A Systems Biology Approach to Elucidate the Process of Blastocyst Implantation

Debabrata Ghosh   and   Jayasree Sengupta

Department of Physiology, All India Institute of Medical Sciences, New Delhi, India

Introduction

A linear application of simplistic, largely deterministic and logically reliable model of operations has, in past, held the notion that three primary modules are integral to the physiological process of blastocyst implantation in the human: endometrial competence, adequate progesterone priming, and viable endometrium – embryo dialogue (1, 2). However, blastocyst implantation is not a highly programmed process; it is rather a complex physiological process. Like any complex biological process, blastocyst implantation is dependent on non-linear interaction of multiple modules and motifs with specific time and space contexts. These operational characteristics collectively attribute to the emerging property of a robust order in the system (3). Our knowledge about the process underlying the control system of blastocyst implantation is very thin (4). We now propose that a large scale time course study of transcript profiles in adequately hormone primed competent endometrium in the presence of age- and stage-matched synchronous and viable embryo may divulge the nature of interaction networks of transcriptomes and their factorial regulation. We believe that deduction of such a large scale expressional networks dynamics shall provide a better picture of the process and possibly open up novel areas of basic, strategic and translational research in blastocyst implantation. We also provide the proof of principle by using deductively selected gene products based transcript profiles in receptive endometrium of the rhesus monkey.

Cartesian model of blastocyst implantation

According to the Cartesian paradigm, blastocyst implantation is a process that is determined by linear and direct interaction of three major factors in the human. They are endometrial competence, adequate progesterone priming, and endometrium – embryo dialogue. Accordingly, progesterone priming allows endometrium to acquire sufficient secretory maturation in a self-limiting manner. Thus, luteal phase progesterone plays the role of a prime mover for endometrial receptivity to blastocyst implantation. In case, time synchronous embryo-derived or analogous input-set is not available, primed endometrium breaks into menstrual loss (5). On the other hand, progesterone dependent secretory maturation makes uterine luminal environment hospitable to support the growth and differentiation of preimplantation stage embryo. Time synchronous interaction between endometrium and embryo renders fine adjustments of endometrial receptivity allowing for blastocyst implantation (1, 2). A few paracrine factors have been shown to be involved in this scheme of action. The details of the schema have been given elsewhere (2).

Despite its simplistic and deterministic edges, the major problem of this model is that it fails to accommodate the following observations like:

Address for correspondence :   D. Ghosh, Department of Physiology, All India Institute of Medical Sciences, New Delhi – 110 029, India; E-mail: debabrata.ghosh1@gmail.com

(i) Occurrence of successful implantation despite relative progesterone starvation (6),

(ii) Occurrence of delayed implantation (7),

(iii) A subset cases of implantation failure with no apparent anomaly in endometrium and ovarian functions (8), and

(iv) Generally low implantation rate in *in vitro* implantation and embryo transfer (IVF-ET) (7, 9).

We believe the above-mentioned phenomena may be addressed only when we start understanding the physiological complexity of the control process functional towards blastocyst implantation. It is becoming increasingly evident that a knowledge base of gene expressional networks in endometrium is a pre-requisite to elucidate the nature of the above-mentioned control process of blastocyst implantation (10).

There are indeed a few human reports wherein transcripts profile of mid-luteal stage endometrium has been compared with that of proliferative stage or early luteal stage endometrium to delineate the role of progesterone in establishing endometrial receptivity for embryo implantation (11–17). Table I gives a summary of the reported studies. Albeit interesting data emerged from these studies, this model suffers from following critical limitations.

(1) These studies did not address the time course of endometrial trancriptomics during pre- to peri-implantation window.

(2) The potential impact of pre-implantation embryo derived signal on the transcriptomics of progesterone dominated endometrium has not been explored in these studies.

(3) These studies did not explore endometrial transcriptomics during window of implantation subjected to relative progesterone starvation by effective progesterone antagonism in pregnancy cycle. It has been earlier demonstrated that mifepristone administered at a relatively small dose (2 mg/kg body weight) in the early luteal stage rendered endometrium phenotypically non-receptive during the implantation window resulting in implantation failure without any discernible change in serum concentrations of estradiol and progesterone (18, 19).

Collectively, there has been no substratum for any serious attempt in the past to employ systems biology approach to elucidate endometrial receptivity in the human.

Multi-modular study design to delineate regulatory transcriptome networks

Thus we propose to perform experiments for a comparative analysis of transcript profiles in implantation stage endometrium and construct the time course dynamics of expressional networks operational in the process of endometrial receptivity. Since this study design cannot be applied in human subjects for ethical, practical and technical constraints, we employed the rhesus monkey as the primate model. In brief, expressional transcriptomics using cDNA based arrays for both ~400 and ~1200 separate gene products in endometrial samples collected from fecund cycles of rhesus monkeys on days 4 and 6 after ovulation with and without retrievable synchronous viable embryo (morula to blastocyst stages) and with or without mifepristone (2 mg per kg body weight) treatment on day 2 after ovulation was conducted (Table I). All the gene products selected were assumed to be involved in endometrial physiology and pathophysiology based on available reports. We have reported for the first time that an overt differential regulation of a set of genomic expression in implantation stage endometrium under adequate progesterone action and in the presence of stage- and age-synchronized embryo is an integral process (20-22).

Table I : Previous array-based studies of endometrial receptivity in human and monkey.

Reference	Subject (No. of gene products)	Array experiment design (Sample size)
Kao et al., 2002 (11)	Normally cycling human (~12,000)	Between mid-proliferative (n= 4) and mid-secretory (n=7) phases
Carson et al., 2002 (12)	Noramelly cycling human (~12,000)	Between early (n=3) and late (n=3) secretory phases
Borthwick et al., 2003 (13)	Normally cycling human (~60,000)	Between mid-proliferative (n=5) and mid-secretory (n=5) phases
Catalano et al., 2003 (45)	Human endometrial explant culture (~1000)	Mid-secretory phase (n=5) endometrial explants treated with 10^{-9} M E_2 plus 10^{-7}M medroxyprogesterone acetate and 10^{-9} M E_2, 10^{-7} M medroxyprogesterone acetate plus 10^{-6} M RU486
Riesewijk et al., (14)	Normally cycling human (~12,000)	Between paired samples (n=5) collected 2003 during early and mid secretory phases
Ace and Okuliz 2004 (46)	Ovariectomized hormone simulated rhesus monkey (~12,000)	Normal proliferative, day 13 (n=3) and mid secretory days 21(n=3) and day 23 (n=3)
Ghosh and Sengupta, 2005 (20)	Cycling rhesus monkey (~400 custom made)	Fecund receptive versus fecund non-receptive (following RU486) on day 6 after ovulation (n=12)
Talbi et al., 2006 (17)	Normally cycling human (~55000)	Mid-late proliferative (n=5), early (n=3), mid (n=8) and late (n=6) secretory phases of cycle
Catalano et al., 2007 (47)	Normally cycling human (16000)	Mid-secretory endometrial samples with no treatment (control; n=15) and with mifepristone (200 mg) treatment (n=9)
Sherwin et al., (48)	Adult female baboons (8000)	Mid-secretory phase samples with either 2007 no treatment (control; n=2) or rhCG (1.25 IU/h) treatment (n=2) during days 5-10 after ovulation
Ghosh et al., 2009 (21)	Cycling rhesus monkey (~400 custom made)	Fecund receptive versus fecund non-receptive (following RU486) on days 4 and 6 (n=28) after ovulation
Najwa, 2009 (22)	Cycling rhesus monkeys (~1200 custom made)	Fecund receptive versus fecund non-receptive (following RU486) on days 2-8 (n=72) after ovulation

Transcriptomic pattern in the implantation stage receptive endometrium

Based on our cDNA based array results (Table I), we have constructed predictive pattern of transcriptomes using annotated structural characteristics of transcripts that displayed more than two fold changes in significant analysis of microarray in implantation stage endometrium relative to the one that has been subjected to progesterone starvation by low dose early luteal phase mifepristone rendering it inhospitable for embryo to implant, and to zero-embryo sojourn, respectively. The underlying assumption was that all the three conditions, namely progesterone adequacy plus zero-embryo (group 1), progesterone adequacy plus synchronous embryo (group 2), and progesterone starvation plus synchronous embryo (group 3) yield different process products (23) at the endometrial transcript networks level, and that the transcript networks connectivity and assortments follow explicit principle of mathematical engineering (24-26). It is also assumed that any one of the processes can be targeted to analyze (predict) using other two processes as probes (predictons) under the above-mentioned format (27, 28). It was also assumed that the predictive analysis follow the basics of Shannon informatics including the

modules like signal, noise and hidden layer, as well as, the principle of entropization of the underlying coding process (29). It means that the coding process in the given issue of control process of blastocyst implantation relates to the form and size of endometrial transcriptomes quantitatively, and is not necessarily related to corresponding and proportionate proteome products.

