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2 **Centrosome Reduction during Gametogenesis and Its**
3 **Significance**
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58 Abstract

59 Animal spermatids and primary oocytes initially have typical centrosomes comprising pairs of
60 centrioles and pericentriolar fibrous centrosomal proteins. These somatic cell-like centrosomes
61 are partially or completely degenerated during gametogenesis. Centrosome reduction during
62 spermiogenesis comprises attenuation of microtubule nucleation function, loss of pericentriolar
63 material and centriole degeneration. Centrosome reduction during oogenesis is due to complete
64 degeneration of centrioles that leads to dispersal of the pericentriolar centrosomal proteins, loss
65 of replicating capacity of the spindle poles, and switching to acentrosomal mode of spindle
66 organization. Oocyte centrosome reduction plays an important role in preventing parthenogenetic
67 embryogenesis and balancing centrosome number in the embryonic cells.

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73 **Introduction**

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75 Despite the fact that the pioneer work on centrosomes was carried out in spermatocytes and
76 oocytes well over 100 years ago [1] and despite the fact that most of the recent knowledge on
77 centrosomal biology has come from investigations performed on eggs or egg extracts, gametic
78 centrosomes are understood less than their somatic cell counterparts. It had been hypothesized
79 that oocytes lack centrosomes, while spermatozoa contain centrosomes that become functional
80 in the oocyte cytoplasm during fertilization (Boveri 1901, translated by Wilson 1924 [1]). Further
81 research showed that spermatozoa retain centrioles but lose most of the pericentriolar
82 centrosomal proteins whereas the oocytes lose centrioles while retaining a stockpile of
83 centrosomal proteins [reviewed in 2, 3]. The male and female gametes degenerate centrosomes
84 in a reciprocal manner so that after fusion their centrosomal components complement each other
85 generating a functional zygotic centrosome. The hypothesis of reciprocal centrosome
86 degeneration in male and female gametes is valid in many animal species, but not in all cases.
87 Recent findings have shown that sperm centrioles degenerate completely or incompletely in
88 different animal species and oocytes of some animals can regenerate functional centrosomes by
89 themselves, leading to birth of parthenogenetic offspring. In light of new data, the significance of
90 centrosome loss in gametes needs to be re-evaluated. Moreover, it has been an intriguing
91 question how the bipolar spindles are organized in animal oocytes and early embryos of some
92 species in the absence of standard centrosomes. In the last decade, much has been learned
93 about the acentrosomal pathway of microtubule polymerization and bipolar spindle organization.
94 These findings have been derived mainly from experiments performed in cell-free amphibian and
95 invertebrate egg extracts and centrosome/centriole-free cell systems. However, the mechanisms
96 of spindle organization in the cell-free extract and mammalian oocytes may not be the same.
97 Recent research on acentrosomal mode of spindle organization is discussed in the present
98 review.

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100 Structural and Functional Definitions of the Centrosome

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102 A typical centrosome consists of a pair of centrioles associated with fibrous pericentriolar
103 material [4]. Typical centrioles are cylinder-shaped structures, made up of nine symmetrically
104 oriented microtubular triplets, measuring 0.5 μm in length and 0.2 μm in diameter [5, 6]. The two
105 centrioles are associated with each other in an orthogonal orientation with the axis of the newly
106 formed (daughter) centriole crossing the axis of the older (mother) centriole. The mother
107 centrioles have flap-like appendages at the distal end, cone-shaped and striated depositions
108 called the appendages on the outer wall while the daughter centrioles have “cartwheel”
109 organization in the proximal lumen [6]. The fibrous material is confined around the mother
110 centrioles performing microtubule nucleating function.

111 The centriolar cylinder and the fibrous pericentriolar material comprise more than 100
112 different types of proteins [7-9]. Among them, the γ -Tubulin Ring Complexes (γ TuRC) comprising
113 γ -tubulin and accessory proteins are directly involved in microtubule nucleation [10, 11]. γ TuRC
114 are embedded in the pericentriolar matrix [12], possibly anchored by the centrosome protein
115 pericentrin [reviewed in 13]. Several cytoplasmic and nuclear proteins associate with
116 centrosomes in a microtubule dependent or independent manner. Dynein and proteins coupled
117 with dynein move to the centrosome through microtubular tracks by minus-end directed motor
118 activity [14]. Some proteins localize to centrosomes only during dividing stages [8]. NuMA
119 [nuclear-mitotic apparatus protein] is a nuclear protein during interphase, but associates with
120 centrosomes during spindle assembly after nuclear envelope breakdown [15]. Some newly
121 discovered centriolar/centrosomal proteins have been described in recent reviews [16, 17].

122 Unlike other cellular organelles, centrosomes do not have a definite shape, size, or limiting
123 boundary. Their shapes and activities change dynamically during different stages of the cell cycle.
124 The centrosome structure is highly variable in different cell types and organisms. Homologous
125 organelles are Spindle Pole Bodies (SPBs) of yeast [18], basal bodies of flagellates [19],
126 deuterosomes of ciliated epithelial cells [20], blepharoplasts of lower plants [21], etc. Due to

127 extremely diverse forms in different types of cells, it has been problematic to propose a
128 universally applicable definition of the centrosome.

