Research on early mammalian development in India

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ABSTRACT Historically, research in India on early mammalian development had only begun, rather modestly, in the last century, unlike the USA and UK. In India, initial studies were on gonadal and reproductive tissue development and function and they were limited to anatomical and histological characterization. This was followed by research on fertility regulation and contraception. Since the 1960s, a major initiative took place regarding endocrine biochemistry and the use of antifertility agents in inhibiting gonadal function and early development. Post-independence, the Indian government’s funding support enabled universities and institutions to embark on various research disciplines in biology but with no particular emphasis on developmental biology per se. Subsequently, India made significant progress in the area of mammalian reproduction and development, but not specifically in the core aspects of developmental biology. Reasons for this could be due to the nation’s compulsion to invest and embark on socio-economic and infrastructure development and on research involving family planning methods for reversible-affordable contraceptives to curtail population growth. With regard to the latter, biologists were involved in hormone-based contraception research. During this pursuit, insights were achieved into basic aspects of the development of gonads, gametes and embryos. Notwithstanding this, in the post-1980s through to the present time, Indian scientists have contributed to (i) the understanding of the cellular and molecular regulation of early development, (ii) developing genetically modified mouse models, (iii) using assisted reproductive technologies, generating mammalian progeny, including humans and (iv) deriving pluripotent stem cell lines for developmental studies. This article provides a perspective on the past and current status of early mammalian development research in India.

KEY WORDS: gametogenesis, embryogenesis, implantation, early pregnancy

Introduction

The history of mammalian developmental biology and embryology research reveals very interesting facts in the context of both the global and the Indian scenario. In this article, we provide an initial description of the global perspective, followed by the status of Indian research on early mammalian development and a discussion on India-specific issues on mammalian reproduction and development research. We avoided information on the non-mammalian development model organisms since they are covered in other chapters of this journal issue.

Historically, it is acclaimed that the origin of embryology is traditionally associated with Aristotle who was the first to define a basic problem that dominated embryology (Needham, 1959). In the early 1300s, Mondino was one of the earliest anatomists who performed dissection of human embryos and contributed to the beginning of embryo-uterine biology research (Mondino, 1478). Similarly, in the late 1400s, the contribution of Leonardo da Vinci was significant pertaining to quantitative observations on human embryonic development (Needham, 1959). This is followed by William Harvey who brought in the dynamic conception of embryology (De Felici and Siracusa, 2000) and research continued through the 17th century (Needham, 1959). During this era, mammalian embryology remained a purely descriptive discipline and experimental embryology had only begun with studies on fertilization and early development in mammalian models, such as rabbits and bats (Van Beneden and Julin, 1880). However, the turning point of modern mammalian embryology was only when Malpighi and Spallanzani began making microscopic observations of embryos being experimented (Needham, 1959).

In the early-mid part of the 20th century, a great revolution occurred in experimental embryology research in the USA, UK and

Abbreviations used in this paper: ART, assisted reproductive technology; EGFP, enhanced green fluorescent protein; ICM, inner cell mass; IVF-ET, in vitro fertilization+embryo transfer; TE, trophoderm.
elsewhere. During this period, many discoveries were made which include (i) gamete function and fertilization (Needham, 1959; Mulnard, 1986), early embryonic cell lineage commitment (Gardner and Papaionnou, 1975) and earliest development of primordial germ cells (PGCs) in the mouse (McLaren, 1992); (ii) generation of mouse chimeras (Gardner, 1968; McLaren, 1976) and their application in genetics, immunology and other allied areas (Mintz, 1974); (iii) genetic modification of the mouse genome via transgenic (Constantini and Lacy, 1981; Palmiter et al., 1982; Papaioannou, 1998) and knock-out approaches (Thomas and Capecchi, 1987); (iv) establishment of pluripotent stem cell (PSC) lines (Evans and Kaufman, 1981), in vitro development of early post-implantation embryos and (v) embryo cloning technologies (Wilmut et al., 1997).

While these achievements were made by many developmental biologists, of particular mention in this context is the outstanding contributions made by Anne McLaren for over half a century (Fig. 1). Dr. McLaren was a pioneer in mouse embryology and she made a few visits to India (IISc; under the Indo-UK exchange visitors program) and infused, in part, the early development research initiative in India. In this article, we provide a brief historical and contemporary perspective on early mammalian development and reproduction research in India.

