Effects of Addition of Gonadotropin in the Media of Oocyte Maturationon the Embryo Cleavage

by Epy M. Luqman

Submission date: 20-Dec-2022 11:08AM (UTC+0800)
Submission ID: 1984823249
File name: f_Addition_of_Gonadotropin_in_the_Media_of_Oocyte_Maturation.pdf (496.3K)
Word count: 4861
Character count: 25470

Effects of Addition of Gonadotropin in the Media of Oocyte Maturation on the Embryo Cleavage

A. A. M. N. Kasman[1], B. Santoso[2], E. M. Luqman[3], Widjiati[4*]

1. Faculty of Health Science, Universitas Muhammadiyah Mataram. Mataram, Indonesia.
2. Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga. Surabaya, Indonesia.
3. Division of Veterinary Anatomy, Department of Veterinary Science, Universitas Airlangga. Surabaya, Indonesia.
4. Division of Veterinary Anatomy, Department of Veterinary Science, Universitas Airlangga. Surabaya, Indonesia.

Abstract

This research was performed to find the effects of addition of gonadotropin hormone in oocyte maturation in vitro on embryo cleavage. Gonadotropin hormone such as hMG 0,75 µg/ml and PMSG 15 IU were used in each oocyte maturation in vitro. Maturation media in this research was divided into 4 treatments such as Tc0, Tc+, Tc+hMG, and Tc+PMSG. Each treatment obtained 6 replicates. Oocyte maturation was carried out in each 100 µl TC 199 incubated for 24 hours. Staining of oocyte mature with aceto orcein and in vitro insemination were carried out in the middle of rosset. The research result showed that the average percentage of mature oocyte after maturation was 16.67% in the Tc0, 30% in the Tc+, 80% in the Tc+hMG, and 83.33% in the Tc+PMSG. Percentage of cleavage embryo was 5% for Tc0, 11.67% for Tc+, 43.33% for Tc+hMG, and 46.67% for Tc+PMSG. The result of correlation test on mature oocyte and cleavage was 0.932, the result of ANOVA test showed a significant difference, and the result of LSD test showed that there was difference between treatment groups of Tc0 and Tc+ compared to treatment group of Tc+hMG and Tc+PMSG, in both maturation and cleavage. This research concluded that the addition of gonadotropin obtained from hMg and PMSG into oocyte maturation media affected the increase in mature oocyte percentage and cleavage, there was no different result between the addition of hMG and PMSG on the percentage of mature oocyte and cleavage, and there was a correlation of mature oocyte on cleavage.

Experimental article (J Int Dent Med Res 2021; 14(1): 412-418)
Keywords: Gonadotropin, oocyte, in vitro maturation, cleavage.
Received date: 22 July 2020 **Accept date:** 29 September 2020

Introduction

The development of in vitro fertilization technique particularly relating to optimization of the use of reproductive organ as a source to obtain eggs and sperm cell has important role in improving embryo quality, which until today it is still being studied and developed. One related to in vitro fertilization is the process of maturation of oocytes in vitro which is one of stages in fertilization events to obtain quality embryo[1].

Oocyte maturation in vitro requires a media that is able to be functioned as a place to supply nutrient and at the same time also as a place to remove nutrient metabolite contained in the media needed for metabolism[2]. The addition of hormone in the maturation media is needed for the development of oocytes to improve oocytes quality so that oocytes potential for fertilization and embryonic development can be improved[3]. Imperfect completion in the process of maturation in vitro leads to disruption in oocytes growth that certainly affects embryo development. The addition of hormone in the media of oocyte maturation in vitro is meant to improve the oocyte quality so that the fertility rate will increase when fertilization is carried out. Hormonal factors are important for proliferation and differentiation that specifically interact among molecules, therefore the hormonal addition in maturation media allows oocyte to be more able to live in their environment for the purpose of embryonic development[4].

Hormone that is often added in the oocyte maturation media is gonadotropin hormone such

Corresponding author:
Widjiati
Departemen of Veterinary Anatomy
Faculty of Veterinary Medicine Universitas Airlangga Surabaya, Indonesia, 60115
E-mail: widjiati@fkh.unair.ac.id

as follicle stimulating hormone (FSH) and luteinizing hormone (LH) secreted by pituitary gland[5]. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are the main and primary gonadotropin hormone responsible for oocyte development[6]. The main signal transduction pathway is activated by FSH binding to membrane receptor in granulosa cell[7], meanwhile LH has important role in follicular maturation and ovulation induction, as well as androgen synthesis stimulation by theca cell[8]. However, other research states that the use of gonadotropin has not been able to increase maturation and potential of embryonic development, leads to disruption on embryo development, and the use of improper gonadotropin concentration leads to negative effects[9].

Today, gonadotropin has been used to treat infertility. Gonadotropin hormone has important role during oocyte development, so it is necessary to explore the effects of addition of gonadotropin hormone in oocyte maturation in vitro on embryonic development for the development of science in the field of assisted reproductive technology. This research facilitates the readers to understand the effects of addition gonadotropin hormone in oocyte maturation media in vitro on embryonic development.

Materials and methods

hMG Making

As much as 100 ml female urine was collected in the morning and centrifuged within 1.000 x g speed for 15 minutes with 4° C. This centrifugation aims to separate the metabolite cells from the discarded precipitate. The supernatant was filtered and put into an Erlenmeyer flask by utilizing filter paper. The procedures above were repeated until as much as 50 ml supernatant was collected. Next, the urine was used in the glycoprotein isolation examination procedure. It needed 200 µl urine sample, homogenized by adding PBST-PMSF 5 times in the urine sample. The supernatant was put into microtube and was sonicated for 10 minutes, processed in the vortex and centrifuged within 3.000 x g speed for 15 minutes. Cold absolute ethanol in a ratio of 1:1 was added, then it was incubated inside the refrigerator for 1 hour or overnight. The liquid was then centrifuged within 5000 x rpm speed for 15 minutes, the precipitate was dried to eliminate the smell of ethanol and was added with 20 mM tris-Cl buffer. After that protein isolate was obtained and SDS-PAGE examination was performed.

PMSG Making

The blood of 3.5 months pregnant local race horse was taken through jugular vein. The blood was left in room temperature for 3 hours, the edge of a tube in contact with blood was stabbed and left until 24 hours. It was then centrifuged for 15 minutes within 1000 x rpm speed to separate the serum part from the frozen blood. The supernatant was separated as a ready-to-use serum after went through the 0.2 µm millipore filter or can be stored in -20o C if not used.

Oocyte Collection

Oocyte is originated from local cow ovaries that had entered puberty for more than 18 months. The ovaries were cut in a slaughterhouse and brought hygienically into the laboratory by using thermos in 0,9 (w/v) NaCl containing penicillin G (75µg/ml) and streptomycin sulfate (50µg/ml) maintened at 35-37°C. Cows oocytes was aspired toward secondary follicle (antral) with 2-5mm section by using a 10 ml syringe of 18 G needles which contained 2 ml of oocyte wash medium.

In Vitro Maturation

Maturation was done in every 100 µl TC199 containing ten oocytes which covered with mineral oil (Sigma) in disposable 36 mm plastic petri dish (Nunclon, Denmark). The incubation lasted for 24 hours within 5 % CO_2 incubator with 38,5° C temperature, and 95-99% humidity (Thermoline, USA). The oocyte temperature used 18 G needles associated with 3-5 ml syringes with 500 - 1000 µl oocyte wash medium. Aspiration was done on secondary follicle (2-5 mm diameter). Each treatment was inserted with ten oocytes in treatment medium; Tc0, Tc+, Tc+hMG, and Tc+PMSG with at least six repetitions each. The hMG dosage was 0.75 µg/ml, while the PMSG dosage was 15 IU (10%).

Aceto Orcein Staining

The procedure of collecting egg maturity level data was done through aceto orcein *staining* solution 1%. The matured oocyte was placed in a glass object with a pipette that had been washed with 0.5% *hyaluronidase enzyme* beforehand to eliminate it from its cumulus cell and rewashed with oocyte wash medium. Next, it was fixed in a 90% acetic acid solution and 70% ethanol in a ratio of 1:3 for 2-3 days, then

covered with a lid supported with Vaseline at the edge of the glass object. The fixed oocyte was stained with aceto orcein solution 1% and left for 2-3 minutes and rinsed with 90% acetic acid, glycerin, and 70% ethanol in a ratio of 1:1:3. Then, the *mature* oocyte development was analyzed under the inverted microscope. Mature oocyte confirmation with chromosome identification reached the metaphase II stage and/or the appearance of polar body I.

In Vitro Fertilization

The sperms used were the local cows' sperms frozen in a container with liquid nitrogen. The sperm were thawed, washed, and centrifuged at 850 rpm for 10 minutes twice. The next step was swams up for 15-20 minutes, and around 30 – 40 µl from the surface of the samples were taken and transferred into the middle part on rosset medium. In the middle rosset medium, 100 µl EBSS associated with 5-6 drops 10 µl of treatment was put on its centripetally on 35 mm plastic petri dish. On the centripetal of rosset medium, there was oocyte that had been maturedin the incubator on each treatment medium.

Cleavage Observation

The cleavage observation under the inverted microscope was done after the oocytes were fertilized by spermatozoa for 48 hours in Earle's Balanced Salt Solution (EBSS) medium to see the correlation from the amount of matured oocyte.

Data Analysis

The data analysis used Analysis of Variance (ANOVA). If there was any significant difference, it was then followed with Least Significant Difference (LSD) test to determine the significantly different treatment value.

Results

Oocyte Maturation Level in Maturation Medium

Oocyte maturation level observation in treatment medium was done by using staining preparation with aceto orcein staining solution 1 %. Mature oocyte data was based on polar body I percentage which formed and/or reached the state of chromosome up to the metaphase II stage from nuclear meiosis cleavage as seen in table 1.

The statistical analysis using one-way ANOVA, it showed significant differences (P<0.05) regarding the addition of treatment on maturation medium on mature oocyte formation percentage. To determine the percentage of mature oocyte differences between treatment groups, LSD was conducted, the result was shown in table 3.

Medium	Replicates (n)	Mature oocytes (%)
Tc0	6	16,67 ± 8,16
Tc+	6	30 ± 10,95
Tc+hMG	6	80 ± 12,65
Tc+PMSG	6	83,33 ± 15,06

Table 1. Mean ± SD mature oocyte proportion in maturation medium for 24 hours.

Different value among treatment groups (LSD)	
Tc 0 vs Tc +	13,33
Tc 0 vs (Tc+hMG)	63,33*
Tc 0 vs (Tc+PMSG)	66,67*
Tc + vs (Tc+hMG)	50,00*
Tc + vs (Tc+PMSG)	53,33*
(Tc+hMG) vs (Tc+PMSG)	3,33

Table 2. Least Significant Difference (LSD) among treatment groups on the number of oocyte maturation.
LSD (0,5) = 14,42. Description: * significant difference (P<0.05).

The statistical analysis using one-way ANOVA and followed by LSD showed significant difference between Tc0 and Tc+ treatment group compared to Tc+hMG and Tc+PMSG treatment group on cleavage percentage.

Cleavage

The rate of embryo cleavage of each treatment in oocyte maturation media followed by in vitro fertilization can be seen in Table 3. To determine the effects of each treatment on maturation media on embryo cleavage, the data were transformed into the ANOVA table. The results can be seen in the Table 4. Embryo experiencing and not experiencing cleavage after maturation and fertilization can be seen in figure 3.

Based on statistical analysis using oneway ANOVA, it was showed that there was significant difference result, where (P<0.05) on the addition of hormone in maturation media to the percentage of cleavage. In order to find the difference in the percentage of cleavage among treatment groups, then LSD test was carried and the result was presented in table 5.

Maturation Media	Replicates (n)	Cleavage
Tc 0	6	5 ± 5,48
Tc +	6	11,67 ± 4,08
Tc + hMG	6	43,33 ± 5,16
Tc + PMSG	6	46,67 ± 8,16

Table 3. Proportion of Mean ±SD cleavage after spermatozoa insemination for 48 hours.

The result of statistical analysis using one-way ANOVA that was followed by LSD test showed a significant difference result in the treatment groups of Tc0 and Tc+ compared to the treatment groups of Tc+hMG and Tc+PMSG on the percentage of cleavage.

