Short Communication

Ketapang Leaf Extract Enhances Cumulus Cells Expansion in Bovine Oocyte Maturation *In Vitro*

Budi Purwo Widiarso*, Rohman Abidin, Muhamad Rusliyadi, Dewi Pranatasari

Polytechnic of Agricultural Development Yogyakarta-Magelang, Jl. Magelang-Kopeng Km 07 Tegalrejo Magelang, Central Java, 56192, Indonesia.

Abstract | The aim of this research was to determine the effect of ketapang (*TerminaIia catappa L.*) leaf extract supplementation on enhancing cumulus cells expansion in bovine oocyte maturation *in vitro*. This research was conducted at Embryo Transfer Institution Cipelang Bogor. Data analysis used in this research was a Completely Randomized Design (CRD) with four treatments, supplementation with ketapang leaf extract at a dose of P0 0 mg/ml, P1 7 mg/ml, P2 10 mg/ml, P3 13 mg/ml and 6 repetitions. Variables in this study include the success rate of oocyte maturation in *vitro*, characterized by expansion *of the cumulus oophorus*. The procedures used include ketapang leaf extraction, ovary aspiration, oocyte selection, oocyte washing and *in vitro oocyte maturation*. Data were analyzed using *Kruskal-Wallis* which was then followed by *Less Significant Difference* (LSD) *post-hoc test* and *independent sample T-test* analysis to determine whether supplementation of ketapang leaf extract in *in vitro maturation medium* can increase the expansion of cumulus cells. . The level of significance or accuracy used in this research is P=0.05. The variable of this research is the expansion of cumulus ooporeoophorus cells. The research procedure is to make ketapang leaf extract using the maceration method. The results of this study revealed that Supplementation of Ketapang Leaf Extract (*Terminalia catappa* L) has an effect (P<0.05) on oocyte cumulus expansion at a dose of 10 mg/ml P2 and 13 mg/ml P3 for *in vitro* maturation.

Keywords | Ketapang leaves, Oocytes, Cumulus cells, Maturation

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***Correspondence** | Budi Purwo Widiarso, Polytechnic of Agricultural Development Yogyakarta-Magelang, Jl. Magelang-Kopeng Km 07 Tegalrejo Magelang, Central Java, 56192, Indonesia; **Email:** budipw2000@yahoo.com
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INTRODUCTION

The Central Statistics Agency (2024) reports that Indonesia's beef cattle population is 11.75 million heads and 478.05 thousand dairy cattle. The beef cattle population in Indonesia is still unable to meet the community's beef needs. It is known that data from the Ministry of Agriculture regarding beef imports in 2023 will reach 20,115.22 tons per month and beef imports will reach 18,520.62 tons of beef weight. Indonesia has been trying to reduce import quotas and increase the beef cattle population by improving genetic quality. Improving the genetic quality of cattle in Indonesia continues to be developed in three ways, namely Artificial Insemination (AI), Embryo Transfer (TE) and Sperm Sexing. These three methods of embryo transfer have advantages compared to the other two. TE only requires nine months of waiting, if the livestock successfully becomes pregnant after TE the livestock has produced pure breed seeds. It was found that Transfer Embryo has an advantage compared to the other two in TE by only waiting nine months, if the animal successfully becomes pregnant after TE the animal has produced pure breed. Reproductive biotechnology regarding artificial insemination based on Pranatasari *et*

al. (2016), synchronization of lust, super ovulation efforts and the use of technology regarding *in vitro* fertilization or (IVF) which is based on (Widyastuti, *et al*., 2015) provides many benefits for human life especially in developing the livestock industry in Indonesia (Daoed *et al*., 2013).

Widayati *et al*. (2014) explained that at the stage of the sperm fertilization process for egg cells, *in vitro* maturation is very important before the IVF process is carried out because at this stage the success rate is greatly influenced by the quality and maturity of the oocytes when *in vitro* oocyte maturation is carried out. The level of oocyte maturation is assessed based on two criteria, namely nuclear maturation and cytoplasmic maturation (Nurcahyo and Ciptono, 2013). The nuclear maturation process which is related to RNA synthesis activity is characterized by a change in the nucleus from the *dipIotene phase* to metaphase II (YuInawati, 2006). *CumuIus oophorus* expansion is one indicator of successful oocyte maturation Ciptadi *et al*. (2011), according to Widayanti (2010) and Adifa *et al*. (2010) who stated that expansion of seI in *cumuIus oophorus* is a sign of oocyte maturity. The general cell plays a role in the growth and maturation of oocytes, as shown by the relationship between the expansion rate of the general cell and the quality of oocyte maturation Baszary *et al*. (2012).

Ketapang leaves are shade plants (Istarina *et al*., 2015) which are usually planted in urban areas, parks and can grow wild on riverbanks and beaches. Ketapang tree or *TerminaIia catappa* L. is a coastal plant with quite wide coverage in the area. This plant originates from tropical India and then spread to Southeast Asia. In Indonesia, the ketapang plant is often found along roadsides as an ornamental tree and as a shelter (Nopitasari *et al*., 2014).

In general, *TerminaIia catappa L*. contains *tannins (punicaIin, terfIavin A* and *B, tergaIin, tercatin, kebuIagin, geranin, granatin B, coriIagin), fIavonoids (isovitexin, vitexin, tritintrin),* (Sirait *et al*., 2021). Ketapang leaves contain phyavonoids, saponins, triterpenes, diterpenes, phenoIic compounds and tannins (PauIy, 2001).

Purwani *et al*. (2015) said that *TerminaIia catappa* L is one of the plants with potential antibacterial compounds containing secondary metabolites, namely tannins, flavonoids and saponins. The phytoestrogen content in ketapang leaves in the form of flavonoids can be used to help increase oocyte maturation today in line with (Sirotkin and Harrath, 2014) which states that phytoestrogens are compounds derived from nature with the same structure, activity and affinity as estrogen found in mom's body. One of the largest groups of compounds that have phytoestrogen activity are phytoestrogens such as *genistein, kaempferoI, daidzein* and others (Ma'arif *et al*., 2019). Phytoestrogen compounds are able to exert their activity after binding to their receptors (ERdependent) or other pathways (ERIndependent). Meanwhile, estrogen itself in the mother is a hormone for the formation process up to the release of the ovum or folliculogenesis. The aim of this research was to determine the effect of ketapang (*TerminaIia catappa* L.) leaf extract supplementation on enhancing cumulus cells expansion in bovine oocyte maturation *in vitro*.

MATERIALS AND METHODS

Oocyte collection aspiration and washing

Oocytes were obtained from the ovaries of limosin and simmental cattle at the Bogor animal slaughterhouse. As soon as possible after the cattle is slaughtered, the ovaries are placed into a vacuum flask containing 0.9% NaCL medium treated with antibiotics at a temperature of 31-34 ºC (optimum temperature for storing ovaries) (Widayati *et al*., 2014). Oocytes that have been collected from the ovaries are transferred into disposable TCDs that have been filled with DBPS slowly to avoid damage to the oocytes and CumuIus cells. After obtaining the oocytes, the oocyte selection process is then carried out under a stereo microscope in the medium used for washing the oocytes, based on the following identification, the quality of the oocytes is divided into 4 types including: Grade A with the best quality which has Cumulus specifications with a dense layer of more than three layers. and homogeneous oopIasma, Grade B has a dense cumulus layer consisting of one to 3 layers and homogeneous oopIasma, a rough appearance and a darker colored pesticide zone, Grade C has a cumIous layer that is not so dense or sparse and the shape of the oopIasma is random Irregular, dark layers, Grade D oocytes without a layer of enveloping cumulus surrounding the egg cell can be called glabrous (Amer *et al*., 2008). The oocytes used in the research were oocytes with quality or grade A and B, while oocytes with quality C and D were not used. E observation was carried out under a stereo microscope. Oocytes resulting from aspiration were washed once in DPBS, then washed twice in TCM-199. Oocyte washing, which is carried out two to three times, is useful for cleaning dirt and seIs that are picked up and that are not used during the *in vitro* oocyte maturation process. The dirt that is picked up can cause damage to the maturation medium by reducing the pH from neutral to more acidic, so that the medium is not isotonic.

In vitro oocyte maturation and cumulus cell expansion

In vitro oocyte maturation process prepared by 1 TCD. Oocyte maturation was carried out using control maturation medium and supplementation of ketapang leaf extract in the form of droplets or drops of 15 µ m for 5 oocytes, then oil mineral is poured into the TCD so that all

surfaces of the TCM-199 drop are submerged, oil mineral serves to prevent contamination and makes it easier to handle the oocytes. Before use, the maturation medium is kept in an incubator for at least one hour. Oocytes that have been washed with DPBS media are transferred into the TCM-199 drop at the TCD *dispenser*. Oocyte placement must be in accordance with the treatment to be given, namely disposable TCD marked with point one for P0, point two for P1, point 3 for P2, point 4 for P3. Oocyte maturation is carried out by culturing oocytes in TCM-199 medium which is placed in an incubator at a temperature of 38 ºC with 95% humidity and 5% CO_2 pressure for 1 x 24 hours. Oocytes that have been incubated in a CO_2 incubator for a period of 1 x 24 hours are then evaluated for maturity by observing the expansion of their total cells. The level of expansion of cumulus cells was measured from the difference in distance between cumulus cells before immaturation and after maturation in the same oocyte and the same area, measuring the expansion of cumulus cells using an inverted microscope.

Terminalia catappa L extraction

5 kg of leaves were obtained, and washed, then air-dried until the water that came off them when washed disappeared, followed by placing them in an incubator at a temperature of 75 ºC after the water content of the leaves reached approximately 15 up to 20% of the leaf selection, Ketapang leaves are selected which have a perfect shape and even color, after that the process is smoothed with a blender so that the resulting ketapang leaf powder is then sifted, after sifting the result is smooth ketapang leaf powder, weigh the powdered leaf extrcat weighing 100 grams then put it in In a beaker, powdered leaf extract is soaked in 96% ethanol, the ratio of simplicia and ethanol is 1:3, get 100 grams ofpowdered leaf extract, then add 300 milliliters of ethanol then stir until homogeneous. Then close the beaker tightly and cover it with a cloth so that it doesn't get exposed. After leaving the sunlight for 24 hours, it was filtered using filter paper, this process was repeated for 3 days. In this process, 850 ml of solution was obtained, then evaporated using a water bath with a temperature of 65º until the solution turned thick. This process took 1-7 days. by obtaining a thick liquid of 15 ml. Before being mixed into the maturation medium, the extract is first diluted with ultra pure water with the formula:

$$M_1.V_1 = M_2.V_2$$

Experimental design

This research was carried out at the Cipelang Livestock Embryo Center, Bogor. using a Completely Randomized Design (CRD) with 4 (four) treatments supplementation with ketapang leaf extract at a dose of P0 0 mg/ml, P1 7 mg/ml, P2 10 mg/ml, P3 13 mg/ml The treatments used in this research include:

Treatment	Cumulus cells expansion
P0	TCM maturation media - 199
P1	TCM maturation media – 199 + dose of ketapang leaf extract 7 mg/ml
P2	TCM maturation media – 199 + dose of ketapang leaf extract 10 mg/ml
P3	TCM maturation media – 199 + dose of ketapang leaf extract 13 mg/ml

Data analyzes

Data were analyzed using Kruskall Wallis which was then followed by Less Significant Difference (LSD) post-hoc test and independent sample T-test analysis to determine whether supplementation of ketapang leaf extract in in vitro maturation medium can increase the expansion of cumulus cells. The level of significance or accuracy used in this research is P = 0.05. The variable of this research is the expansion of cumulus oopore cells. The research procedure is to make ketapang leaf extract using the maceration method.

RESULTS AND DISCUSSION

Cumulus cell expansion before maturation

Before treatment, preparations are made including searching for ovaries at the animal Slaughterhouse. The ovaries obtained from cull cows, then stored in vacuum falsk containing Ringer's Lactate medium mixed with the antibiotic solution Streptomycin. The ovaries had a diameter of 3 to 8 mm. The ovaries from the animal slaughterhouse are then washed using Ringer's Lactate before aspiration or suction of the oocytes from the ovaries. The aspiration method is in accordance with research by Widayati *et al.* (2014). As soon as possible after the cattle is slaughtered, the ovaries are inserted into the vacuum flask containing Ringer Lactate medium treated with antibiotics at a temperature of 31-34 ºC (optimum ovarian storage temperature). Oocyte collection is carried out by aspirating oocytes from the ovaries with DPBS solution media, aspiration using a 5 ml syringe which has previously been filled with 1 ml DPBS solution with an 18 G needle so as not to damage the oocytes,

After aspiration the oocytes collected in the syringe are inserted into the TCD has been filled with DPBS solution, then selection/grading of oocytes that have been aspirated from the ovaries is carried out under a stereo microscope. After the oocytes are obtained, maturation will be carried out, washing the oocytes three times by transferring them into the DPBS solution.

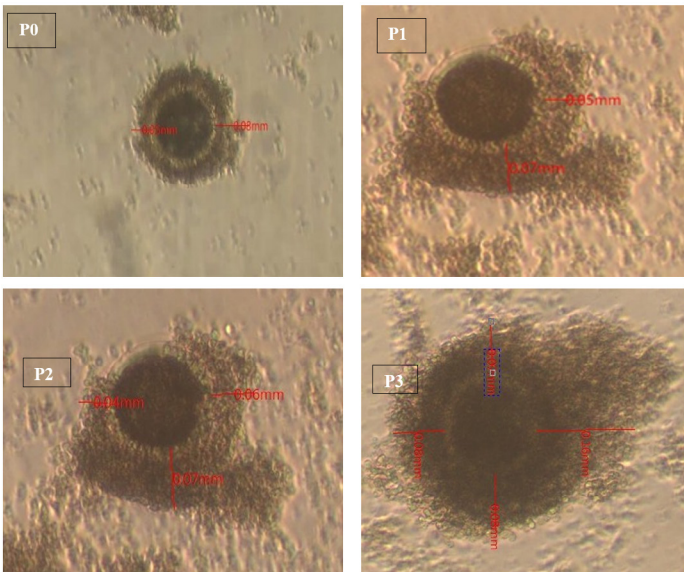

Figure 1: Oocytes before maturation, 40x magnification.

Figure 1 showed that Ketapang leaves extract suplementated could cumulus cells expansion before maturation significantlly especially P3 and P2 on P0 ($P<0.05$). There is a widening of the cumulus cells in P1, P2, and P3 compared to the cumulus cells in P0. Cumulus cell expansion is one of the determinants of the quality of oocytes resulting from in vitro maturation This widening is because supplementation with ketapang extract contains antioxidants so it can prevent oxidative stress due to reactive oxygen species (ROS) created at the in vitro maturation stage which diffuse through cumulus cells. Ketapang leaf extract may contain the hormones estrogen, FSH and LH which support the *in vitro* maturation process (Ekayanti *et al*., 2020). The expansion of cumulus oopores in oocytes is caused by the active compound GSH contained in ketapang leaf extract (*Terminalia catappa* L) which is transported by the oocyte during development in the ovary. Glutathione or GSH is a natural reservoir that can be quickly used by cells to defend against oxidative stress. The expansion of cumulus cells occurs coincident with the occurrence of meiosis.Cumulus cells are stimulated by FSH and growth factors to produce and excrete hyaluronic acid which causes cumulus cell expansion (Mahdiyah, 2006). Hyaluronic acid plays an important role during oocyte culture and embryo development until the embryo transfer process (Mohammed *et al*., 2019). Hikmah (2017) also added that 20% of follicular fluid in bovine oocyte maturation medium can increase cumulus cell expansion. Cumulus cells have an important role in the development and maturity of oocytes. Cumulus cells serve as distributors of oocyte nutritional materials and as synthesizers of protein and acetate into cholesterol. Cholesterol is used as a precursor to steroid hormones which function to stimulate the oocyte maturation process. When the cumulus cells do not expand, malnutrition occurs and results in maturation failure (Widjiati *et al*., 2020). At the oocyte maturation stage (Figure 1), Cumulus has a function as a mediator for energy transport, micronutrients, carrier molecules for oocyte development and hormones needed for the process of oocyte development and maturation through gap junction communication (Rahma *et al*., 2020). This is in line with Gordon (2003) that the presence of Cumulus cells that surround immature oocytes can produce a cellular state that is suitable for the oocyte maturation process because the oocyte and Cumulus cells experience a joint metabolism which produces nutrients which have an important role during the oocyte maturation process.

Cumulus cell expansion after maturation

After maturation in a CO_2 incubator for 24 hours using TCD along with treatment at each dose, it was removed to calculate the expansion of cumulus cells from the zona pellucida to the outermost point of the cumulus layer. The way to calculate cumulus expansion is to measure the distance from the right zona pellucida to the outermost point of the right cumulus cell and the left zona pellucida to the outermost point of the left cumulus cell, then add them up and divided by 2 and then find the measurement results.

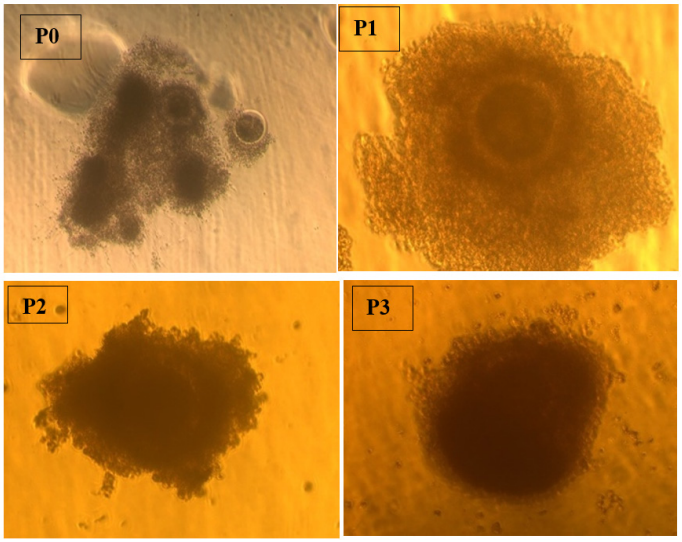

Figure 2: Oocytes after 24 hours of maturation, 40x magnification

The treatment with the addition of *Indigofera* sp. flour caused More livestock experience estrus than livestock that do not experience estrus, P2 shows that estrogen levels in livestock are higher compared to P1 and P3 because phytoestrogens successfully bind to estrogen receptors in the body, so that more livestock in P2 experience estrus. In accordance with research by Daniela *et al*. (2013), isoflavones or phytoestrogens can bind to estrogen receptors as part of hormonal activity, causing a series of reactions that benefit the body. The oocytes to be used are measured using an inverted microscope first to determine the initial width of the cumulus oopore before maturation,

then marking is carried out on the TCD which will be used for the *in vitro* oocyte maturation process. The oocytes obtained were divided into four groups, namely the control group and 3 treatment groups. The control group (P0) was placed in 100 µm TCM-199 maturation medium. P1 was placed in 100 µm maturation medium and 7 mg/ml ketapang leaf extract was added which was dropped 15 µm. P2 with 100 µm maturation media and added 10 mg/ml ketapang leaf extract which was dropped 15 µm. P3 with 100 µm maturation media and added 13 mg/ml ketapang leaf extract which was dropped 15 µm. After that, the pre-treatment data was obtained and then analysis of the pre-treatment data was carried out to see the homogeneity of the data to be studied. Samples with homogeneous data results were then matured in a CO_2 incubator for 24 hours at a temperature of 38 °C and a CO_2 content of 5%. The incubation time was set at 24 hours based on several previous studies including Novitasari *et al.* (2022); Ciptadi *et al.* (2011); Adifa *et al.* (2010) explained that oocyte incubation was carried out for 24 hours. Another duration was carried out by Daoed *et al.* (2013) who explained in their method that incubation was carried out for 22 hours, but the variables observed were different, namely only the percentage of maturation, not cumulus cell expansion.

