

Evaluation of the developmental competence and chromosomal compliment of mouse oocytes derived from in-vitro growth and maturation of preantral follicles

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Abstract

Purpose To evaluate the developmental potential and aneuploidy rates of in-vitro versus in-vivo grown and matured mouse oocytes.

Methods Mice were superovulated to obtain in-vivo matured oocytes. Mouse preantral follicles were also mechanically isolated and cultured in-vitro. In-vitro fertilization (IVF) was performed and fertilization, cleavage, and morula/blastocyst formation rates were compared between groups. Cytogenetic analysis was used to compare oocyte aneuploidy rates and aneuploidy characteristics in the developing embryos.

Results In-vivo oocyte maturation resulted in higher IVF fertilization, cleavage, and morula/blastocyst formation

rates versus in-vitro follicle culture (96.4% versus 78.5%, $p < 0.001$; 95.3% versus 77.4%, $p < 0.001$; 94.1% versus 76.9%, $p < 0.001$). Total aneuploidy rates were higher in embryos derived from in-vitro matured oocytes versus those grown in-vivo (4.0% versus 1.3%, $p < 0.05$).

Conclusions Results indicate a reduced developmental competency of in-vitro matured oocytes. The data also highlight an increased susceptibility to meiotic errors in early stage follicles undergoing in vitro culture.

Keywords Aneuploidy · Follicle · In-vitro · Maturation · Mosaicism · Oocyte

Capsule Impaired developmental capacity and errors in proper chromosome segregation are observed in mouse oocytes generated from in-vitro preantral follicle culture versus those developed in-vivo.

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Introduction

In most mammalian species, oocytes enter meiosis during fetal development and remain arrested at prophase-I until follicular recruitment [1]. Subsequent oocyte development involves two principle processes: nuclear and cytoplasmic maturation [1]. The former relates to the resumption of meiosis and subsequent progression to a nuclear mature oocyte, which is arrested at the metaphase-II stage until fertilization. The latter is associated epigenetic modifications and accumulation of maternal factors supporting fertilization and early embryonic development [2, 3].

A number of studies have led to the successful maturation of oocytes outside of the ovary with the use of various in-vitro follicle maturation approaches [1, 4–7]. These relatively new techniques have garnered much attention due to their potential application in assisted reproductive technologies. The capability to produce mature oocytes from primary and primordial follicles would bypass the need for expensive and sometimes dangerous superovulation. Furthermore, in conjunction with success-

ful ovarian tissue cryopreservation, in-vitro oocyte maturation may also constitute a potential source for fertility preservation, particularly in patients undergoing cancer treatment.

Animal studies have recently demonstrated increasing efficacy of preantral follicle in-vitro growth and maturation [1–3, 5–8]. Progress in humans has been somewhat limited due to the failure of similar animal-derived culture systems to bring human oocytes to full maturation [6, 9–11]. Additionally, even the more successful animal studies demonstrate a reduced developmental competency of in-vitro matured oocytes versus those matured in-vivo. These observations are exemplified by a recent study citing significantly reduced in-vitro fertilization, cleavage, and blastocyst formation rates of in-vitro matured mouse oocytes [1]. Additionally, in-vitro matured oocytes may be prone to meiotic errors, evidenced by a report of higher total aneuploidy rates [7]. Collectively, these studies suggest a reduced developmental competency and an increased susceptibility of meiotic errors exist in oocytes derived from in-vitro maturation.

While much is known regarding the reduced capacity of in vitro matured oocytes to undergo embryogenesis and subsequent development to live offspring, studies have failed to elucidate overall aneuploidy rates and specific ploidy patterns at specific stages of embryogenesis in those embryos derived from in-vitro matured oocytes. The purpose of this study was to evaluate the developmental potential and aneuploidy rates of in vitro versus in vivo matured mouse oocytes. Additionally, the overall aneuploidy rates and ploidy patterns at specific stages of embryogenesis are evaluated in embryos derived from in-vitro matured oocytes.

Materials and methods

Animal and chemicals

All protocols and treatments were performed in accordance with institutional animal care and use committee (IACUC) approval. F1 hybrid (C57BL/6 X DBA/2) mice were bred in an on-site laboratory facility. The mice were housed in a temperature and light controlled room at 23–25°C, on a 12-h light/dark cycle (on at 6 A.M. and off at 6 P.M.) and fed with pellet food and water. All the chemicals and reagents were purchased from Sigma (St. Louis, MO, USA), unless otherwise specified.

