exist, then the research question will be answered...
using a two-tailed independent t-test. If a statistically significant mean difference is found to exist, then an Analysis of Covariance (ANCOVA) will be conducted controlling for baseline data.

Effect size will be calculated using Cohen’s d and coefficient of determination ($r^2$). A significance value of 0.05 will be used for this study.

The questions that will be answered at the conclusion of the study:

- What is the survival rate and growth rates of hairs with respect to the out of body time?
- Is there a statistically significant mean difference between these 4 study groups as per their various out of body time?

References:


