Impact of Superovulation And In Vitro Fertilization On LINE-1 Copy Number And Telomere Length In C57BL/6J Mice Blastocysts

Thalita S Berteli (thalitabertelli@gmail.com)
Department of Obstetrics and Gynecology, New York University https://orcid.org/0000-0002-5445-0053
Fang Wang
Department of Obstetrics and Gynecology, New York University https://orcid.org/0000-0001-8752-8139
Fabiana B Kohlrausch
Department of Obstetrics and Gynecology, New York University https://orcid.org/0000-0001-5992-2905
Caroline M. Da Luz
University of Sao Paulo https://orcid.org/0000-0001-6733-8266
Fernanda V. Oliveira
University of Sao Paulo https://orcid.org/0000-0001-7534-0876
David L. Keefe
Department of Obstetrics and Gynecology, New York University https://orcid.org/0000-0001-6704-6343
Paula A. Navarro
University of Sao Paulo https://orcid.org/0000-0003-2368-4188

Research Article

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Abstract

Objective: Millions of babies have been conceived by IVF, yet debate about its safety to offspring continues. We hypothesized that superovulation and in vitro fertilization (IVF) promote genomic changes, including altered telomere length (TL) and activation of the retrotransposon LINE-1 (L1), and tested this hypothesis in a mouse model.

Material and methods: Experimental study analyzing TL and L1 copy number in C57BL/6J mouse blastocysts in vivo produced from natural mating cycles (N), in vivo produced following superovulation (S), or in vitro produced following superovulation (IVF). We also examined the effects of prolonged culture on TL and L1 copy number in the IVF group comparing blastocysts cultured 96 hours versus blastocysts cultured 120 hours. TL and L1 copy number were measured by Real Time PCR.

Results: TL in S (n=77; Mean: 1.50± 1.15; p=0.0007) and IVF (n=82; Mean: 1.72± 1.44; p<0.0001) exceeded that in N (n=16; Mean: 0.61± 0.27). TL of blastocysts cultured 120 hours (n=15, Mean: 2.14± 1.05) was significantly longer than that of embryos cultured for 96 hours (n=67, Mean: 1.63 ± 1.50; p=0.0414). L1 copy number of blastocysts cultured for 120 hours (n=15, Mean: 1.71± 1.49) exceeded that of embryos cultured for 96 hours (n=67, Mean: 0.95 ± 1.03; p=0.0162).

Conclusions: Intriguingly ovarian stimulation, alone or followed by IVF, produced embryos with significantly longer telomeres compared to in vivo, natural cycle-produced embryos. The significance of this enriched telomere endowment for the health and longevity of offspring born from IVF merit future studies.

Introduction

Telomeres are non-coding, repetitive DNA sequences of TTAGGG located at the termini of linear chromosomes [1]. It protects the end of chromosomes by forming single strands that invade the telomeric double helix to create loops, called t-loops. Together with associated proteins, telomeres prevent chromosome ends from being recognized as double-stranded DNA breaks, thus maintaining chromosomal stability [2]. With each cell division, telomere length (TL) shortens due to the inability of the DNA polymerase enzyme to complete synthesis if the lagging strand at chromosomal ends [3]. When cells divide, they cannot replicate approximately 50 base pairs of the lagging DNA strand at chromosome ends [4]. In most cells, this limits the number of divisions that a cell can tolerate because when telomeres reach a critical size, senescence and programmed cell death occur [5].

After fertilization, TL is reset in mice and humans. Mice oocyte telomere length is surprisingly short but significantly lengthens between the zygote and 2-cell stages [6]; despite the minimal telomerase activity, the enzyme capable of reconstituting telomere repeats [6, 7]. Maximum telomere shortening occurs in utero and during the first year of life (presumably due to the rapid cell division), then slows in adultness [7]. The telomere attrition rate is faster in males because females have higher levels of estrogen (17 b-estradiol), which binds to the promoter for the gene encoding TERT, the catalytic subunit of telomerase.

Estrogen also induces manganese superoxide dismutase MnSOD, an enzyme that scavenges free radicals [7], which shorten telomeres even in non-dividing cells.

The rate of telomere shortening and also the accumulation of short telomeres with age vary among species. TL in humans starts with 10–15 kb at birth and shorten about 70 bp each year. TL in Mus musculus C57BL6 is 40–50 kb at birth and decreases 100 times faster than TL in humans [8]. In some cells, telomerase adds telomeric tandem repeats during DNA replication [9]. In mice, telomerase is activated, and telomeres lengthen in the inner cell mass (ICM) of the blastocyst [8]. In vitro expansion of mouse embryonic stem (ES) cells made out from the ICM results in additional telomere lengthening, doubling the TL from its length in the ICM. This process is associated with the loss of heterochromatic marks [8]. This telomere lengthening occurs in the absence of variations in the telomerase gene [8].

Telomeres reconstitute during early development even in parthenotes, consistent with sperm telomere-independent elongation [10, 11]. Telomeres also elongate in embryonic stem cells produced by somatic cell nuclear transfer [11] and induced pluripotent stem cells [12]. Even telomerase null mice elongate telomeres after activation [6]. Alternative Lengthening of Telomeres (ALT) [13], a recombination based-mechanism of telomere elongation previously observed only in cancer cells [14], contributes to telomere elongation in preimplantation embryos [12]. Markers of ALT, including sister chromatid exchange and nuclear localization of proteins involved in double-strand DNA break repair, are found in cleavage but not blastocyst stages of development [15]. Thus, telomeres can elongate independently of telomerase and sperm but dependent on a double strand DNA-based mechanism involving telomere sister chromatid exchange (T-SCE) [12].

Telomere attrition in oocytes contributes to abnormal meiotic spindles [15] arrested, and fragmented embryos [16], decreased chiasmata and synapsis [17], and infertility [18]. At the molecular level, critically short telomeres trigger a DNA damage response that leads to senescence or apoptosis [19, 20].

Accumulation of critically short telomeres leads to aging and aging-associated diseases in mice and humans [18, 21–25]. Telomere shortening from cell division and/or oxidative damage compromises chromosome end protection [26]. Impaired telomere function triggers DNA damage response and activation of the tumor suppressor protein p53 [26]. P53 plays a critical role in enforcing senescence and apoptotic responses to dysfunctional telomeres [26]. Loss of p53 creates a permissive environment in which critically short telomeres are inappropriately joined to generate end-to-end chromosomal fusions [26]. These fused chromosomes result in cycles of chromosome fusion-bridge-breakage, which can fuel cancer initiation, especially in epithelial tissues, by facilitating changes in gene copy number [26].

DNA damage caused by telomere dysfunction also induces transcription of transposable elements, which, in turn, can cause additional damage by producing active transposases and new insertion events [27]. The mammalian genome hosts several types of transposable elements, which represent up to 40% of the genome and can propagate and insert into new locations in the genome. Long interspersed element 1 – LINE-1 (L1), is the only autonomous, active element in humans where it comprises 17% of the human genome [28]. In a study with hamster ovaries, non-functional telomeres serve as a substrate

for the retrotransposition of L1, independent of the endonuclease. This pathway appears to be linked to L1 endonuclease loss. Transposition to telomeres is increased in cells that express a dominant negative allele (TRF2) that disrupts TL [29].

Because both TL and L1 are exquisitely sensitive to environmental perturbation and essential for early murine embryogenesis [30, 31] we questioned whether superovulation or in vitro fertilization (IVF) could alter TL and/or activation of L1 in mouse blastocysts. We compared L1 copy number and TL among C57BL/6J mice blastocysts produced in vivo from natural mating cycles (N), in vivo following superovulation (S), or in vitro following superovulation (IVF). We also examined the effects of prolonged culture on TL and L1 in the IVF group.

Material And Methods

Mouse strain and animal handling

C57BL/6J female mice (N=50) at 8 weeks of age and C57BL/6J males (N=20) with confirmed fertility [32] were maintained in the Animal Care Facility of the University of São Paulo before experiments. During this period, the animals were kept on a 12-hour light-dark cycle, controlled temperature, with water and feed *ad libitum*, following animal care guidelines.

Superovulation protocol

For superovulation, mice were injected intra-peritoneally with 5 IU of equine chorionic gonadotropin (eCG; Novormon - Syntex SA - Buenos Aires, Argentina) followed 48 hours later by 5 IU of human chorionic gonadotropin (hCG; Chrorulon - Syntex SA, Buenos Aires, Argentina).

Experimental design

To investigate effects of superovulation and in vitro fertilization (IVF) on TL and L1 copy number of mice blastocysts, three groups were studied: 1) Natural (N) group: To eliminate the effect of ovarian stimulation and in vitro fertilization on outcomes, blastocysts were produced in vivo from females in the natural cycle (N); 2) Stimulated (S) group: Blastocysts produced in vivo after ovarian stimulation (S); and 3) IVF group: Blastocysts produced in vitro from stimulated cycles using fresh oocytes (IVF) - (Figure 1, Table 1). All IVF procedures were performed in the Experimental Laboratory of the Human Reproduction Sector of the Clinical Hospital of the Ribeirao Preto Medical School, University of São Paulo (HCFMRP/USP) and the PCR experiments were performed at Department of Obstetrics and Gynecology, New York University, Langone Medical Center, New York, NY, United States.

Table 1
Descriptive data of embryo morphology at the time of fixation:

GROUP	N embryos	RECOVERY TIME	MORPHOLOGY
Natural (N)	16	Approximately 74h	H, E, B1, B1, B1, B1, B1, E, E, E, E, E, E, B1, B1, B1
Superovulation (S)	77	Approximately 74h	E, E, E, B1, B1, E, E, E, E, H, B1, B1, B1, B1, B1, B1, E, B1, E, E, E, E, E, E, E, E, E, B1, B1, B1, B1, B1, E, E, E, E, E, E, E, E, E, E, E, E, E, B1, B1, B1, B1, B1, E, E, H, H, H, H, H, H, H, B1, B1, B1, B1, B1, B1, B1, B1, B1, B1, E, E, E, E, E, E, E, E
Fresh IVF group (IVF)	82	96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 120h, 120h, 120h, 120h, 120h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 120h, 120h, 120h, 120h, 120h, 120h, 96h, 96h, 96h, 96h, 96h, 120h, 120h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 120h, 120h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h, 96h.	E, E, B1, B1, B1, H, H, B1, H, E, E, B1, B1, H, H, H, HT, H, HT, H, H, H, H, E, H, H, H, H, E, H, E, E, E, B1, B1, H, H, H, H, E, E, E, H, H, HT, E, H, H, H, E, B1, B1, H, H, E, H, H, H, H, B1, B1, B1, H, H, H, H, E, B1, H, H, H, E, E, H, H, H, B1, B1, B1, B1, B1, B1.

Note: N: number, B1 is a inital blastocyst, E is the expanded blastocyst with thin zona pellucida ,trophectoderm and internal cell mass well defined; H: is the hatching blastocyst that is expanded with initial eclosion. HT: is the blastocyst completely ecloded with no zona pellucida. 74h is an estimate of embryo development time and collection since we are not able to know exactly the mating time in the in vivo groups (N and S). The classification was performed under a Nikon SMZ 745 stereomicroscope with 7,5x zoom.

Natural mating group

Male mice with confirmed fertility were mated overnight with one female (estrous positive) and vaginal plugs were recorded at 0.5-day post-coitum. Euthanasia was performed by cervical dislocation and blastocysts were collected by uterus flushing approximately 74 hours later using Embryomax M2 medium (Millipore sigma). Blastocysts were washed 3 times in HTF homemade medium and fixed in 2ul PVP/PBS and kept at -80^{o} C until analysis.