We report here that endometrial transcriptomes show reduction of gain in expressional variability in the presence of stage synchronous, viable embryo as evidenced by marked loss of differentially displayed gene expression between group 2 and group 1 endometrium on day 6 after ovulation (15) as compared to that on day 4 after ovulation [640] in ~1200 gene product arrays. It is generally believed that lowering of gain is a way by which a complex coding process enhances its determinism, albeit at the cost of inherent vulnerability. As shown in Fig. 1A, networks analysis of our 1200 gene products data also revealed a small-world type pattern of transcript connectivity in the presence of adequate progesterone plus viable embryo (termed as *sufficient ecosystem*). It appears that the expressional-networks under progesterone as the prime mover bears considerable potential toward evolving small-world transcript networks depending on the nature and the course of high dimension attractor, namely embryo-derived signals, applied onto it.

In the real world, trajectories of a sustained, non-transient, dynamical system tend to cease by dissipation unless some driving force or an attractor is in operation. Thus, an attractor typically provides a set of constraints to a system to make it evolve as a dynamical system (see reference 3 for details). Stage-synchronous pre-implantation embryo (PIE) behaves like a high dimension attractor, thus, it out-limits dissipative processes in progesterone dominated endometrium. The PIE mediated attractor properties converge onto the progesterone determined properties in the implantation stage endometrium in physiologically responsive manner towards endometrial receptivity and blastocyst implantation.

The example of differential regulation of mucin in implantation stage endometrium by sex steroid hormones and implanting blastocyst clarify the above-mentioned issue of prime mover action of progesterone and attractor function PIE (30, 31). It has been shown that human endometrial epithelial cells under progesterone dominance secrete mucin that protects the maternal endometrium from parasitic adhesion and invasion, while implanting blastocyst down-regulates mucin to facilitate its implantation (31). This behaviour is species specifically selected depending on larger ecological and evolutionary coordinates (30), and also not mediated by single embryo-derived factor (32).

As explained in Fig. 2, small-world-ness emerges as the result of replacing a fraction (p) of links of a dimensional lattice interpolating between a regular lattice (p=0) and a random graph (p=1) (33, 34). Such networks are characterized by high degrees of clustering and short path lengths. It results in the emergence of a physiological process that appears to be both deterministic and vulnerable (35). The administration of a high affinity progesterone antagonist (like mifepristone having ten times more affinity to progesterone receptor than progesterone itself), thus, can inject significant noise into the expressional networks (Fig. 1B), making the coding process entropized and resulting in failure of blastocyst implantation (18, 19). The example of endometrial immunology with or without progesterone antagonism explains the issue of noise-effect on cellular signaling in implantation stage endometrium. While natural killer (NK) cells show high migratory ability into human implantation stage endometrium because of progesterone-dependent and possibly embryo-derived signals, administration of anti-progestin, mifepristone, however resulted in higher migration of macrophage and neutrophils with no change in
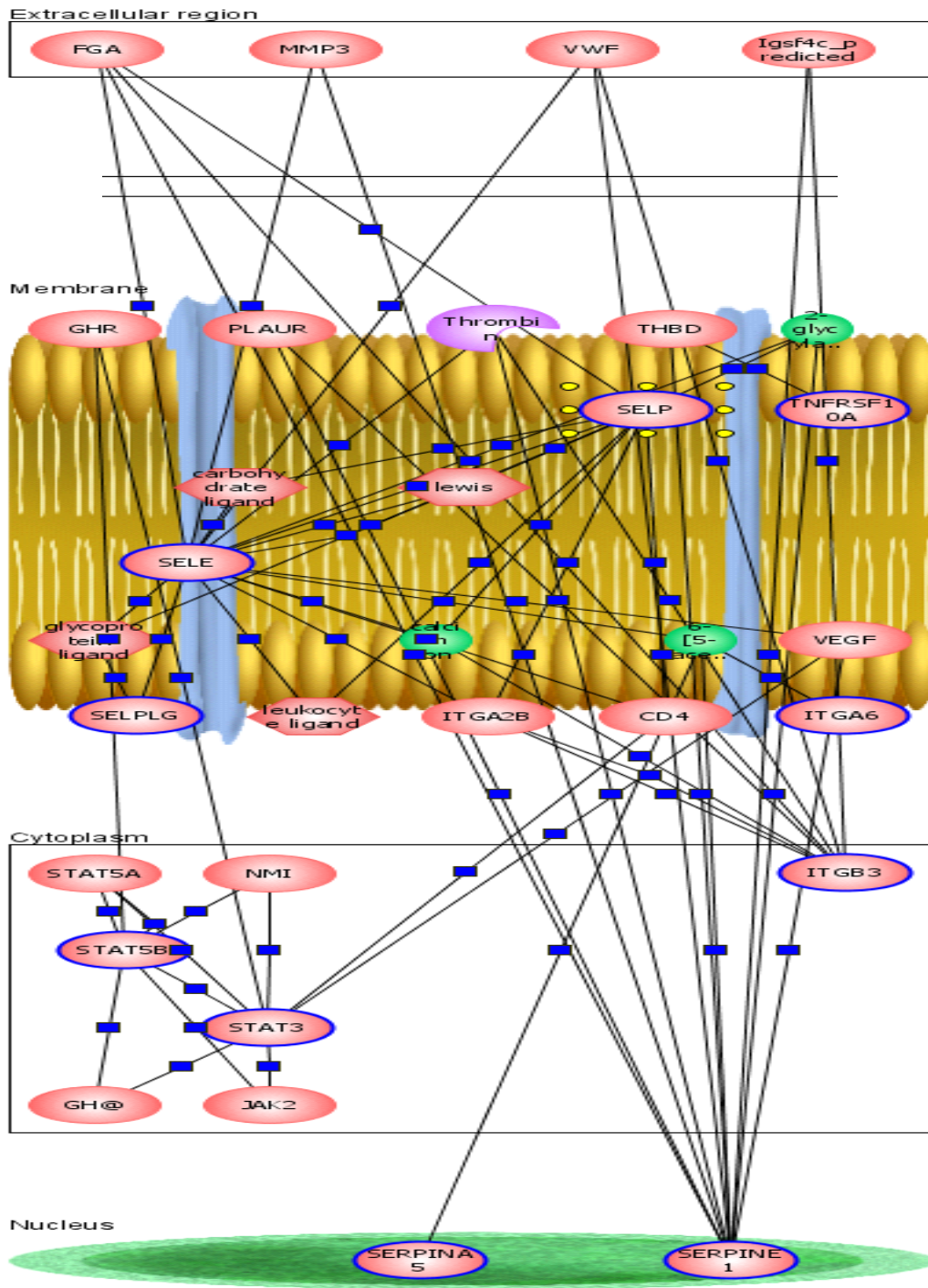
Fig. 1(A)