Female mice used for preantral follicle isolation and subsequent in-vitro culture were 12–14 days old. Female mice superovulated to obtain oocytes and embryos were two to four months of age. Male mice used for mating and sperm harvesting for IVF were two to four months of age.

Superovulation was induced in virgin females with an IP injection of PMSG (5 IU; Calbiochem, Cat. 367222) at 8 A.M., followed by a subsequent injection of hCG (5 IU; Calbiochem, Cat. 230734) 48 h later. Oocyte recovery was achieved 13 h post-hCG injection by mechanical harvest from the ampullae after sacrificing female mice with CO₂.

Follicle culture and oocyte in-vitro maturation

The in-vitro follicle culture technique used in this study was modified from that reported by Liu et al. [1]. Mechanically isolated follicles (100–130 µm diameter) were cultured in 10 µl droplets of the culture medium and covered with sterile mineral oil. A total of 20 droplets were placed in each 60×15 mm-dish. The culture medium consisted of α-MEM medium with Glutamax (Invitrogen Corporation, Cat. 32561-037), supplemented with 5% heat-inactivated fetal calf serum (FCS), 5 µg/ml insulin, 5 µg/ml transferrin, 5 ng/ml selenium (ITS; BD Biosciences, 354350), 100 mIU/ml recombinant hFSH (Gonal-F, Serono), 50 mIU/ml penicillin, and 50 µg/ml streptomycin. An additional 10 µl of culture media was added to each droplet on day-2 of culture. Beginning on day-4, half of the culture media in each droplet was replaced with fresh medium every other day. Follicle cultures were maintained for 10 days at 37°C under an atmosphere of 5% CO₂ and 20% O₂ (balance N₂).

On the morning of day-10, the culture medium in each droplet was replaced with maturation medium [α-MEM medium supplemented with 5% FCS, 2.5 U/ml of hCG (Calbiochem, Cat. 230734) and antibiotics] and cultured for an additional 16 hours. At the end of this in-vitro maturation interval the oocytes were collected, washed, and transferred to media droplets and prepared for IVF (80–100 oocytes per droplet).

In-vitro fertilization (IVF)

Male mice were sacrificed one hour prior to IVF insemination. The vas deferens was removed from the male mice and transferred to 1 ml IVF medium (α-MEM medium supplemented with 3 mg/ml bovine serum albumin (BSA) and antibiotics). Sperm were carefully teased into the medium and the vas deferens was discarded. Sperm were capacitated for 1-h at 37°C under an atmosphere 5% CO₂, 20% O₂, and balanced N₂.

Insemination was achieved by adding a proper volume of sperm suspension to each IVF droplet. A final sperm concentration of 2×10^5 was used to inseminate each droplet. The IVF insemination was conducted at 37°C under an atmosphere 5% CO₂ and 20% O₂ (balance N₂). IVF was allowed to proceed for a period of 4-to-6 h, at which time all oocytes were washed with IVF medium.

Fertilization assessment and embryo culture

Pronuclei (PN) were observed twelve hours post-insemination using an inverted microscope ($\times 400$ magnification). During this evaluation, the oocytes/zygotes were sorted according to the number of visible pronuclei. The oocytes/zygotes were sorted into three categories: unfertilized oocytes (0-PN), two-pronuclear zygotes (2PN), and three or more-pronuclear zygotes (3PN). During the evaluation, the inseminated oocytes were held in a drop of IVF medium (50 μ l) alongside three additional IVF medium drops (50 μ l), which were used for sorting different zygote categories.

Fertilized oocytes (2PN and 3PN zygotes) were washed twice with KSOM medium (Specialty Media, Cat MR-106) and transferred to separate KSOM droplets under oil (2PN and 3PN zygotes were cultured separately). In-vitro embryo culture involved the culture 10 to 20 embryos in each media droplet for approximately 4.5 days. Developmental data was collected 1.5, 2.5, 3.5 and 4.5 days post-insemination and included fertilization, cleavage, and morula/blastocyst formation rate observations in each of the treatment groups.

Chromosome spread and staining

Embryo blastomeres were arrested at the metaphase stage in order to evaluate ploidy characteristics in the developing embryos. Embryos were arrested at either 20 or 50 h post-insemination. All embryos were arrested at the two-cell stage when evaluated at 20 h and the majority of them were five- to eight-cell embryos when arrested at 50 h. At these time points, the embryos were transferred to KSOM medium supplemented with 0.05 μ g/ml colcemid, which facilitated the metaphase arrest. To determine colcemid incubation time, five additional embryos were incubated in KSOM medium without colcemid. Embryos exposed to colcemid were ready for the chromosome spread when the control embryos displayed a doubling in cell number.