Ovarian stimulation and in-vivo or in-vitro fertilization

Female mice were injected intra-peritoneally with 5 IU of equine chorionic gonadotropin (eCG; Novormon - Syntex SA - Buenos Aires, Argentina) followed by 5 IU of human chorionic gonadotropin (hCG; Chrorulon - Syntex SA, Buenos Aires, Argentina), 48 hours later. Male mice with confirmed fertility were mated

overnight with one female and euthanasia for blastocysts collection and fixation were performed as described above in natural mating group. To investigate the effect of S plus in vitro fertilization (IVF group), epididymis were isolated, washed in M2 medium and punctured, and spermatozoa were capacitated for 1-2 h at 37° C, 5% CO2 in HTF homemade medium. Simultaneously, cumulus-oocyte cell complexes (COCs) were liberated from the oviducts of superovulated females using M2 medium and immediately transferred to other plate with pre-equilibrated HTF homemade medium covered by mineral oil (Irvine Scientific) at 37° C, 5% CO2. COCs were denuded with Hyaluronidase enzyme (Irvine Scientific). Denuded oocytes (n=936; 10 replicates) were submitted to Partial zone dissection (PZD) as previously described by Nakagata et al. [33]. After capacitation, oocytes were inseminated with spermatozoa for 3 h at 37° C, 5% CO2 in CARD MEDIUM (homemade HTF plus reduced glutathione, GSH - Sigma 4251). Oocytes were washed in HTF homemade medium and cultivated for 96 hours and 120 hours in continuous culture media (KSOM, Cosmo bio co., LTD), incubated at 37^{o} C with 5% CO_2. Blastocysts were fixed and stored as previously described.

Telomere length and LINE-1 copy number assay

Blastocyst genomic DNA was obtained by adding 2 µL 2x Lysis buffer ((100 ul mM trisHCL ph: 7.4; 300mM NaCl; 0.8 mM EDTA, 2% NP-40; 5mM dithiothreitol (DTT)) to a PCR micro tube and heated at 75°C for 10 min in the “cool down step” protocol for room temperature for 5 minutes. A single-cell TL assay (SC- qPCR) [34] was used to measure TL and L1 copy number in mouse blastocysts. A key feature of this assay is a pre-amplification step, performed before quantitative polymerase chain reaction (qPCR). Primers were synthesized by Integrated DNA Technologies according to the assay described below.

Briefly, pre-PCR was performed using DNA Polymerase Hot Start Version (TAKARA). The reactions were set up by aliquoting 21 µL of master mix into the PCR microtubes containing 2 µl of mouse blastocyst genomic DNA. Each reaction was set up with 2.5 µL 10x PCR buffer, 2.5 µL 2.5 mM dNTP, 0.16 µL DNA polymerase, 0.5 µL each of telomere forward and reverse primer (10 µM, TeloF: 5' CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT 3'; TeloR: 5' GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT 3'), 0.5 µL each of reference genes forward and reverse primer (10 µm, B1F: 5' GCACCTTTAATCCCAGCAC 3 ' e B1R: 5' TGAGACAGGGTTTCTCTGTA 3'), L1-Orf1 forward and reverse primer (10 µM, L1F: 5' GAACCAAGACCACTCACCATCA 3'; 10 µM L1R: 5' CCCTGGACTGGGCGAAGT 3’), 0.5 ul of each 5s rDNA forward and reverse primer (10 µM, 5sF: 5' GATCTCGGAAGCTAAGC 3; 10 µM, 5sR: 5' TACAGCACCCGGTATT 3) and water to a 21 µL final volume. Thermal cycler reaction conditions were set at 94°C for 5 min followed by 15 cycles of 94°C for 30 s, 60°C annealing for 30 s and extension at 72°C for 30 s, with a final extension for 10 min at 72°C. PCR products were purified following the protocol of the purification kit (Agencourt AMPure XP beads) [35] and were eluted in 100 µL of molecular grade water.

Finally, the purified products from each blastocyst were aliquoted into each well of a 96-well plate to perform the quantitative real-time PCR. Each reaction included 10 µL 2× SYBR Green mix (Bio-Rad), 1 µL each of 10 µM forward and reverse primers used in the pre-amplification step, 4 µL molecular grade water

and 5 µL genomic DNA to yield a 20-µL reaction. DNA samples were placed in a 96-well plate for telomere primers and reference gene primers, L1 primers and reference gene primers, respectively. A Bio-Rad thermocycler (CFX96) was used with reaction conditions of 95°C for 10 min followed by 40 cycles of data collection at 95°C for 15 s, 60°C annealing and extension for 1 minute). Each sample was run in triplicate along with a target-specific non-template control (NTC). Mouse embryonic stem cells were used as positive controls in each plate to assure concordance between plates. After thermal cycling and DNA amplification, CFX manager software was used to generate standard curves and Ct values for mouse telomere and reference gene (mB1) signals. For L1 copy number mouse 5srDNA was used as reference gene. To ensure high reproducibility of samples, only assays with real-time PCR efficiencies between 95 and 105% and intra-assay coefficient of variation (CoV) less than 1% were included in the analysis.

Statistical analysis

Following log transformation, analysis of variance with Tukey post-test compared TL and L1 among the 3 groups. Students t test compared TL and L1 between embryos cultured for 120 vs. 96 hours in the IVF group. P-value <0.05 was considered significant. Analyses were performed with SAS 9.4.

Results

In vitro fertilization and development of embryos derived from fresh oocytes (IVF group)

In the IVF group, 10 replicates produced a fertilization rate of 90.52% (95% CI: 85.19-95.85), blastocyst formation rate at 96 hours of 61.90% (95% CI: 52.62-71.19) and cumulative blastocyst rate (96 plus 120 hours) of 76.19% (CI: 68.04- 84.34).

Comparison of telomere length in mouse blastocysts from N, S and IVF group

TL of blastocysts from N group (n=16; Mean: 0.61± 0.27) was significantly shorter than S group (n=77; Mean: 1.50± 1.15; p=0.0007) and IVF group (n=82; Mean: 1.72± 1.44; p<0.0001). TL in S group (n=77; Mean: 1.50± 1.15) did not differ from the IVF group (n=82; Mean: 1.72± 1.44; p=0.3237; power of the test: 84%; Figure 2).

Comparison of L-1 copy number in mouse blastocysts from N, S and IVF group

L1 copy number of blastocysts from N group (n=16; Mean: 0.80± 0.31) did not differ from S (n=77; Mean: 1.23± 0.75; p=0.1386) and IVF group (n=82; Mean: 1.09± 1.16; p=0.6709, Figure 3).

Comparison of telomere length and L-1 copy number in mouse in vitro produced blastocysts from 96 hours and 120 hours of development

TL of blastocysts recovered at 96 hours (n=67, Mean: 1.63 ± 1.50) was significantly shorter than that of embryos recovered at 120 hours of embryo culture (n=15, Mean: 2.14± 1.05, p=0.0414, Figure 4A). L1 copy number of blastocysts recovered at 96 hours (n=67, Mean: 0.95 ± 1.03) was significantly lower from that of embryos recovered at 120 hours of embryo culture (n=15, Mean: 1.71± 1.49; p=0.0162, Figure 4B).