CumuIus expansion is a variable from research that is calculated numerically using an inverted microscope. The first test carried out was the basic assumptions, namely normality and homogeneity. The norm test is carried out with the aim of finding out whether the data is normally distributed or not. In this research, Shapiro Wilk was used because the samples used were less than 50 samples with a significance level of <0.05. Data can be said to be normally distributed if the P value is <0.05.

Table 1: Cumulus cells expansion in various treatments.

Ketapang leaves extract	0- hour immature (mm)	24- hour mature (mm)	Cumulus cells expansion (mm)
P0	0.174 ± 0.144	0.182 ± 0.182	0.104 ± 0.087[a]
P1	0.191 ± 0.790	0.255 ± 0.852	0.064 ± 0.052[a]
P2	0.121 ± 0.073	0.348 ± 0.371	0.227 ± 0.076[b]
P3	0.232 ± 0.295	0.292 ± 0.292	0.206 ± 0.104[b]

[a,b] different superscripts indicate significant differences (p<0.05).

Based on Table 1, it can be seen that the sig. < 0.05 which means there is a significant difference in each treatment. FIavonoids are included in natural phenolic compounds which can be used as antioxidants. In previous research conducted by Pandya *et al.* (2013), testing flavonoids derived from Ketapang leaves by extraction using the 96% ethanol maceration method resulted in a flavonoid content of 51.67 mg. /g, Erukainure *et al.* (2011) also stated that the higher the amount of flavonoid content of an ingredient, the higher its antioxidant performance, the function of antioxidants themselves is to neutralize free radicals which are capable of causing further reactions which can cause oxidative stress which can damage cells and tissues, this is in line with Kikuzaki et *et al.* (2002) and Sibuea *et al.* (2008). Which states that due to the huge influence of free radicals on human health, the body needs an intake that contains a compound, namely an antioxidant which is able to capture and neutralize these free radicals so that further reactions that cause oxidative stress can stop and cell damage can be avoided or induced. a disease can be stopped.

The results of the study revealed that supplementation with Ketapang leaf extract had an effect on increasing the expansion of cumulus cells. There was a significant difference with a significance value of p <0.05, namely between the control group and P2 with a sig value. 0.005 < 0.05 and P0 against P3 with a sig value of 0.044 < 0.05. Cumulus cells have an important role in the development and maturity of oocytes. At the stage of oocyte maturation, Cumulus has a function as a mediator for energy transport, micronutrients, carrier molecules for oocyte development and hormones needed for the process of oocyte development and maturation through gap junction communication, in line with Gordon (2003) that there are Cumulus cells that surround the oocyte. immature, a cellular state that is suitable for the oocyte maturation process is capable of being produced because the oocyte and Cumulus cells undergo a joint metabolism which produces nutrients which have an important role during the oocyte maturation process. Intensification of the expansion of the cumulus oophorus in oocytes is formed due to the active compounds contained in ketapang (*Terminalia catappa* L) leaf extract which has systematic antioxidant action so that it can prevent oxidative stress due to reactive oxygen species (ROS) created at the *in vitro* maturation stage which diffuse through the cells. cumulus, in Hanna *et al.* (2008) also stated that in the early process of embryo development, Glutathione or GSH reserves transported by the oocyte are obtained during development in the ovary. GSH is a natural reservoir that can be quickly used by cells to defend against oxidative stress. It can be seen in the results in Table 1 which explains that Ketapang leaf extract at a dose of 7 mg/ml does not have a significant impact on the expansion of cumulus cells, however, when supplemented with Ketapang leaf extract at a dose of 10 mg/ml and 13 mg/ml there is a significant difference. significantly than the control treatment. The antioxidants in this study were obtained from ketapang leaf extract which is able to neutralize ROS in a cell, because if there is a surge in ROS in a cell it can cause oxidative damage which makes *in vitro* maturation not get maximum results and can cause the death of the oocyte before *it matures* in line with the opinion Di Meo *et al.* (2016) stated that high

