The process of blastocyst implantation at the endometrium is considered analogous to a "Proinflammatory" response with the consequent involvement of several key inflammatory mediators. These mediators have been found to be very essential for the trophoblast invasion process and may act on COX-2 to regulate the prostaglandin (PG) synthesis or may act directly as signaling molecules.

The key molecule that regulates the inflammatory cascade for prostaglandin synthesis in the body is the 85KD protein "cytosolic phospholipase A_2" ($cPLA_2$). $cPLA_2$ mobilization within a cell is initiated by the influx of intracellular calcium. Moreover, the trigger for this influx is believed to be under control of the Hypothalamus-Pituitary-Adrenal (HPA) axis. Thus, an indepth study of the underlying mechanism of control of the HPA axis on the Ca^{++} channel that leads to the mobilization of $cPLA_2$ and subsequent PG synthesis in the endometrium during the peri-implantation period was undertaken.

The original hypothesis proposed that the adrenals play an important role in the regulation of the calcium channels in the uterine epithelium. These channels are responsible for the instantaneous rise in micromolar concentrations of intracellular calcium which in turn leads to the activation and mobilization of cytosolic phospholipase A_2. These activated enzyme molecules move to the intracellular membranes and act on the phospholipids to release arachidonic acid which further proceeds to produce prostaglandins whose role in implantation is already well established.

To study this cascade, inhibition was attained ***at the adrenal level; at the calcium channel level and at the level of $cPLA_2$***. The overall outcome of the entire research endeavour is partly in line with and several times in contrast with previously well accepted results. The following key conclusions may be drawn from our experiments at this point (Fig. 48):

1. Calcium channels play a significant role in the control of the implantation process.
2. Calcium channel blockers may be used as anti-implantation agents when administered at the implantation site 10-12 hours prior to implantation.
3. Various calcium channel blockers are selective in their role and diltiazem has shown the best activity in our experiments.

4. Diltiazem in its therapeutic dose after oral administration does not display this activity and its anti-implantation effect is observed only after a localized administration.
5. Adrenals do not hold a direct control over the implantation mechanism though it does interfere with the normal functioning of the estrus cycle.
6. Adrenals seem to have a regulatory role over the functioning of the calcium channels leading to its indirect control over the implantation machinery.
7. Cytosolic phospholipase A_2 ($cPLA_2$) which is already known to be activated under the influx of intracellular calcium plays a direct significant role in the implantation pathway.
8. Localization of $cPLA_2$ is prominent along the uterine luminal epithelial lining at the peri-implantation period emphasizing its increased activity during implantation.
9. The entire calcium mediated activation process is independent of the estrogen surge in the uterus prior to implantation and hence may be under a different stimulatory factor arising from the hypothalamus-pituitary-adrenal (HPA) axis.

Applications and Future Prospects

Unraveling the mechanism of any reproductive pathway satisfies two main aims, namely,

- Providing a possible explanation for unexpected infertility issues
- Locating novel targets for contraceptive research

This project was aimed at satisfying both these goals.

The above results indicate that any problem at the level of either the calcium channels or the enzyme $cPLA_2$ may lead to an unexplained implantation failure and may hence be an important cause of infertility worth studying further. On the other hand, both calcium channels and $cPLA_2$ have evolved as important contraceptive targets as a result of this study. Routinely used calcium channel blockers like diltiazem may be studied further to standardize their dose and mode of administration to be used as potential contraceptives. Anti-$cPLA_2$ agents may

also be researched upon further to develop novel molecular contraceptives. Thus, this study provides two novel contraceptive targets in females without interference with the hormonal system. Use of such non-hormonal agents at a very low dose in the intra uterine devices or any other favorable delivery systems could be studied further for direct delivery at the implantation sites which may provide a new direction to contraceptive research.

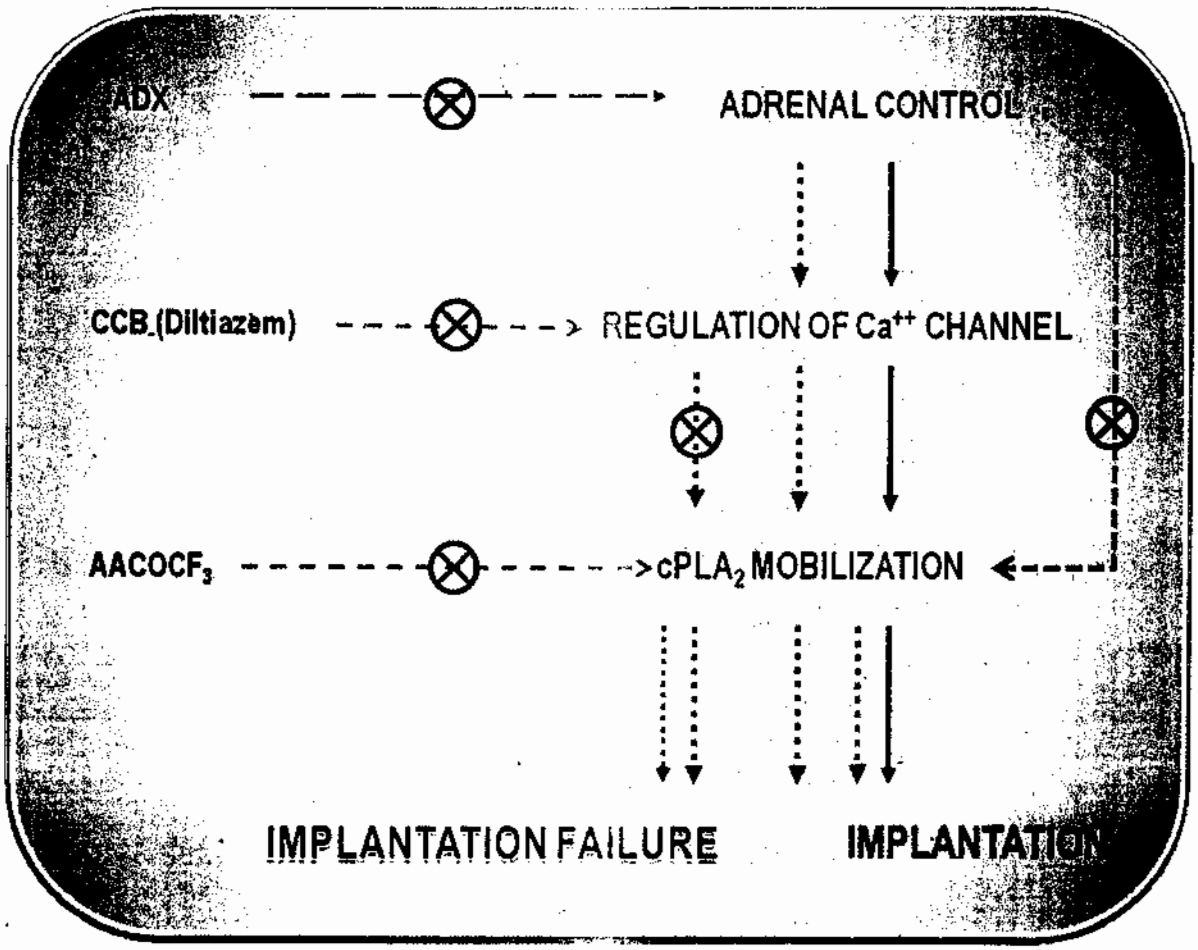

Figure 48: Summary of experimental outcomes