129 Alike structural plasticity, centrosomes perform varied functions. They are the major
130 Microtubule Organizing Centers (MTOCs) of cells. Centrosomes emanate microtubule asters
131 during interphase and spindle microtubules during dividing stages. In cycling cells, the
132 centrosomes duplicate into two before entry into mitosis [22], split and form the poles of bipolar
133 spindles during prometaphase. This ensures inheritance of a complete functional centrosome to
134 each daughter cell. Ultrastructurally centrosome duplication is reflected by the centriole
135 replication [5, 6, 23]. At the G1/S transition, the mother and daughter centrioles form globular
136 fibrous material, called the procentriole, associated with the proximal region [6, 22]. They grow
137 into daughter centrioles during S and G2 periods finally forming two pairs of centrioles that are
138 indicative of duplicated centrosomes. The microtubular triplets of the original centrioles do not act
139 as template but serve as a site where the procentriole is laid down. Centriolar microtubules grow
140 within the fibrillar matrix of the procentriole, seemingly around a cartwheel structure. The
141 centrosomes also play an important role in cytokinesis [24, 25]. During spermiogenesis, the distal
142 centrioles form microtubular axonemes of the sperm tails. The basal bodies/centrioles of ciliated
143 epithelia generate motile cilia. It has been argued that the formation of the motile apparatus in
144 male gametes is the universally conserved and indispensable function of the centriole [26].
145 During fertilization, except in rodents, the sperm centrosome assembles a microtubular aster. The
146 female pronucleus moves towards the sperm pronucleus along the astral microtubules [27, 28]
147 using a dynein motor system [29, 30]. Centrosomes house many key molecules of cell cycle
148 regulation [31-37], the observations leading to speculation that centrosomes may trigger a
149 phosphorylation cascade resulting in entry into mitosis. Cells in which centrosomes are depleted
150 by microsurgery or laser ablation are able to complete mitosis and G1 stage, but become
151 arrested at the G1/S transition [24, 38] suggesting that the centrosomal integrity is crucial for
152 entry into S phase.

153 Centrosomes may be involved in several other cellular functions in addition to those
154 described above [39]. Centrosome functions are crucial for eukaryotic cells. Abnormal

155 centrosomal activity leads to genomic instability [40, 41]. Normality of centrosomes is strictly
156 maintained in the cycling cells by intimate coordination between the nuclear and centrosomal
157 cycles, ensured by coordinated checkpoint control mechanisms at the G1/S [24, 42], G2/M and
158 M/G1 transitions [43]. When cells exit the mitotic cycle and undergo differentiation, the
159 centrosomes undergo profound changes to accommodate the specialized morphological and
160 functional requirements.

161 Male and female gametes are highly specialized cells that are produced after a prolonged
162 dictyate stage, meiotic cell divisions and intricate morphogenesis. Centrosomes undergo
163 degeneration and profound modification during the final stages of gametogenesis to meet the
164 specific needs of gametic functions and fertilization. In the following sections, centrosome
165 reduction in male and female gametes and their significance will be described in detail.

166

167 **Male Gametogenesis**

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169 The primary and secondary spermatocytes show typical somatic cytoplasmic organization.
170 Mouse spermatocytes possess the major centrosomal proteins such as centrin [44], γ -tubulin and
171 pericentrin [G Manandhar and G Schatten, unpublished observations]. Male meiotic spindles of
172 most animal species display two centrioles in each pole [45]. Centrosomes are structurally and
173 functionally intact until the end of meiosis.

174 The haploid cells formed after meiotic divisions are round spermatids. Morphogenesis of fully
175 differentiated spermatozoa from the round spermatids (spermiogenesis) takes place in the
176 seminiferous tubules in close association with Sertoli cells. During this process, the spermatids
177 shed the excess cytoplasm as residual bodies. Morphologically fully formed spermatozoa are
178 released into the seminiferous tubule lumen, the process being called spermiation. Further
179 maturation takes place in the epididymis. Mammalian spermiogenesis has been reviewed
180 elsewhere [46-48].

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182 **Centrosome Reduction during Spermiogenesis**

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184 The round spermatids have apparently intact centrosomes comprising centrioles and
185 centrosomal proteins (Fig. 1A). The spermatid centrosomes are gradually inactivated and partially
186 or fully degenerated, the phenomenon termed as the centrosome reduction [49]. It has been
187 systematically studied in mice and rhesus monkeys. Centrosome degeneration occurs in three
188 stages: loss of microtubule nucleating function, loss of centrosomal proteins and disintegration of
189 centrioles. Spermatid centrosomes cease to nucleate microtubules at the early stage, before
190 displaying any signs of physical degeneration. γ -Tubulin is discarded in the residual bodies during
191 the spermiation stage (Fig. 1B) [50]. Centriolar disintegration begins in the testis and continues
192 through the epididymis. Mouse spermatozoa completely lose γ -tubulin, centrin and centrioles [44,
193 50]. Complete centriolar degeneration has also been reported in rat spermatozoa (Fig. 1C) [51].
194 Non-rodent mammalian spermatozoa degenerate centrosomes partially. Mature spermatozoa of
195 rhesus monkeys lose γ -tubulin but retain centrin [52]. The proximal centrioles remain intact in
196 sheep [53], bull [54], rhesus monkeys [55] and humans [56, 57] but the distal centrioles undergo
197 various degrees of degeneration in several species (Fig. 1D) [58-61]. Rhesus and human
198 spermatozoa show highly degenerated distal centrioles with 50% of their microtubular triplets lost
199 [62]. Among the remaining, the majority is collapsed, and their A-tubules are filled with dense
200 material. The central lumen of the residual distal centriole is occupied by proximally extended
201 microtubular duplex of the axoneme.

202 The mammalian model of sperm centrosome reduction also holds true in lower animals.
203 *Xenopus* spermatozoa do not display γ -tubulin [63, 64] but possess some other centrosomal
204 proteins such as pericentrin [63], CTR2611 [64] and Spc98p [65]. *Drosophila* spermatocytes
205 display γ -tubulin, centrosomin and centrin that are discarded from the mature spermatozoa [66].
206 Centrioles degenerate to various extents in different lower animals [45]. Snail (*Lymnaea*
207 *stagnalis*) spermatozoa lose both centrioles at maturity [67]. The proximal centriole disappears
208 during prometaphase II, so that the round spermatids possess only one centriole. During late
209 spermiogenesis, the microtubular cylinder of the centriole is replaced by nine amorphous columns

210 [67]. In insects, centrioles either disappear during spermiogenesis [68] or become modified into
211 various "acentriolar" structures [69]. Centrioles have been shown in *Xenopus* [64, 70] and
212 *Drosophila* spermatozoa (Fig. 1E) [71].