amounts of ROS are capable of causing oxidative damage. When oocytes mature, the surge in ROS will increase, in maturing oocytes there will be an escalation in the creation of ROS due to the level of oxygen absorption to create energy in oxidative phosphorylation in mitochondria in Rocha *et al*. (2016) also revieled that the increase in ROS during *in vitro* maturation was greater than *in vivo*. An increase in ROS that is too high than physiological needs can disrupt the maturation process and oocytes can die, in line. Giving the extract at a dose of 10 mg/ml and 13 mg/ml was able to have a good impact on oocyte maturation, the indications of which were that the cumulus cells expanded well and the antioxidant content in the ketapang leaf extract was able to offset the increase in ROS that occurred to the level of homeostasis. The extract at a dose of 7 mg/ml did not have a significant impact because the antioxidant capacity required for oocyte maturation was not sufficient. This is correlated with the statement from Erukainure *et al*. (2011). Increasing the ability of oocyte maturation can be added with various substances and compounds into the *in vitro maturation media* , this was claimed by Barakat *et al*. (2018). The sign that the oocyte is experiencing maturity is marked expansion of the cumulus cells, while the expansion of the cumulus cells themselves is stimulated by follicle stimulation hormone or FSH and growth factors to produce and release hyaluronic acid (HA) which causes expansion Gordon (2003). Dose of 10 mg/ml of ketapang leaf extract is more effective than 7 mg/ml in supporting oocyte maturation because: Contains more active antioxidant and hormone-like compounds, increases protection against oxidative stress,stimulates molecular pathways that are important in oocyte maturation, and has a stronger synergistic effect (Novitasari *et al*., 2022). This is in accordance with the assumption that the oocyte maturation process is a response to the LH surge and HA secretion from cumulus cells. The action of HA by binding to a protein (CD44) is responsible for the inhibition of apoptosis, proliferation, resumption of meiosis and maturation of the oocyte cytoplasm (cumulus expansion). Cumulus cells play an important role in oocyte maturation, influencing the continuation of meiosis and cytoplasmic maturation. The expansion of cumulus cells and maturation of the oocyte nucleus is related to the developmental capability of the oocyte, this is in line with research from Aghaz *et al*. (2015) which states that cumulus expansion and nuclear maturation (MII stage) are important factors in determining the developmental capability of the oocyte. Giving a dose of ketapang leaf extract to the *in vitro* maturation medium of 10 mg/ml in treatment 2 showed significantly different results, the statistical test results were 0.005 with a p value < 0.05 and was the best result from this study even though there was also a significant treatment in treatment 3 with The statistical test result is 0.044 with a p value <0.05, but the P2 result is smaller than the significance value <0.05. The possible reason for this to occur is due to differences in the quantity of active compounds present in each ketapang (*Terminalia catappa* L) leaf extract in the maturation medium of this study which is not the same which causes the effects given to also experience differences and if the absorption of the nutrients provided is not the same then the food will also affect the on the results of the expansion of cumulus cells and vice versa, if the absorption or administration of extracts in the maturation media is too high, it is feared that it will eliminate ROS at physiological levels needed to signal oocyte maturation, in line with Baszary *et al*. (2012) who state that cumulus cells are mediators that provide energy transport, micronutrients and carrier molecules for oocyte development and mediate the effects of hormones on the oocyte cumulus cell complex. In other research, the use of flavonoids in extraction using ethanol to be supplemented in the oocyte maturation medium in *vitro* also showed good results by suppressing ROS below physiological levels which was able to make the expansion of cumulus cells in the oocyte maturation medium provide a better effect than without administration. treatment. Syamsudi *et al*. (2020), however in previous studies the doses given were greater, namely 10, 15 and 20 mg/ml because the flavonoid content in the extract was smaller, namely 27 mg/ ml in Moringa leaf extract compared to the flavonoid content in Ketapang leaf extract of 51.67 mg/ml Pandya *et al*. (2013).

It is anticipated that this research will demonstrate that a small dose can yield a more effective outcome; however, further testing is required to identify the minimum and maximum doses to assess the toxicity and effectiveness of Ketapang leaf extract (*Terminalia catappa* L).

This research shows that the supplementation of Ketapang leaf extract (*Terminalia catappa* L) has a significant effect ($P < 0.05$) on the expansion of beef cattle oocyte cumulus cells in in vitro maturation media in increasing the maturation of beef cattle oocytes with P2 treatment. 10 mg/g and P3 at a dose of 13 mg/g, with the best results in treatment P2. In general, the results of this research can be used as a basis for applying the addition of ketapang leaf extract (*Terminalia catappa* L) to *in vitro* embryo production to improve the quality of oocyte maturation before *in vitro* oocyte fertilization.

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NOVELTY STATEMENT

Ketapang leaves contain flavonoids, saponins, triterpenes, diterpenes, phenolic compounds, and tannins. Ketapang leaves contain phytoestrogens in the form of flavonoids that can be used to help improve oocyte maturation. The novelty of this study lies in the use of ketapang leaf extract in the maturation medium to enhance the expansion of cumulus cells in beef cattle oocytes in vitro. Previous studies have mostly used hormones to enhance cumulus cell expansion during in vitro maturation.

AUTHOR'S CONTRIBUTION

Budi Purwo Widiarso: Wrote the original manuscript, performed formal analysis and writing, reviewed and edited the manuscript.
Rohman Abidin: Conducted research and obtained data sources.
Muhamad Rusliyadi: Conducted discussions and investigations.
Dewi Pranatasari: Analyzed data, reviewed, and edited the manuscript.
All authors are responsible for reading and approval of the final manuscript.

Ethical approval

All stages of the research were approved by the Ethical Committee of Gadjah Mada University (number 00118/04/LPPT/IX/2017).

