

Cytogenetics of *In vitro* Matured Oocytes - A Review

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Abstract: The field of cytogenetics is concerned with the study of the structure and properties of chromosomes, their behavior during mitosis and meiosis in reproduction and their influence on the phenotype of the organism. The word “chromosome” was introduced from the Greek language meaning “colored body”. Quality of oocytes is one of the important factors affecting the successful rate of *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) techniques. The occurrence of chromosomal aberrations in oocytes matured *in vitro* is an important factor affecting the *in vitro* production of embryos. Oocytes meiosis is very sensitive to endogenous or exogenous factors, which could lead to chromosomally abnormal oocytes. Abnormalities occurring during gametogenesis and the first stages of development play a significant role in infertility, *in vitro* fertilization failure and fetal loss. The following areas of cytogenetics will be discussed in more detail; meiosis in female, factors affecting meiotic maturation, chromosome abnormalities in oocytes as diploidy and aneuploidy.

Key words: Chromosome Abnormalities • Meiotic Maturation • Diploid Oocytes • Aneuploidy

INTRODUCTION

Chromosomal abnormalities and abnormal embryonic development have previously been observed with human [1-3] and animals [4-6] *in vitro* fertilization. Chromosomal abnormalities may arise not only after fertilization but even earlier during meiotic maturation of oocyte in culture. They arise as a result of nondisjunction during the first or second meiotic division in the ovum [7] or spermatozoon [8].

Oocytes are most remarkable cells. They are the only cells, which can form a new individual after fertilization. During maturation, oocytes undergo changes in nuclear status that involve exit from diplotene stage of the first meiotic prophase, known as germinal vesicle stage (GV) and progression to the metaphase II stage with extrusion of the first polar body [9]. The direct observation of the chromosomes is a more reliable mean of defining the stage of nuclear maturation [10]. Although the presence of the first polar body is a sign of oocyte maturation, its absence is not decisive mean to determine the stage of meiosis this is because the polar body may not be extruded from the ooplasm or may be obscured by the cumulus complex and

/ or ooplams [11]. Moreover, Hyttel *et al.* [12] indicated that the first polar body is often degenerated seven hours after the LH peak. This suggests that the stability of the polar body changes overtime.

Metaphase-II stage was found earliest at 8 hrs but it started appearing in high percentage from 16 hrs of culture and reached a peak value of 92.10 percent at 24 hrs of *in vitro* culture [13]. The cytogenetic analysis of *in vitro* matured buffalo oocytes carried out by Mahmoud and Nawito [14] revealed that the percentage of the matured oocytes (TI +MII stage) ranged from 71.77 to 80.64% at 22-24 hrs culture period and from 75.68 to 90.42 at the culture period of 25-28 hrs. Sosnowski *et al.* [15] classified the oocytes at telophase-I and metaphase-II as matured for IVF.

It is generally accepted that mammalian oocytes are frequently suffering from chromosome segregation errors during meiosis I, which have severe consequences, including pregnancy loss, developmental disorders and mental retardation. New cytogenetic techniques, based on molecular and immunofluorescent analyses, are allowing a better description of meiotic processes, including gamete production [16].

Meiosis in Female: Meiosis a Greek word meaning reduction consists of two successive cell divisions following one round of DNA replication. Meiosis gives rise to four haploid cells from a single diploid cell. This type of cell division is characteristic of germ cells. Meiosis up to the diplotene stage occurs in the fetal ovary. During the first meiotic division, maternal and paternal genes are exchanged before the pairs of chromosomes are divided into two daughter cells. The second meiotic division occurs without being preceded by DNA synthesis and nuclear reformation. The two meiotic divisions of the oocyte are asymmetrical, resulting in expulsion of polar bodies. Meiosis in each female germ cell results in a single egg and two polar bodies [9]. The ova of mammals enter meiosis early in fetal development and then become arrested at the diplotene stage of prophase until meiosis is resumed just before ovulation [9]. Meiotic maturation involves nuclear progression from dictyate of first meiotic prophase to metaphase II [17,18].