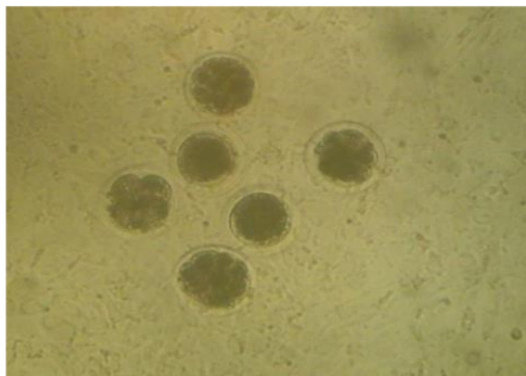

Figure 1. Embryo experiencing and not experiencing cleavage after maturation and fertilization with 100X magnification

	Sum of Squares	df	Mean Squares	Fcount	F-table 0,05
Beetween Groups	8233.33	3	2744.44	78.41 *	3,10
Within Groups	700.00	20	35.00		
Total	8933.33	23			

Table 4. Oneway ANOVA, the effect of treatment on cleavage.
Note: * there was a significant difference result where (P<0,05).

Different Value among treatment groups (LSD)	
Tc 0 vs Tc +	6,67
Tc 0 vs (Tc+hMG)	38,33*
Tc 0 vs (Tc+PMSG)	41,67*
Tc + vs (Tc+hMG)	31,67*
Tc + vs (Tc+PMSG)	35,00*
(Tc+hMG) vs (Tc+PMSG)	3,33

Table 5. LSD test among treatment groups on the cleavage.
LSD (0,5) = 7,13 Note: * there was significant difference result where (P<0,05).

Mature Oocyte and Cleavage Correlation.

To determine the correlation between mature oocyte and cleavage occurrence, hence, the data was transformed into ANOVA table and followed by statistical analysis using Pearson correlation, in which the result could be seen in table 1. Statistical analysis using Pearson correlation on table 6 showed strong correlation between maturation and cleavage.

	MATURE	CLEAVAGE
MATURE	1	.932(**)
CLEAVAGE	.932(**)	1

Table 6. Mature oocyte and cleavage correlation test.
Description: ** very significant difference (P<0.01).

Discussion

In vitro fertilization is a process of combining eggs and sperm cell to form a new individual outside the body of female mammalian. There are many factors affecting the success of in vitro fertilization, such as optimization media environment condition, oocyte treatment during in vitro fertilization, treatment of spermatozoa during washing and in vitro capacitation, as well as embryo breeding after fertilization. To have optimum media environment, the condition must be resembled in vivo environment and has mechanical, physical, and biochemical function ensuring oocyte and embryo sustainability in vitro.

Mammalian oocyte maturation process is characterized by a change in the morphology of the cell nucleus, a change from immature oocytes (diplotene phase) to the mature stage (metaphase II) so that it is ready to be fertilized. The results of this research showed that oocyte maturation in the maturation media with the addition of FSH and LH led to the increase in the percentage of mature oocytes and provided positive effects on cleavage after in vitro fertilization. This result was in line with the result of previous research that gonadotropin was needed for oocyte competence[10].

The maturation result and cleavage in the treatment group of Tc0 and Tc+ that was significantly difference compared to Tc+hMG dan Tc+PMSG might be caused by bioactivity ratio of FSH: LH contained in hMG and PMSG[11]. In addition, it also could be caused by the molecule structure of α and β subunit chain that was

different in hMg and PMSG[12] as a comparison in human with FSH and LH composition consisted from the same α subunit, which was 92 amino acid, while FSHβ subunit consisted of 110 amino acid and LHβ subunit consisted of 121 amino acid [13]. hMG treatment was not significantly different compared to PMSG on the mature oocyte percentage, it might be because bioactivity in FSH and LH was the same, even though their composition and molecule structure of α and β were different, so that biological response produced was still able to stimulate oocyte maturation.