Discussion

This study shows that ovarian hyperstimulation, alone or followed by IVF, produced blastocysts with significantly longer telomeres than in vivo, natural cycle-produced embryos. In addition, telomere length and L1 copy number of in vitro produced embryos cultured for 120 hours exceeded those from embryos cultured for 96 hours.

To the best of our knowledge this is the first report of effects of ovarian hyperstimulation and IVF on genomic architecture during early development. Schaetzlein et. al. reported that TL is determined at the morula to blastocyst transition by a telomerase-dependent mechanism. Telomere elongation is restricted to this stage of development and restores telomeres of fibroblast-derived cloned bovine embryos to normal length [36]. Analyzing bovine preimplantation embryos they found a similar TL in morula derived from in vivo and in vitro fertilization suggesting that the in vitro culture per se had no significant effect on TL [36] in agreement with our findings.

In contrast, we report that ovarian hyperstimulation, alone or followed by IVF, produced embryos with significantly longer telomeres compared to in vivo, natural cycle-produced embryos. Ovarian hyperstimulation was the main factor responsible for these changes, since no statistical difference was found in TL between S and IVF group (p: 0.3237; power of the test: 84%). In addition, TL in blastocysts cultured for 120 hours was significantly longer than that of embryos cultured for 96 hours and L1 copy number of blastocysts cultured for 120 hours exceeded that of embryos cultured for 96 hours, demonstrating that extending in vitro embryo culture had an effect not only on TL but also on inducing an increase in L1 copy number.

Barbara McClintock's Nobel Prizing winning research previously identified activation of retrotransposons as a response to stress [37]. A derivative hypothesis is that stress from ovarian hyperstimulation [38–40] and/or embryo culture activates transcription of L1. Our findings that prolonged embryo culture increased L1 copy number are consistent with Professor McClintock's model. Intriguingly, however, embryo culture at earlier stages and ovarian hyperstimulation did not affect L1 copy number in mouse embryos, but did elongate telomeres. These two findings could be related. Recently we demonstrated a role for L1 in telomere elongation in preimplantation embryos [41]. Also, in cultured cells activation of retrotransposition leads to de novo L1 insertions at chromosome ends [42]. Activation of retrotransposition and integration of retroelements at telomeres has been reported in cells that survive mutations in telomere function [29, 43]. *Drosophila* use retrotransposons rather than telomeric repeats to protect chromosome ends [44]. *Drosophila* lack telomerase, but three telomere-specific retrotransposons

maintain chromosome ends [45]. Future studies should elucidate the mechanisms underlying telomere elongation induced by ovarian superovulation and the potential role of L1 in this effect.

Most in *vitro* experiments suggest that oxidative stress accelerates telomere shortening [46–48], but telomere elongation also has been reported. The intensity of the stress may determine its effect on TL [49]. Mishra et. al. reported disruption of normal telomere interactions, leading to loss of the looped chromosomal configuration [49]. In 112 infertile men, seminal ROS and 8-isoprostane levels were increased compared to controls. TL measured by real time PCR correlated positively with reactive oxygen species (ROS) levels, consistent with mild oxidative stress as a cause of telomere lengthening. Severe oxidative stress, on the other hand, shortened sperm TL.

Superovulation and in vitro fertilization had no significant effect on L1 copy number in our study, but extended embryo culture duration increased L1 copy number. Carmignac et. al., also found no effect of hyperstimulation on TE transcription around the preimplantation period, but in vitro culture increased TE expression at the blastocyst stage compared to in vivo development. By covering the transmission and mobilization of a transgenic L1 transposon, they also found that in vitro fertilization may alter the Mendelian rate of paternal heritage [50]. In vitro culture had an impact genome-wide on the embryo cultured in amino-acid-poor medium such as M16, showing an aggravated upregulation of TEs compared to amino acids rich medium (KSOM) [50]. Changes in expression levels from ovarian hyperstimulation also were observed in some L1 subfamilies (*L1 type f* and *L1 type tf*) at the blastocyst stage [50].

While low-dose hormone did not affect the L1 methylation levels, superovulated mouse females treated with high-dose hormone showed decreased L1 methylation levels in blastocysts [51], suggesting that superovulation may disturb the balance between methylation and demethylation on L1. The mechanisms involved in this process need further evaluation.

Millions of babies have been born following IVF, yet debate continues about its safety to offspring. We found genomic effects of ovarian stimulation and prolonged in vitro embryo culture in mice, including telomere elongation and L1 activation. Since both TL and TEs can modulate gene expression, and show unique regulatory dynamics during the preimplantation period [50], the consequences of ovarian hyperstimulation and IVF for embryonic gene expression merit further study. Future studies should not only validate our findings using other methodologies to examine TL and L1 activation, but also examine the effects of these changes on TL and TE activity on longevity and cancer risk in IVF offspring.

Declarations

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Availability of data and material (data transparency): The data underlying this study will be shared on reasonable request to the corresponding author.

Code availability (software application or custom code): Not applicable.

References

1 Iqbal K, Kues WA, Baulain U, Garrels W, Herrmann D, Niemann H (2011) Species-Specific Telomere Length Differences between Blastocyst Cell Compartments and Ectopic Telomere Extension in Early Bovine Embryos by Human Telomerase Reverse Transcriptase. Biol Reprod 84: 723–733. DOI: 10.1095/biolreprod.110.087205.

2 O'Sullivan RJ, Karlseder J. Telomeres: Protecting Chromosomes against Genome Instability (2010) Nat Rev Mol Cell Biol 11: 171–181. DOI: 10.1038/nrm2848.

3 Ozturk S, Sozen B, Demir N. Telomere Length and Telomerase Activity during Oocyte Maturation and Early Embryo Development in Mammalian Species Telomeres Includes (2014) Mol Hum Reprod 20: 15–30. DOI: 10.1093/molehr/gat055.