213 Why do centrosomes degenerate during spermiogenesis? The answer to this question is
214 largely speculative. Centrosome degeneration has been studied in some experimental models. In
215 cultured cells, centrosomes degenerate when they are physically damaged by X-rays [72], laser
216 irradiation [73] or treated with antimetabolic drugs [74]. Centrosome degeneration in cultured cells
217 exposed to anti-glutamylated tubulin antibody closely mimics spermatogenic centrosome
218 reduction. The antibody binds to the glutamylated sites of tubulin making them inaccessible to the
219 centriolar organizer proteins resulting in disintegration of centrioles. Centriole loss is
220 accompanied by the disjunction of centrosomal material from the pericentriolar region and
221 scattering into the cytoplasm. Analogous centrosome degeneration in gametes may be related to
222 depletion of cytoplasmic reserves of centrosomal constituents, since the synthetic activities of the
223 nuclei are totally shut down during the late spermiogenesis stages. In *Chlamydomonas* and
224 *Paramecium*, microtubular triplets of the basal bodies/centrioles degenerate due to deficiency of
225 δ -tubulin [75, 76], ϵ -tubulin [77, 78], Bld10-p [79], Vfl1 [80], etc [17]. It is very likely that centriole
226 degeneration during animal spermiogenesis could be related to deficiency of homologous
227 molecules. The role of a ubiquitin-proteasome system in centriole degeneration is also an
228 interesting field to be investigated [81]. Pericentrin and Spd-2 are involved in recruiting the
229 centrosomal materials around the pericentriolar lattice in frog egg extract and nematode
230 embryonic cells [82, 83]. Conversely, a lack of these proteins could be implicated in disjunction
231 and/or loss of pericentriolar material from the degenerating centrioles of gametogenic cells. The
232 existence of divergent molecular pathways of pericentriolar material disjunction and centriolar
233 disintegration would explain why these two events are temporally separated during
234 spermiogenesis. Centrioles of various differentiating somatic cells lose pericentriolar material and
235 cease to function as MTOC before degeneration [49]

236 There is no proven explanation why spermatozoa of some animal species lose both
237 centrioles while other species only partially degenerate the distal one. Perhaps the distal

238 centrioles are derived from the mother centrioles, which are one cell cycle generation older than
239 the proximal centrioles [6] and hence are more vulnerable to degeneration. Molecular markers of
240 the old (mother) and new (daughter) centrioles have been recently described [84-87] that might
241 help to track the 'aging' or 'senescence' of the centrioles during spermiogenesis.

242

243 **Significance of Sperm Centrosome Reduction**

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245 A widely accepted hypothesis about centrosomal inheritance during animal fertilization
246 assumes that the male gamete contributes two centrioles that organize a functional zygotic
247 centrosome by recruiting centrosomal proteins from the oocyte cytoplasm [63, 64, 88]. The
248 centrioles duplicate during the pronuclear stage making two pairs of centrioles, equivalent to two
249 centrosomes [89, 90]. The duplicated centrosomes with centriolar duplexes organize the
250 cleavage spindles [91]. Hence, the sperm centrioles are the progenitor of all centrioles of the
251 offspring.

252 The finding that centrioles are not present in all animal spermatozoa is intriguing. Because
253 many invertebrate species and rodents eliminate both centrioles during spermiogenesis,
254 centrioles must originate *de novo* in embryos of those animals. In non-rodent mammals including
255 monkeys and humans, the distal centrioles exist in highly degenerated and modified form. A
256 question was raised whether such residual distal centrioles would regenerate and/or replicate in
257 the zygotes [49, 62].

258 Centriole biogenesis in embryonic cells is yet an unresolved enigma in cell biology. As
259 described above, new centrioles originate from procentrioles that are associated with the proximal
260 region of the mature centrioles but not from the microtubular triplets of the centrioles. New
261 centrioles also can arise *de novo* without being associated with a pre-existing centriole. In ciliated
262 epithelia, the basal bodies originate from deuterosomes [92, 93] that are dense aggregates of
263 fibrous substances surrounded by granular material in which several developing procentrioles are
264 embedded [94]. In fertilized mouse embryos and parthenogenetic rabbit embryos, centrioles arise
265 during the blastocyst stage by *de novo* synthesis [95, 96]. The precursor bodies of new centrioles

266 of those embryonic cells are similar to procentrioles or deuterosomes [96, 97]. Evidently the
267 procentriole-like bodies of the embryonic cells form solitary centrioles that replicate in orthogonal
268 manner afterward [97]. These observations gave rise to a question, if centrioles can originate *de*
269 *novo*, why has the geometrically intricate orthogonal mode of replication been evolutionarily
270 conserved? Perhaps, it serves a very important purpose by strictly regulating centriole replication
271 in a $2 \rightarrow 4$ manner, equivalent to $1 \rightarrow 2$ centrosomes in each cell cycle that is crucial for bipolar
272 spindle formation. Parthenogenetically activated sea urchin and insect eggs form multiple
273 centrioles [98-100] possibly due to the lack of control on the initiation site. Cells can switch to a
274 default pathway of *de novo* synthesis when their existing centrioles are experimentally destroyed
275 but in this mode cells cannot restrain over the number of the resurrected centrioles [101]. Genes
276 that control the fidelity of centriole replication are beginning to be discovered [102-104].