### Status of Research on Reproduction and Developmental Biology in India

#### Early phase

In India, the emergence of early mammalian development research, as a discipline, occurred alongside the general progress of the natural science in a few academic departments in universities/Institutes involved in research on mammalian reproduction and development (historical period)

#### Table 1

| SL No. | University/Institute (Year of establishment) | Departments involved in MRDR | Area of Research |
|--------|---------------------------------------------|-----------------------------|-----------------|
| 1      | Madras Christian College, Chennai (1837)   | Zoology (1885)              | Endocrinology   |
| 2      | University of Calcutta, Calcutta (1867)    | Zoology (1919)              | Reprod. endocrinol. |
| 3      | University of Mumbai, Mumbai (1985)        | Life sci. (>1857)           | Histopathology, Reproduction |
| 4      | University of Madras, Chennai (1877)       | Zoology (1927)              | Herbal contraceptive biology |
| 5      | University of Lucknow, Lucknow (1893)     | Zoology (1921)              | Endocrinology   |
| 6      | Aligarh Muslim University, Aligarh (1975)  | Zoology (1966)              |Development |
| 7      | Panjab University, Chandigarh (1882)       | Zoology (>1882)             | Reprod. physiology |
| 8      | Bangalore University, Bangalore (1886)     | Zoology (1909)              | Reproduction |
| 9      | Allahabad University, Allahabad (1887)     | Zoology (1906)              | Reprod. toxicol., Endocrine disruption |
| 10     | Ind. Vet. Res. Inst., Izatnagar (1889)     | Anim.genet. (1941)          | Livestock reproduction |
| 11     | Ind. Inst. of Sci., Bangalore (1909)       | Biochemistry (1921)         | Development |
| 12     | Banaras Hindu University, Varanasi (1916)  | Zoology (1921)              | Dev., Implantation, Early pregnancy |
| 13     | University of Mysore, Mysore (1916)        | Zoology (1945)              | Reprod. Physiol., Development. Fertility |
| 14     | University of Delhi, Delhi (1922)         | Zoology (1947)              | Reproduction. Endocrine disruption |
| 15     | Nagpur University, Nagpur (1923)           | Zoology (1963)              | Reprod. Physiol. |
| 16     | Seth G. S. Med. Coll., Mumbai (1926)       | Reprod. physiol. (1965)     | Reproduction, Fertility research |
| 17     | Dr. B.R. Ambedkar Uni., Agra (1927)        | Zoology (1981)              | Mol. cell boil. Endocrinology |
| 18     | Annamalai Uni., Chidambaram, (1929)        | Zoology (1931)              | Endocrinology |
| 19     | Ind. Inst. of Chem.Biol., Kolkata (1935)   | Cell boil.& physiol.        | Reprod. Physiol. |
| 20     | University of Rajasthan, Jaipur (1947)     | Zoology (1960)              | Reproduction. Fertility, Contraceptive res. |
| 21     | Kamtak University, Dharwad (1949)          | Zoology (1959)              | Dev., Early pregnancy, Reprod. toxicol. |
| 22     | Cent. Drug Res. Inst., Lucknow (1951)      | Endocrinol div.(1965)       | Reprod. endocrinol., Reprod. toxicol. |
| 23     | Ind. Cancer Res. Inst., Mumbai (1952)      | Biology division            | Reprod. physiol., Endocrinol. |
| 24     | All Ind. Inst of Med.Sci. New Delhi (1956)| Reprod. boil. (1966)        | Reprod. endocrinol., Fert. res. |

Abbreviation: MRDR, mammalian reproduction and development research; SL No., serial number.

#Universities/Institutions listed belong to the period between 1830-1960.
Early mammalian development in India

TABLE 2

INDIAN UNIVERSITIES/INSTITUTIONS INVOLVED IN RESEARCH ON MAMMALIAN REPRODUCTION AND DEVELOPMENT (HISTORICAL TO CONTEMPORARY PERIOD*)