4 Vasilopoulos E, Fragkiadaki P, Kalliora C, Fragou D, Docea AO, Vakonaki E, Tsoukalas D, Calina D, Buga AM, Georgiadis G, Mamoulakis C, Makrigiannakis A, Spandidos DA, Tsatsakis A. The Association of Female and Male Infertility with Telomere Length (Review). (2019) Int J Mol Med 44: 375–389. DOI: 10.3892/ijmm.2019.4225.

5 Armstrong CA, Tomita K. Fundamental Mechanisms of Telomerase Action in Yeasts and Mammals: Understanding Telomeres and Telomerase in Cancer Cells. Open Biol 7. DOI: 10.1098/rsob.160338.

6 Liu L, Bailey SM, Okuka M, Muñoz P, Li C, Zhou L, Wu C, Czerwiec E, Sandler L, Seyfang A, Blasco MA, Keefe DL. Telomere Lengthening Early in Development (2007) Nat Cell Biol 9: 1436–1441. DOI: 10.1038/ncb1664.

7 Thilagavathi J, Venkatesh S, Dada R. Telomere Length in Reproduction (2013) Androl 45: 289–304. DOI: 10.1111/and.12008.

8 Varela E, Muñoz-Lorente MA, Tejera AM, Ortega S, Blasco MA. Generation of Mice with Longer and Better Preserved Telomeres in the Absence of Genetic Manipulations (2016) Nat Commun 7. DOI: 10.1038/ncomms11739.

9 Jiang S. Telomeres and Telomerase. Methods in Molecular Biology. 2017.

10 Liu L, DiGirolamo CM, Navarro PAAS, Blasco MA, Keefe DL. Telomerase Deficiency Impairs Differentiation of Mesenchymal Stem Cells (2004) Exp Cell Res 294: 1–8. DOI: 10.1016/j.yexcr.2003.10.031.

11 Huang J, Okuka M, Lu W, Tsibris JCM, McLean MP, Keefe DL, Liu L (2013) Telomere Shortening and DNA Damage of Embryonic Stem Cells Induced by Cigarette Smoke. Reprod Toxicol 35: 89–95. DOI: 10.1016/j.reprotox.2012.07.003.

12 Keefe DL (2020) Telomeres and Genomic Instability during Early Development. Eur J Med Genet 63. DOI: 10.1016/j.ejmg.2019.03.002.

13 Adjaye J, Daniels R, Bolton V, Monk M (1997) CDNA Libraries from Single Human Preimplantation Embryos. Genomics 46: 337–344. DOI: 10.1006/geno.1997.5117.

14 Draskovic I, Vallejo AL.] 1 (2013) Telomere Recombination and Alternative Telomere Lengthening Mechanisms. Front Biosc vol. 18. 2013. DOI: 10.2741/4084.

15 Liu L., Blasco M.A., Keefe D.L (2002) Requirement of Functional Telomeres for Metaphase Chromosome Alignments and Integrity of Meiotic Spindles. Eur Mol Biol Organ 3: 230–234. DOI: 10.1093/embo-reports/kvf055.

16 Keefe DL, Franco S, Liu L, Trimarchi J, Cao B, Weitzen S, Agarwal S, Blasco MA (2005) Telomere Length Predicts Embryo Fragmentation after in Vitro Fertilization in Women - Toward a Telomere Theory of Reproductive Aging in Women. In:. Am. J. Obstet. Gynecol., vol. 192. Mosby Inc 1256–1260. DOI: 10.1016/j.ajog.2005.01.036.

17 Liu L, Franco S, Spyropoulos B, Moens PB, Blasco MA, Keefe DL (2004) Irregular Telomeres Impair Meiotic Synapsis and Recombination in Mice. Proc Nat Ac Sc U.S.A 101(17), 6496–6501. DOI: 10.1073/pnas.0400755101.

18 Lee H-W, Blasco MA, Gottlieb GJ, Horner II JW, Greider CW, DePinho RA (1998). Essential Role of Mouse Telomerase in Highly Proliferative Organs. Nat. 392(6676) 569-574. DOI: 10.1038/33345.

19 Collado M, Blasco MA, Serrano M (2007) Cellular Senescence in Cancer and Aging. Cell. 130: 223–233. DOI: 10.1016/j.cell.2007.07.003.

20 Deng Y, Chan SS, Chang S (2008) Telomere Dysfunction and Tumour Suppression: The Senescence Connection. Nat. Rev. Cancer 8: 450–458. DOI: 10.1038/nrc2393.

21 Kong CM, Lee XW, Wang X (2013) Telomere Shortening in Human Diseases. FEBS J 280: 3180–3193. DOI: 10.1111/febs.12326.

22 Blasco MA, Lee H-W (1997) Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA, Cell 91(1) 25-34. DOI: 10.1016/S0092-8674(01)80006-4.

23 Armanios MY, J-L Chen J, Cogan JD, Alder JK, Ingersoll RG, Markin C, Lawson WE, Xie M, Vulto I, Phillips III JA, Lansdorp PM, Greider CW, Loyd JE (2007) Telomerase Mutations in Familieswith Idiopathic Pulmonary Fibrosis. N Engl J Med. 356: 1317–1326. DOI: 10.1056/NEJMoa066157.

24 Michael T. Hemann, Margaret A. Strong, Ling-Yang Hao, Carol W. Greider (2001) (The Shortest Telomere, Not Average Telomere Length, Is Critical for Cell Viability and Chromosome Stability. Cell 107(1) 67-77. DOI: 10.1016/S0092-8674(01)00504-9.

25 Yamaguchi H (2007) Mutations of Telomerase Complex Genes Linked to Bone Marrow Failures. J Nippon Med Sch 74: 202–209. DOI: 10.1272/jnms.74.202.

26 Artandi SE, Attardi LD (2005) Pathways Connecting Telomeres and P53 in Senescence, Apoptosis, and Cancer. Biochem. Biophys. Res Commun 331: 881–890. DOI: 10.1016/j.bbrc.2005.03.211.

27 De Cecco M, Criscione SW, Peckham EJ, Hillenmeyer S, Hamm EA, Manivannan J, Peterson AL, Kreiling JA, Neretti N, Sedivy JM (2013) Genomes of Replicatively Senescent Cells Undergo Global Epigenetic Changes Leading to Gene Silencing and Activation of Transposable Elements. Aging Cell 12: 247–256. DOI: 10.1111/acel.12047.

