article prof. Herry Agoes Hermadi et al

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Date: Friday, June 22, 2018 at 09:56 PM GMT+7

Yth. Dok Havan

Bersama ini saya kirim revisi yang diminta o/ Philippine Journal Vet Med dan tambahan references (Green High light)

a.n. Prof. Herry Agoes Hermadi et al

Trm ksh

Dr. Erma Safitri

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Comments on Papers

THVMIC09
Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women at 1.5 - 3.5 Months for Manipulate *In Vitro* Maturation of Madura Cattle Oocytes
Title

- Transfer the month of pregnancy to Materials and methods
- Delete the word "Manipulate"

Abstract

- Indicate the objective of the study.
- State briefly the methodology in study 1 and 2 e.g. methods of extraction and calculation of hCG, added supplements in TCM 199.
- Indicate the results on maturation rate between treated and control groups (reached MII)
- Include your conclusion and implication of the findings.

Introduction

- Add related literatures (previous studies or researches) on the use of hCG in animals specifically on follicular growth and development whether in vivo or in vitro.
- Objective of the study must be stated clearly.
- Indicate any implications in the livestock industry.
- What is the nature of the problem being addressed by the study?
- What is novel in the study? Why should you conduct the study?
- What is the hypothesis of the study? What are the possible applications of the results of the study?

Materials and Methods

- Present and describe appropriately the methods used in both studies (Cite literatures).
- Just include the major materials, reagents and equipment's used.
- Indicate the number(s) of source(s) of urine samples.
- Discuss your selection criteria on the size of cumulus oocyte complexes before aspiration and maturation.
- Indicate the supplementations of TCM-199 used for maturation.
- Describe properly the manner or what concentration of hCG was added to the maturation medium.
- Explain further why did you preferred to use cumulus-free oocytes. In animal models, cumulus cells are very important in the maturation of oocytes in vivo and in vitro.
- Indicate the treatments used and the number of replicates.

- Describe properly the manner of using aceto-orcein technique in assessing maturation rate. As far as I know, it is not being incorporated in a maturation medium
- Describe properly how maturation rate was evaluated or considered in this study, nuclear and cytoplasmic maturation?
- For all equipments, materials, kits and media used, pleased specify generic name, brand name, manufacturer and address.

Results and Discussion

- Present your data properly in Tables or in Figures.
- There is very little discussion. Needs more in depth interpretation and discussion of the results. Compare the results to other previous researches.
- Last figures reflects not a matured oocytes, it is the developmental kinetics of the oocytes, which is not the objective of this research.
- The conclusion statement is not part of the objective of the study.
- What is the conclusion of the study?
- What are the recommendations of the study?

References

- All references should be written in English or translated in English.

General comments

- The paper should be subjected to intensive English language editing.
- The paper cannot be considered for publication in the PJVM since the subject matter is outside the scope of the journal.
- If the authors can focus only on the *in vitro maturation* in cattle, then this can be considered as a research note.
- Printing of the paper necessitates payment of US$150 for one coloured page.

Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women for In Vitro Maturation of Madura Cattle Oocytes

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ABSTRACT

The purpose of this study was to prove that human Chorionic Gonadotropin (hCG) from urine of pregnant women can be used for in vitro maturation of Madura cattle oocytes. Methods of the research was devided three stage, First stage: isolation, identification and purification of hCG from urine pregnant women at 1.5 to 3.5 months with charcoal aid in ultra-centrifugation 4 ° C by using sephadex g-100 coloums chromatography. Performing characteristics for identification of hCG protein were using sds-page and then purification of hCG protein; Second stage: Elisa level calculation of hCG from urine pregnant women at 1.5 to 3.5 months, Third stage: in vitro maturation of Madura cattle oocytes. The results: the molecular weight of hCG at 37 and 22 kDa and then hCG level with Elisa calculation from pregnant women urine at 1.5 and 3.5 months age with 10 times deposition was average level of 27,333 m iu/l and 105,667 m iu/l. Furthemore statistical analysis with t test was showed no significant difference ($p > 0.05$) between the control group with patent hCG (Chlorulon Intervet Holland product) = $91.15^{a} \pm 8.57$ and hCG for pregnant women urine at 1.5 - 3.5 months = $90.54^{a} \pm 12.86$ for maturation of

Madura cattle oocytes in vitro. The conclusions : hCG from urine of pregnant women at 1.5 – 3.5 months can be used for in vitro maturation of Madura cattle oocytes

Keywords: hCG, invitro maturation of Madura beef cattle oocytes, urine of pregnant women

INTRODUCTION

Pregnancy tests, quantitative blood tests and the most sensitive urine tests in humans usually detect hCG between 6 and 12 days after ovulation (Wilcox et al., 1999). However, it should be taken into account, but that total hCG levels may vary within a very wide range within the first 4 weeks of pregnancy, leading to incorrect results during this period (Butler et al., 2001). A 35% increases over 48 hours is proposed as a minimum increment consistent with an appropriate intrauterine pregnancy (Kirk et al., 2013).

Human Chorionic Gonadotropin (hCG) is not of a specific species meaning that although the resulting urine of pregnant women still has a therapeutic effect on receptive women or patients. Santibañez et al. (2014), investigate the intrauterine injection effect of hCG may be directly involved in the invitro fertilization (IVF) process in human before embryo transfer. Human chorionic gonadotropin (hCG) is a hormone produced by the placenta after implantation (Cole, 2009; Gregory and Finlay, 1999). The presence of hCG was detected in several pregnancy tests (hCG pregnancy strip test).

Human chorionic gonadotropin is a glycoprotein consisting of 237 amino acids with a molecular mass of 36.7 kDa, approximately 14.5 αhCG and 22.2 kDa βhCG (Tenant, 2011) it is heterodimeric, with α (alpha) subunits identical to luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and β subunit (beta) unique to hCG. The α subunit (alpha) is an old amino acid, the β subunit of hCG gonadotropin (beta-hCG) contains 145

amino acids, encoded by six high homologous genes composed tandem and pairs on chromosome 19q13.3 - CGB (Agrawal et al., 2000; Canfield et al., 1987; Steel and Torrie, 1995).

hCG treated in female infertility when compared with recombinant human FSH on the comparison of oocyte collection results and embryo development results are very satisfactory. The usage of 85% oocyte hCG reaches the metaphase phase at IVF treatment 2 to see if there is an additional benefit of giving FSH in addition to hCG priming in vitro maturation program (IVM). Three-five cycles were treated with 75 IU rFSH for 6 days, and 33 cycles were not treated given hCG 10 000 IU 36 hours before oocyte retrieval in vitro and fertilized by ICSI, and the resulting embryo is replaced on day 2 or 3. A total of 1528 immature oocytes are found. The overall maturation and fertilization rate are 74.2 and 72.8%, respectively. After embryo transfer, 23 pregnancies resulted (33.8%) (Ng et al., 2001).

The use of hCG in animals specifically on follicular growth and development in vivo or in vitro. Due to its similarity to LH, hCG may also be used clinically to induce ovulation in the ovaries. As the most abundant biological source is women who are currently pregnant, some organizations collect urine from pregnant women to extract hCG. Comparative follicles begin to occur simultaneously but many cases become atresia. During the luteal phase of the follicular growth cycle where one of them becomes the dominant follicle. Biochemical changes in the development of subordinate follicles to dominant follicles when analyzed for chemical changes in contrast to the principle are FSH, LH and receptor (Zemitis et al., 2014).

The study of molecular hCG, level of development of follicologenesis occurs very dependent of the influence of gonadtrotopin hormone, antral folliculi into responssible to FSH-LH. Inhibin, Activin, Insulin Like Growth Factor I (IGF I) and protein walls (bonds with proteins) have effect directly on granulosa cells and Techa cells can stimulate follicular growth and

storeidogenesis (Gardner et al., 2004). Injection of 1000 IU hCG exogenous within 24 hours of sow post partum (farrowing) induced ovulation in 41%-75% at 7 to 10 days after injection (Armstrong et al., 1999; Kirkwood et al., 1999). Some researchers mention that, postpartum sow ovaries have potentially estrogenic medium follicles (4 to 5 mm) and some sows exhibit estrous behavior (De Rensis et al., 1993; Sestis and Britt, 1993; Sestis and Britt, 1994; Langendijk et al., 2007). But the postpartum estrus observed at 2 to 4 days post farrowing is anovulatory, likely due to an inability to generate a preovulatory LH surge (Sestis and Britt, 1993).

Until now, medical experts rely solely on activity dependent on LH from late oocyte maturation and thus Take it for granted that the natural waves of mid-cycle FSH are biologically excessive. However, it is now time to question this paradigm. The evidence from clinical research suggests that in an undetermined subset of patients, the LH and FSH waves are doubly advantageous compared to the LH spike in the form of a human chorionic gonadotrophin (hCG) trigger. Double waves can be triggered by gonadotrophin-releasing hormone agonist boluses causing endogenous LH and FSH flare ups and resembling natural waves of concomitant hCG gonadotrophin to ensure adequate exposure to LH activity (Shahar and Peter, 2010). Further research is needed to characterize of hCG from urine of pregnant women and then can be used for in vitro maturation of Madura cattle oocytes

The Research purposes are extracted hCG and perform hCG separation in urine of pregnant women 1.5 to 3.5 months gestational age, characterization and identification of molecular weight of hCG protein and hCG biological potential test on maturation level of Madura cattle oocytes.

MATERIALS AND METHODS

Materials and Research Tools

First stage: isolation, identification and purification of hCG from urine pregnant women

In the first study requires a healthy pregnant urine sample taken from urine collected from 1.5 - 3.5 months pregnant women. Number of pregnant women are healthy collected from 50 women from home clinic midwife Mrs Darmun on Gogor street Surabaya, East Java, Indonesia. Sample preparation of pregnant women urine who were taken in the morning as much as 100 ml per person were centrifuged at 3000 rpm for 15 min with temperature 4 ° C This centrifugation aims to separate the metabolite cells and the sediment part is removed. Furthermore, urine was added activated charcoal powder (charcoal) with a dose of 30 mg/100 ml and centrifugation 3000 rpm at 4ºC for 20 minutes (Green and Leake, 1987), usually used to remove organic contaminants, and to purify. The two mixtures are stirred until homogeneous. The results of the supernatant are filtered to clearness and fed into the erlenmeyer tube using filter paper. The above procedure is performed to obtain a 50 ml supernatant. Furthermore, it is incorporated into the sephadex G-100 filtration. device (mini protein gel, biorad production), then running buffer is poured on chamber electrophoresis (David, 2009). The purpose of charcoal delivery for pregnant urine removal from toxic substances, dyes and steroid hormones, absorbs and inactivates organic chemicals. Furthermore, hCG isolates obtained from pregnant urine extract after characterization and isolation using SDS-PAGE 12% and elusi techniques (Gam and Latif, 2005). Put the running gel into the SDS-PAGE tool through the wall roughly less than the upper limit. Next add butanol approximately 1 ml, leave for 25 minutes. Then the butanol is discarded after the gel freezes and cleansed with PBS, dried with Whatman paper. Next comb is taken and cleaned and the remnants of gel with e buffer.

Second stage: Elisa level calculation of hCG from urine pregnant women at 1.5-3.5 months

There are two Principle of test the hCG Elisa Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (Mahaputra and Mustafa, 2003). The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site of the hCG molecule. An aliquot of sample containing endogenous hCG is incubated in the coated well. After washing, a second incubation follows with enzyme conjugate, which is an anti-hCG antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off, the amount of bound peroxidase is proportional to the concentration of hCG in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of hCG in the sample (Kariman et al., 2011).

Third stage: in vitro maturation of Madura cattle oocytes

Investigate hCG biological potential test on the maturity level of In Vitro maturation madura beef cattle oocytes. Maturation of oocytes in this research was devided two group, the control group : Maturation oocytes cow in TCM 199 + PMSG 0.5 IU + hCG 0.5 IU Chorulon intervet (patent hCG Holland product) compared with group treatment : Maturation oocytes cow in TCM 199 + PMSG 0.5 iu + hCG 0.5 IU from pregnant woman.

After the ovaries from slaughterhouses are washed 2 3 times with physiological saline solution and placed in a glass above the water bath at 37°C, then the ovaries are taken one by one using sterile tweezers and the surface is dried with sterile tissue paper. Using a 10 ml sterile disposable syringe and an 18 G syringe filled with 1.5 ml of OWS (Oocyt Washing Medium) medium. The suction is done by stabbing the adjacent part of the ovary parenchyma in the next follicle bubble and then the needle tip is directed to the follicle follicle, which is approximately 5

mm in diameter near the point of needling (without removing the needle first). After all the fluid of the follicle is inhaled or if the syringe contains 3 4 ml of follicle fluid, the needle is removed and the follicular fluid is carefully inserted in the test tube to avoid mechanical damage to the oocyte and stored in a water bath. It is then waited until the oocyte drops to the bottom of the tube, then the precipitate is evaluated by placing on a large petri dish and examined under a stereo microscope (Mahaputra and Mustofa, 2003).

