Electronic Supplementary Material (ESI) for Biomaterials Science.
This journal is © The Royal Society of Chemistry 2022

Supplementary Information

Melatonin loaded PLGA nanoparticles effectively ameliorate the in vitro maturation of deteriorated oocytes and the cryoprotective abilities during vitrification process.

Sujin Lee[a], *Hye Jin Kim*[a], *Hui Bang Cho*[a], *Hye-Ryoung Kim*[a], *Sujeong Lee*[a], *Ji-In Park*[a], *and Keun-Hong Park*[a*]

[a]Department of Biomedical Science, College of Life Science, CHA University, 6F, CHA Bio-Complex, 335 Pangyo-ro, Bundang-gu, Seongnam-si, 134-88, Republic of Korea

Corresponding Author:

***Keun-Hong Park, Ph.D.**

Professor

Department of Biomedical Science,

College of Life Science, CHA University, 6F,

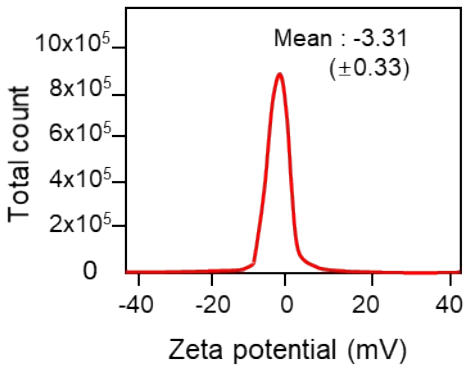
CHA Bio-complex, Sampyeong-Dong, Bundang-gu,