Nuclear maturation refers to the progression of the oocyte nucleus from the germinal vesicle to the metaphase II stage. Nuclear maturation involves germinal vesicle breakdown (GVBD), condensation of chromosomes, metaphase I spindle formation, separation of the homologous chromosomes with extrusion of the first polar body and arrest at metaphase II [19]. The nuclear membrane starts to fold, the nuclear pores disappear and then the nuclear membrane undergoes fragmentation and rapidly disappears [20]. It appears that nuclear maturation follows the same pattern *in vivo* and *in vitro* [21]. Nuclear maturation involves changes in protein synthesis patterns [22]. Bovine oocytes undergo marked changes in the patterns of protein synthesis GVBD *in vitro* and *in vivo*, whereas oocytes that remain at GV stage have consistent protein synthesis patterns [23, 24].

The ability of the oocyte to complete meiosis is known as meiotic competence. Meiotic competence is acquired gradually during follicular growth. Oocytes firstly acquire the capacity to undergo GVBD and chromosome condensation, then further follicular development is required to acquire the ability to progress to the metaphase I [25] and finally they acquire the ability to reach metaphase II [26].

Bovine oocyte maturation involves structural as well as functional changes. The nuclear maturation of oocytes is reflected by structural changes of the chromatin; whereas the functional changes of oocytes during final maturation (cytoplasmic maturation) is evaluated indirectly by their ability to undergo fertilization and

embryonic development [27-29]. Lucidi *et al.* [29] recorded that a correct nuclear maturation (the ability of the germ cell to resume meiosis) and cytoplasmic maturation of the oocytes was essential for normal fertilization and male nucleus decondensation to occur and thus permit subsequent embryo development.

Van Blerkom *et al.* [30] found that the optimal duration of *in-vitro* maturation of oocytes was 32, 30 and 24 hrs for sheep, goat and buffalo. In bovine, Lorenzo *et al.* [31] demonstrated that the immature oocytes at the time of collection were in the germinal vesicle stage (GV) that the highest maturation rate was at 24 hrs of culture. Kim *et al.* [32] found that the percentage of oocytes reached the MII stage of maturation range from 51.3% after 16 hrs of culture to 86% at 28 hrs in cumulus intact bovine oocytes.

In buffalo, Dewit *et al.* [33] observed that oocytes with metaphase I (MI) from 6 to 24 hrs, peaking at approximately 12 hrs after the start of maturation, the percent of oocytes with MII increased from zero at 12 hrs to approximately 75% at 24 hrs after start of maturation. Panyarachun *et al.* [34] and Mahmoud and Nawito [14] demonstrated that 24 hours maturation were sufficient for maturation of buffalo oocytes to cause both nuclear and cytoplasmic changes. Also, a period of 24 hrs is necessary for a bovine oocyte to complete nuclear maturation [35]. However, the acquisition of developmental competence for fertilization and the ability of the egg to develop progressively after fertilization appear to be related to the organization of the cytoplasm at GV stage.

Meiosis in all mammalian species is triggered by the preovulatory LH surge but the timing of nuclear maturation differs substantially according to species. Germinal vesicle breakdown (GVBD) requires 2 hrs in mice [36], 3-4 hrs in rabbits [37], 7-8 hrs in ruminants [21] and about 20 hrs in human, primate and pig oocytes [38]. Oocytes of fox and dog, however, remain in the germinal vesicle (GV) stage under 48 hrs after the peak of the LH surge [39] or hCG injection [40]. These observations indicate that differences in the timing of nuclear maturation of mammalian oocytes are mainly caused by the time required for GVBD, but the underlying reasons for the species differences are not clear.

Factors Affecting Oocyte Meiosis: The hot climate was found to be adverse, affecting the meiotic chromosomes, as represented by the decreasing of telophase I and metaphase II oocytes [41, 42]. Jongbloet *et al.* [43] concluded that the seasonal disturbance of preovulatory

ripening of the ovum emerges as a possible cause of the first and second meiotic non-disjunction. In addition, Leibfried- Rutledge *et al.* [44] reported that high ambient temperature and humidity have a deleterious effect on oocyte capacity for maturation and fertilization *in vitro*. Similarly, Rutledge *et al.* [45] and Al-Kantanani *et al.* [46] in cattle and Hammam *et al.* [47] in sheep, recorded that the proportion of oocytes and cleaved embryos that developed to blastocysts was lower in the warm seasons compared with the cool season. Mahmoud and Eashra [48] reported that the number of oocytes showing meiotic chromosomes, which are represented by telophase I and metaphase II, was significantly ($P < 0.01$) reduced in summer and autumn than in winter and spring. The highest incidence of diploid oocytes was recorded in spring while the lowest was reported in autumn.