Observation on oocytes, some suctioned follicle fluid is poured into a petri dish and examined under a stereo microscope and when the oocyte has been found, the oocyte is taken with a modified pastry pipette that is the same diameter as the oocyte, and placed on another smaller petri dish containing washing medium. The oocyte is then examined again under a microscope to determine its quality (Mahaputra and Mustafa, 2003). After determining the quality of oocytes all the oocytes were first washed in washing medium as much as 3-4 times and the final washing with Tissue Culture Medium 199 (TCM 199) as much as 2.5-3.0 ml. Then oocytes with no cumulus debris are transferred into the maturation medium in the form of drops of 100 µl each in a petri dish of 20 oocytes each. Total oocytes used in this study 10 oocytes prepared 2 hours earlier in incubator C02 5%. Maturation mediums are made in sterile Petri dishes of 35 mm in drops of 4 drops, each of which drops 100 µl in volume. In each drop of medium can be cultured as much as 5 oocytes and then covered with mineral oil. The culture of the three mediums was carried out in an incubator containing 5% C02 with a humidity of 95-100% and a temperature of 390C. Each incubated medium was carried out for 24 hours. Added 1% aceto orcein stain to determine the maturity level of egg cell (Chen et al., 2013).

The procedure for collecting data on egg maturity is by performing 1% Aceto orcein staining as follows: eggs that have been harvested, placed on the object glass and covered with

glass cover, then fixed in fixative solution for 48 hours. The preparation is then dyeed with Aceto orcein 1% for 2-3 minutes. Then washed with washed solution and eggs that have been stained then viewed under an inverted microscope. Assessment of egg maturity level with Germinal Vesicle (GV), Germinal Vesicle Break Down (GVBD), Metaphase I and Metaphase II criteria (de-Oliviera et al., 2016). Purpose of oocytes matured in vitro are capable of resuming meiosis, extruding a first polar body, and undergoing fertilization (Figure 2).

Design and Statistical Analysis

The data of identification, characteristic, isolation of hCG and level calculation of hCG from urine pregnant women at 1.5 to 3.5 months were processed descriptively. Data obtained from the number of eggs mature that have been done due to the addition of hCG control from Chorulon intervet (patent hCG Holland product) and from from woman pregnant were analyzed by t test (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Isolation, identification and purification of hCG from urine pregnant women

From the results of this study The molecular importance of hCG corresponds to the result of SDS-PAGE 12% are in the urine of pregnant women bands 37 kDa and 22 kDa (Figure 1). The other research the bands that were detected in no-reduced fractionally desialylated-hCG sample were 43.5, 38.5, 29.45 and 20.85 kDa, which after being lessen, run as uncompounded pledge at 35.2 kDa (Gam and Latiff, 2005).

Elisa level calculation of hCG from urine pregnant women at 1.5 to 3.5 months

The total level samples hCG age 1,5 and 3,5 month with 10 times collected of urine human pregnancies ammonium sulphate precipitated Concentration of hCG in this reseach was analyzed by average Level hCG 27,333 mIU/mL (1,5 month) 105,66 (3,5 months) and Measurement of hCG provides a specific test for pregnancy. Stability Lyophilized hCG although stable at room temperature for 3 weeks, should be stored between 2-8°C (Suthar, 2008). It is advised to reconstitute the lyophilized hCG in sterile 18M-cm H2O at a concentration of 1000 IU/ml, which can be further diluted to other aqueous solutions. Although, Stability Lyophilized hCG stables at rank temperature for 3 weeks, should be stored between 2-8°C (Suthar and Shah, 2009).

In vitro maturation of Madura cattle oocytes

Purpose of oocytes matured in vitro are capable of resuming meiosis, extruding a first polar body, and undergoing fertilization (Figure 2). In this research, maturation of cow oocytes in TCM 199 + PMSG 0.5 IU + hCG 0.5 IU from Chorulon intervet (patent hCG Holland product) = $91.15^{a} \pm 8.57$, compared maturation cow oocytes in TCM 199 + PMSG 0.5 iu + hCG 0.5 IU from woman pregnant = $90.54^{a} \pm 12.86$, the use of t test in statistical analysis showed no significant difference between the both ($p > 0.05$) (Table 1). It means hCG from pregnant woman can be used for maturation process of cow oocytes.

Observation of the oocyte collected from the suctioned follicle fluid and poured into a petri dish and examined under a stereo microscope. Furthermore the oocyte that has been mature with hCG (control and from pregnant woman), then taken with a modified pastry pipette of the same diameter as the oocytes, and placed on another smaller petri dish which is smaller contains washing medium. The oocytes are then examined again under a microscope to determine its

quality (Wang et al., 2011). The results of the Suthar (2008) study suggest that, Transvaginal Oocytes Recovery (TVOR) and succeeding IVEP in eight HF x Sahiwal crossbred oxen. For that he used TCM-199 + eCG + hCG, m-TALP and m-SOF as in vitro maturation, fecundation and educate media, respectively. They found 94 % maturation 64% fertilization and intermammary sulcus rate, respectively.