Seongnam-si, Republic of Korea

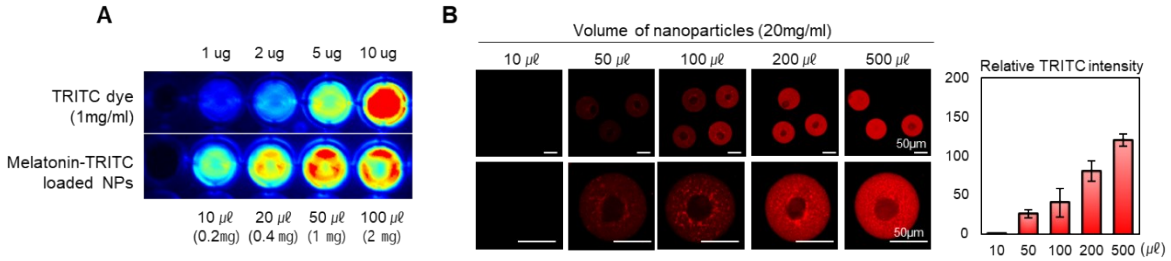
E-mail address: pkh0410@cha.ac.kr

Supplementary Figures 1-7

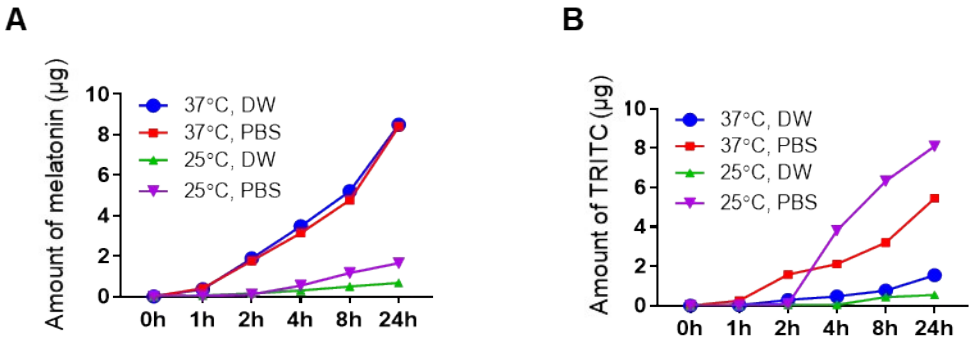
Supplementary Tables 1-2



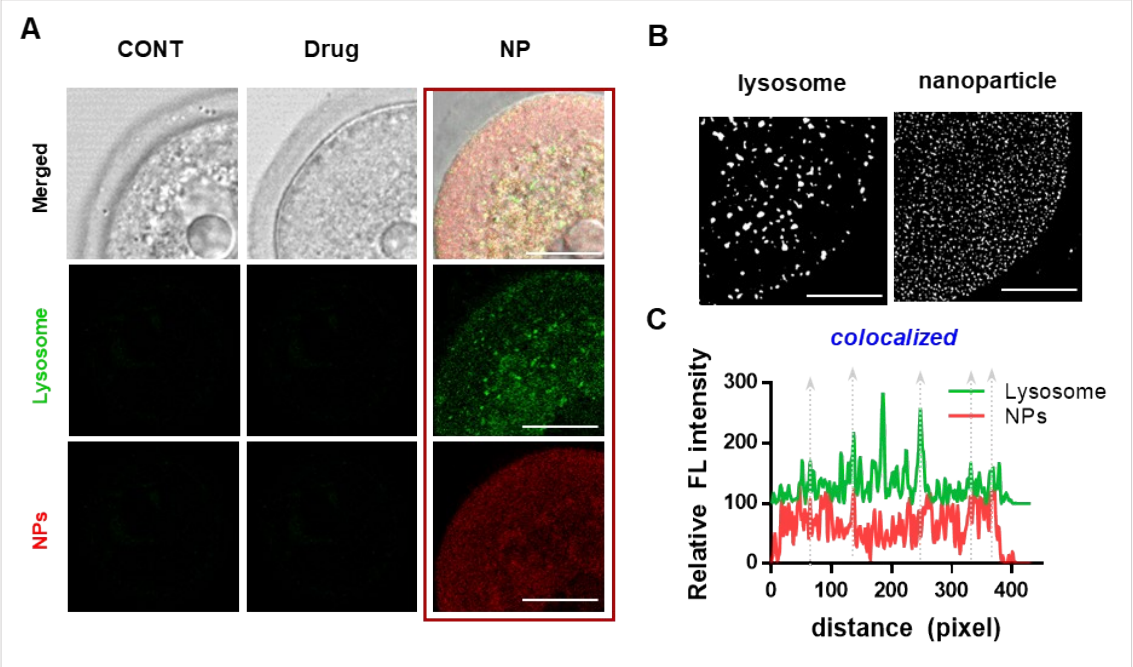
Supplementary Figure 1. Zeta potential of melatonin/TRITC-loaded PLGA NPs; related to Figure. 1 - Measurements were taken triplicate and the values represent the mean (± s.d.).



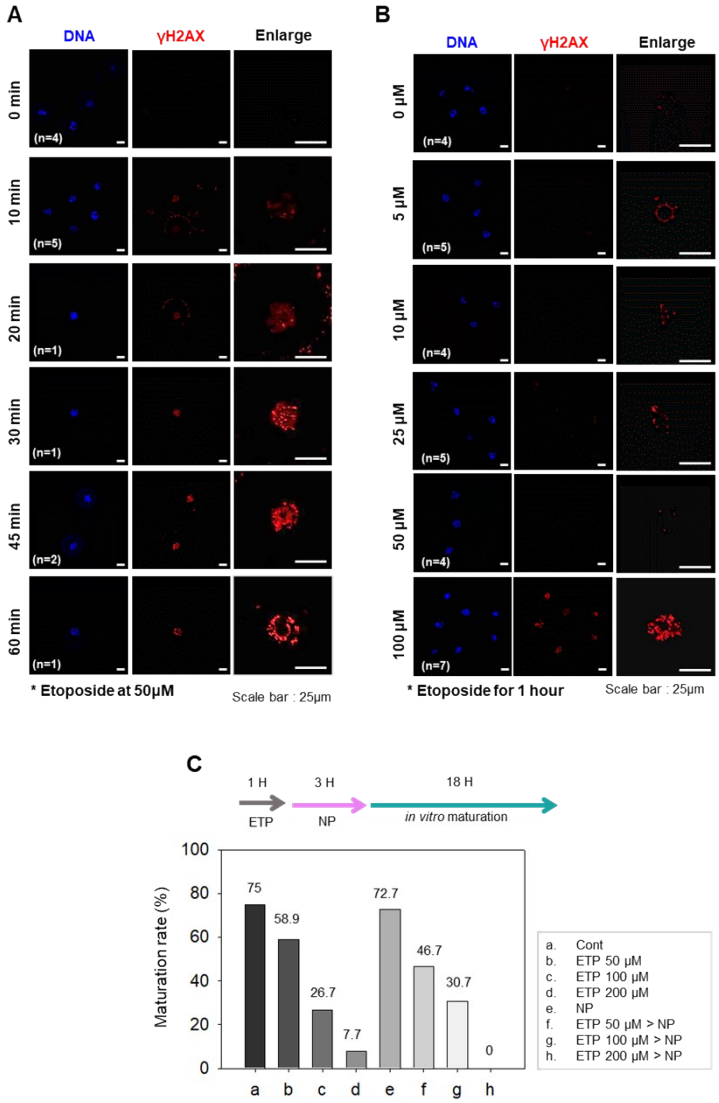
Supplementary Figure 2. Fluorescent properties of NPs and delivered them to oocyte; related to Figure. 1 – (A) Fluorescence intensity of TRITC dye and TRITC loaded in NPs were detected using Gel Doc system and images were obtained using Image Lab software. **(B)** Confirmation of delivered NPs using loaded tracer, TRITC and quantification of fluorescence intensity by treatment concentration. Measurements were taken after 3 h of NPs treatment and performed triplicates, bars represent the mean value ± s.d. Scale bar: 50 μm.