Combination of the role of FSH and LH contained in hMG and PMSG supported classical theory of two cells, the theory of two gonadotropins during folliculogenesis-stereodogenesis stating that LH stimulated androgen production in theca cells, which was subsequently converted into estrogen by stimulating activity of FSH in granulosa cells [14]. There were many researchs have showed FSH ability to stimulate oocyte maturation in vitro using various dosage[15]. LH had role in facilitating the recruitment of folliculogenesis supported by several research [16]. LH has been widely considered as gonadotropin responsible for the onset of the return of meiosis[17]. Other research reported that the effect of LH added in the maturation media activated the binding of EGF and EGF receptor, in which it led to the activation of ERK1/2 required to not only facilitate oocyte to achieve metaphase II stage, but also form effective pronuclear[18]. However, only FSH was considered as gonadotropin responsible for inducing in vitro maturation on cumulus-wrapped oocyte [19]. This is the reason why only cumulus-wrapped oocyte that was able to reach mature stage in vitro, it was because FSH receptor was located in cumulus cell. The uneven uniformity of the cumulus wrapping oocyte at the time of collection from the follicle and continued in vitro maturation was one of the causes why there were oocytes that had not yet reached matured stage although the maturation media was added by gonadotropin[20]. Besides, it was also caused by condition of oocyte collected was not able to grow individually, so it could not survive in the culture media[21].

The addition of concentration of gonadotropin combination obtained from the result of extraction and fertilization of menopausal women in the maturation media was significantly able to increase the percentage of oocyte reaching metaphase II, normal configuration of syndrome, normal chromosome regulation, cortical granule migration, and mitochondrial aggregation[22]. PMSG is the most glycolysis pituitary glycoprotein group in mammalian placenta with both hormone subunits containing N linked and O linked glycolysis sides[23]. The high sialic acid content in PMSG greatly increased half-life compared to other glycoprotein hormones. This also can cause the maturation and cleavage rates produced by PMSG to be slightly higher compared to the media added by hMG, although it was not significant. PMSG also had luteinizing activity that was important for the optimal development of follicles in the pre-ovulatory phase[24]. Other research reported that the addition of gonadotropin from serum resulted in the high level maturation[25].

The results of this study indicate that the addition of FSH and LH in hMG and PMSG into oocyte maturation influences the embryo division. The addition of FSH and LH combination in maturation media by utilizing TCM-199 could increase in vitro fertilization rate and embryo division[26]. Supplementation with gonadotropin in maturation media could boost the capacity of oocyte development into embryonic phase, and play an essential role in oocyte competence into metaphase II, and does not prompt the escalation of a chromosome abnormality. Gonadotropin in maturation media acts as an autocrine regulator and paracrine which also involved in protein steroidogenesis and synthesis during embryonic development[27]. Oocyte without the addition of FSH and LH in maturation media results significantly fewer division rates and tend to experience worse development. The observation on oocyte rate that successfully split after the in vitro fertilization and incubation for 48 hours resulted in different divisions level, i.e., divisions were starting at two cells, four cells, and eight cells. The division rate diversity in this study is allegedly due to the different levels of oocyte maturation during fertilization. The low rate of embryo division in this study is probably due to the oocyte being fertilized in the early stages of maturation. Oocytes that are not fully mature could not split completely, or the process will be inhibited when fertilized. Perfect in vitro fertilization occurs when the division of oocyte core reached the metaphase II during

spermatozoa penetration[28]. The low cleavage rate hit in this study could be due to the low rate of fertilization that is influenced by spermatozoa mobility which must experience capacitation and acrosome reactions, and the media environment during in vitro fertilization and division[29].

The result of correlation test between matured oocyte and cleavage provided positive effects or strong correlation. It showed that the maturation results affected cleavage result, in other words, maturation result was directly proportional with cleavage result. This was in accordance with the result that the more perfect of the level of oocyte maturation, the more oocyte would be fertilized and divided, and the more embryo would grow to reach blastocyst stage. Other research also reported that the addition of FSH and LH hormone in the oocyte maturation media was significantly correlated with the increase in embryonic cleavage after in vitro fertilization [30]. This research concluded that the addition of PMSG and hMG hormones contained FSH-LH to maturation media can be used as alternative hormones for oocyte maturation in vitro. Oocyte competence reached maturity and the embryonic cleavage stage was stimulated by the coordination of FSH and LH action. This research emphasizes the complexity of gonadotropin transduction signal and its receptor during oocyte maturation in vitro so that it still needs further in-depth studies on gene expression levels related to ligand and receptor interactions as a result of addition of different types and concentrations of gonadotropins to oocyte competence.

