

Editorial

Human centriole: origin, & how it impacts fertilization, embryogenesis, infertility & cloning

The centriole (cenrosome) has been an enigma in cell biology for nearly 120 years; some cells have it while others do not. Oocytes, certain rodents, flies and plants do not have centrioles. Now we know the origin of centrioles from the sperm cell at fertilization in humans¹⁻³ and in most animals from roundworms to primates, that obey Boveri's rule⁴. Unfortunately, Boveri's brilliant work was not recognized for nearly a hundred years and how it impacted on infertility⁵. Its role in mitosis of the embryo organizing the first mitotic spindle initiating the process of human development (egg activation) is now well established^{6,7}. Centriole is also involved in organizing the cytoskeleton, establishes cell polarity, and is involved in cytokinesis during the cell cycle. The centrosomal cycle is closely integrated with the chromosomal cycle in embryonic and somatic cells. In essence, it controls the cell cycle and cell division in most cells. Like chromosomes, centrioles are self-replicating organelles which duplicate during interphase, when they are located close to the nucleus^{2,8}. If all other organelles of the cell are removed, the centriole should occupy the center of the cell, hence was referred to as the cell centrum⁴. The process of egg activation by sperm and initiation of embryonic cleavage was little understood, until the role of the centriole in humans was discovered¹.

Transmission electron microscopy (TEM) is the best way to study centrioles, which are minute organelles (~20 microns), barrel-shaped and presenting a unique '9+0' organization of microtubules (MT) resembling a pin-wheel. These are usually surrounded by pericentriolar material (PCM) which nucleates MT in somatic cells. Such ensembles are called a centrosomes (centriole+PCM) and becomes functional in the oocyte only after fertilization, when the sperm centrosome forms a sperm aster around duplicated centrioles – now called the zygote centrosome⁷.

Centrioles have been traced in all stages of pre-implantation embryogenesis and also in embryonic stem cells and the sperm centriole is undoubtedly the precursor of centrioles in all foetal and adult somatic cells^{2,6}. The maternal centrosome is absent or greatly reduced in humans, unlike in mice which has a dominant maternal centrosome without centrioles. Hence mice can develop parthenogenetically to term. Only one dominant, functional centrosome, paternal or maternal, is required to ensure normal development. The inheritance of 2 centrioles, as in dispermic fertilization, leads to abnormal development in humans^{6,7}.

The sperm centriole is located in its neck within a 'black box', flanked by the segmental columns, just beneath the basal plate, proximal to its nucleus. It has very little paternal PCM, demonstrable by TEM, and is not functional. There are normally 2 centrioles, mother and daughter, aligned perpendicular to each other in an orthogonal configuration in spermatids. Developing spermatogonia and oogonia have two functional centrioles (diplosomes) in their centrosomes, showing the typical "9+0" organization of microtubule triplets – common to all somatic cells. During spermiogenesis, the distal centriole forms the axoneme of the sperm tail and becomes a vestige, whilst the proximal remains close to its nucleus and is inherited by the embryo^{8,9}. Thus both are products of the same mother centriole. In oogonia, both centrioles are lost or reduced during oogenesis.

Attempts have been made to unravel the molecular structure of sperm and somatic centrosomes^{7,10}. The sperm centrosome has specific regulatory proteins - centrin, pericentrin, gamma tubulin, associated with disulphide bonds, sulphhydryl and phosphates among other molecules. The zygote centrosome has duplicated centrioles and maternal gamma tubulin is

further added to the PCM in the ooplasm to make it a functional centrosome, when the centrosome forms the sperm monoaster. Both centrioles (mother and daughter) then duplicate again and move to opposite poles establishing a bipolar spindle (bipolarization) at syngamy, the first mitosis of the human embryo^{2,6}. This ensures the cleavage of 2 equal blastomeres in the first cell cycle. Centrin is a universal, centrosomal protein playing a key role in centriolar duplication and occurs in PCM in fibers linking centrioles to one another. Recent biochemical studies reveal that the centrosome is a polyfunctional, multiprotein, cell complex in somatic cells¹⁰.