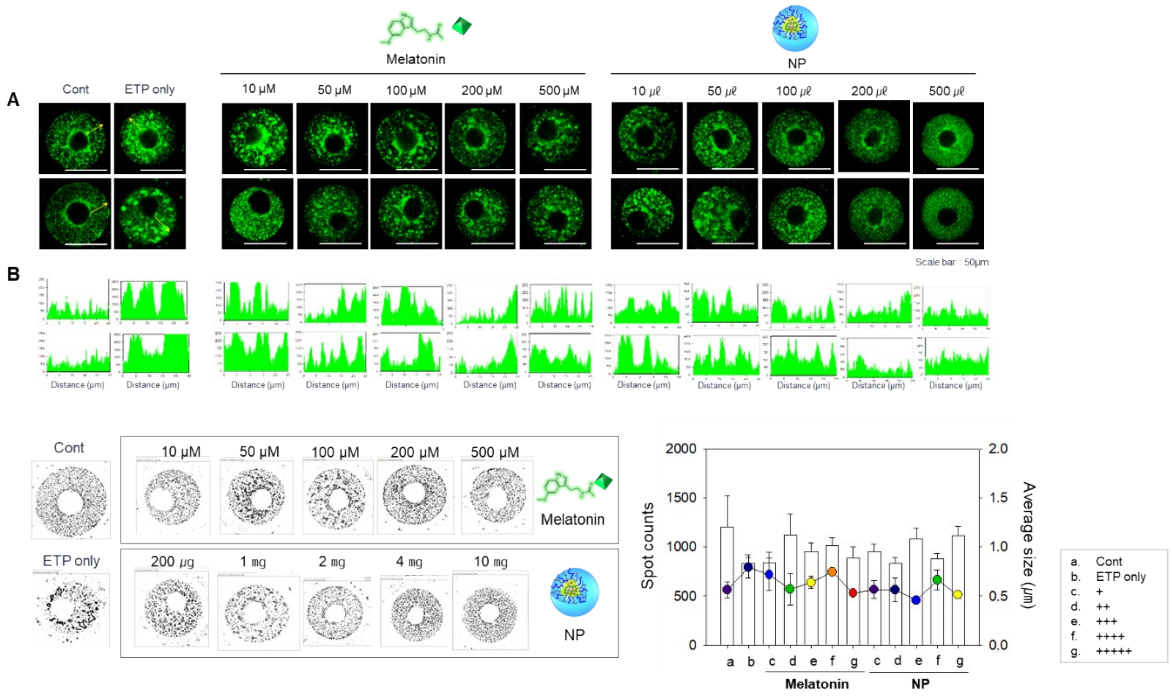
Supplementary Figure 3. Cumulative release profiles of melatonin and TRITC by NPs dispersed in PBS or DW at 25°C or 37°C; related to Figure. 1 - (A) Melatonin and **(B)** TRITC present in supernatant of NP dispersed solution were measured using microplate reader via absorbance and fluorescence, respectively. Each sample was measured n=3 replicate and symbols represent the mean value.