In dairy cattle and beef cattle industry was perfected the application of the first reproductive biotechnology, i.e. artificial insemination (AI) - a great success story and also remains the user of embryo transfer technology (ETT). In addition, newly the researchers taking interest to embraced the field of Transvaginal Oocyte Recovery (TVOR) and in vitro product (IVEP) of embryos. IVF condition the starting point for the production of generativ material for a many of sophisticated reproduction techniques contain sperm microinjection into oocyte that have been maturated. In several countries commercial IVF facilities are already being service by cattle ET operators. Various research groups have reported on modification of TVOR (Suthar and Shah, 2009).

CONCLUSIONS:

From the results of this study can be concluded that: The molecular weight of hCG is 37 and 22 kDa corresponds to the result of SDS-PAGE 12%. Elisa level calculation of pregnant women hCG pregnancy at 1.5 and 3.5 months age with 10 times deposition was average level of 27,333 m iu/l and 105,667 m iu/l. The use of t test in the statistical analysis showed no significant difference ($p>0.05$) between the control group of the Holland hCG chorulon intervet patent product and hCG from urine of pregnant women at 1.5 – 3.5 months. It means hCG from

urine of pregnant women at 1.5 – 3.5 months can be used for in vitro maturation of Madura cattle oocytes.

ACKNOWLEDGMENTS

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Table 1. Rate of Maturation of Oocyte

Types and Doses of hCG	n	Means (±SD)

TCM 199 + PMSG 0.5 IU + hCG 0.5 IU Chorulon Intervet (patent hCG Holland product)	50	91.15[a] ± 8.57
TCM 199 + PMSG 0.5 iu + hCG 0.5 IU from woman pregnant	50	90.54[a] ± 12.86

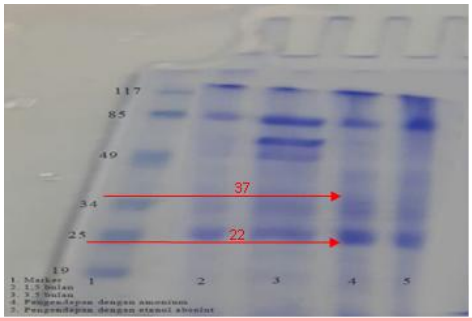

Figure 1. Sds-page hCG in the urine of pregnant women 37 and 22 Kda

A **B** **C** **D**

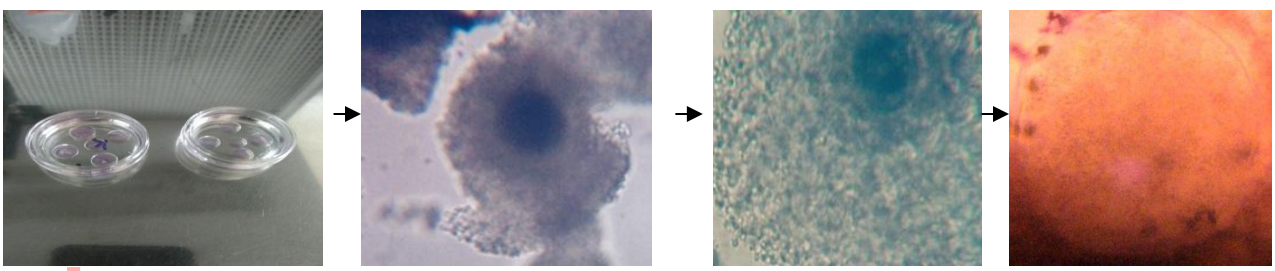

Figure 2. The Changes mechanism of the invitro maturation culture of madura beef cattle oocytes, drops until osits immature, oocytes mature to metaphase egg cells.

A. Madura beef cattle oocyte
B. Oocyte immature
C. Oocyte mature
D. Mathapase egg cell

ANSWER FOR COMMENTS (Herry Agoes Hermadi et al)

From: Safitri Erma (rma_fispro@yahoo.com)

To: vmic@fkh.unair.ac.id

Date: Thursday, July 19, 2018 at 12:14 AM GMT+7

Dear Dok Havan

Di bawah ini saya lampirkan ANSWER FOR COMMENTS from Philippine Journal a.n Herry Agoes Hermadi et al . .