Meiotic competence is closely correlated with oocyte size, which in turn is correlated with follicle size [49]. The size of the antral follicle at which the oocyte acquires meiotic competence is species-specific [50]. Bovine oocytes acquire the ability to complete GVBD and meiosis by the time the antral follicle reaches 2-3 mm in diameter [51,52]. Meiotic competence is also related to oocyte diameter, since bovine oocytes must have a diameter of 110 μm to complete nuclear maturation to the MII stage [51,53].

Cohan and Hunter [54] concluded that fetal oocytes did not mature *in vitro* as well as cow oocytes. After IVM, more cow oocytes matured to MII than did fetal oocytes (93.7% vs. 26.95; $p < 0.05$) and they suggested that the low meiotic competence of fetal oocytes could be attributed to their being at earlier stages of GV development before *in vitro* maturation. Also, Kazim and Hunter [55] found that there was a difference ($p < 0.05$) between fetal and cow oocytes for *in-vitro* maturation (80.1 % vs. 92.0 %) and that more fetal oocytes (12%) were observed at GV and MI stage than cow oocytes (2.3%) after 24 hours of IVM.

Cumulus cells might be a good indicator for an oocytes ability to undergo meiosis I *in vitro* and that the developmental problems of denuded oocytes were due to deficient cytoplasmic maturation [56]. Maturation rate represented by the percentage of oocytes reaching Telophase I and Metaphase II stages was higher ($p < 0.005$) in oocytes with cumulus cells than without cumulus cells in buffalo [57].

Age may be another factor that affects the oocytes *in vitro* maturation and fertilization [58]. The increase of age may have significant effects on meiotic stages of immature camel oocytes. Mahmoud *et al.* [59] reported that 25.70 ± 0.38 % and 21.3 ± 0.91 % of oocytes at the

time of collection from ovary *in vitro* had already undergone some nuclear maturation and were at GVBD stage or early condensed stage in young and adult camels respectively.

Oocyte maturation is the first and most critical step towards successful *in vitro* embryo production. The culture medium and selection of protein supplements and hormones for IVM play an important role in the subsequent maturation rate and embryonic development following IVF [60]. Several factors such as addition of FSH, LH and their combination to culture media had been considered for maximizing success [61].

Diploidy in Oocytes Matured *in vitro*: The *in vitro* fertilization now offers the opportunity to study the chromosome abnormalities in gametes. The diploid oocyte has been found to be a more frequent abnormality observed in *in-vitro* matured oocytes. The chromosome complement of oocytes after *in vitro* maturation was studied in buffalo [13,14,62], bovine [15,17, 48, 63] and camel [64]. Different meiotic stages and diploid oocytes in some farm animal are shown in Figs. 1, 2 and 3. The most common chromosomal abnormality in oocytes was diploid metaphase II. Diploid ova arising from non disjunction at meiosis II of oogenesis were believed to be responsible for most of the triploid embryos [65,66]. The finding of McFadden *et al.* [67] demonstrated that 75% of the examined human triploid fetuses were of digynic origin while only 25% were diandric.

Oocytes with the diploid number of chromosomes have been observed with various frequencies according to the species, in human: 8.3% [68] and 9.9% [69], in pig: 14.4% [70], in horse: 2.7% [71], in buffalo: 5.33% [72], in bovine: 10.7% [73], 3.1% [63] and 11.5% [74]. The diploid oocyte has been found to be a more frequent abnormality than the diploid spermatocyte which does not exceed 1%. It was recorded that diploid sperm cells occurred with a frequency of 0.05% in cattle [75], 0.09% in human [76] and 1% in rabbits [77].