| SL No. | University/Institute (Year of establishment) | Departments Involved in MRDR | Area of research |
|--------|-------------------------------------------|-------------------------------|-----------------|
| 1      | Ind. Veter. Res Inst, Izatnagar (1889)    | Animal reproduction          | Livestock repro., Early dev. |
| 2      | Ind. Inst. of Sci., Bangalore (1909)      | MRDG                          | Pre-implantation dev., Hum.emb. biomarkers |
| 3      | Nat. Dairy Res. Inst, Karnal (1923)       | Animal biotechnology          | Embryo biotechnol., Fert. Res., ART |
| 4      | Seth G. S. Med College, Mumbai (1926)     | Reproductive physiology      | Reprod. Fertility research |
| 5      | Ind. Inst. of Chem. Biol. Kolkata (1935)  | Cell biology & physiology    | Placental dev. Trophoblast boil. |
| 6      | Cent. Drug Res. Inst. Lucknow (1951)      | Endocrinology division        | Reprod. toxicol. & implant. boil. |
| 7      | Ind. Cancer Res. Cent. Mumbai (1952)     | Experimental embryology      | Reprod. cancer boil., Development |
| 8      | Kasturba Med. Coll. Manipal (1953)        | Clinical embryology           | Human embryogenesis, Early dev. |
| 9      | All Ind. Inst. of Med. Sci. New Delhi (1956) | Reproductive biology      | Reprod. endocrinol., infertility |
| 10     | Nat. Inst. for Res. in Reprod. Health, Mumbai (1970) | Primate biology division | Endometral & Implantn. biol |
| 11     | Nat. Inst. of Fam. Plann. New Delhi (1977) | Biomedical division          | Reprod. health |
| 12     | Cent.for Cell. & Mol. Biol. Hyderabad (1977) | Reprod. unit & LaCONES      | Infertility, Dev. genet., ART in big cats |
| 13     | University of Hyderabad, Hyderabad(1974)  | Animal biology               | Reprod. endocrinol. & toxicol. |
| 14     | Int. Cent. for Gen. Eng. & Biotech. New Delhi (1983) | Medical biotechnology      | Mammalian boil. |
| 15     | Nat. Inst. of Immunol. New Delhi (1982)   | Life sci. & biotechnology    | Trophoblast dev. & differentiation |
| 16     | Rajiv Gandhi University, Itanagar (1984)  | Zoology                      | Herbal repro. med. & contraception |
| 17     | Rajiv Gandhi Cent. for Biotech.,TV puram (1990) | Sperm-embryo-Omics unit | Fert. Spermatogenesis & implantation boil. |

Abbreviations: ART, assisted reproductive technology; LaCONES, Laboratory for the Conservation of Endangered Species; MRDR, mammalian reproduction & development research; MRDG: molecular reproduction development & genetics; SL No., serial number.

* Universities/Institutions listed belong to the period between 1880s through 1960s (as listed in Table 1) and until the present time.

Historically, a few activities in reproductive biology research took place in a few of the oldest universities and institutes (see Tables 1 and 2). Here, we list twenty four universities and institutions, believed to have initiated research on mammalian reproductive biology and development, at some time during the early part of the last century. We have segregated the list of universities and institutes belonging to the (i) historical (1830-1960) and (ii) historical through contemporary (1960s-till date) time periods in Tables-1 and -2, respectively.

Earliest Indian reports indicate that a few clinical observations were made on the human fetal development, arising from obstetrical findings in hospital settings (Narasimhamurty, 1948; Rao, 1948). These reports describe the malformation of foetus (Ayyar, 1949) and premature infants (Janaky, 1950), segmentation of the fetal spinal cord, brain developmental disorders (Rao, 1946, 1948) and ovarian cyst formation (Singh, 1926). During this early period, there was no experimental embryology-driven research in any mammalian species. Moreover, published later-reports indicate that research was predominantly on the reproductive biology (Sarkar, 1945; Rao, 1950) and endocrinology (Sampath kumar, 1950) of rodents and farm-animals (Vaidyanathan, 1950). These early reports do not point to the exact time as to when core developmental biology research began in India. This trend continued since the later part of 1960s through the current period.

Contemporary phase

Since post-independence (1947), cell and developmental biology research in Indian universities and institutes was very limited. Traditionally, these establishments (Table 1) were engaged in classical disciplines like botany, zoology, animal nutrition, and taxonomy. They solely served for the purpose of under/post-graduate education. Only in the late 1960s, Indian universities and institutes (Table 2) began mammalian reproduction and development research and that too, much of it was pursued in only a few institutions and universities (Table 2). The research focused majority on three areas viz., (i) reproduction and reproductive endocrinology, (ii) fertility and contraceptive studies and (iii) human reproductive health disorders, albeit to a limited extent. In the 1970s and through the early 1980s, there was a boost on the country’s infrastructure and resource capabilities. This enabled many biologists to study cells and tissues in the developmental biology context. Consequently, major research took place primarily with the endocrine physiology and reproductive biology areas with only a peripheral interest to study fundamental aspects of developmental biology.

A major reason for the late start of research on mammalian early development is that the Government of India (Goi), post-independence period, had majorly embarked on the nation building involving development of various socio-economic, industrial, agriculture and rural-cum-urban sectors. Relatively, the much required levels of efforts and the quantum of money for the education and research sectors in general and, building institutions dedicated to specific areas of reproduction and development research in particular has only began in the late 1960s. Later, new generation institutions
with modern research facilities developed but, the country did not establish any dedicated research institute(s) or centre(s) on mammalian development. However, with the support of UGC, Family Planning Foundation, ICMR, ICAR and later DBT, a substantial infrastructure and research funds were invested to create centres for advanced research in reproductive biology in institutions such as IISSC, AIIMS, IVRI (Izatnagar), NIRRH, CDRI, IICB and a few state universities in Jaipur, Chennai and Delhi (see Table 2). Currently, many of these and the new establishments (Table 2) have been pursuing contemporary research in various aspects of developmental biology involving a variety of laboratory animal models (basic research) and other species that are economically and ecologically important “flagship” species (applied research).