In 1991, we postulated a hypothesis that if a defective male centrosome is inherited by a human oocyte, it might lead to abnormal cleavage and compromise embryonic development¹¹. Such centrioles are present in poorly motile or immotile sperm, commonly used in intracytoplasmic sperm injection (ICSI). Since the distal centriole gave rise to the sperm axoneme during spermiogenesis, it is logical to suppose that the proximal centriole that is inherited by the embryo could cause aberrant mitosis as both are derived from the same mother centriole in spermatids. Our hypothesis was based on defects observed in the proximal centriole in the neck of sperm from motile, poorly motile, and immotile sperm samples⁹. Normal centriolar configuration is essential to ensure duplication and the functionality of the centrosome to organize embryonic spindles. A variety of chromosomal aberrations have been documented in early human embryos such as aneuploidy, polyploidy and mosaicism¹² that could be partly attributed to centrosomal dysfunction. There is now increasing clinical evidence to support this hypothesis that poor sperm means poor embryos. Centrosomal defects can lead to failure in fertilization, and cause embryonic arrest through the formation of abnormal spindles and the accumulation of chromosomally abnormal cells that derive from them¹³. These are some of the causes of early embryo loss in assisted reproduction causing infertility.

We have documented centrosomes in embryonic stem cells (ESC) and their role in cell division and cell polarity^{14,15}. These ESC are pluripotent, diploid, self-reproducing cells, derived from the inner cell mass (ICM) of the blastocyst and are capable of differentiating into all 3 germ layers, thus to any cell type of the human body. These are also capable of spontaneous differentiation and in essence, are somatic

cells with typical centrosomes, though with a much more simplified structure than ICM. This we alluded to cell de-differentiation¹⁴. It is now possible to induce pluripotency (IPS) in adult somatic cells, like skin to produce stem cells¹⁶.

One of the challenges of modern reproductive technology is therapeutic cloning and reproductive cloning in mammals, including primates. Cloning or somatic cell nuclear transfer (SCNT) into enucleated oocytes to produce blastocysts, ESC and offspring has not been very successful, even in mice or monkeys¹⁷. There are problems with centrosomal compatibility, cell cycle asynchrony, nuclear reprogramming and genomic imprinting after SCNT. In SCNT, a diploid somatic cell is electrofused with or injected into a haploid enucleated oocyte and activated to develop as during parthenogenesis. The oocyte is at metaphase II of meiosis, while the somatic cell is at interphase of mitosis with 2 centrioles, closely associated with its nucleus. Further the oocyte centrosome is absent or inactive, while the somatic centrosome is functional and presumably forms the bipolar spindle to initiate mitosis in the cloned cell. Abnormal MT formations were reported in monkey SCNT constructs but not in the bovine constructs¹⁷. Further, oocyte mitochondria are also disorganized after electrofusion¹⁵. These aberrations would explain some of the failures of cloning for therapeutic purposes or for animal reproduction like the sheep Dolly.

It is paramount that the fundamental concepts of human fertilization and embryogenesis be clearly understood and applied to the new technologies of assisted reproduction. Foremost is the process of egg activation by sperm soluble factors, such as oscillin that initiates the Ca⁺⁺ transient in the fertilized egg and, of course, the centrosome which undoubtedly plays a crucial role in humans and large mammals. Having learnt some lessons from SCNT (cloning), we need to be very cautious in generating stem cells by directed differentiation (IPS) or de-differentiation for cell therapy to treat diseases. The body's immune system has to be also contended with, lest there be tissue rejection, sooner or later. Possibly patient-specific somatic cells, such as cheek cells (skin cells) with the same genetic and centrosomal make up, could be easily harvested to generate cells for therapy. It must be also realized that tissues work in concert in the human body and not in isolation.

Future research in centrosomes should be directed toward unravelling its molecular structure in gametes

and embryos using specific protein probes and antibodies, as we do in stem cells. Combining these techniques with TEM is the gold standard for both centrosomal and ESC research. We also need to assess centrosomal and nuclear defects in sperm and follow this up with clinical outcomes after *in vitro* fertilization (IVF) and ICSI. With over 400 IVF clinics with qualified clinicians and embryologists, India should be able to generate ESC for therapeutic purposes.

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