28 Belan E (2013) LINEs of Evidence: Noncanonical DNA Replication as an Epigenetic Determinant. Biol Dir 8:22. DOI: 10.1186/1745-6150-8-22.

29 Morrish TA, Garcia-Perez JL, Stamato TD, Taccioli GE, Sekiguchi JA, Moran J V (2007) Endonuclease-Independent LINE-1 Retrotransposition at Mammalian Telomeres. Nat 446 (7132): 208–212. DOI: 10.1038/nature05560.

30 Beraldi R, Pittoggi C, Sciamanna I, Mattei E, Spadafora C (2006) Expression of LINE-1 Retroposons Is Essential for Murine Preimplantation Development. Mol Reprod Dev 73: 279–287. DOI: 10.1002/mrd.20423.

31 Kohlrausch FB, Berteli TS, Wang F, Navarro PA, Keefe DL (2021) Control of LINE-1 Expression Maintains Genome Integrity in Germline and Early Embryo Development. Reprod Sci DOI: 10.1007/s43032-021-00461-1.

32 Dutta S, Sengupta P (2016) Men and Mice: Relating Their Ages. Life Sci 152: 244–248. DOI: 10.1016/j.lfs.2015.10.025.

33 Nakagata N, Okamoto M, Ueda O, Suzuki H (1997) Positive Effect of Partial Zona-Pellucida Dissection on the in Vitro Fertilizing Capacity of Cryopreserved C57BL/6J Transgenic Mouse Spermatozoa of Low Motility. Biol. Reprod. 57 (5): 1050–1055. DOI: 10.1095/biolreprod57.5.1050.

34 Wang F, Pan X, Kalmbach K, Seth-Smith ML, Ye X, Antumes DMF, Yin Y, Liu L, Keefe DL, Weissman SM (2013) Robust Measurement of Telomere Length in Single Cells. Proc. Natl. Acad. Sci. U. S. A. 110 (21). DOI: 10.1073/pnas.1306639110.

35 Cawthon RM (2009) Telomere Length Measurement by a Novel Monochrome Multiplex Quantitative PCR Method. Nucleic Acids Res. 37 (3): 1–7. DOI: 10.1093/nar/gkn1027.

36 Schaetzlein S, Lucas-Hahn A, Lemme E, Kues WA, Dorsch M, Manns MP, Niemann H, Rudolph KL (2004) Telomere Length Is Reset during Early Mammalian Embryogenesis. Proc Natl Acad Sci U. S. A. 101(21) 8034-8038. DOI: 10.1073/pnas.0402400101.

37 McClintock B (1950) The Origin and behavior of mutable loci in maize. Proc. Natl. Acad. Sci. U. S. A. 36: 334–355.

38 Lin E, Li Z, Huang Y, Ru G, He P (2021) High Dosages of Equine Chorionic Gonadotropin Exert Adverse Effects on the Developmental Competence of IVF-Derived Mouse Embryos and Cause Oxidative Stress-Induced Aneuploidy. Front. Cell Dev Biol 8: 1–19. DOI: 10.3389/fcell.2020.609290.

39 Chia-Hung Chou, Shee-Uan Chen, Chin-Der Chen, Chia-Tung Shun, Wen-Fen Wen, Yi-An Tu, Jehn-Hsiahn Yang (2020) Mitochondrial Dysfunction Induced by High Estradiol Concentrations in Endometrial Epithelial Cells. J. Clin. Endocrinol. Metab. 105: 126–135. DOI: 10.1210/clinem/dgz015.

40 De Melo AS, Rodrigues JK, Jordão AA, Ferriani RA, Navarro PA (2017) Oxidative Stress and Polycystic Ovary Syndrome: An Evaluation during Ovarian Stimulation for Intracytoplasmic Sperm Injection. Repr 153: 97–105. DOI: 10.1530/REP-16-0084.

41 Navarro P, Wang FH, Robinson LG, Pimentel R, Radjabi R, Kramer Y, Keefe D (2017) Zidovudine Inhibits Telomere Elongation, Increases the Transposable Element Line-1 Copy Number and Compromises Mouse Embryo Development. In:. Fertil. Steril., vol. 108. e154–e155. DOI: 10.1016/j.fertnstert.2017.07.466.

42 Servant G, Deininger PL (2016) Insertion of Retrotransposons at Chromosome Ends: Adaptive Response to Chromosome Maintenance. Front Genet 6: 1–14. DOI: 10.3389/fgene.2015.00358.

43 Scholes DT, Kenny AE, Gamache ER, Mou Z, Curcio MJ (2003) Activation of a LTR-Retrotransposon by Telomere Erosion. Proc. Natl. Acad. Sci. U. S. A. 100: 15736–15741. DOI:10.1073/pnas.2136609100.

44 Saint-Leandre B, Nguyen SC, Levine MT (2019) Diversification and Collapse of a Telomere Elongation Mechanism. Genome Res. 29: 920–931. DOI: 10.1101/gr.245001.118.

45 Pardue M Lou, DeBaryshe PG (2011) Retrotransposons That Maintain Chromosome Ends. Proc Natl Acad Sci U. S. A. 108 (51): 20317–20324. DOI: 10.1073/pnas.1100278108.

46 Von Zglinicki T (2002) Oxidative Stress Shortens Telomeres. Trends Biochem. Sci 27: 339–344. DOI: 10.1016/S0968-0004(02)02110-2.

47 Von Zglinicki T, Pilger R, Sitte N (2000) Accumulation of Single-Strand Breaks Is the Major Cause of Telomere Shortening in Human Fibroblasts. Free Radic Biol Med 28: 64–74. DOI: 10.1016/s0891-5849(99)00207-5.

48 Richter T, Zglinicki T von. A (2007) Continuous Correlation between Oxidative Stress and Telomere Shortening in Fibroblasts. Exp Gerontol 42 (11): 1039–1042. DOI: 10.1016/j.exger.2007.08.005.

49 Mishra S, Kumar R, Malhotra N, Singh N, Dada R (2016) Mild Oxidative Stress Is Beneficial for Sperm Telomere Length Maintenance. World J Methodol 6 (2): 163. DOI: 10.5662/wjm.v6.i2.163.