277 Preservation of proximal centrioles and degeneration of distal centrioles in non-rodent
278 mammalian and some invertebrate spermatozoa suggests that centriolar pairs are asymmetrically
279 replicated during fertilization. For example, in sea urchins the proximal centriole replicates in a
280 normal fashion. The fibrous, globule-like residual distal centriole also assembles a new centriole
281 without resurrecting its own microtubular triplets [89]. Perhaps a similar mode of centriole
282 replication is valid in non-rodent mammals during fertilization. This could be the reason why one
283 pole of the first cleavage spindle of sheep zygotes possesses two centrioles, while at the other
284 pole only one exists [91]. Similar distribution of centrioles was also observed in monospermic
285 androgenetic eggs [91]. An extensive electron microscopic study of human zygotes has failed to
286 find centriolar duplexes in all spindle poles during the first cleavage [56, 57]. Nevertheless,
287 centriolar duplexes occur invariably in all late stage embryonic cells [95, 105].

288 These studies indicate that the loss of one or both spermatozoan centrioles does not impede
289 in the regeneration of new centrioles in embryos. As discussed above, new centrioles originate
290 from the procentrioles associated with the mature centrioles, not from the centrioles themselves.
291 Hence, degeneration of sperm centriolar microtubules might not affect their ability to organize
292 procentrioles and support generation of new centrioles. The hypothetical 'polar organizers' of
293 starfish eggs/embryos described by Sluder and colleagues [106-108] may be equivalent to the

294 procentrioles of the cycling cells. They colocalize with centrioles or exist independently when
295 centrioles are absent. A typical example of *de novo* regeneration of centrioles is seen in *Lymnaea*
296 *stagnalis* zygotes. The spermatozoa introduce basal bodies that are devoid of microtubular
297 triplets [68]. New centrioles arise from deuterosome/procentriole-like structures independent from
298 the sperm basal bodies [109]. Despite the oocytes' ability to generate centrioles without male
299 contribution, replication mediated by a preexisting mature or residual centriole is seemingly more
300 efficient.

301

302 **Female Gametogenesis**

303 The primary oocytes are formed long before birth but remain arrested in diplotene stage until
304 puberty. At the beginning, they are small (10-20 μm) and are surrounded by a single layer of
305 follicular cells. They are also called the primary follicles. Though arrested at meiotic prophase, the
306 primary oocytes are synthetically active with interphase-like decondensed chromatin, distinct and
307 large nucleoli. The arrested meiotic nuclei are also called the germinal vesicles. The primary
308 oocytes grow enormously reaching up to 70-150 μm in diameter.

309 In mammals, the primary oocytes resume meiosis I division in response to luteinizing
310 hormone (LH) secreted by the pituitary. Among the stockpile of arrested oocytes, only fully-grown
311 ones are competent to respond to LH at a given time to the developmental stimulus. The
312 stimulated oocytes complete meiosis I division by forming secondary oocytes and 1st polar
313 bodies. The secondary oocytes enter meiosis II but become arrested again at metaphase II
314 stage. In most mammals, the metaphase II oocytes are released from the follicles and become
315 ready for fertilization.

316

317 **Centrosome Reduction during Oogenesis**

318

319 The main bulk of knowledge in this area has come from studies in invertebrates and lower
320 vertebrates such as *Drosophila*, sea urchin, clam, starfish and *Xenopus*. Mammalian oocyte
321 centrosome studies are sporadic and scanty, mainly due to technical difficulties in obtaining

322 enough material for biochemical or cytological studies or for experimental manipulations. For
323 comparison, a typical rodent would yield less than 30 oocytes after induced superovulation and
324 oocyte manipulations have to be done under delicate environmental conditions and nutritional
325 media. In contrast, millions of eggs can be obtained from a spawning sea urchin simply by
326 injecting 0.5 M KCl solution into the coelom and eggs can be studied or manipulated in seawater.
327 With the presently available methods, it is very difficult to obtain sufficient numbers of early stage
328 mammalian oocytes, without somatic cell contamination for biochemical analysis or high-
329 resolution microscopic studies. In the following section, we will analyze the fundamental events of
330 centrosome reduction in phylogenetically diverse animals in order to derive generalized
331 conclusions.

332 A remarkable event of centrosome reduction during animal oogenesis is the loss of centrioles
333 (Fig. 2). Pre-meiotic and meiotic stages of mouse oocytes have been thoroughly studied using
334 electron microscopy. Oogonia and fetal oocytes display normal centrioles until pachytene stage,
335 but lack them in the subsequent stages (Fig. 2A, B, E) [95]. During the germinal vesicle
336 breakdown (GVBD) stage in mouse oocytes, multiple perinuclear MTOCs appear [95, 110] that
337 gradually coalesce to form the poles of metaphase spindles. Centrioles are not observed in the
338 perinuclear MTOCs or spindle poles of the meiotic oocytes [95]. Besides in mice, lack of
339 centrioles in the metaphase II spindle poles has been documented in rabbits [111], cows [105],
340 sea urchins [89], *Xenopus* [112], humans [113] and several other species (Fig. 2E) [reviewed in
341 95].

342 Centrosomes of *Drosophila* oocytes help to transport nutrients from the surrounding nurse
343 cells before being degenerated. In the beginning they nucleate microtubules along which the
344 inactive centrioles of the surrounding nurse cells migrate into the oocyte [114] and organize a
345 large MTOC [115]. The microtubules emanating from the aggregated centrioles grow into nurse
346 cells through the ring canals [114], directing the nutrients and mRNA flow into the oocyte from the
347 nurse cells [114, 115]. In the course of degeneration, the centriolar aggregate eventually loses
348 pericentriolar material and ceases microtubule-nucleating function [116]. Finally in GV stage,
349 MTOCs disperse into the cytoplasm and disappear beyond detection. The final fate of centrioles

350 has not been investigated at the ultrastructural level, but they are presumed to disintegrate
351 completely before the oocytes enter meiosis [116, 117].