Simultaneously, the GoI (since the early 1990s), also embarked on creating enabling advanced and state-of-the-art research facilities such as cell and molecular biology laboratories, transgenic and gene targeting (knockout) facilities both for the mouse and for the farm-animals. Thereafter, high-end and high-resolution microscopic imaging national facilities were also created. These major national initiatives, in big-measures, have significantly boosted research capabilities on early mammalian development, employing a variety of high-end cell, molecular biological and genetic tools. Since then, Indian scientists began contributing to early mammalian development research with impactful outcomes (see Table 3).

India’s contribution to Gamete biology and Embryo Development Biology

As described below, academic research contributions were made in areas of gamete biology, preimplantation embryo development, implantation biology with embryo- endometrial cross talks and placental development and differentiation. Moreover, molecular regulators involved in the management of human fertility were also investigated. While specific details on the research contributions of individual Indian scientists in the area of early mammalian development is beyond the scope of this article, in the following section, a brief overview and a glimpse is provided on research profiles of India’s mammalian developmental biology.

**Gamete development and function**

Research on gametogenesis was initiated in mid part of the last century. Studies were mainly on gamete physiology and regulation of gametogenesis and fertility control measures in terms of promoting or inhibiting fertilization using rodents (Raj and Moudgal, 1970; Gaur and Talwar, 1975; Talwar et al., 1979) and non-human primate models (Moudgal et al., 1971; Anand Kumar et al., 1980,1982).

Experimental approaches involved gonadectomy models (Rao and Talwar, 1972; Lohiya and Dixit, 1974), reproductive endocrinology (Mukku and Moudgal, 1970; Singh et al., 1971), *in vitro* culture of reproductive tissues and their derived cell types (Talwar and Sharma, 1975; Paul et al., 1978) and reproductive toxicological studies (Kar et al., 1967). Besides, work was also carried out on screening of Indian plants for antifertility effect (Satyavati, 1984) and on endocrine disruptors (Batra, 1966). During later part of the last century and the early part of this century, India contributed to the area of spermatogenesis and sperm function involving the molecular regulation of male germ (stem) cell development and differentiation (Mitra and Shivaji, 2004; Seshagiri et al., 2007; Parab et al., 2015; Dumasia et al., 2016) and the ontogeny of differentiation-associated signalling systems during germ cell development (Chandrasekhar et al., 2011; Bhattacharya et al., 2012; Majumdar et al., 2012).

Using rodent models and the human, Indian scientists contributed to the understanding of the capacitation-associated changes in sperm protein phosphorylation and their functional significance in sperm hyper-activation phenomenon (Ain et al., 1995, 1999; Mitra and Shivaji, 2004; Mariappa et al., 2006; Seshagiri et al., 2007; Mariappa et al., 2010; Chandrasekhar et al., 2011; Sagare-Patil and Modi, 2017; Sagare-Patil et al., 2017). Besides, the functional significance of progesterone and HSP90 involved in sperm hyper-activation was determined (Sagare-Patil et al., 2017) and the molecular causes of male factor infertility (Majumdar et al., 2012; Basu et al., 2018). A number of infertility-associated gene mutations (known and novel ones) were determined (Suryavathi et al., 2008; Kattri et al., 2011; Indu et al., 2015; Colaco and Modi, 2018; Sudhakar et al., 2018). Besides, the significance of genomic imprinting, its expression and those of estrogen receptors (ESR-1 and ESR-2) during spermatogenesis and sperm function was established (Dumasia et al., 2016; Kumar et al., 2018).

In contrast to studies on male germ cell biology, research on ovarian development had only been very sparse. Investigators examined genetic polymorphisms associated with a few ovarian endocrine disorders, associated with aberrations in follicular development and ovulation in the human (Ambekar et al., 2013). Experimental systems were established for the ovarian follicular development using gonadotropins in order to optimize achieving multiple ovulations and production of multiple preimplantation embryos (Seshagiri et al., 2001; Sukesh et al., 2017). Regulatory mechanisms were also elucidated with respect to ovarian corpus luteum development and/or demise in farm animals (Mondal et al., 2016). For more information on the contributions of Indian scientists, please refer to the references provided.
al., 2011; Shah et al., 2014b) and primates (Priyanka et al., 2009; Sukesh et al., 2017). In farm animals, veterinary and basic scientists have optimized in vitro conditions to achieve oocyte maturation, fertilization and embryo development, particularly in economically-profitable species such as the cattle (Saini et al., 2015) and buffalos (Madan et al., 1991; Shah et al., 2014a). In consolidation, the above contributions have indeed boosted animal (veterinary) biotechnology research sector (Wakchaure et al., 2015). Interestingly, there was always cross-fertilization among the fields of reproductive biology, fertility regulation of laboratory and farm animals and the human (in)fertility management studies.