Fig. 1: The small-world-ness of endometrial transcript networks in sufficient ecosystem (ie., adequate progesterone and viable preimplantation stage embryo) on day 6 after ovulation (A), and its entropization by administration of low dose high affinity anti-progestin (mifepristone, 2 mg/kg body weight) (B) is seen. The probable physiological significance of observations as shown in (A) and (B) has been discussed in the essay.
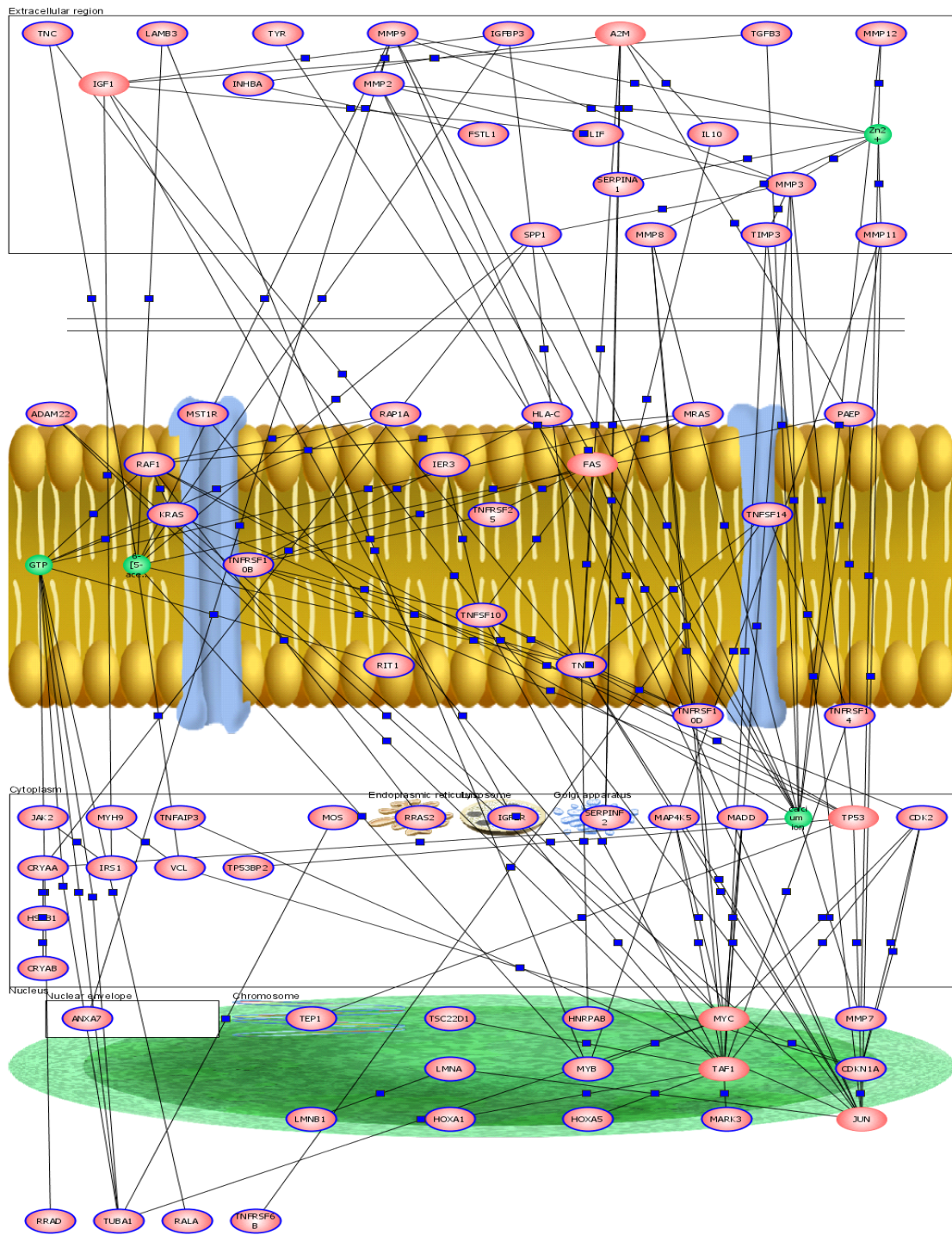
Fig. 1(B)