Conclusions

The study was that the addition of gonadotropin obtained from hMg and PMSG into oocyte maturation media affected the increase in mature oocyte percentage and cleavage, there was no different result between the addition of hMG and PMSG on the percentage of mature oocyte and cleavage, and there was a correlation of mature oocyte on cleavage.

Acknowledgements

The researcher thanks to Faculty of Medicine of Universitas Airlangga that have been facilitated the implementation of this research.

Declaration of Interest

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

References

1. Ding N, Liu X, Jian Q, Liang Z, Wang F. Dual trigger of final oocyte maturation with a combination of GnRH agonist and hCG versus a hCG alone trigger in GnRH antagonist cycle for in vitro fertilization: A Systematic Review and Meta-analysis. Eur. J. Obstet. Gynecol. Reprod. Biol. 2017; 218: 92–98. DOI: 10.1016/j.ejogrb.2017.09.004
2. Hatırnaz Ş, Ata B, Hatırnaz ES, Dahan MH, Tannus S, Tan J, Tan SL. Oocyte in vitro maturation: A systematic review. Turk Jinekoloji ve Obstetrik Dernegi Dergisi. 2018; 15(2) 112–125. DOI: 10.4274/tjod.23911
3. Abbara A, Clarke SA, Dhillo WS. Novel concepts for inducing final oocyte maturation in in vitro fertilization treatment. Endocr. Rev. 2018; 39(5): 593–628. DOI: 10.1210/er.2017-00236
4. Luciano AM, Sirard MA. Successful in vitro maturation of oocytes: a matter of follicular differentiation. Biol. Reprod. 2018; 98(2): 162–169. DOI: 10.1093/biolre/iox149
5. Filatov M, Khramova Y, Parshina E, Bagaeva T, Semenova M. Influence of gonadotropins on ovarian follicle growth and development in vivo and in vitro. Zygote. 2017; 25(3): 235–243. DOI: 10.1017/S0967199417000168
6. Lu C-L, Wang T-R, Yan L-Y, Xia X, Zhu X-H, Li R, Zhao H-C, Yan J, Yin T-L, Jin H-Y, Zhang Y, Zhang W-X, Feng H-L, Qiao J. Gonadotropin-Mediated Dynamic Alterations During Bovine Oocyte Maturation In Vitro. Biol. Reprod. 2014; 91(2): 44-58. DOI: 10.1095/biolreprod.114.117945
7. Casarini L, Crépieux P. Molecular Mechanisms of Action of FSH. Front Endocrinol. 2019; 10: 305-310. DOI: 10.3389/fendo.2019.00305
8. Liu X, Qiao P, Jiang A, Jiang J, Han H, Wang L, Ren C. Paracrine regulation of steroidogenesis in theca cells by granulosa cells derived from mouse preantral follicles. BioMed Res. Int. 2015; 2015: 1-8. DOI: 10.1155/2015/925691
9. Edwards LJ, Kind KL, Armstrong DT, Thompson JG. Effects of recombinant human follicle-stimulating hormone on embryo development in mice. Am J Physiol Endocrinol Metab. 2005; 288(5): 845–851. DOI: 10.1152/ajpendo.00398.2004
10. Blaschka C, Sánchez-Guijo A, Zimmer B, Stöhr J, Kotarski F, Grothmann H, Hartmann MF, Wudy SA,Wrenzycki C. Temporal expression pattern of steroid-metabolizing enzymes in bovine COC during in vitro maturation employing different gonadotropin concentrations. Theriogenology. 2029; 131: 182–192. DOI: 10.1016/j.theriogenology.2019.03.028
11. Alvarez RH, Natal FLN, Ribela MTCP, De Almeida BE, De Oliveira JE, Bartolini P. Physical-chemical and biological characterization of different preparations of equine chorionic gonadotropin. J. Vet. Sci. 2016; 17(4): 459–465. DOI: 10.1016/j.theriogenology.2019.03.028
12. Murphy BD, Martinuk SD. Equine chorionic gonadotropin. Endocr. Rev. 1991; 12: 1 27–44.
13. Narayan P, Ulloa-Aguirre A, Dias JA. 2018. Gonadotropin Hormones and Their Receptors. Elsevier Inc. DOI: 10.1016/B978-1-4557-2758-2.00002-0
14. Short RV. Steroids in the follicular fluid and the corpus luteum of the mare. J Endocrinol. 1962; 24: 59–63. DOI: 10.1677/joe.0.0240059
15. Lee HS, Seo YI, Yin XJ, Cho SG, Lee SS, Kim NH, Cho SK, Kong IK. Effect of follicle stimulation hormone and luteinizing hormone on cumulus cell expansion and in vitro nuclear maturation of canine oocytes. Reprod. Domest. Anim. 2007; 42(6): 561–565. DOI: 10.1111/j.1439-0531.2006.00818.x