352 Whereas mammalian and insect oocytes lose centrioles during pachytene arrest, echinoderm
353 and mollusk oocytes degrade them during meiotic divisions (Fig. 2A, D, G). In starfish oocytes,
354 the meiosis I spindle poles contain two centrioles, but the meiotic II spindle poles have only one
355 [118]. Hence, the two centrioles received by the secondary oocytes do not duplicate before
356 meiosis II division probably due to the lack of S period. However, during that period, the
357 unduplicated centriolar pair separates, each localizing to the opposite poles of the meiosis II
358 spindle. Consequently, the fully formed eggs receive one centriole (Fig. 2G) [118]. What happens
359 to the centrioles retained by the eggs is yet to be investigated. Most probably they rapidly
360 degenerate as evidenced by the fact that the asters formed in parthenogenetically activated sea
361 urchin eggs do not encompass centrioles during early stages [119, 120]. Similar mode of centriole
362 degeneration has been reported during oogenesis of *Mytilus edulus* [121] and crayfish [122].

363 The mode of centriole loss during oogenesis of pulmonary snail *Lymnaea stagnalis* is midway
364 between the *Drosophila* and sea urchin types (Fig. 2A, C, F). *Lymnaea* oocytes possess
365 centrioles [109] but do not duplicate before entering the dividing phase of meiosis. Hence, the
366 meiosis I spindle poles contain one centriole. The secondary oocyte inherits one centriole that is
367 distributed to the meiosis II outer spindle pole and extruded with the meiosis II polar body. Finally
368 the mature eggs are devoid of centrioles (Fig. 2F) [109]. Interestingly, the inner pole of
369 metaphase II spindle engulfs the sperm basal body, but its amorphous cylindrical structure does
370 not resemble a centriole [67].

371 The molecular mechanism of oocyte centriole degeneration is totally unknown as in the case
372 of spermatid centriole degeneration nevertheless; they could be similar.

373

374 **Significance of Oocyte Centrosome Reduction**

375

376 Dispersal of Centrosomal Proteins

377 Probably as a direct consequence of centriolar degeneration, oocytes lose the structural
378 integrity of the centrosomes. Due to the absence of centrioles, the oocyte's centrosomal material
379 does not aggregate into unified foci. The transitional stages of centrosomal protein dispersal due
380 to centriole loss have been shown by rapidly fixing the mouse oocytes and pursuing correlative
381 light and electron microscopic studies [123]. The pre-GVBD oocytes possess two large
382 multivesicular aggregates (MVA) containing γ -tubulin. At the onset of maturation, the MVA are
383 fragmented, transformed into smaller MTOCs and translocated to the GV [123]. In dividing stage
384 oocytes, the proximal ends of meiotic spindle microtubules coalesce into multiple bundles around
385 the poles. Hence, the spindle poles appear as rings [124, 125] or flat structures [126-128] and the
386 spindles appear barrel-shaped [126, 129]. Mouse oocytes do not acquire centrioles from
387 spermatozoa after fertilization and hence the centrosomes remain dispersed as the cytoplasmic
388 MTOCs, forming multiple asters during the pronuclear stage. The centrosomal proteins like 5051,
389 pericentrin and γ -tubulin are localized to the astral foci [124, 126, 128]. At mitotic entry, the asters
390 disappear and the mitotic spindle forms from the perinuclear microtubules [126, 129]. In non-
391 rodent mammalian zygotes and sea urchins, the sperm centrioles organize a dominant MTOC by
392 recruiting dispersed oocyte centrosomal proteins [2, 63, 88, 130]. Oocytes that normally form a
393 sperm aster after fertilization display cytoplasmic asters or microtubular network if activated
394 parthenogenetically [130, 132].

395 In *Drosophila*, the meiosis II spindles are formed in tandem [133]. The inner pole of the outer
396 spindle and the outer pole of the inner spindle form a common central body, analogous to a
397 merged common pole [134]. The central body is a disc-shaped structure formed by accumulation
398 of several centrosomal proteins like γ -tubulin [133, 135], centrosomin, [136] and CP190 [137], but
399 lacks a centriole. None of these centrosomal proteins are observed in the proper spindle polar
400 region [69].

401 Despite the lack of definite centrosomes, animal oocytes possess a variety of centrosomal
402 proteins [reviewed in 2, 3]. Investigation of the oocyte's centrosomal proteins has been mostly
403 done by immunocytochemistry using mono- and polyclonal antibodies. Positive detection of
404 putative centrosomal proteins relies upon the presence of microscopically recognizable punctate

405 structures in the oocytes. The soluble cytosolic proteins or proteins that are extracted during
406 processing would not be revealed by immunocytochemistry. The oocyte's centrosomal proteins
407 may exist in a dispersed form due to the lack of centriole and pericentriolar lattice. The majority of
408 cellular γ -tubulin and centrin are present in soluble form [138, 139] that can be detected by
409 immunoblotting or other molecular and biochemical techniques. Specificity of antibody reaction
410 poses further problems in immunocytochemistry. An antibody produced against a particular
411 isolated organelle or peptide from one organism may not cross-react with the homologous
412 structures of other organisms. Most of the antibodies have been studied in one or few animal
413 species, not covering phylogenetically diverse species [3], thus precluding a generalization.