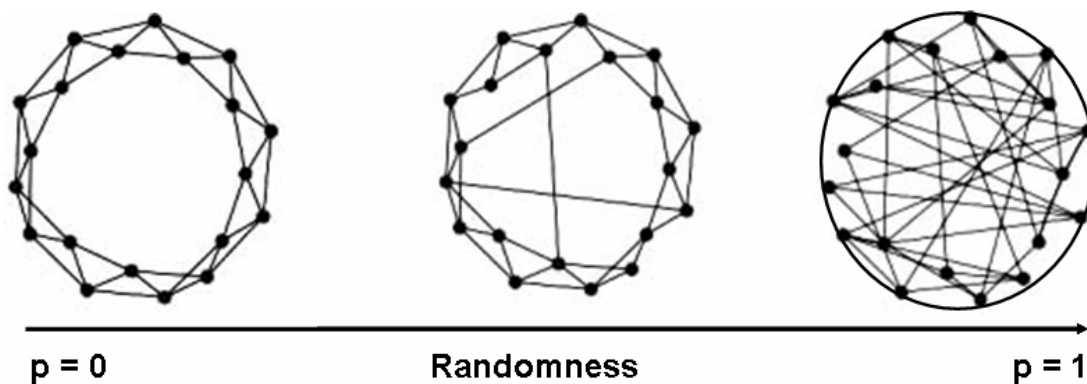p = 0   Randomness   p = 1

Fig. 2: Two spectrums of networks. In many biological networks, clusters of nodes are assorted into tight clusters and maintain relatively short path links or across the networks, giving rise to a small-world in the networks. The small-world-ness emerge as the result of replacement of a fraction (p) of links of a dimensional lattice interpolating between the two limiting cases, a regular lattice (p=0) and a random graph (p=1). Such networks are characterized by high degrees of clustering and short path lengths. See Almaas, 2007 (26) for details.

NK cells in early pregnancy stage endometrium along with the termination of pregnancy (36-38).

In lieu of conclusion

The aim of the essay was not to draw any final conclusion. It is rather an attempt to examine blastocyst implantation in the primate as a process that can be explored from the view point of systems biology. We believe that there are two kinds of futurism in the presented endometrial transcriptomic networks application with specific reference to our approach and interpretation of endometrial receptivity.