Embryo development and function

Mammalian experimental embryology research was initiated relatively late in India vis-a-vis western countries. Much of the Indian initial studies took place in a few institutions i.e., IISc, AllIMS, NIRM, and in universities at Varanasi and Jaipur (Tables 1,2). Earliest research on development involved examining anti-estrogen effect on blastocyst development and implantation (Sengupta et al., 1981, 1983) and on the endocrinology and paracrinology of blastocyst implantation in rodents and non-human primates (Sengupta et al., 1989; Ghosh et al., 1997). Reports also showed the role of steroids on endometrial receptivity during embryo implantation in various model organisms such as the mouse (Sengupta et al., 1979, 1981), hamster (Prasad et al., 1980; Sengupta et al., 1983) and the rhesus monkey (Sengupta et al., 1989).

Later, studies were carried out on the cellular and molecular regulation of fertilization (Gupta et al., 2012) and pre- and peri-implantation embryo development (Seshagiri et al., 2009; Roy and Seshagiri, 2013; Modi and Bhartiya, 2015; Roy and Seshagiri, 2016; Seshagiri et al., 2016). The expression and functional significance of oocyte ZP proteins and their functional role in the context of sperm fertilization were established both in the bonnet monkey and in the human (Jeetanandani et al., 1998; Gupta et al., 2012). In the early developmental processes, involvements of endocrine and paracrine regulators, for example sex-steroid hormone analogues/antagonist, cytokines and growth factors were investigated (Rosario et al., 2005; Ghosh and Sengupta, 2014; Seshagiri et al., 2009, 2016). Epigenetic regulatory mechanisms of imprinted genes during embryo implantation were studied (Kedia-Mokashi et al., 2011; Bhusane et al., 2016). Interesting insights were also provided into the post-implantation developmental processes, involvements of endocrine and paracrine regulators, for example sex-steroid hormone analogues/antagonist, cytokines and growth factors were investigated (Rosario et al., 2005; Ghosh and Sengupta, 2014; Seshagiri et al., 2009, 2016). Epigenetic regulatory mechanisms of imprinted genes during embryo implantation were studied (Kedia-Mokashi et al., 2011; Bhusane et al., 2016). In vitro conditions to achieve oocyte maturation, fertilization and embryo development, particularly in economically-profitable species such as the cattle (Saini et al., 2015) and buffalos (Madan et al., 1991; Shah et al., 2014a). In consolidation, the above contributions have indeed boosted animal (veterinary) biotechnology research sector (Wakchaure et al., 2015). Interestingly, there was always cross-fertilization among the fields of reproductive biology, fertility regulation of laboratory and farm animals and the human (in)fertility management studies.

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Sontakke et al., 2009). Moreover, many non-invasive methods and tools were developed for measurement of biomarkers and hormone metabolites (Umapathy et al., 2007b; Kumar et al., 2014; Ghosal et al., 2012, 2013; Pokharel et al., 2017). These achievements have enabled Indian scientists to monitor reproductive- and stress-status of endangered animals endemic to India, for example, Asian elephants.

For more than a couple of decades, we have been studying early mammalian development particularly with three dynamic developmental phenomena viz., (i) sperm hyper-activation involving molecular dissection of capacitation-associated sperm protein tyrosine phosphorylation changes critical for sperm fertilization (Ain et al., 1999; Mariappa et al., 2006; Seshagiri et al., 2007; Mariappa et al., 2010), (ii) cellular and molecular regulation of development and hatching of blastocysts (Seshagiri et al., 2003, 2009, 2016) and (3) establishment of blastocyst-derived embryonic stem (ESC) lines (Devgan and Seshagiri, 2003; Devgan et al., 2003) and the cell lineage-specification and functional differentiation (Verma et al., 2017). We established the first Indian EGFP-expressing transgenic green mouse (GU-2 and GU-3) lines (Singh et al., 2012) and subsequently, derived two PSC lines viz., the GS-2 ESC (Singh et al., 2012) and the N9 iPSC (Abbey et al., 2019) lines. Figure 2 highlights the EGFP transgene-expressing GU-2 mice with at its various stages of embryo development, the PSC mouse lines generated and the ESC-reconstituted chimeric mouse embryo produced. These research resources are being used by us as well as by others having our resources, elsewhere in the country.