Diploidy accounts for the most frequently observed anomalies in mammalian oocytes and the failure of the first polar body extrusion is thought to be the main mechanism contributing to this phenomenon [78]. Several factors affecting the incidence of diploid cells *in vitro* have been identified in domestic animals: IVM medium composition, temperature, the time of culture, the diameter of follicle and oocyte, the genotype of oocyte donor [79, 80]. In the pig, however, diploidy was influenced by the donor genotype at the RYR1 locus. The TT genotype was associated with a higher rate of diploidy [81].

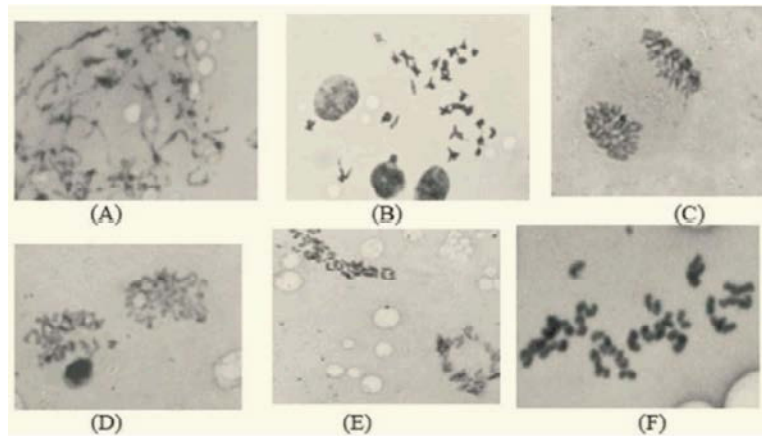


Fig. 1: Blffalo oocytes at germinal vesicle break down with a condensation of chromatin(A), metaphase I stage (B), anaphase I stage showing complete homologous (C), telephase I stage showing two group of equally spread homologous chromosomes (D), Metaphase II note the normal haploid number and t first polar body chromosomes have undergone degeneration (E). Metaphase II stage (F). Adapted from Mahmoud *et al.*, 2010

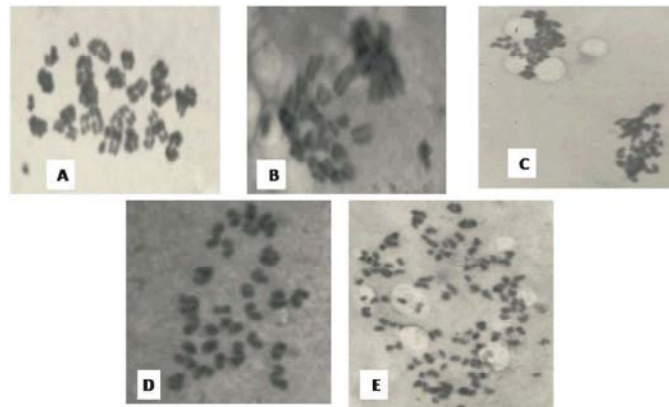


Fig. 2: Cattle oocytes at mataphase I stage (A), anaphase I stage showing complete homologous segragation of chormosomes (B), telophase I stage showing two groups of equally spread homologous chromosomes ©, metaphase ii stage (D), diploid metaphase II stage (E) . Adapted from mahmoud and eashra, (2004)

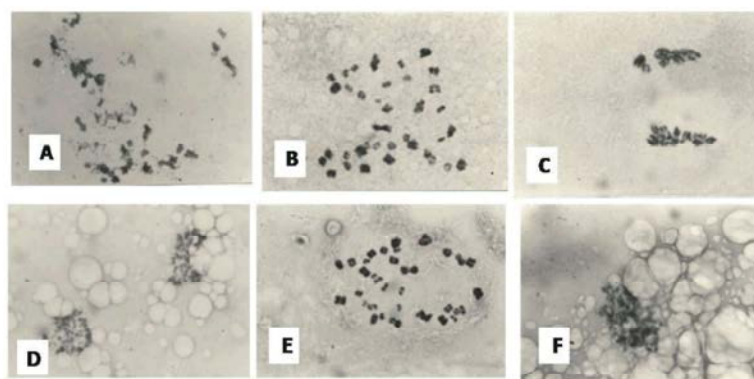


Fig. 3: Chromosome configuration of camel oocytes. (A) Post germinal vesicle breakdown stage showing isolated chromatin mass. (B) oocyte at metaphase I stage of meiosis note the bivalent chromosome. (C) complete homologous segregation of chromosomes at anaphase I. (D) oocyte at telophase I stage of meiosis showing two group of equally spread homologous chromosomes. (E) oocyte at metaphase II . Note the normal haploid number. (F) undefined metaphase. Note chromatin degeneration. Adapted from Mahmoud *et al.*, 2003