Firstly, our proposed model should be further examined using high throughput systems biology approach especially to assess the veracity and biological tangibility of the assumptions and tools suggested. Secondly, the usefulness of the model may further be checked by introducing different grades of movers, attractors, as well, noises in timed manner. For example, we have demonstrated that intra-uterine administration of oil and thread may provide embryo-analogous but distinguishable attractor function in the uterus of the rhesus monkey (39). Furthermore, local and systemic application of progestin agonists and antagonists and other chemical and physical agents during early pregnancy may provide mover and noise functions of different qualities (36, 37, 40-42). It appears possible from the present model that high affinity anti-progestin may cause insufficiency in endometrial receptivity from increment in overall gain along with attenuation of signal-noise ratio despite cohorts of specific signals being accentuated (37, 42).

There exists a limitation in the proposed model approach. We have proposed in the present study that the complex transcriptomics in endometrium following interplay between embryo and endometrium under adequate hormonal milieu resulting in successful implantation process is associated with transformation of the expression networks to a small-world pattern with potential high determinism and vulnerability (20-22). However, the application of broad based systems biology approach is always limited in a low throughput study performed on selected number of gene products as employed in the present study design. Furthermore, our study employed PCR products of human genome retrieved from human genome project. Rhesus macaque genome project has made PCR products of monkey genes available in the open access research domain

only very recently (43). Although there is good parsimony of 93% in genomic sequence between the rhesus monkey and the human, the orthology among genes of two species are discrete (44). Thus, the data obtained using rhesus monkey RNA and PCR products of 400 and 1200 genes from human genome bear a bias of false detection. We believe that, with the rhesus monkey as the non-human primate model and with robust tools of systems biology at our disposal, the present model should be further explored using high throughput global gene expressional array technology for the rhesus monkey to investigate the systems biological basis of informational networks of endometrial transcriptomes in the process of endometrial receptivity for blastocyst implantation and that it shall yield future leads having high strategic significance in human reproduction.

Finally, it is notable that understanding of the key factors in endometrial receptivity has been a subject of intense study using non-primate and primate (human and non-human subjects) models for nearly six decades. While significant advancement has been made in this area, there have been limited attempts to systematically analyze and integrate the cohorts of factors in the context of the principal actors namely, embryo, endometrium and the hormonal milieu in an integrated manner using systems biology approach and robust tools like high throughput transcriptomics and proteomics, and computational modeling. The present article addresses this issue by developing transcriptomic networks of gene expressions of primate (macaque) endometrium in: (i) presence or absence of embryo but with normal hormonal milieu, and (ii) presence of embryo but with and without progesterone starvation (caused by early luteal phase low dose mifepristone administration resulting in insufficiency in endometrial receptivity). Our study reveals that presence of a viable embryo introduces small-world type transcriptomic networks in the competent and adequately progesterone primed endometrium. This results in a physiological

process to support blastocyst implantation. However, validation of the proposed principle of transcriptomic networks shall emerge from the study of high throughput endometrial transcript networks involved in the implantation process. We believe that the proposed systems biology approach to understand the endometrial physiology in the process of blastocyst implantation shall help to explain phenomena like delayed implantation, as well as, idiopathic failure of implantation in natural set-up and following IVF-ET.

Acknowledgements

References

1. Ghosh D, Sengupta J. Recent developments in endocrinology and paracrinology of blastocyst implantation in the primate. *Hum Reprod Update* 1998; 4: 153–168.

2. Ghosh D, Sengupta J. Endocrine and paracrine correlates of endometrial receptivity to blastocyst implantation in the human. *Indian J Physiol Pharmacol* 2004; 48: 6–30.

3. Kauffman SA. The Origins of Order. Oxford University Press, New York, 1993, pp. 441–522.

4. Macklon NS, Geraedts JPM, Fauser BCJM. Conception to ongoing pregnancy : the 'black box' of early pregnancy loss. *Hum Reprod Update* 2002; 8: 333–343.

5. Finn CA. Implantation, menstruation and inflammation. *Biol Rev Camb Philos Soc* 1986; 61: 313–328.

6. Edwards RG. Physiological and molecular aspect of implantation. *Hum Reprod* 1995; 10: 1–13.

7. Rogers PAW, Murphy CR. Uterine receptivity for implantation : human studies. In : Yoshinaga K (Ed), Blastocyst Implantation, Adams Pub, Boston, MA, 1989; pp. 231–238.

8. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med* 2001; 345: 1400–1408.

9. Schwarzler P, Zech H, Auer M, Pfau K, Gobel G, Vanderzwalmen P, Zech N. Pregnancy outcome after blastocyst transfer as compared to early cleavegestage embryo transfer. *Hum Reprod* 2004; 19: 2097–2102.