50 Carmignac V, Barberet J, Iranzo J, Quéré R, Guilleman M, Bourc'his D, Fauque P (2019) Effects of Assisted Reproductive Technologies on Transposon Regulation in the Mouse Pre-Implanted Embryo. Hum Reprod 34: 612–622. DOI:10.1093/humrep/dez020.

51 Liang X, Cui X, Sun S, Jin Y, Heo YT, Namgoong S, Kim N (2013) Superovulation Induces Defective Methylation in Line-1 Retrotransposon Elements in Blastocyst. 11:69. DOI: 10.1186/1477-7827-11-69.

Figures

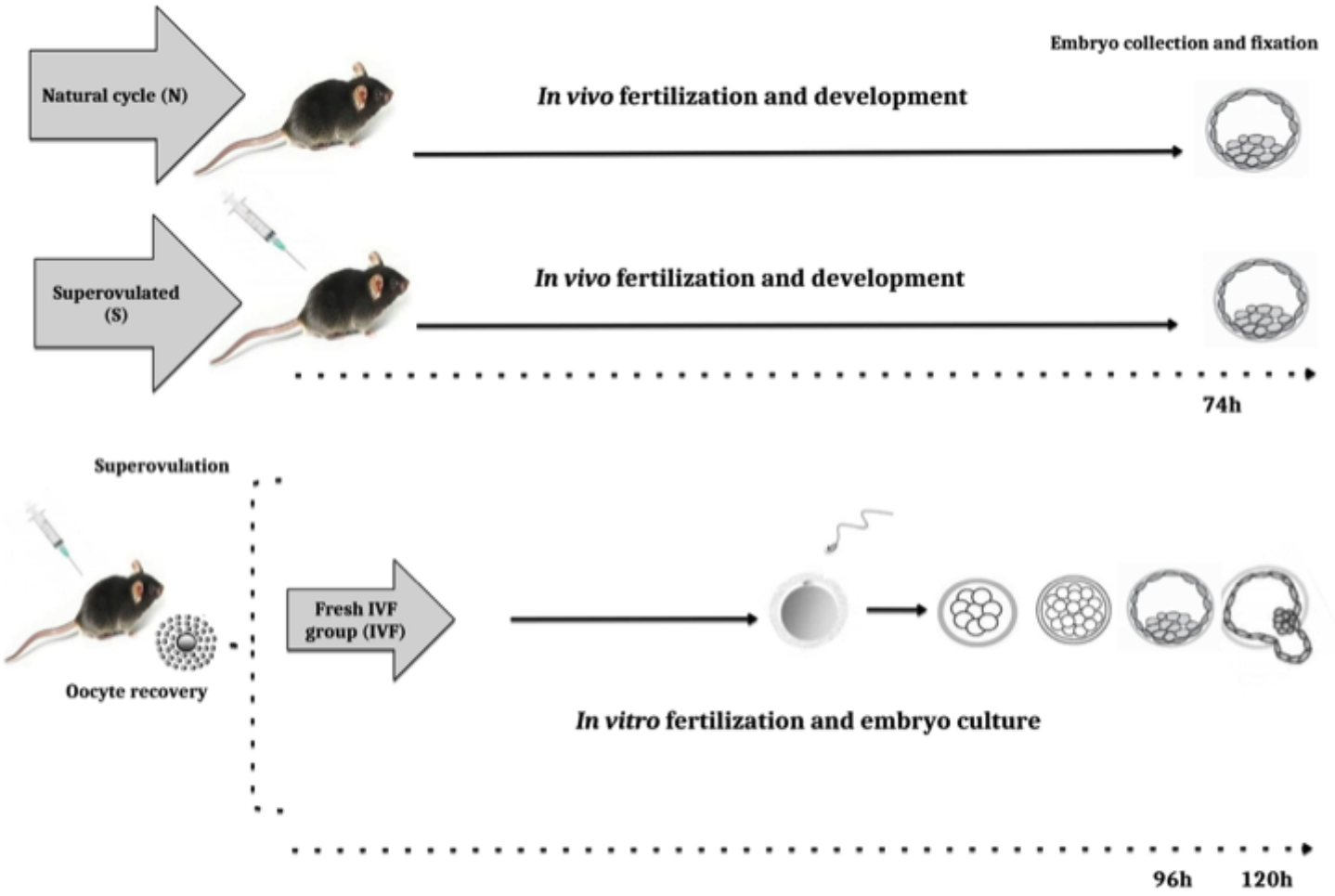

Figure 1

Experimental design: Three groups were defined related to their type of conception. Group N is the control, naturally conceived group. Group S represents the embryos that were derived from hormonally stimulated oocytes (eCG, hCG), and that had undergone natural fertilization and *in vivo* development. Group IVF is the i*n-vitro* fertilization group including superovulation plus collection of oocytes, IVF and embryo culture. Embryos were collected at blastocyst stage of development in all of three experimental groups. ***Note:*** 74h is an estimate of embryo development time and colletion since we are not able to know precisely the mating time in the in vivo groups (N and S).

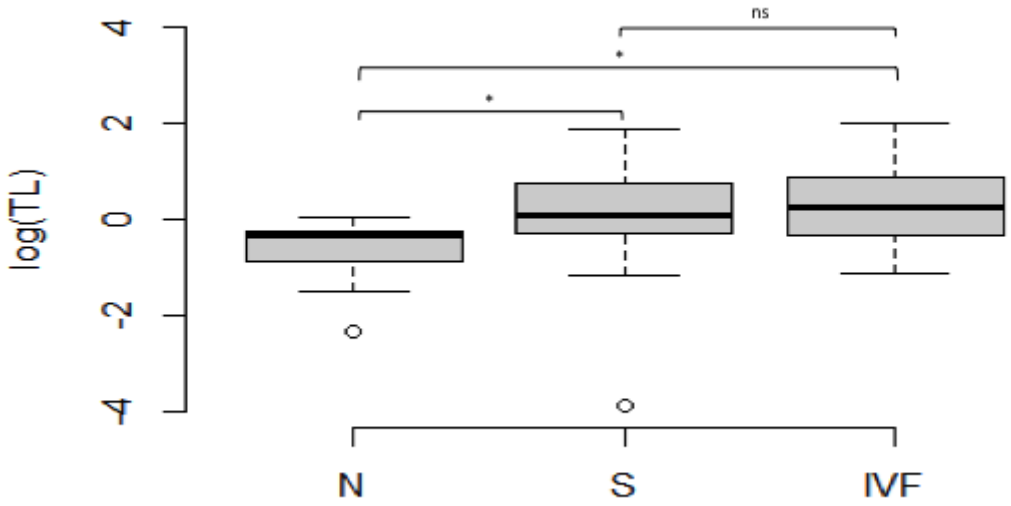

Figure 2

Logarithmic distribution of the TL in mouse blastocysts between Natural cycle (N), Superovulated (S) and, IVF group. *Note*: The groups (N, S and IVF) were compared statistically with each other *p < 0.05; ns indicate a non-significant difference; n is the number of blastocysts included in each group.