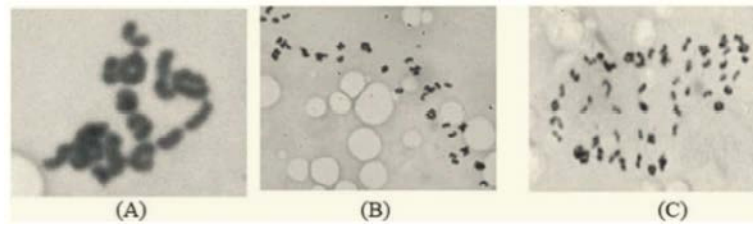


Fig. 4: Buffalo oocytes chromosomes showing hypohaploidy (A), hyperhaploidy (B), diploid number (C) adapted from Mahmoud *et al.*, 2010

Aneuploidy in Oocytes Matured *in vitro*: Aneuploidy is a common phenomenon observed in mammalian gametes [82,83]. It is well documented, especially in humans, in which the rate of aneuploid germ cells is quite high in oocytes; 4-57.7%, estimated overall frequency 13% [84] and in spermatozoa (up to 10%; [85]). The overall, non-disjunction rate in oocytes of domestic animals ranges between 2% and 7%, in a relatively low number of analyzed cells [86,71]. In buffalo, Mahmoud *et al.* [57] reported that aneuploidy percentage were nearly identical for oocytes with cumulus ($4.6 \pm 0.8\%$) and for denuded oocytes ($5.6 \pm 1.1\%$). Aneuploid oocytes when fertilized give rise to aneuploid embryos, which usually die during early pregnancy. The frequency of aneuploid embryos in domestic animals does not exceed 2% [71]. Aneuploid germ cell may be attributed to the chromosome non-disjunction at the first or second meiotic division during gametogenesis or anaphase lag. However, there were also reports of aneuploid bull primary spermatocytes that arose because of a non-disjunction process in mitotic cleavage of spermatogonia [87].

Oocyte meiosis is very sensitive to endogenous and exogenous factors that could result in oocytes with chromosomal abnormalities. Aneuploidy may be affected by factors that appear in the *in vitro* maturation and *in vitro* fertilization system. It has been shown that media used for *in-vitro* oocyte maturation may cause maturation delay and aneuploidy [88]. Moreover, patient-related factors as infertility history, female age and stimulation regimens contribute to aneuploidy [89]. When preovulatory mammalian oocytes are exposed to chemicals that alter microtubular structure and function, increased frequencies of metaphase I (MI) and diploid metaphase II (MII) oocytes are usually found in the ovulated oocytes. These MI and diploid oocytes have undergone a delay in their rate of oocyte maturation and are usually accompanied by aneuploid MII oocytes [90]. Aneuploid buffalo oocytes are shown in Fig. 4.

The vast majority of animal studies on aneuploidy induction in germ cells represent cause and effect data. Specific studies designed to evaluate possible gender

differences in induction of germ cell aneuploidy have not been found. Human gametogenesis is uniquely and gender-specific susceptible to errors in chromosome segregation. Overall, between 1% and 4% of sperm and as many as 20% of human oocytes have been estimated by molecular cytogenetic analysis to be aneuploid [91]. Maternal age remains the paramount aetiological factor associated with human aneuploidy [92]. The majority of extra chromosomes in trisomic offspring appears to be of maternal origin resulting from nondisjunction of homologous chromosomes during the first meiotic division. Differences in the recombination patterns between male and female meiosis may partly account for the striking gender- and chromosome-specific differences in the genesis of human aneuploidy, especially in aged oocytes [91].

CONCLUSION

Oocyte chromosome abnormality can present a considerable genetic burden for future generations. Thus, additional research is definitely needed to investigate the differences in farm animals abnormality induced by exogenous factors as well as to identify the underlying mechanisms. This information would be valuable for both basic science and clinical treatment related to fertility.

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