Continuing our research with regard to peri-hatching developmental events, we systematically examined the expression and function of blastocyst hatching promoting and enabling molecular regulators and the associated zonalysins viz., cathepsins, required for hatching (Mishra and Seshagiri, 2000; Sireesha et al., 2008). Recently, we showed the critical functional roles of COX-2 (Roy and Seshagiri, 2013) and NF-κB (Roy and Seshagiri, 2016) in blastocyst development and hatching. One of the striking cell biology phenomena we demonstrated was the timed-appearance of trophectodermal projections during the hatching process with the intimate association of hatching-enabling molecular regulators examined (Seshagiri et al., 2009). Many of these exciting findings have immense clinical relevance in the context of human blastocyst development and implantation, potentially leading to a viable and successful pregnancy outcome.

Impact of early mammalian development research in India

India’s contribution in developmental biology research, though modest, have had significant impact (see Table 3) in terms of (i) understanding basic biology of gamete and embryo development and function (Seshagiri et al., 2007, 2009; Chandrashekar et al., 2011; Dumasia et al., 2016; Seshagiri et al., 2016; Kumar et al., 2018), (ii) establishing genetically-modified mouse models (Sathish Kumar et al., 1994; Devgan et al., 2003; Devgan and Seshagiri, 2003; Dhup and Mujumdar, 2008), (iii) derivation of PSC lines (Mandal et al., 2006; Kumar et al., 2009; Inamdar et al., 2009; Singh et al., 2012; Kumar et al., 2011; Manian et al., 2018; Abbey et al., 2019) and (iv) using ART, generation of mammalian progeny (Shah et al., 2014a; Saini et al., 2015), including humans, by ART approaches (Mukherjee et al., 1978; Hinduja and Anand Kumar, 1988; see also Table 3).

These achievements particularly with the current availability of immortalized cell lines from reproductive tissues and the PSC lines are expected to further enhance the capacity of early mammalian development research endeavours in the country. In view of the national need to boost animal biotechnology research, in the last four decades, ICAR, CSIR and the DBT have provided major funding in order to improve fertility and enhance production of economically-profitable elite-breeds of farm animals. Generally speaking, the most significant impact, arising out of any early development research, is the birth of “test tube” progeny (conception in dishes). In this regard, India is credited to have produced world’s first (i) cloned buffalo (Shah et al., 2014a) and a calf via IVF-ET (Saini et al., 2015). Excitingly, Indian scientists also produced one of the endangered species viz., “spotty”, the spotted deer (Sontakke et al., 2009). Much of these achievements in reproductive biotechnology have been possible with enabling funding and infrastructure support systems of the GoI. India will greatly benefit if the gene-modification and transgenic technology approaches...
are exploited in the context of farm animals, in term of production of elite breeds and for bio-pharmaceuticals. This is possible with the support of GoI, its national institutions and jointly with animal biotechnology private entrepreneurs.

Another aspect that requires attention in the Indian context is the primate research and its contribution to early developmental biology. Earlier periods (1970s and 1980s) have contributed substantially to primate reproduction research (Moudgal et al., 1971; Anand Kumar et al, 1980, 1982; Seshagiri and Adiga, 1987). However, in the last decade, with the exception of a few studies (Rosario et al., 2005; Ganguly et al., 2008; Priyanka et al., 2009; Sukesh et al., 2017), the pace of primate research has rapidly diminished and it is almost stopped. Over time, the trajectory of primate research activity had several road blocks. This is owing to various reasons such as (i) dwindling numbers of experimental primatologists, (ii) unsustainability of cost-intensive primate research facility, (iii) changes in national policies- priorities of research resources, (iv) sensitivities associated with non-feral primates being housed in research establishments, (v) stringent measures in seeking ethics approvals to conduct research on non-human primates and (vi) such other national-societal and public concerns. Nevertheless, not-with-standing these facts, non-human primate (biomedical) research can indeed address a number of human (reproductive) health problems. Ironically, India is uniquely endemic to one of the largest native primate resources. It is hoped that in the immediate future, a re-visit is required to boost primate research with a flexible and pragmatic planning, with the involvement of (inter)-governmental and private-biotechnology R & D sectors.

Despite this India-specific limitations on primate reproduction and development alluded to above, it is remarkable that India contributed to a land-mark achievement and the capability to produce one of the first IVF human-primate “test tube” baby (Mukherjee et al., 1978; Hinduja and Anand Kumar, 1988). Arising out of the reproduction and development (clinical) research capabilities is the consistently successful generation of several of “test tube” babies by ART. Therefore, it is desirable to describe India-context-wise additional aspects pertaining to ART and birth of humans in the following dedicated section.