Generative AI and AI-assisted technology statement

The authors state that in writing the manuscript, they did not use generative artificial intelligence (a type of artificial intelligence technology that can generate various types of content, including text, images, audio, and synthetic data. Examples include ChatGPT, NovelAI, Jasper AI, Rytr AI, DALL-E, and others) or artificial intelligence-supported technology in the writing process prior to submission

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Adifa NS, Astuti P, Widayanti DT (2010). The effect of adding chorionic gonadotropin to the maturation medium on the ability of maturation, fertilization and embryo development *in vitro* in Ettawa Cross-Broed Goats. Bull. Petern., 34(1): 8-15. https://doi.org/10.21059/buletinpeternak.v34i1.101

Aghaz F, Hajarian H, Shabankareh HK, Abdolmohammadi A (2015). Effect of sericin supplementation in maturation medium on cumulus cell expansion, oocyte nuclear maturation, andsubsequent embryo development in Sanjabi ewes during the breeding season. Theriogenology, 84: 1631–1635.https://doi.org/10.1016/j.theriogenology.2015.08.013

Amer HA, Hegab AO, Zaabal SM (2008). Effects of ovarian morphology on oocyte quantity and quality, granulosa cells, in vitro maturation, and steroid hormone production in buffaloes. Anim. Reprod., 5: 55-62.

Barakat IAH, Alajmi RA, Zoheir KMA, Salem LM, Al-Hemidiy AR. (2018). Gene expression and maturation evaluation of sheep oocytes cultured in medium supplemented with natural antioxidant source. South Afr.J. Anim. Sci., 48(2): 261-265. https://doi.org/10.4314/sajas.v48i2.7

Baszary CDU, Sumitro BS, Djati MS, Widjajanto E (2012). Effect of epidermal growth factor (EGF) Induction on Cx43 protein during cumulus cell expansion. J. Kedokteran Hewan. 6(1): 36-40.

Cholisoh Z, Utami W (2008). Radical capturing activity of 70% ethanol extract of jengkol seeds (*Archidendron jiringa*). Pharmacon, 9(1): 33-40.

Ciptadi G, Susilawati T, Siswanto B, Karima HN (2011). Effectiveness of gonadotropin hormone enhancers in mSOF maturation medium on oocyte maturation levels. J. Ternak Tropika. 12(1): 108-115.

Daniela CV, Cateno P, Barbara M, Filipo D, Salvatore S (2013). Isoflavones: Estrogenic activity, biological effect and bioavailability. Eur. J. Drug Metab. Pharmacokinet., 38(1): 15-25. https://doi.org/10.1007/s13318-012-0112-y

Daoed DM, Ngadiyono N, Widayati DT (2013). Effect of fetal calf serum supplementation on the *in vitro* maturation ability of cow oocytes. Bull. Petern., 37(3): 136-142. https://doi.org/10.21059/buletinpeternak.v37i3.3077

Di Meo S, Reed TT, Venditti P, Victor VM (2016). Role of ROS and RNS sources in physiological and pathological conditions. Oxidat. Med. Cell. Long., pp. 1-40. https://doi.org/10.1155/2016/1245049

Ekayanti MK, Angger TP, Muhammad G, Ennytantini S, Supri OY (2020). Supplementation of follicular fluid in in vitro maturation media for Garut sheep (*Ovis aries*) oocytes. Pros. Sem. Nas. Masy Biodiv. Indon., 6(1): 557-561.

Erukainure OL, Oke OV, Ajiboye AJ, Okafor OY (2011). Nutritional qualities and phytochemical constituents of Clerodendrum volubile, a tropical non-conventional vegetable. Int. Food Res. J., 18(4): 1393-1399.

Gordon I (2003). Laboratory production of cattle embryos, University Press, Cambridge. https://doi.org/10.1079/9780851996660.0000

Hanna C, Long, Hinricchs K, Westhusin M, Kraemer D (2008). Assessment of cannie oocyte viability after transportation and storage under different conditions. Anim. Reprod. Sci., 105(3-4): 451-456. https://doi.org/10.1016/j.anireprosci.2007.12.009

Hikmah N (2017). Effect of addition of cow ovarian follicular fluid on the quality of *in vitro* maturation of goat oocytes. [skripsi]. Fakultas Peternakan Universitas Brawijaya, Malang

Istarina D, Khotimah S, Turnip M (2015). Antibacterial activity of methanol extract of ketapang fruit (*Terminalia catappa* Linn.) against the growth of *Staphylococcus epidermidis* and *Salmonella typhi*. Protobiont, 4: 98-102.

Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, and Taniguchi

H (2002). Antioxidants properties of ferulic acid and its related compound, J. Agric. Food Chem., 50(7): 2161-2168. https://doi.org/10.1021/jf011348w

Ma'arif B, Mirza DM, Hasanah M, Laswati H, Agil M (2019). Antineuroinflammation activity of n-butanol fraction of marsilea crenata presl. in Microglia HMC3 cell line. J. Basic Clin. Physiol. Pharmacol., 30(6). https://doi.org/10.1515/jbcpp-2019-0255

Mahdiyah R (2006). Effect of addition of FSH and LH in maturation medium on maturation of cow oocytes *in vitro*. [Skripsi]. Animal Husbandry, Agriculture Facullty of Diponegoro University. Semarang.

Mohammed AEN, Al-Suwaiegh S, Al-Shaheen T (2019). Effect offolicullar fluid component on oocyte maturation and embryodevelopment *in vivo* and *in vivo*. Adv. Anim. Vet. Sci., 7(5): 346-355. https://doi.org/10.17582/journal.aavs/2019/7.5.346.355

Nopitasari, Amilia N, Muhdarina N (2014). Carbonization of Ketapang leaf waste for biosorption of Cr (VI) in Water. Ind. Che. Acta, 5(1): 30-35.