Chromosomes were spread using a slight modification of the method described by Roberts et al. [12]. Briefly, three to five embryos were treated with 0.4 ml hypotonic solution (1% sodium citrate) on a nine-well watch glass covered with parafilm (10 min). The embryos were fixed in 0.4 ml of an ethanol/water/acetic acid mixture (6:5:1) until zona pelucida disappearance (approximately 30 s). The embryos were transferred into the second fixative (3 to 1 ethanol/acetic acid) and incubated for 5 min. The embryos (one to five at a time) were gently transferred to a clean plain glass slide along with 20 μ l of fixative. The slides were immediately placed inside a chamber with 50–60% humidity and allowed to dry. The slides were cured for two days on warming plate (37°C). The C-banding procedure was applied as described by Salamanca and Armendares [13].

Cytogenetic analysis

Numerical chromosomal analysis was performed on a compound light microscope under $\times 1,000$ magnification. Inclusion/analysis criteria included the following:

1. The blastomere was included for analysis when all the chromosomes completely separated from the chromosomes of the other blastomeres. If the blastomeres overlapped, they were included in the analysis only when the overlapped chromosomes presented enough differential in chromosomal condensation to be distinguished from each other.
2. Whenever chromosome number was less than 40, a lower amplification lens was used to evaluate a wider area on the slide to ensure no chromosomes spread out of the field of view.
3. Blastomeres were not included if chromosome overlap significantly impaired identification of each chromosome.
4. Tetraploidy was recorded when all chromosomes showed the same condensation level and spread evenly in one group.
5. The embryo was taken into account only when 2 metaphase cells were available to be analyzed.

Statistical analysis

Oocyte IVF fertilization rates and IVF cleavage, morula/blastocyst formation rates were compared between in-vivo and in-vitro oocyte maturation groups using χ^2 analysis. Aneuploidy comparisons were also compared between groups using χ^2 analysis. Differences were considered significant in the p value was less than 0.05.

Results

In the first portion of the study, 222 in-vivo matured oocytes and 107 in vitro grown and matured oocytes were inseminated via in-vitro fertilization (Table 1). The in-vivo matured oocyte group displayed a significantly increased fertilization rate (96.4%–214/222) versus the in-vitro maturation group (78.5%–84/107; $p < 0.001$, Table 1). Embryos derived from in-vivo matured oocytes cleaved at significantly higher rates than those derived from in-vitro maturation (95.3% (204/214) versus 77.4% (65/84), $p < 0.001$, Table 1). Similarly, morula/blastocyst formation rates for embryos derived from in-vivo maturation were significantly increased versus those matured in-vitro [94.1% (192/204) versus 76.9% (50/65), $p < 0.001$, Table 1].

The second portion of the study was directed towards the cytogenetic analysis of oocytes and embryos derived from

Table 1 In-vitro fertilization and embryo development characteristics

	MII Oocytes (N)	Fertilized [N (%)]	Two-cell embryos [N (%)]	Morula/blastocysts [N (%)]
In-vivo matured oocytes	222	214(96.4) ^a	204 (95.3) ^b	192 (94.1) ^c
In-vitro grown oocytes	107	84 (78.5) ^a	65 (77.4) ^b	50 (76.9) ^c

Fertilization, cleavage, and morula/blastocyst formation rates were compared between the in-vivo and in-vitro matured oocyte group using χ^2 analysis ($p < 0.001$ for all rates)

^a Fertilization rate

^b Cleavage rate

^c Morula/blastocyst formation rate

in-vivo and in-vitro oocyte maturation. In this segment of the study, a total of 2560 follicles were cultured for 10 days with a 93% (2380) survival rate. After final maturation, 1,933 oocytes were recovered, but because the majority of these were in complex with cumulus cells, oocyte maturation stages were identified after IVF insemination and denudation of the oocytes. A total of 1,419 oocytes were at the MII stage and 1,022 (72.3%) of them displayed evidence of fertilization: 875 with 2PN and 147 with 3PN.