Human development and assisted reproductive technology (ART) in India

The most notable contribution is the 1st human IVF baby “Durga” birth in India (Mukherjee et al., 1978; Anand Kumar et al., 1986; Anand Kumar, 1997; Tables 3-4). Its timing indeed coincided with the birth of the world’s 1st test tube baby, “Louise Joy Brown” (Steptoe and Edwards, 1978). While the birth of the India’s 1st IVF baby “Durga” was achieved by Dr. Subhash Mukherjee (Fig. 3A), almost after about eight years, Drs. Indira Hinduja (Fig. 3B) and TC Anandkumar (Fig. 4) were credited to have achieved the next and the earliest scientifically documented IVF baby “Harsha” (Anand Kumar et al., 1986; Hinduja and Anand Kumar, 1988). These early India’s achievements, nevertheless, witnessed varied views and debates (Anand Kumar, 1997; Dhawale, 2014; Bharadwaj, 2016). The genesis of India’s earliest human IVF-births, its publicity in the 1980s and with a few visits to India of Prof. Robert G. Edwards, the 2010 Nobel Prize awardee, (Fig. 4) have opened up a huge research scope and a revolution in the treatment of human infertility in India. Ironically, this advancement has failed to catch up with research on human reproduction and development.

Strikingly, India has been one of the world’s leading countries contributing to human “test-tube” babies through ART (Malhotra et al., 2013). It is estimated optimistically that close to a million IVF babies are born in India contributing to approximately one fifth-sixth of babies globally born (current estimate: approx. 6 million) by ART approaches (Blövin et al., 2007; Bharadwaj, 2016). India has in fact made deep inroads into medical tourism in reproductive medicine, providing a whole range of sophisticated ART approaches. Table 4 provides an overview of the current status on human embryo development technologies achieved globally and its timing of comparison with the practices in India. In terms of providing the state-of-the-art ART technologies, India has not lagged behind the western countries and it has always caught up with the rest of the world (Table 4). This flourishing infertility management health care enterprise has been constantly growing. Ironically, India is unable to catch up with the core-research on early human development.
research that could provide mitigating strategies to overcome early embryonic loss and infertility.

However, India has not caught up with a few new and exciting ART approaches, for example tri-parental origin of offspring (Amato et al., 2014) and genome editing of babies (Meiliana Selvaraj et al., 2014). A number of reasons could be attributed to this such as the limitation and/or lack of expertise in gamete-derived organelle micromanipulation, non-availability of other sub-cellular and molecular techniques, the lack of sufficient number of patient-pool and the cost-factor. Besides, a major sticking issue in ART practices in the Indian context is the surrogacy. Though the Indian Surrogacy (Regulation) Bill was passed in 2016, with its own share of controversies, the GoI has recently banned commercial surrogacy in India.

India requires a research program on human reproduction and development

While the above described examples of achievements, in animal embryo biotechnology and human procreation strategies, are highly significant in its own measure, the quantum of research output, admittedly, is grossly inadequate and, it is not commensurate to what other western countries have been contributing thus far. In this context, an aggressive and a robust national program on human reproduction and development is urgently required.

Notably, India is second only to China in terms of the most populous country with an estimate of 1.35 billion people and it is on the rise (WPP, 2019). Associated with this massive growth and the socio-economic and environmental and ecological burdens and challenges, life-style disorders have been on the rise in the Indian population. This is particularly affecting individuals in the prime-reproductive age group and the working-class segment. They are burdened with a huge impact on their poor reproductive health status. These include impaired gonadal development and functions, aberrant reproductive endocrine disorders, life-style diseases-associated sub/in-fertility disorders and reproduction-associated genetic disorders. To mitigate these, there is an urgent need to understand cellular and molecular controls of early human development and in applicable human surrogate animal models. There is a need to develop diagnostic methods and interventional therapeutic strategies. Besides, enhanced translational research capacities are required. All these are hugely possible in India in view of the enabling systems in place. Besides, India has a huge availability of precious clinical reproductive tissue resources, i.e., “spare” reproductive tissues and/or gametes/embryos; they could be potentially and judiciously used for research, with informed donor consent and IEC approvals.

How can India internationally-catch up with developmental biology research

First, there is a critical need for Indian scientists to contribute towards basic and translational research on early mammalian (human) development with particular emphasis on human reproductive health. In academia, at this time, research in developmental biology is carried out only in select-institutions such as IISc, NIIRRHH, AIIMs, NII, CCMB, RGC, to indicate a few. Central and state universities should be empowered to expand programs on teaching and research in developmental biology discipline. There should be policies to encourage inter-institutional and inter-national collaborations on early development research with enabling resource provisions.