414

415 Loss of Centrosomal Reproductive Capacity

416 Centriole degeneration in oocytes serves an important purpose by eliminating the
417 reproductive capacity of the maternal centrosome that in turn helps to balance the centrosome
418 number in zygotes and embryos. The reproductive capacity of centrosomes is correlated to the
419 number of centrioles they contain. Two centrioles in the centrosome ensure full reproductive
420 capacity, i.e., capability of producing a bipolar spindle in the subsequent division. The presence of
421 one centriole results in half reproductive capacity that can produce only a monaster. These
422 conclusions have been derived from a series of experiments in sea urchin [140] and starfish
423 oocytes/zygotes [107-109]. By treating with mercaptoethanol, the dividing stage sea urchin
424 zygotes can be blocked at a disorganized metaphase stage [140]. If the blockage is continued for
425 duration of one cell cycle, the mother and daughter centrioles of each spindle pole split apart and
426 acquire independent centrosomal function. Upon reversal of the blockage the zygotes re-enter
427 mitosis, the four centrioles together organize a tetrapolar spindle with one centriole at each pole.
428 The zygote divides into four blastomeres. Since each blastomere inherits a single centriole, their
429 centrosomes cannot split into two, consequently forming monopolar spindles in the next mitosis.
430 They enter into interphase without undergoing division, however, the centrioles subsequently
431 replicate and form bipolar spindles in the following mitosis [107].

432 A simple correlation between the loss of centrioles and reproductive capacity of the
433 centrosome holds true in sea urchin type oocytes [118]. The meiosis I spindle poles contain two
434 centrioles capable of producing two centrosomes by splitting, leading to the formation of a bipolar
435 spindle in meiosis II division. Since the centrioles do not replicate before meiosis II division, each
436 meiotic II spindle pole possesses only one centriole (Fig. 2G). The mature egg retains one
437 centriole of the inner pole of meiosis II spindle. One centriolar centrosome of the meiosis II
438 spindle poles cannot form bipolar spindles. Moreover, unlike zygotic centrioles, the oocyte
439 centrioles lose the ability to replicate. As a consequence, they are capable of forming only
440 monasters in the subsequent division cycles [118]. The reproductive capacity of meiotic spindle
441 poles was directly demonstrated by microinjecting them into pronuclear stage zygotes [106]. The
442 centrosomes of meiosis I spindle poles containing two centrioles double in the subsequent
443 mitosis and produce bipolar spindles while the meiotic II spindle pole centrosome containing one
444 centriole fails to reproduce resulting in monopolar spindles. These observations also indicate that
445 the oocyte centrosomes lose reproductive capacity between meiosis I and II divisions [108].

446 A correlation between the centriole number and centrosome replication is not valid in all
447 cases. Even in sea urchins, the microinjected metaphase I spindles sometimes failed to produce
448 bipolar spindles [106], indicating degradation of the reproductive capacity of their centrosomes.
449 When injected into zygotes, the first polar bodies containing two centrioles assemble monopolar
450 spindles in a similar manner as the second polar bodies possessing one centriole [141].
451 Apparently, functional degeneration (ability to duplicate) of centrioles takes place before the
452 structural degradation (reduction in number).

453 Loss of centriole and elimination of the reproductive potential of oocyte centrosome are
454 crucially important for successful fertilization and cleavages. The zygotic centrosome organized
455 around the sperm centrioles ensures pronuclear apposition, leading to close congregation of the
456 male and female chromosomes into a single metaphase plate and bipolar spindle formation
457 during embryonic mitoses. As evident, any extra centriole if retained in the oocyte would organize
458 superfluous centrosomes interfering with pronuclear apposition and normal bipolar mitosis. On
459 the other hand, the evolutionary strategy to retain centrioles in spermatozoa is justified by the fact

460 that they need centrioles until the late spermiogenesis stage to generate axonemes of the tail.
461 Centriole degeneration in oocytes confers evolutionary advantage by accommodating exogenous
462 centrioles introduced by the spermatozoa while maintaining the balance of centrosome number in
463 the zygote. Mouse oocytes do not receive centrioles from spermatozoa after fertilization, yet their
464 centrioles are completely eliminated as in other mammals. Independent evolutionary pathways of
465 oocyte and sperm centriole degenerations as seen in mice, suggest that the oocyte centriole
466 degeneration might be involved in other vital functions (described below) in addition to
467 accommodating sperm centrioles after fertilization.

468

469 Checkpoint Control of Parthenogenesis

470

471 Besides delivering the haploid male genome, spermatozoa perform additional vital functions
472 by triggering the embryonic cell cycle and providing essential centrosomal components to the
473 oocytes. Unfertilized oocytes can be activated by various physical or chemical stimuli [142, 143]
474 and their female haploid genome can be diploidized, but the embryonic development does not
475 proceed to term. One of the reasons could be the inability of the parthenogenetic oocytes to
476 organize normal spindles due to the lack of a functional centrosome [144]. In *Xenopus*,
477 microinjected exogenous centrosomes can function as zygotic centrosomes and induce
478 successful parthenogenesis. It was shown that the centrosomes from various cell types are
479 capable of inducing parthenogenesis [145-147] lest they contain intact and replication competent
480 centrioles [148, 149]. These observations implicate that the oocyte centrosome reduction has
481 evolved as a control mechanism to suppress parthenogenetic development. Due to the loss of
482 functional autonomy, the oocyte centrosome cannot initiate or successfully complete normal
483 embryonic cleavages without being supplemented by a fertilizing spermatozoon.

484 Yet many insect species are obligatory or facultative parthenotes [150]. Successful
485 parthenogenesis depends upon the oocyte's ability to generate complete and functional
486 centrosomes in the absence of one supplied by a spermatozoon. Parthenogenetically activated
487 oocytes form multiple cytoplasmic asters [69, 150-152] containing centrosomal proteins and

488 centrioles [100]. The asters behave like typical centrosomes by replicating and splitting [100].
489 Among the multiple astral centrosomes, two become associated with the female pronucleus and
490 form the mitotic spindle while the others degenerate. In some stick insect species, the
491 spermatozoa do not contribute centrioles [153], so eggs replenish all the components of the
492 zygotic centrosome. For this reason, parthenogenetic and fertilized mitotic spindles are
493 assembled in a similar manner and with equal efficiency [154]. This in turn has resulted in a
494 widespread occurrence of parthenogenesis in stick insects.