10. Spencer TE, Sandra O, Wolf E. Genes involved in conceptus – endometrial interactions in ruminants : insights from reductionism and thoughts on holistic approaches. *Reproduction* 2008; 135: 165–179.

11. Kao LC, Tulac S, Lobo S, Imani B, Yang JP, Germeyer A, Osteen K, Taylor RN, Lessey BA, Giudice LC. Global gene profiling in human endometrium during the window of implantation. *Endocrinology* 2002; 143: 2119–2138.

12. Carson DD, Lagow E, Thathiah A, Al-Shami R, Farach-Carson MC, Vernon M, Yuan L, Fritz MA, Lessey B. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. *Mol Hum Reprod* 2002; 8: 871–879.

13. Borthwick JM, Charnock-Jones DS, Tom BD, Hull ML, Teirney R, Phillips SC, Smith SK. Determination of the transcript profile of human endometrium. *Mol Hum Reprod* 2003; 9: 19–33.

14. Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, Pellicer A, Mosselman S, Simon C. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. *Mol Hum Reprod* 2003; 9: 253–264.

15. Horcajadas JA, Riesewijk A, Mertin J, Cervero A, Mosselman S, Pellicer A, Simon C. Global gene expression profiling of human endometrial receptivity. *J Reprod Immunol* 2004; 63: 41–49.

16. Mirkin S, Arslan M, Churikov D, Corica A, Diaz JI, Williams S, Bocca S, Oehninger S. In search of candidate genes critically expressed in the human endometrium during the window of implantation. *Hum Reprod* 2005; 20: 2104–2117.

17. Talbi S, Hamilton AE, Vo KC, Tulac S, Overgaard MT, Dosiou C, Le Shay N, Nezhat NC, Kempson R, Lessey BA, Nayak NR, Giudice LC. Molecular phenotyping of human endometrium distinguishes menstrual cycle phases and underlying biological processes in normo-ovulatory women. *Endocrinology* 2006; 147: 1097–1121.

18. Gemzell- Danielson K, Swahn ML, Svanlander P, Bygdeman M. Early luteal phase treatment with mifepristone (RU486) for fertility regulation. *Hum Reprod* 1993; 8: 870–873.

19. Ghosh D, Sengupta J. Anti-nidatory effect of a single, early post-ovulatory administration of mifepristone (RU 486) in the rhesus monkey. *Hum Reprod* 1993; 8: 552–558.

20. Ghosh D, Sengupta J. Target oriented anti-implantation approaches for pregnancy interception: experiences in the rhesus monkey model. *Contraception* 2005; 71: 294–301.

21. Ghosh D, Sharkey AM, Charnock-Jones DS, Smith SK, Sengupta J. Effect of low dose mifepristone administration on day 2 after ovulation on transcript profiles in implantation stage endometrium of rhesus monkeys. *Reproduction* 2009; 138: 357–370.

22. Najwa AR. A study of the molecular basis of endometrial receptivity for Blastocyst implantation in primates. Doctoral (Ph.D.) Thesis, 2009, All India Institute of Medical Sciences, New Delhi.

23. Balazsi G, Oltvai ZN. Sensing your surroundings : how transcription-regulatory networks of the cell discern environmental signals. *Sci STKE* 2005; 282: pe20.

24. Alon U. Biological networks : the tinkerer as an engineer. *Science* 2003; 303: 1866–1867.

25. Milo R, Itzkovitz S, Kashtan N, Levitt R, Shen-Orr S, Ayzenshtat I, Sheffer M, Alon U. Superfamilies of evolved and designed networks. *Science* 2004; 301: 1538–1542.

26. Almaas E. Biological impacts and context of network theory. *J Exper Biol* 2007; 210: 1548–1558.

27. Strogatz SH. Exploring complex networks. *Nature* 2001; 410: 268–277.

28. Shmulevich I, Saarinen A, Yli-Harja O, Astola J. Inference of genetic regulatory networks via best-fit extensions. In : Zhang W, Shmulevich I (Eds), *Computational and Statistical Approaches to Genomics.* Kluwer Academic, Boston, MA, 2002, pp. 197–210.

29. Shannon CE. Communication in presence of noise. *Proc Inst Radio Engineer* 1949; 37: 10–21.

30. Ghosh D, Sengupta J. New perspectives on immunoreecognition of gametes and embryos. Molecular mechanism of embryo-endometrial adhesion in primates: a future task for implantation for implantation biologists. *Mol Hum Reprod* 1998b; 4: 733–738.

31. Meseguer M, Aplin JD, Caballero-Campo P, O'Connor JE, Martin JC, Remohi J, Pellicer A, Simon C. Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod* 2001; 64: 590–601.