Embryos were arrested at metaphase using colcemid treatment and the chromosomes were fixed and spread for cytogenetic analysis. Embryos were arrested for cytogenetic analysis at either 20 h (two-cell stage) or 50 h (five- to eight-cell stage) post-IVF insemination. Blastomeres did not always arrest at metaphase even with increasing colcemid concentration and incubation time. Embryos were also lost during chromosome spreading. Thus, the total number of analyzed embryos was smaller than the embryos obtained after maturation, insemination, and in-vitro culture.

A total of 386 in-vivo oocyte maturation-derived embryos (199× two-cell embryos, and 187× five- to eight-cell embryos) and 326 in-vitro oocyte maturation derived-embryos (224× two-cell embryos and 102× five- to eight-cell embryos) were analyzed. Among the 712 embryos analyzed, 29 embryos (4.1%) were diploid/tetraploid (2n/4n) mosaic

Table 2 Diploidy/tetraploidy (2n/4n) mosaicism in two stages of embryo development

	2-cell embryo 2n/4n mosaicism rate (%)	5- to 8-cell embryo 2n/4n mosaicism rate (%)
In-vivo matured oocytes	1.0 (2/199)*	10.2 (19/187)*
In-vitro matured oocytes	0.9 (2/224)**	5.9 (6/102)**

Mosaicism rates were compared between the in-vivo and in-vitro matured oocyte group using χ^2 analysis and between embryo stages in each group. While no significant difference was detected between oocyte maturation groups, there was a significant increase in five- to eight-cell mosaicism rates compared with that observed in two-cell embryos

* $p < 0.001$

** $p < 0.05$

(Table 2). All the mosaic embryos included one tetraploid cell except three embryos (each at the five- to eight-cell stage), which contained a pair of tetraploid cells. The 2n/4n mosaicism rate increased significantly at the five- to eight-cell embryo stage versus the two-cell stage ($p < 0.001$, Table 2). The difference of mosaic tetraploidy between control and IVM was not significant ($p > 0.05$). Comparison of overall mosaic aneuploidy revealed no difference ($p > 0.05$) between two-cell embryos (0.9%) and five- to eight-cell embryos (2.4%) when combining data for embryos derived from in-vitro and in-vivo matured oocytes.

In a previous study, a higher incidence of triploidy was detected after IVF of in-vivo derived oocytes versus those matured in-vitro [7]. To determine if pronuclei sorting could provide a mechanistic rationale for this discrepancy, the zygotes were sorted 12-hours post-IVF based on zygotic pronuclei number (2PN and 3PN; Table 3).

A total of 326 in-vitro matured oocytes and 386 in-vivo matured oocytes resulting in 2PN zygotes were analyzed for numerical chromosome abnormalities (Table 3). Each of the 2PN zygotes developed into a normal diploid embryo with the exception of four embryos, which were generated in the same IVF cycle. In that particular IVF cycle, three 2PN embryos from the in-vivo oocyte maturation group and one 2PN embryo in the in-vivo maturation group developed into uniform triploid embryos.

A total of 72 embryos (derived from 3PN zygotes) were also evaluated for numerical chromosome abnormalities (Table 3). The majority of these (55/72, 76.4%) developed into embryos with uniform triploidy (Table 3). A smaller percentage of these zygotes developed into normal diploid embryos (16/72, 22%; Table 3). One embryo derived from

Table 3 Ploidy characteristics of embryos derived from 2PN and 3PN zygotes

	Diploidy (%)	Triploidy (%)	Tetraploidy (%)
2PN-derived embryos	322/326 (98.8)	4 ^a /326 (1.2)	0/326 (0)
3PN-derived embryos	16/72 (22.2)	55/72 (76.4)	1 ^a /72 (1.4)

^a Embryos generated from the same IVF cycle.

a 3PN zygote developed with uniform tetraploidy. Interestingly, this zygote and the four other uniform triploid embryos (derived from 2PN zygotes as described above), were generated during the same IVF cycle.

The uniform aneuploidy noted in these data is believed to derive from meiotic errors (numerical chromosome abnormalities from the sperm or oocyte). Conversely, mosaic aneuploidy is believed to derive from mitotic error after zygote formation. The total aneuploidy rate (uniform plus mosaic aneuploidy) in embryos derived from the in-vitro oocyte maturation group (4%) was significantly higher than that of embryos derived from in-vivo matured oocytes (1.3%; $p < 0.05$; Table 4). Uniform and mosaic aneuploidy of embryos derived from in-vitro oocyte maturation were generally higher than that of the embryos derived from in-vivo maturation. However, due to the limited number of embryos available for analysis these differences were not statistically significant ($p > 0.05$; Table 4).