495 Parthenogenetic development is not an evolutionarily preferred pathway of species
496 propagation because it leads to genomic homogeneity that in turn results in the accumulation of
497 genetic anomalies in the population. A typical example of anomalous features of parthenogenetic
498 development is seen in *Sciara* embryos. Normally a spermatozoon introduces a giant basal body
499 containing 60-90 singlet microtubules [155]. During fertilization they are broken down to give rise
500 to MTOCs producing cytoplasmic asters. The cleavage spindles however originate from the
501 perinuclear microtubules. The parthenogenetic oocytes lack asters; nevertheless, they assemble
502 mitotic spindles from the perinuclear microtubules in a similar way as the fertilized oocytes. There
503 is no visible anomaly during the first mitosis, but the parthenogenetic syncytial embryos formed
504 after several division cycles are distinctly abnormal and nonviable. The abnormalities are
505 attributed to the dysfunction of the centrosomal apparatus failing to separate and translocate the
506 daughter nuclei to the cortex [156].

507 The oocyte centrosome becomes active only when sperm penetration fails. Though
508 cytoplasmic asters form in the fertilized and unfertilized *Nasonia* oocytes, the sperm centrosomes
509 always take over the role of zygotic centrosome in the fertilized oocytes. In the absence of sperm,
510 the oocyte asters participate in the cleavage spindle formation [152]. When parthenogenetically
511 activated *Drosophila mercatorum* oocytes are fertilized, the oocyte centrosomes remain in the
512 cortex forming small asters while the sperm centrosomes recruit centrosomal proteins, nucleate
513 astral microtubules and establish efficient interaction with the female pronucleus [100] directing
514 the embryonic development through the fertilized pathway.

515 Mouse oocytes do not receive centrosomes from spermatozoa yet they do not develop
516 through parthenogenesis. According to a viewpoint put forth by Gerald Schatten [2], eutherian
517 mammals have evolved genomic imprinting as a strategy to ensure biparental fertilization and
518 mice represent the vanguard. Genomic imprinting is the differential methylation of maternal and
519 paternal genomes [157-159] so that their expressions in the zygotes complement each other. In
520 other words, without paternal genes the expression of the maternal genome alone cannot ensure
521 normal embryonic development. According to G Schatten, this strategy might have loosened a
522 stringent control over the requirement of a paternal centrosome to initiate embryonic development
523 and that is why parthenogenetic activation or early cleavages are not so uncommon in higher
524 mammals [2]. The parthenogenetic embryogenesis does not proceed to term possibly due to
525 genetic imbalance [160], rather than due to centrosomal abnormality.

526

527 Acentrosomal Mode of Spindle Organization

528

529 The oocytes switch to acentrosomal mode of spindle organization due to centrosome
530 reduction. Acentrosomal spindle assembly is accomplished in two steps: spontaneous nucleation
531 of microtubule around the condensed chromatin, followed by sorting of the randomly oriented
532 microtubules into bipolar spindle. Spontaneous microtubule nucleation is due to localized
533 elevated activity of Ran GTPase protein [161]. The active form of Ran GTPase is GTP-bound and
534 is associated with chromatin because it is generated by the GTP exchange factor, RCC1 [162]
535 that colocalizes with chromosomes. In the downstream of Ran-GTPase activity, TPX2 (target
536 protein for *Xenopus* kinesin-like protein 2) and NuMA are released from the inhibitory association
537 with α - and β -importins [163, 164]. The TPX2 in turn stimulates Aurora A kinase, Eg2 [165],
538 resulting in enhanced spindle assembly [165, 166]. Eg2 may result in phosphorylation of multiple
539 substrates, including kinesin-like protein Eg5 [167] that in turn helps to establish the bipolar
540 spindle. The inactive form of Ran GTPase is GDP-bound, and is distributed in the cytoplasm
541 [168, 169]. The GTPase activating protein found in cytoplasm inactivates Ran by hydrolyzing
542 GTP [170].

543 Various microtubular motors play a role in generating characteristic bipolar spindles by
544 bundling, polar sorting and polar focusing of the randomly nucleated microtubules. Cross-linking
545 BimC motor proteins like Eg5 and Klp61F help in bundling and parallel orienting the microtubules
546 [171, 172]. Kinesin-like proteins (plus-end directed) including Xklp1 and Klp2 orient the
547 microtubular plus-ends towards the chromosomes and minus-ends away [173, 174] thus
548 contributing to bipolar spindle assembly. Centromere associated kinesin-like protein, CENP-E
549 localized to the kinetochore corona fibers [175, 176] captures the microtubular plus-ends, and
550 stabilizes them as kinetochore microtubules [177]. Due to the pushing force of the kinetochore
551 microtubules against the chromosomes and pulling force of the centromere associated kinesin-
552 like motor proteins on microtubules, the chromosomes congregate at the metaphase plate [178].
553 Dynein moves NuMA and various other molecular cargos along the microtubules towards the
554 minus-ends and accumulates in the polar regions [179, 180]. Due to microtubule cross linking
555 characteristics of NuMA [180, 181] and dynein, the minus-ends of the spindle microtubules are
556 tethered together forming foci in the polar region [180, 182, 183]. Some other minus-end directed
557 motor proteins like *Drosophila* Ncd kinesins and Xctk2 are also required for maintaining integrity
558 of the spindle poles [184, 185]. Once the polarized spindles are established, the centrosomal
559 proteins are recruited to the spindle poles [100, 186] that may further promote microtubule
560 nucleation.

561 The mechanism of acentrosomal spindle organization, described above is an attractive
562 hypothesis; however, such evidence is mainly based on experiments done in cell-free *Xenopus*
563 egg extracts. Spindle organization in animal oocytes may be similar to that of *in vitro* assembled
564 spindles in *Xenopus* egg extracts [187, 188] but a strict analogy is not justified considering the
565 fact that they are very different systems [189, see also, 190]. Systematic investigations are
566 necessary to understand how acentrosomal spindles are organized in oocytes.

