## P49 Increased Risk of Early Pregnancy Loss in Blastocyst Implantation with Sub-optimal Uterine Environments in Mice

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Objectives: Uterine milieu for blastocyst implantation is classified into prereceptive, receptive, and nonreceptive phases. In mice, it has been long believed that blastocyst can implant only for a limited period on day 4 of pregnancy or pseudopregnancy (PSP), and the uterus becomes nonreceptive (refractory) and fails to respond to the presence of blastocysts. In this study, we re-examined whether blastocysts can initiate implantation beyond normal "window" of uterine receptivity in mice using embryo transfer (ET) to various days of (PSP).

Materials & Methods: Day 4 blastocysts were transferred to the uteri of recipients in the morning (1000 h) of various days of PSP. Recipients were sacrificed at 0900 h on various days depending on the experimental conditions after ET. The number of implantation sites (IS) was recorded by intravenous injection of Chicago blue dye solution 48 h after ET rather than 24 h as generally performed in previous studies. Artificial decidualization, cell proliferation assay, in situ hybridization were performed with routine protocols to examine the capacity of decidualization, stromal cell proliferation, and gene expression on days 4, 5, and 6 of PSP, respectively.

Results: ET demonstrated that blastocysts can initiate the attachment reaction in day 5 PSPt (PSPt) recipients (48%) as much as in day 4 (52%) only when IS were examined 48 h rather than 24 h after ET. While blastocysts completely fail to initiate the implantation on day 6 of PSP (0%), daily supplementation of progesterone ($P_4$) to day 6 PSPt recipients from day 5 partially maintained receptive state of uterus for blastocyst implantation. Artificial decidualization response to intraluminal oil injection on various days of PSP showed similar patterns of what we observed in ET. Molecular characteristics of uterine receptivity observed on day 4 of PSP, i.e., the profile of uterine cell proliferation and expression pattern of LIF, a uterine receptivity marker, were similarly maintained on day 5 of PSP, but not thereafter. Again, $P_4$ supplementation partially rescued these molecular characteristics of receptive uterus on day 6 of PSP. Whereas blastocyst can initiate implantation on day 5 of PSP as much as on day 4, number of pups born at term from day 5 PSPt recipients (16/130; 12/5%) was significantly lower than that of day 4 PSPt recipients (44/168; 26%).

Conclusion: Blastocysts require longer time to initiate the attachment reaction in sub-optimal uterine environments. Initiation of blastocyst implantation in sub-optimal receptive uterus accompanies poor postimplantation embryo developments and subsequent poor pregnancy outcome.

**Key Words:** Blastocyst Implantation, Uterine Receptivity, Pregnancy Outcome

## P50 Effect of adenosine on mouse oocyte maturation

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Mammalian oocytes are arrested in the diplotene stage of the first meiotic prophase until input signals of LH. The purines, adenosine which are compounds of follicular fluid are to be important inhibitory substances of oocyte maturation. In this study, we have examined effect of adenosine in oocyte maturation and its signaling mediators. Cumulus-enclosed oocytes (CEO) and denuded oocytes (DO) from PMSG primed immature mice were cultured for 15 hr in BWW medium containing different concentration of adenosine (50 uM, 750 uM). The present data demonstrate pulse time inhibition of spontaneous meiosis in both CEO and DO by adenosine. Germinal vesicle breakdown (GVB) is inhibited with concentration dependent manners of adenosine only in CEOs. Adenosine receptors expressed in CEO. Intracellular calcium level of cumulus was extensively increased after adenosine treatment. However, its level was not much changed by adenosine in the GV intact oocytes. Intracellular calcium modulator also inhibited the GVB in the CEO. PKC antagonist accelerated the CEO. Based on these results it is suggested that one of the GV arrest signaling is mediated by calcium along with PKC pathways in CEO. Conclusively *in vivo* condition GV arrested maintained by several factors including cAMP and calcium because DO may has different pathway from CEO.