Novitasari E, Pemayun TGO, Suatha IK, Trilaksana IGNB (2022). Oocyte maturation level of Bali cattle in TCM 199 medium with the addition of hypotaurine. Bull. Vet. Udayana, 14(5): 524-530. https://doi.org/10.24843/bulvet.2022.v14.i05.p12

Nurcahyo H, Ciptono (2013). Oocyte maturation and *in vitro* fertilization using ovarian follicle granulosa cell culture. Laporan Tahunan Hibah Bersaing. Universitas Negeri Yogyakarta. Yogyakarta.

Pandya BN, Tigari P, Dupadahali K, Kamurthy H, dan Nadendla (2013). Antitumor and antioxidant Status of Terminalia catappa against Ehrlich ascites carcinoma in Swiss albino mice. Indian J. Pharmacol., 45(5): 464-469. https://doi.org/10.4103/0253-7613.117754

Pauly G (2001). Cosmetic, dermatological and pharmaceutical use of an extract of *Terminalia catappa*. United States Patent Application no. 20010002265: 1-2.

Pranatasari D, Kustono, Widayati DT (2016). Gonadotropin hormone supplementation in *in vitro* maturation medium to increase the development of stage 4 cell embryos in bligon goats. Bull. Vet., 40(2): 83-91. https://doi.org/10.21059/buletinpeternak.v40i2.9080

Purwani I, Kristanti (2015). *In vitro* potential test of ketapang (*Terminalia catappa*) leave extract against *Aeromonas salmonicida*. J. Appl. Environ. Biol. Sci., 5(7): 1- 2.

Rahma N, Udin Z, Masrizal (2020). Effect of storage time of ovaries on the quantity and quality and *in vitro* maturation rate oocyte of bovine. J. Petern. Indonesia, 22(3): 346-352.

Rocha PS, Goes GR, Diniz AR, Aguiar PH, Machado CR, Vieira LQ (2016). Trypanosoma cruzi needs a signal provided by reactive oxygen species to infect macrophages. PLoS Neglect. Trop. Dis., 10(4): e0004555. https://doi.org/10.1371/journal.pntd.0004555

Sibuea P, Raharjo S, Santoso U, Noor Z (2008). The role of surfactant micelles on the partitioning of antioxidant and the oxidative stability of oil-in-water emulsion. J. Teknol. Industri Pangan, 19(1): 47-47.

Sirait NM, Senjaya AA (2021). Chemical compound of *Terminalia catappa* L. as hemostatic agents in post tooth extraction. J. Health Med. Sci., 4(4): 28-39. https://doi.org/10.31014/aior.1994.04.04.189

Sirotkin AV, Harrath AH (2014). Phytoestrogen and their effects. Eur. J. Pharmacol., 741: 230–236. https://doi.org/10.1016/j.ejphar.2014.07.057

Statistic Centre Agency (2024). Livestock in 2024. ISSN/ISBN : 2714-8416. Published in December 20 th 2024. Jakarta

Syamsudi NA, Widjiati, Hendarto H (2020). Effect of moringa (*Moringa pterygosperma* Gaertn) leaf extract supplementation on oocyte cumulus expansion in *in vitro* maturation medium. J. Penelitian Kesehatan Suara Forikes, XI: 43-47. https://doi.org/10.33846/sf11109

Widayati DT (2010). Effect of oocyte morphology on embryo development and implantation. Materi kuliah program konsultan fertilitas dan endokrinologi reproduksi (Unpublished), bagian Obsgin. Fakultas Kedokteran, Universitas Gadjah Mada, Yogyakarta.

Widayati DT, Fatmawati DV, Ariesta N, Kustono (2014). Use of follicular fluid in *in vitro* maturation media for bligon goat oocytes. J. Kedokteran Hewan, 8(1): 64-67. https://doi.org/10.21157/j.ked.hewan.v8i1.1263

Widjiati ZF, Darsini N, Hendrawan VF, Luqman EM, Sumitro SB (2020). The role of cumulus in the *in vitro* maturation process towards the maturation level of kacang goats (*Capra aegagrushircus*). J. Int. Dental Med. Res., 13(3): 1211-1216.

Widyastuti R, Setiawan R, Rasad ST (2015). Comparison of the maturity levels of bovine oocyte nuclei after *in vitro* maturation with the addition of 10% artificial serum and 10% fetal bovine serum. J. Ilmu Ternak, 15(2): 28-32.

Yulnawati (2006). Optimalisasi produksi embrio domba secara *in vitro*: Use of CR1aa medium and influence of ovarian reproductive status.Tesis. Sekolah Pascasarjana, Institut Pertanian Bogor. Bogor.

Yulnawati MA, Setiadi, Boediono A (2005). Maturation and fertilization rate of ovine oocytes collected from different status of ovaries. Proceedings, reproductive biotechnology for improved animal breeding in Southeast Asia. Bali, Indonesia. pp. 199-202.