Salam

Dr. Erma Safitri

-

VMIC-09_ Answer for Comments on Papers.docx
18kB

Answer for **Comments on Papers**

THVMIC09

Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Women at 1.5 - 3.5 Months for Manipulate *In Vitro* Maturation of Madura Cattle Oocytes

Title

- Transfer the month of pregnancy to Materials and methods
- Delete the word "Manipulate"

Answer: Yes, we have revised the title

Abstract

- Indicate the objective of the study.
- State briefly the methodology in study 1 and 2 e.g. methods of extraction and calculation of hCG, added supplements in TCM 199.
- Indicate the results on maturation rate between treated and control groups (reached MII)
- Include your conclusion and implication of the findings.

Answer: Yes, we have revised abstract in accordance with suggestions

We have added the concusion of the study :

The conclusions : hCG from urine of pregnant women at 1.5 – 3.5 months can be used for in vitro maturation of Madura cattle oocytes

Introduction

- Add related literatures (previous studies or researches) on the use of hCG in animals specifically on follicular growth and development whether in vivo or in vitro.
- Objective of the study must be stated clearly.
- Indicate any implications in the livestock industry.
- What is the nature of the problem being addressed by the study?
- What is novel in the study? Why should you conduct the study?
- What is the hypothesis of the study? What are the possible applications of the results of the study?

Answer: We have added answer for your questions about introduction.
Our revised our manuscript in green highlight

Materials and Methods

- Present and describe appropriately the methods used in both studies (Cite literatures).
- Just include the major materials, reagents and equipment's used.
- Indicate the number(s) of source(s) of urine samples.
- Discuss your selection criteria on the size of cumulus oocyte complexes before aspiration and maturation.
- Indicate the supplementations of TCM-199 used for maturation.

- Describe properly the manner or what concentration of hCG was added to the maturation medium.
- Explain further why did you preferred to use cumulus-free oocytes. In animal models, cumulus cells are very important in the maturation of oocytes in vivo and in vitro.
- Indicate the treatments used and the number of replicates.

- Describe properly the manner of using aceto-orcein technique in assessing maturation rate. As far as I know, it is not being incorporated in a maturation medium
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Results and Discussion
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- Last figures reflects not a matured oocytes, it is the developmental kinetics of the oocytes, which is not the objective of this research.
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Answer: We have revised the references, all references have written in English or translated in English

General comments
- The paper should be subjected to intensive English language editing.
- The paper cannot be considered for publication in the PJVM since the subject matter is outside the scope of the journal.
- If the authors can focus only on the *in vitro maturation* in cattle, then this can be considered as a research note.
- Printing of the paper necessitates payment of US$150 for one coloured page.

Answer: We have revised our manuscript
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Human Chorionic Gonadotropin (hCG) from Urine of Pregnant Womenfor *In Vitro* Maturation of Madura Cattle Oocytes

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ABSTRACT

The purpose of this study was to test whether human chorionic gonadotropin (hCG) from urine of pregnant women can be used for *in vitro* maturation of Madura cattle oocytes.Urine samples were collected fromfifty (50) healthy pregnant women gestating for 1.5 to 3.5 months.Molecularweights of hCG were 37kDA and 22 kDa. HCGlevels measured via ELISAhad 10 times the deposition?, withan average of 27,333 m IU/l at 1.5 months and 105,667 m IU/l at 3.5 months, respectively. T-test showed no significant differencein oocyte maturation ratebetween the control group with patenthCG(91.15[a] ± 8.57) and hCG from pregnant women(90.54[a] ± 12.86) at P>0.05.This study illustrates that hCG from pregnant women at 1.5 to 3.5 months can aid in *in vitro* maturationof Madura cattle oocytes.

Keywords:hCG, *invitro* maturation,Madura beef cattle, oocytes, urine of pregnant women

INTRODUCTION

Pregnancy tests, quantitative blood tests and the most sensitive urine tests in humans usually detect hCG between 6 and 12 days after ovulation (Wilcox *et al.*, 1999). However, it should be considered that total hCG level varies a lotwithin the first four weeks of pregnancy, which makes tests susceptible to error(Butler *et al.*, 2001). A 35% increasein hCG level over 48 hours is the proposed minimum increment, consistent with an appropriate intrauterine pregnancytest (Kirk *et al.*, 2013).