32. Horne AW, Lalani E–N, Margara RA, White JO. The effects of sex steroid hormones and interleukin-1-beta on MUC1 expression in endometrial epithelial cell lines. *Reproduction* 2006; 131: 733–742.

33. Watts DJ, Strogatz SH. Collective dynamics of 'small world' networks. *Nature* 1998; 393: 440–442.

34. Barabasi AL, Alberts R. Emergence in scaling in random networks. *Science* 1999; 286: 509–512.

35. Sporns O, Tononi G. 2002. Classes of network connectivity and dynamics. *Complexity* 2002; 7: 28–38.

36. Druckmann R, Druckmann MA. Progesterone and the immunology of pregnancy. *J Steroid Biochem Mol Biol* 2005; 97: 389–396.

37. Milne SA, Henderson TA, Kelly RW, Sauders PT, Baird DT, Critchley HOD. 2005. Leukocyte populations ans steroid receptor expression in human first-trimester decidua ; regulation by antiprogestin and prostaglandin E analog. *J Clin Endocrinol Metab* 2005; 90: 4315–4321.

38. Carlino C, Stabile H, Morrone S, Bulla R, Sorianai A, Agostinis C, Bossi F, Mocci C, Sarazani F, Tedesco F, Santoni A, Gismondi A. Recruitment of circulating NK cells through decidual tissues : a possible mechanism controlling K cell accumulation in the uterus during early pregnancy. *Blood* 2009; 111: 3108–3115.

39. Ghosh D, Bell SC, Sengupta J. Immuno-histological localization of insulin- like growth factor binding protein-1 in primary implantation sites and trauma-induced deciduomal tissue of the rhesus monkey. *Placenta* 2004; 25: 197–207.

40. Ghosh D, Lalitkumar PGL, Wong V, Dhawan L, Rosario JF, Hendrickx AG, Lasley BL, Overstreet JW, Sengupta J. Effect of vaginally administered fumagillin on the morphology of implantation stage endometrium in the rhesus monkey. *Contraception* 2001a; 63: 95–102.

41. Ghosh D, Dhawan L, Lalitkumar PGL, Wong V, Rosario JF, Hendrickx AG, Lasley BL, Overstreet JW, Sengupta J. 2001b. Effect of vaginally administered (Ala[8,13,18])-magainin II amide on the morphology of implantation stage endometrium in the rhesus monkey (*Macaca mulatta*). *Contraception* 2001b; 63: 335–342.

42. Lalitkumar PG, Sengupta J, Ghosh D. Endometrial tumor necrosis factor alpha (TNFalpha) is a likely mediator of early luteal phase mifepristone-mediated negative effector action on the preimplantation embryo. *Reproduction* 2005; 129: 323–335.

43. Wallace JC, Korth MJ, Paeper B, Proll SC, Thomas MJ, Magness CL, Iadonato SP, Nelson C, Katze MG. 2007. High-density rhesus macaque oligonucleotide microarray design using early-stage rhesus genome sequence information and human genome annotations. *BMC Genomics* 2007; 8: 28 [doi: 10.1 186/1471-2164/8/28].

44. Rhesus Macaque Genome Sequencing and Analysis Consortium. Evolutionary and biomedical insights from the rhesus macaque genome. *Science* 2007; 316: 222–233.

45. Catalano RD, Critchley HO, Heikinheimo O, Baird DT, Hapangama D, Sherwin JRA, Charnock-Jones DS, Smith SK, Sharkey AM. Mifepristone induced progesterone withdrawal reveals novel regulatory pathways in human endometrium. *Mol Hum Reprod* 2007; 13: 641–654.

46. Ace CI, Okulicz WC. Microarray profiling of progesterone-regulated endometrial genes during the rhesus monkey secretory phase. *Reprod Biol Endocrinol* 2004; 2: 54 [doi:10.1186/1477-7827-2-54].

47. Catalano RD, Yanaihara A, Evans AL, Rocha D, Prentice A, Saidi S, Print CG, Charnock-Jones DS, Sharkey AM, Smith SK. 2003. The effect of RU486 on the gene expression profile in an endometrial explant model. *Mol Hum Reprod* 2003; 9: 465–473.

48. Sherwin JRA, Sharke AM, Cameo P, Mavrogianis PM, Catalano RD, Edassery S, Fazleabas AT. 2007. Identification of novel genes regulated by chorionic goandotropin in baboon endometrium during the window of implantation. *Endocrinology* 2007; 148: 618–626.