Discussion

The purpose of this study was to evaluate the developmental potential and aneuploidy rates of in vitro versus in vivo grown/matured mouse oocytes. Additionally, we sought to evaluate the overall aneuploidy rates and ploidy patterns at specific stages of embryogenesis in embryos derived from in-vitro matured oocytes.

The culture system used for in-vitro follicle culture originally was described by Liu et al. [1] who demonstrated that, versus other follicle culture approaches, it may improve mature oocyte yields and developmental competence of those gametes. In a previous study, we also evaluated the efficacy of this particular culture system in tandem with two different approaches of follicle isolation and found this to be a superior approach [7]. In the present study, 10-day follicle culture yielded high follicle survival rates (93.0%), modest oocyte recovery rates (75.5%), and a mature oocyte yield of 73.4% of the total number of recovered oocytes. These results were consistent to previous reports using similar culture systems [1, 14].

Table 4 Uniform, mosaic, and total aneuploidy rates among embryos derived from in-vitro and in-vivo matured oocytes

	Uniform aneuploidy (%)	Mosaic aneuploidy (%)	Total aneuploidy (%)
In-vivo matured oocyte group	1/386 (0.3) ^a	4/386 (1.0) ^b	5/386 (1.3) ^c
In-vitro matured oocyte group	6/326 (1.8) ^a	7/326 (2.1) ^b	13/326 (4.0) ^c

^{a,b} z-test, $p > 0.05$; ^c z-test, $p < 0.05$

The developmental competence of oocytes grown and matured in-vitro was significantly compromised versus those matured in-vivo. This conclusion is evidenced by the reduced fertilization, cleavage and morula/blastocyst formation rates observed in the in-vitro group. These results are consistent with previous follicle culture studies in mice [1–3, 5–8]. Thus, while gross nuclear maturation is achieved at normal rates, cytoplasmic maturity and the acquisition of various factors involved in the developmental potential of these oocytes may be compromised in the in-vitro culture environment. It should be noted that mice used for preantral follicle isolation and in-vitro culture were only 12–14 days old, while mice used for in-vivo follicle growth were 2 to 4 months old. This difference in age may account for differences observed in oocyte developmental competence.

On the subject of cytogenetic perturbations, there is conflicting evidence suggesting in-vitro culture of early stage follicles may render the oocyte susceptible to disruption in chromosome cohesion, kinetochore function, spindle assembly, or other events that contribute to proper chromosome segregation.

Indeed, in the present study, total aneuploidy rates were significantly increased in embryos derived from in-vitro grown and matured oocytes versus those developed in-vivo. These data are consistent with two studies indicating an increase in aneuploidy rates and chromosome misalignment of in-vitro cultured preantral follicles [7, 15]. Additionally, the data presented here resembles the incidence of aneuploidy and polyploidy of two-cell mouse embryos derived from IVF after in-vitro follicle culture, reportedly between 3.0% and 6.0% [10].

Meanwhile, other studies have reported no difference in aneuploidy rates between in vitro cultured preantral follicles and in vivo controls [16]. When evaluated separately, mosaic and uniform embryo aneuploidy rates derived from in-vitro versus in-vivo follicle culture did not differ significantly. There was a notable trend towards an increase in these ploidy characteristics in the in-vitro culture group. It is possible these data may reach a level of significance via increasing study power through sample size augmentation.

Another notable result was the increased incidence of diploid/tetraploid (2n/4n) mosaicism after the third mitotic division. Specifically, we noted a significant rise at the five to eight cell embryo stage versus the two-cell stage. This 2n/4n mosaicism likely manifests due either to a failure of cytokinesis or from cell fusion [17]. Indeed, tetraploid cells have been generated experimentally by applying cytokinesis inhibitor and electrofusion [18]. Additionally, the early cleavage divisions in human and mouse embryos have been shown to be error-prone and could contribute to the formation of mosaic embryos [19].

Studies also suggest that 2n/4n mosaicism is a normal feature of the later-stage embryos and that there is a non-random allocation of the tetraploid cells within the embryo [17, 20–23]. Ruangvutier et al. [17] described the presence of tetraploid cells in a significant proportion of arrested cleavage-stage human embryos (6/20) and at an even higher proportion of human blastocyst stage embryos (14/19). High rates of 2n/4n mosaics have also been identified in other farm animals including a 50.5% incidence in sheep blastocysts and a 41.5% incidence in bovine embryos [24, 25]. Furthermore, the non-random allocation of 4n cells to different developmental lineages and a specific selection against tetraploid cells may act to exclude these polyploid cells from the epiblast by mid-gestation [20, 22].