Second, although a few researchers are active in this discipline of developmental biology, India is yet to achieve a much required “critical mass” in early development and embryology and developmental biology research. Based on the published literature on mammalian development and the websites of established institutes engaged in this discipline, it appears that only about 15-20 investigators are involved in mammalian developmental biology research. Besides, in the last five decades, the number of India-originated publications in this core-discipline is only about 300 plus. This reflects a highly diminished state of India’s contribution in developmental biology research involving mammalian species. This is despite the avail-

TABLE 4
STATUS OF EARLY HUMAN EMBRYO DEVELOPMENT RESEARCH ACHIEVED IN THE ART SECTOR

| SL No. | Activity                                      | Global                          | India                           |
|-------|----------------------------------------------|---------------------------------|---------------------------------|
| 1     | Gonadotropins and endocrine regulators used in ART | Trounson et al., 1981; Fleming et al., 1982 | Mukherjee et al., 1978          |
| 2     | IVF Technique                                | Edwards et al., 1996            | Mukherjee et al., 1978          |
| 3     | Live births by:                             |                                 |                                 |
|       | (a) Fresh IVF-ET                           | Steptoe & Edwards, 1978         | ----                            |
|       | (b) Cryopreserved-ET                       | Trounson & Mohr, 1983; Mukherjee et al., 1978 | Hinduja and Anand Kumar, 1988 |
|       | (c) Assisted-hatching-ET                   | Cohen et al., 1990              | Panik et al., 1996              |
|       | (d) ICSI-ET                                | Palermo et al., 1992            | ----                            |
|       | (e) Vitriﬁed-ET                           | Gordts et al., 1990             | ----                            |
| 4     | Embryo quality score practice               | Scott, 2003; Gardner and Schoolcraft, 1999a,b | (Protocol of Gardner and Schoolcraft, 1999a,b) |
| 5     | Embryo biopsy & PGS                        | Handyside et al., 1989; De Boer et al., 2002 | Selvaraj et al., 2016          |
| 6     | Embryo viability biomarkers                 | Gardner and Schoolcraft, 1998   | Seshagiri et al., (unpublished) |
| 7     | Babies born till date (through ART)        | 5 Million - Bovin et al., 2007  | 1 Million                      |

Abbreviations: ART, assisted reproductive technology; ET, embryo transfer; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; PGS, pre-implantation genetic screening; SL No., serial number.
ability of a sizable number of freshly qualified (post)graduates and PhD degree holders in the “young” India population.

Third, there are no established dedicated organizations for developmental biology research. Ironically, this discipline does not gain importance as one of the dedicated centres in biological or life sciences in any establishments. There is an urgent need to implement various strategies to augment this important research discipline in order to expand the scope of research in modern developmental biology and reproductive medicine research in India. It is not a big-ask, if the GoI establishes a few national Institutes dedicated to human reproduction and development.

Lastly, another strategy to boost the activity is to vitalize and empower professional societies to make a strong pitch to GoI policy makers. Learned scientists and stake holders have indeed initiated a few Indian societies to foster cell and developmental biology research. These include Indian Society of Cell Biology (ASCB, 1975), Indian Society of Developmental Biology (ISDB, 1977) and Indian Society for Study of Reproduction and Fertility (ISSRF, 1988). In reproductive medicine branch, the two societies of relevance are the Indian Society for Assisted Reproduction (ISAR, 1991) and Academy of Clinical Embryologists (ACE, 2011). These professional organizations, in consultation with GoI and willing-philanthropists could raise funds that could substantially boost much needed India’s R & D capabilities on early mammalian (human) development program and, its potential translation to manage human development and reproductive health issues.

Conclusions

In India, the genesis of early mammalian development research could be traced back to the mid-part of the previous century. Initial research was on reproductive biology and fertility regulation programs. They contributed to basic understanding of development using various mammalian model organisms. Later, GoI provided major inputs on infrastructure development to facilitate modern cell and developmental biology research in public funded institutes and universities. Coupled with this, new and overlapping generations of scientists numbers engaged in reproduction and developmental research have grown. This has produced significant progress in reproduction and development research discipline and it contributed to cellular and molecular regulation of gametogenesis and early embryogenesis. Now, India is positioning well with a good knowledge-base, technical expertise and a high-end research facility to majorly embark on mammalian reproduction and development research programs. Desirably, this could be augmented by GoI in terms of (i) strategic new initiative in this research discipline with massive infusion of research funds, (ii) establishment of dedicated institutions for early mammalian (human) development and (iii) encouragement of national and international collaborative programs. In the future, these major initiatives would have huge impact in this discipline of biology with a huge translational potential in animal biotechnology, clinical translational research involving stem cells, human reproduction and development and lastly, in bio-pharma and biotech industry sectors, involved in clinical diagnostics and product development.

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