16. Mak SMJ, Wong WY, Chung HS, Chung PW, Kong GWS, Li TC, Cheung LP. Effect of mid-follicular phase recombinant LH versus urinary HCG supplementation in poor ovarian responders undergoing IVF – a prospective double-blinded randomized study. Reprod. Biomed. Online. 2017; 34(3): 258–266. DOI: 10.1016/j.rbmo.2016.11.014
17. Mattioli M, Bacci ML, Galeati G, Seren E. Effects of LH and FSH on the maturation of pig oocytes in vitro. Theriogenology. 1991; 36(1): 95–105. DOI: 10.1016/0093-691x(91)90438-j
18. Zhang YL, Liu XM, Ji SY, Sha QQ, Zhang J, Fan HY. ERK1/2 Activities Are Dispensable for Oocyte Growth but Are Required for Meiotic Maturation and Pronuclear Formation in Mouse. J. Med. Genet. 2015; 42(9): 477–485. DOI: 10.1016/j.jgg.2015.07.004
19. Samartzi F, Tsakmakidis I, Theodosiadou E, Vainas E. Effect of porcine and ovine FSH on nuclear maturation of pig oocytes in vitro. Reprod Domest Anim. 2008; 43(2):153–156. DOI: 10.1111/j.1439-0531.2007.00868.x
20. Virant-Klun I, Bauer C, Ståhlberg A, Kubista M, Skutella T. Human oocyte maturation in vitro is improved by co-culture with cumulus cells from mature oocytes. Reprod. Biomed. Online. 2018; 36(5): 508–523.DOI: 10.1016/j.rbmo.2018.01.011
21. Aziz NAA, Osman NA, Bidin H, Embong WK, Hashim NH. Influence of Early Apoptosis Incidence on In Vitro Maturation of Bovine Oocytes. APCBEE Procedia. 8, 2014; 272–276
22. Sha W, Xu BZ, Li M, Liu D, Feng HL, Sun QY. Effect of gonadotropins on oocyte maturation in vitro: an animal model. Fertil Steril. 2010; 93(5):1650–1661. DOI: 10.1016/j.fertnstert.2009.03.003
23. Barrios-De-Tomasi J, Timossi C, Merchant H, Quintanar A, Avalos JM, Andersen CY, Ulloa-Aguirre A. Assessment of the in vitro and in vivo biological activities of the human follicle-stimulating isohormones. Mol. Cell. Endocrinol. 2002; 186(2): 189–198. DOI: 10.1016/s0303-7207(01)00657-8
24. Ruman JI, Pollak S, Trousdale RK, Klein J, Lustbader JW. Effects of long-acting recombinant human follicle-stimulating hormone analogs containing N-linked glycosylation on murine folliculogenesis. Fertil Steril. 2005; 83(4): 1303–1309. DOI: 10.1016/j.fertnstert.2004.12.027
25. Puri G, Chaudhary SS, Singh VK, Sharma AK. Effects of fetal bovine serum and estrus buffalo serum on maturation of buffalo (Bubalus bubalis) oocytes in vitro. Vet World. 2015; 8(2):143–146. DOI: 10.14202/vetworld.2015.143-146
26. Wang X, Tsai T, Qiao J, Zhang Z, Feng H L. Impact of gonadotropins on oocyte maturation, fertilization and developmental competence in vitro. Reprod. Fertil. Dev. 2014; 26(5): 752–7. DOI: 10.1071/RD13024
27. Zhang M, Ouyang H, Xia G. The signal pathway of gonadotrophins-induced mammalian oocyte meiotic resumption. Mol. Hum. Reprod. 2009; 15(7): 399–409. DOI: 10.1093/molehr/gap031
28. Silva GM, Brito IR, Sales AD, Aguiar FLN, Duarte ABG, Araújo VR, Vieira LA, Magalhães-Padilha DM, Lima LF, Alves BG, Silveira LBR, Lo Turco EG, Rodrigues AP, Campello CC, Wheeler MB, Figueiredo JR. In vitro growth and maturation of isolated caprine preantral follicles: Influence of insulin and FSH concentration, culture dish, coculture, and oocyte size on meiotic resumption. Theriogenology; 90 (1): 32–41. DOI: 10.1016/j.theriogenology.2016.10.026
29. Loren P, Sánchez R, Arias ME, Felmer R, Risopatrón J, Cheuquemán C. Melatonin scavenger properties against oxidative and nitrosative stress: Impact on gamete handling and in vitro embryo production in humans and other mammals. Int. J. Mol. Sci. 2017; 18(6): 1–17. DOI: 10.3390/ijms18061119
30. Zhang X, Zerafa A, Wong J, Armstrong DT, Khamsi F. Human menopausal gonadotropin during in vitro maturation of human oocytes retrieved from small follicles enhances in vitro fertilization and cleavage rates. Fertil Steril. 1993; 59(4): 850–853. DOI: 10.1016/s0015-0282(16)55871-3