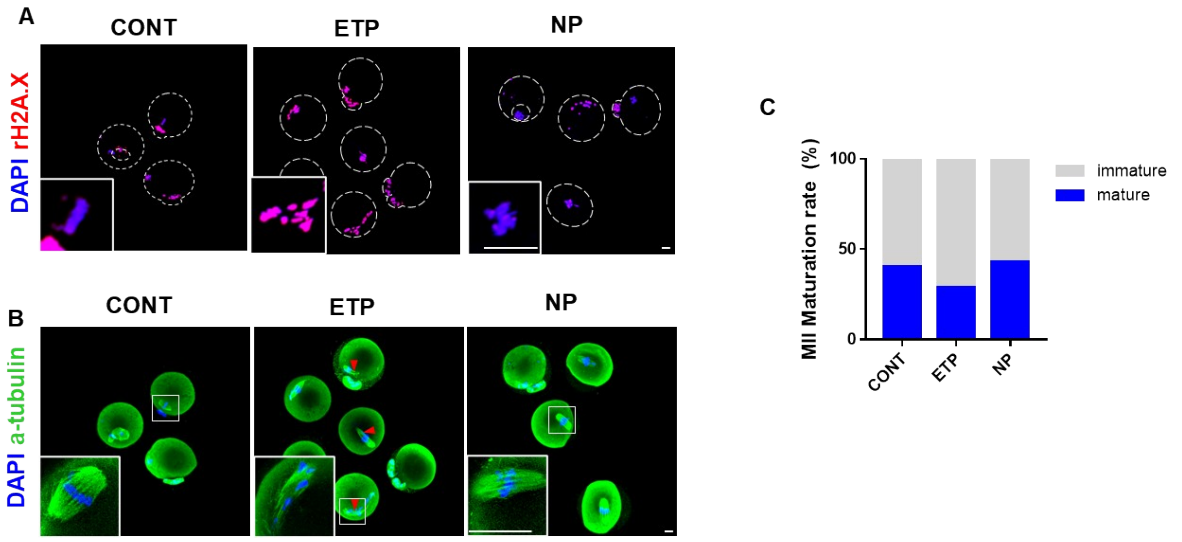
Supplementary Figure 4. Comparison of melatonin drug and nanoparticle-mediated delivery patterns; related to Figure. 1 – (A) CLSM images of oocytes after melatonin delivery. **(B)** Grayscale images of lysosome and nanoparticle in NP-treated oocyte. **(C)** Fluorescence intensity profiling of NP-treated oocytes. Gray arrow lines indicate that colocalized region of lysosome and NPs. Scale bar: 20 μm.



Supplementary Figure 5. Evaluation of oocyte DNA damage degree and maturation rate according to ETP concentration and treatment time; related to Figure. 2 - (A) Representative images after treatment ETP at 50 μM for various duration. **(B)** Representative images after treatment ETP at various concentration for 1 h. Oocytes were labeled with DAPI (blue) and γH2A.X (red, as DNA damage marker). Number(n) means the number of oocytes in the region of interest (ROI). Scale bar: 25 μm. **(C)** Maturation rate after ETP and or NP treatment then in vitro maturation. Maturation rate were calculated from the ratio of the total number to the number of MII oocytes. Measurements were performed duplicate and bars represent the mean value.



Supplementary Figure 6. Mitochondrial distribution of oocyte and quantitative analysis after various treatment; related to Figure. 3 - (A) CLSM image of oocyte mitochondria stained with mitotracker green. Scale bar: 50 μm. **(B)** Fluorescence profiling graphs along the direction indicated by the yellow arrow in Cont group in A. **(C)** Post-processing images of MitoTracker stained oocytes converted into particle form using ImageJ. **(D)** Bars represent the mean value of spot counts (number of mitochondria) and the symbols are mean value of average size (mitochondrial size) ± s.d.



Supplementary Figure 7. Assessment of the degree of damage of post-thaw oocyte after 1 h LN_2 storage; related to Figure. 5 – (A) Detection of DNA damage by γH2A.X level using CLSM. DAPI (blue) and rH2A.X (red). **(B)** Evaluation of spindle morphologies. α-tubulin (green), DAPI (blue) and severe defect of spindle (red arrowhead). **(C)** Assessment of maturation of post-thaw oocytes after in vitro maturation for 16-18 h. Scale bar: 20 μm.