Human chorionic gonadotropin (hCG) is not specific-species and limited to human hormones, *i.e.*, urine from pregnant women also holds therapeutic effects for receptive animals?In fact, Santibañez *et al.*(2014) suggested thatintrauterine injection effect of hCG may be directly involved in*invitro* fertilization(IVF) process in humansbefore embryo transfer. HCG is a hormone the placenta produces after implantation (Gregory and Finlay, 1999; Cole, 2009) andcan be this can detected in several pregnancy tests.

Human chorionic gonadotropin is a glycoprotein composed of 237 amino acids. The molecular mass of intact, α(alpha) and , ß(beta) hCGare 36.7, 14.5 and 22.2kDA, respectively().It is heterodimeric withalphasubunits identical to luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and beta subunit unique to hCG. The α subunitcontains 92 amino acids, while ß subunit contains 145 amino acids, encoded by six high homologous genes composed of tandems and pairs on chromosome 19q13.3 - CGB (Canfield *et al.*, 1987; Steel and Torrie, , Agrawal *et al.*, 2000).

HCG treatment, in terms of embryonic development and oocyte collection, has been shown to be a morepromising treatment to female infertilitycompared with recombinant human FSH. In the study, 85% of the oocytes reachedmetaphase at IVF treatment 2; consequently, it was tested whether there would be an additional benefit toadministeringFSH on top of hCG in priming *in vitro* maturation (IVM). cycles were treated with 75 IU rFSH for 6 days,while 33 cycles were given 10 000 IU hCG 36 hours

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before oocyte retrieval and fertilization via IVF and ICSI?The resulting embryo was replaced on day 2 or 3. A total of 1528 immature oocytes were found. The overall maturation and fertilization rateswere 74.2 and 72.8%, respectively. After embryo transfer, 23 pregnancies (33.8%) were recorded (Ng*et al*., 2001).

The use of hCG in animals, specifically on follicular growth and development *in vivo* or *in vitro*,have been investigated? Due to its similarity to LH, hCG may also be used clinicallyto induce ovulation in the ovaries. For this reason, some organizations collect urine from pregnant women to extract hCG. Using hCG, comparative follicles begin to occur simultaneously, but many cases become atresia.During the luteal phase of the follicular growth cycle, one of the follicles become dominant. Notably, biochemical changes in the development of subordinate follicles to dominant follicles, when analyzed for chemical changes, are different fromthose in FSH, LH and receptor (Zemitis*et al*.,2014).

In the study of molecular hCG, folliculogenesis ishighly dependent on the influence of gonadotrophin hormone, antralfolliculi into responssible to FSH-LH??. Inhibin, activin, insulin-like growth factor I (IGF I) and protein walls (bonds with proteins) directly affectgranulosa cells, and techa cells can stimulate follicular growth and steroidogenesis(Gardner *et al*., 2004).Injection of 1000 IU hCGexogenouslywithin 24 hrof sow postpartum farrowing induced ovulation in 41%-75% of the populationat 7th to10th days after injection (Armstrong *et al*., 1999; Kirkwood *et al*., 1999).Some researchers mention thatpostpartum sow ovarieshave potentially estrogenic medium follicles(4 to 5 mm), and some sows have exhibited estrous behavior (De Rensis *et al*., 1993; Sestis and Britt, 1993; Sestis and Britt, 1994; Langendijk *et al*., 2007). But the postpartum estruos behavior observed at 2 to 4 days postpartum farrowing is anovulatory, which is likely due to the inability to generate a pre-ovulatory LH surge (Sestis and Britt, 1993).

Medical experts up to this day have been relying on LH activity-dependent alone in inducingfinal oocyte maturation,which overlooks the redundancy of the natural waves of mid-cycle FSH surge. Thus, this needs to be resolved.Evidence from clinical research suggests that in an undetermined subset of patients, the LH and FSH waves are doubly advantageous compared to the LH spike in the form of a human chorionic gonadotrophin (hCG) trigger. Double waves can be triggered by gonadotrophin-releasing hormone agonist boluses, causing endogenous LH and FSH flare-ups and resembling natural waves of concomitant hCGgonadotrophin to ensure adequate exposure to LH activity(Kol and Humaidan, 2010).Further research is needed to characterize hCG from urine of pregnant women, which can then be potentially usedfor *in vitro*maturation of Madura cattle oocytes.

The purpose of this research was to extract hCG from urine of pregnant women gestating at 1.5 to 3.5 months, characterize and determine the molecular weight of the hCG protein, and perform a biological potential test to determine its effect onthe maturation level of Madura cattle oocytes.