Effects of Addition of Gonadotropin in the Media of Oocyte Maturationon the Embryo Cleavage

ORIGINALITY REPORT

13%	11%	6%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

#	Source	%
1	Submitted to Universitas Airlangga Student Paper	3%
2	ir.unimas.my Internet Source	1%
3	www.cambridge.org Internet Source	1%
4	www.veterinaryworld.org Internet Source	1%
5	docshare.tips Internet Source	1%
6	biomedpharmajournal.org Internet Source	1%
7	"Development of In Vitro Maturation for Human Oocytes", Springer Science and Business Media LLC, 2017 Publication	1%
8	archive.org Internet Source	1%

9	pubmed.ncbi.nlm.nih.gov Internet Source	1 %
10	repository.unair.ac.id Internet Source	<1 %
11	webs.rmutl.ac.th Internet Source	<1 %
12	lrd.yahooapis.com Internet Source	<1 %
13	bioone.org Internet Source	<1 %
14	ro.uow.edu.au Internet Source	<1 %
15	publikasi.polije.ac.id Internet Source	<1 %
16	Liang Chi, Pengcheng Tu, Chih-wei Liu, Yunjia Lai, Jingchuan Xue, Hongyu Ru, Kun Lu. "Chronic Arsenic Exposure Induces Oxidative Stress and Perturbs Serum Lysolipids and Fecal Unsaturated Fatty Acid Metabolism", Chemical Research in Toxicology, 2019 Publication	<1 %
17	M. Mattioli, M.L. Bacci, G. Galeati, E. Seren. "Effects of LH and FSH on the maturation of pig oocytes in vitro", Theriogenology, 1991 Publication	<1 %

#	Source	Similarity
18	Oogenesis, 2013. Publication	<1 %
19	Principles and Practice of Controlled Ovarian Stimulation in ART, 2015. Publication	<1 %
20	e-journal.unair.ac.id Internet Source	<1 %
21	ir.lib.hiroshima-u.ac.jp Internet Source	<1 %
22	C. Grondahl. "Physiology of meiosis-activating sterol: endogenous formation and mode of action", Human Reproduction, 01/01/2003 Publication	<1 %
23	Maxim Filatov, Yulia Khramova, Elena Parshina, Tatiana Bagaeva, Maria Semenova. "Influence of gonadotropins on ovarian follicle growth and development in vivo and in vitro", Zygote, 2017 Publication	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

Effects of Addition of Gonadotropin in the Media of Oocyte Maturationon the Embryo Cleavage

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7