		Number of oocytes (no.)			Percentage of oocytes (%)		
	Total number	GV	GVBD-MI	MII	GV	GVBD-MI	MII
Control	97	11	11	75	11.34	11.34	77.32
ETP only	96	25	42	29	26.04	43.75	30.21
Single drug treatment (DS)	88	26	26	36	29.55	29.55	40.91
Multiple drug treatment (DM)	97	16	24	57	16.49	24.74	58.76
NP	71	13	17	41	18.31	23.94	57.75

Supplementary Table 1. Number and percentage of oocytes at each maturation stage after *in vitro* maturation; related to Figure. 4 – Summary of the total number of oocytes used in the in vitro maturation evaluation experiment.

Figures	Comparisons	P value	P value summary	Method
Figure 2C(b)	Cont vs ETP	0.000812	***	Unpaired t test
	Cont vs DS	2.76E-05	***	Unpaired t test
	Cont vs DM	0.02825	*	Unpaired t test
	Cont vs NP	0.000251	***	Unpaired t test
	ETP vs DS	0.003458	**	Unpaired t test
	ETP vs DM	0.00191	**	Unpaired t test
	ETP vs NP	0.003062	**	Unpaired t test
Figure 2D(b)	Cont vs ETP	<0.0001	****	Unpaired t test
	Cont vs DS	0.0221	*	Unpaired t test
	Cont vs DM	0.0001	***	Unpaired t test
	Cont vs NP	0.0293	*	Unpaired t test
	ETP vs DS	<0.0001	****	Unpaired t test
	ETP vs DM	<0.0001	****	Unpaired t test
	ETP vs NP	<0.0001	****	Unpaired t test
Figure 3D	Cont vs ETP	0.2232	ns	Unpaired t test
	Cont vs DS	0.9073	ns	Unpaired t test
	Cont vs DM	0.1703	ns	Unpaired t test
	Cont vs NP	0.9937	ns	Unpaired t test
	ETP vs DS	0.39493	ns	Unpaired t test
	ETP vs DM	0.08639	ns	Unpaired t test
	ETP vs NP	0.17972	ns	Unpaired t test
Figure 3E	Cont vs ETP	0.319	ns	Unpaired t test
	Cont vs DS	0.0856	ns	Unpaired t test
	Cont vs DM	0.03182	*	Unpaired t test
	Cont vs NP	0.00077	***	Unpaired t test
	ETP vs DS	0.022	*	Unpaired t test
	ETP vs DM	0.00799	**	Unpaired t test
	ETP vs NP	0.00017	***	Unpaired t test
Figure 3F	Cont vs ETP	0.0936	ns	Unpaired t test
	Cont vs DS	0.08123	ns	Unpaired t test
	Cont vs DM	0.43343	ns	Unpaired t test
	Cont vs NP	0.38645	ns	Unpaired t test
	ETP vs DS	0.730922	ns	Unpaired t test
	ETP vs DM	0.20467	ns	Unpaired t test
	ETP vs NP	0.0433	ns	Unpaired t test
Figure 4C	Cont vs ETP	0.3203	ns	Unpaired t test
	Cont vs DS	0.0713	ns	Unpaired t test

	Cont vs DM	0.1402	ns	Unpaired t test
	Cont vs NP	0.1288	ns	Unpaired t test
	ETP vs DS	0.0632	ns	Unpaired t test
	ETP vs DM	0.1342	ns	Unpaired t test
	ETP vs NP	0.125	ns	Unpaired t test
Figure 4G(b)	Cont vs ETP	0.0496	*	Unpaired t test
	Cont vs DS	0.0162	*	Unpaired t test
	Cont vs DM	0.5345	ns	Unpaired t test
	Cont vs NP	0.3829	ns	Unpaired t test
	ETP vs DS	0.262	ns	Unpaired t test
	ETP vs DM	0.2128	ns	Unpaired t test
	ETP vs NP	0.3719	ns	Unpaired t test

Supplementary Table 2. Statistical methods and precise P values; related to Figure. 4 –

Summary of the p values and calculated statistical methods used to quantitative analysis.