While the 2n/4n mosaicism is a frequent characteristic of morphologically normal blastocysts, it has been speculated that this early developmental feature is related only to precursor cells of the later trophoblast cells. Evidence for this hypothesis is provided by two studies identifying tetraploid cells in 30 to 40% of trophoblast cells concurrent with a notable absence of tetraploid cells in the inner cell mass [20, 26]. Thus, the 2n/4n cells may be eliminated or diverted to the placenta as diploid/tetraploid mosaics, which are not commonly observed in subsequent stages of development [17, 21, 27]. Taken together, these studies suggest that the 2n/4n mosaicism manifests during post-zygotic mitotic divisions at the early cleavage stage and may be a normal phenomenon in embryonic development.

Uniform ploidy characteristics were also evaluated in the present study. A higher incidence of uniform polyploidy has been reported in mouse embryos fertilized in vitro (12.8%) versus those fertilized in vivo (1.5% to 6%) [28, 29]. The polyploidy incidence among IVF embryos increases with sperm insemination concentration as well as the PMSG dose used to superovulate the animals [28, 29].

In this study, uniform polyploid was generally observed before the first cleavage division, eliminating the possibility of a mitotic error. The uniform triploidy may either be digynic in origin (due to retention of the second polar body) or a cause of dispermy (where two sperm fertilize the oocyte) [29, 30]. Consistent with our data, Santalo reported that 54.4% of in-vivo triploids and 63.2% of the in -vitro triploids are dispermic in origin. Thus, the uniform triploidy in embryos developing from fertilized 3PN oocytes, is likely due to dispermic fertilization. Meanwhile, the uniform triploidy observed in embryos derived from fertilized 2PN oocytes is likely due to digynic mechanisms.

In humans, dispermic zygotes are characterized by this triploid cytogenetic phenomenon. Sperm provide the centrioles and centrosome which regulate syngamy and the first zygotic division [31]. When an extra sperm is

introduced into oocyte two pairs of active centrioles form within the ovum, resulting in a tripolar spindle and inevitable production of chaotic chromosome distribution and gross aneuploidy [31]. However, mice and other rodents differ from humans in that the functional centrosome is maternally derived. Our results indicate the primary triploidy was relatively stable throughout embryo stages, even at the eight-cell stage when they all displayed uniform triploidy.

We also observed a total of four 2PN zygotes that developed to uniform triploid embryos and one 3PN zygote that developed to a uniform tetraploid embryo. Each of these was generated during the same IVF cycle, using the same semen sample and insemination concentration. A reasonable hypothesis is that these polyploid embryos likely originated due to a meiotic error directly from the gametes themselves.

Conversely, a relatively large proportion (22%) of 3PN zygotes developed to diploid embryos. Postzygotic diploidization of triploids was also described by Grossmann et al. who reported a significant proportion (36%) of 3PN ICSI-derived zygotes that were actually diploid with respect to chromosome number [32]. These results are also consistent with Macas et al. [23] who reported a 32% incidence of postzygotic diploidization of triploids. Postzygotic diploidization of triploids may arise from the abnormal distribution of the maternal chromosomes into two separate sets after extrusion of the first PB [32].

Conclusions

The data demonstrate in-vitro preantral follicle culture has the capacity to generate efficient yields of nuclear mature oocytes capable of in-vitro fertilization and subsequent embryo development. However, these oocytes exhibit impaired developmental competence versus those matured in-vivo. This conclusion is evidenced by reduced fertilization, cleavage and morula/blastocyst formation rates observed in the in-vitro group. Thus, while gross nuclear maturation is achieved at normal rates, cytoplasmic maturity and the acquisition of various factors involved in the developmental potential of these oocytes may be compromised in the in-vitro culture environment. Future studies, including expression profiling, are needed to elucidate the defective mechanisms and precise cellular products affected by the in-vitro culture of preantral follicles. Additionally, the data suggest in-vitro culture results in significant increases in oocyte aneuploidy rates, suggesting early stage follicle culture may render the oocyte susceptible to disruption in chromosome cohesion, kinetochore function, spindle assembly, or other events that contribute to proper chromosome segregation.

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