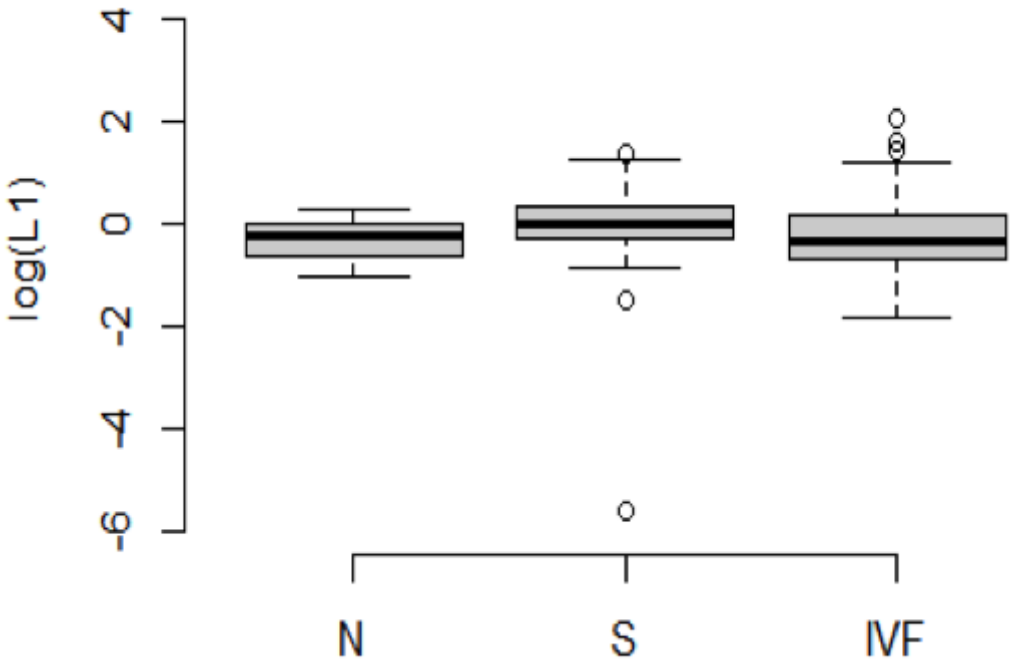

Figure 3

Logarithmic distribution of L1 copy number of mouse blastocysts between Natural cycle (N), Superovulated (S) and, IVF group. *Note*: The groups (N, S and IVF) were compared statistically with each other; n is the number of blastocysts included in each group.

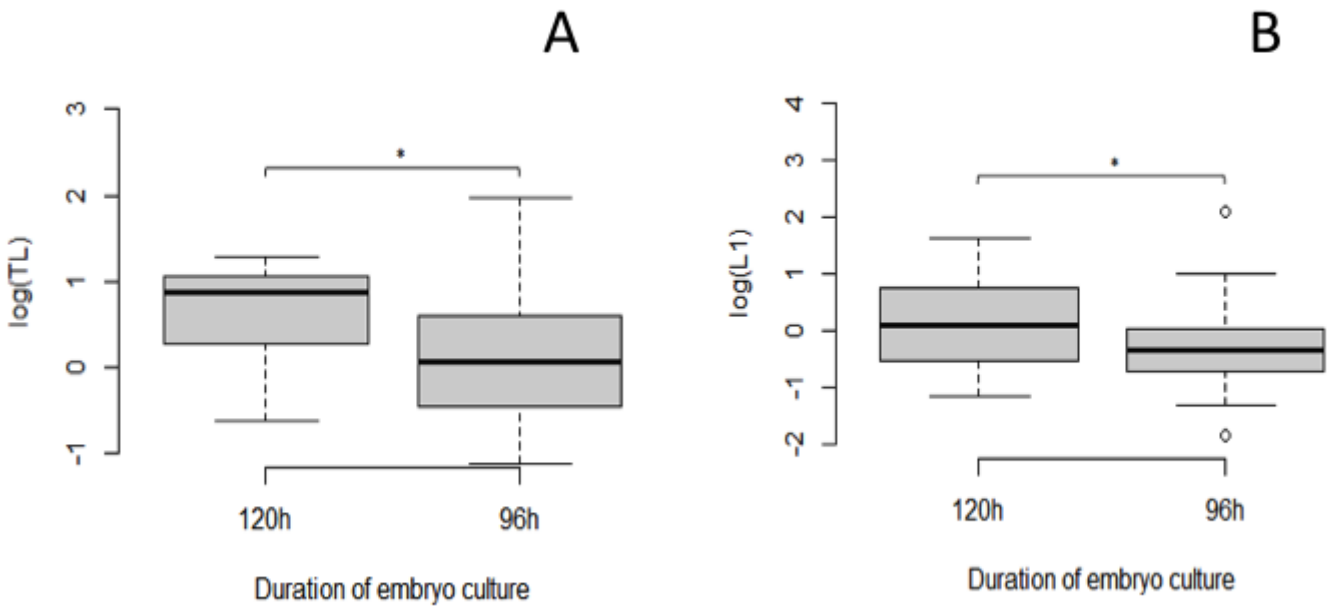

Figure 4

Logarithmic distribution of TL and L1 copy number in mouse *in vitro* produced blastocysts recovered at 96 or 120 hours of embryo culture in IVF group. *Note*: The groups (120 hours and 96 hours) were compared statistically with each other *$p < 0.05$; n is the number of blastocysts included in each group.