567 Centrosome reduction and switching to acentrosomal mode of spindle organization in
568 oocytes have multiple evolutionary implications. Acentrosomal spindle organization serves as a
569 default mode that ensures normal meiotic nuclear divisions and cytokineses in the absence of
570 centrosomes. The acentrosomal spindles have lower fidelity; they can complete meiotic divisions

571 but are unable to initiate or execute normal embryonic cleavages except in some rodent species.
572 Hence, the oocytes are unable to undergo embryogenesis until the centrosomes of fertilizing
573 spermatozoa restore the centrosomal mode of spindle organization.

574

575 Conclusions

576

577 Recent provocative observations indicate that centrosomes are partially or completely
578 reduced during gametogenesis without impairing the essential functions of gametes. Many of the
579 basic questions concerning gametic centrosomal reduction still remain open. Spermatozoan
580 centrioles when present, indisputably play crucial roles during fertilization; nevertheless sperm
581 centriolar microtubules are degenerated in many animal species. In the meantime, variable
582 extents of centriolar degeneration in spermatozoa of closely related animal species have not
583 been understood. The status of the oocyte centrosome is further intriguing. The lack of centrioles
584 in most of the oocytes vividly signifies that they are not required for the oocytes meiotic divisions.
585 While the oocytes switch to default mode of acentrosomal spindle organization, reduced
586 centrosomes in them is evolutionarily implicated in preventing the parthenogenetic
587 embryogenesis and balancing centrosome number after fertilization. The molecular bases of
588 centriolar degeneration during oocyte development and acentrosomal mode of spindle
589 organization remain major unanswered questions.

590 Understanding of the gametic centrosome would bear immediate medical benefit in resolving
591 male infertility, related to putative centrosomal dysfunction. The missing centrosomal component
592 of the infertile spermatozoa is not known yet. When properly characterized, it could be
593 biochemically synthesized and injected into the oocytes in conjunction with IVF or ICSI therapy.

594

595

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600

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Legends

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1224 Fig. 1. Centrosome reduction during spermiogenesis. The male germ cells possess intact
1225 centrosomes containing centrioles and centrosomal proteins until the round spermatid stage (A).
1226 The microtubules of the distal centriole extend as axoneme of the spermatid tail (B). During
1227 spermiation, γ -tubulin and possibly other centrosomal proteins are disjuncted from the centrioles
1228 and discarded with the residual bodies (B). The centrioles are degenerated to various extents in
1229 spermatozoa of different species. Rodent and snail spermatozoa lose both centrioles completely
1230 (C) while primate spermatozoa retain proximal centrioles intact but degenerate distal centrioles
1231 partially (D). *Xenopus* and *Drosophila* spermatozoa possess both centrioles intact (E). The
1232 figures are not drawn to scale.

1233

1234 Fig. 2. Centrosome reduction during oogenesis. The oogonia possess standard centrosomes
1235 containing centrioles and centrosomal proteins (A). The centrioles are either retained or
1236 degenerated during meiotic arrest in different animal oocytes. Mammalian primary oocytes lose
1237 both centrioles completely (B) resulting in acentriolar and anastral poles during meiotic I and II
1238 divisions (E). The pericentriolar centrosomal proteins are dispersed in the oocyte cytoplasm
1239 during non-dividing stage (B) or distributed as concentric poles of the barrel-shaped spindles
1240 during dividing stages (E). In snail primary oocytes, the centrioles are retained, but they do not
1241 replicate during the meiotic arrest (C). As a result, meiosis I spindles possess one centriole at
1242 each pole. The outer centriole is expelled with the 1st polar body. The secondary oocytes inherit
1243 the inner pole centriole but enter into meiosis II division without replicating it. This centriole forms
1244 the outer pole of the meiosis II spindle and is expelled with the 2nd polar body. The mature eggs
1245 are without centriole (F). In starfish primary oocytes, the centrioles duplicate during the dictyate
1246 stage producing four centrioles before beginning the dividing stage (D). The meiosis I spindles
1247 possess two centrioles at each pole. Due to the presence of centrioles, the spindle poles are
1248 compact and astral. After meiosis I division, the oocytes inherit two centrioles but that do not
1249 replicate before the meiosis II division. Hence, the meiosis II spindles have one centriole at each

1250 pole. Consequently the mature eggs retain one centriole from the inner pole (G). The figures are
1251 not drawn to scale.

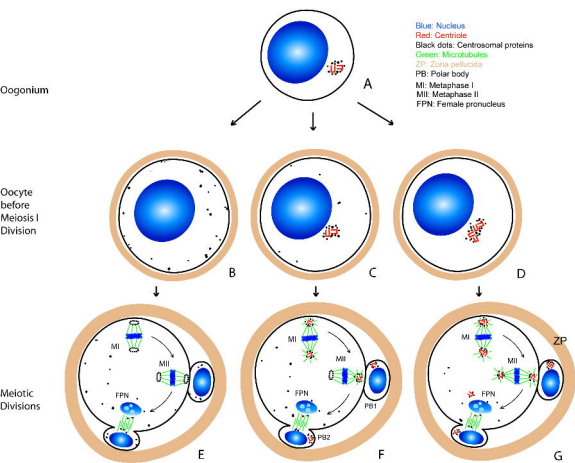
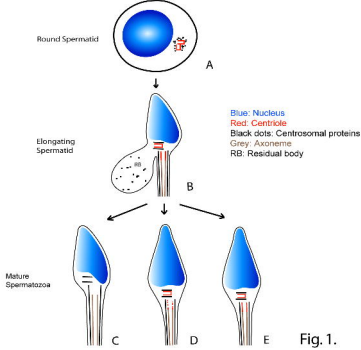


Fig. 2.