MATERIALS AND METHODS

HCG isolation, identification and purification

Urine samples were taken from fifty healthy pregnant womengestating for 1.5 to 3.5 months. Samples were obtained by a midwife from a clinic in Surabaya, East Java, Indonesia.In the morning, 100 ml of urine was collected from each pregnant woman,and these werecentrifuged at 3000 rpm for 15 min at4°C to separate the metabolite cells,30 mg/100 ml activated charcoal powder wasadded, and mixture wascentrifuged at 3000 rpm at 4°C for 20 min (Green and Leake, 1987)until mixture was homogeneous. Charcoal removes toxic substances, dyes, and steroid hormones. It also absorbs and inactivates

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organic chemicals.Supernatant was filtered through an Erlenmeyer tube using a filter paper, producing a 50 ml supernatant. This was poured into aSephadex G-100 filtration device (mini protein gel, biorad production?)and poured into a chamber with running buffer to carry out electrophoresis ().. Furthermore, hCG extract obtained from urine extract was processed through SDS-PAGE and ELISA?(Gam and Latiff, 2005). Arunning gel was insertedthrough the wall roughly less than the upper limit.Around 1 ml of butanol was added, and gel was left for 25 min until it has solidified?Butanol was discarded,and the gelwascleansed with PBS and dried with Whatman paper. Comb was removed and remnants of the gel with buffer were cleaned.

HCG concentration

There are two Principle of test the hCG?ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (Mahaputra and Mustafa, 2003). The microtiter wells were coated with a monoclonal antibody directed towards a unique antigenic site of the hCG molecule. An aliquot of sample containing endogenous hCG was incubated in the coated well. After washing, a second incubation followed with an enzyme conjugate, an anti-hCG antibody conjugated with horseradish peroxidase. After incubation, the unbound conjugate was washed off.The amount of bound peroxidase was proportional to the concentration of hCG in the sample. Having added the substrate solution, color intensity developed was proportional to the concentration of hCG in the sample(Kariman *et al*., 2011).

***In vitro* maturation of cattle oocytes**

Extracted hCGwas used to investigate its potential in inducing*in vitro*maturation in Madura beef cattle oocytes. There were two setups: the control group was given TCM 199 +0.5 IU PMSG + 0.5 IU hCG(Chorulon, Intervet, Holland), while experimental group received the same treatment but with0.5 IU hCG from gestating women.

Ovaries from slaughterhouses were washed 2 to 3 times with physiological saline solution, placed in a glass above water bath at 37°C, taken one by one with sterile tweezers, anddried with sterile tissue paper. Using a 10 ml sterile disposable syringe, samples were washed with 1.5 ml of oocytewashing medium. Suction was done by stabbing the adjacent part of the ovary parenchyma in the next follicle bubble and directing the needle tip to the follicle (approximately 5 mm in diameter) near the point of needling (without removing the needle first). After each follicle fluid was siphoned off (about 3-4 ml), this was carefully transferred into test tubes to avoid mechanical damage to the oocyte, and placed in a water bath. After the oocyte had dropped to the bottom of the tube, precipitate was evaluated by placing it on a large petri dish and examining under a stereo microscope (Mahaputra and Mustofa, 2003).

Once an oocyte was found, this was collected with a modified pastry pipette(of the same diameter as the oocyte), placed on a smaller petri dish with washing medium,and examined again under a microscope to determine its quality (Mahaputra and Mustafa, 2003). Afterwards, all oocytes were washed 3-4 times with washing medium, and was finally washed with 2.5-3 ml of tissue culture medium 199 (TCM 199). Oocytes with no cumulus debris were transferred into the maturation medium,dropping 100 µl each in a petri dish. A total of10? oocytes were used in this study, prepared 2 hours earlier in incubator with 5% CO_2 5%. Four-100 µl drops ofmaturation mediumwereplaced in each 35 mm sterile petri dish.Each drop of medium can culture as much as 5 oocytes.Media werethen covered with mineral oil and placed in an incubator containing 5% CO_2set at 39°C

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and humidity of 95-100% for 24hr. 1% aceto-orcein stain was added to determine maturity level of egg cell(Chen *et al.*, 2013).

To collect data on egg maturity, harvested eggs wereplaced and covered on the glass object,then placed in a fixative solution for 48 hr. These were stained with 1% aceto-orcein for 2-3 min, washed with solution, andviewed under an inverted microscope. Assessment of egg maturity level was based ongerminal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I and metaphase II criteria (deOliviera *et al.*, 2016). Matured oocytes were identified by locating those whichcan resume meiosis (extrudinga first polar body), and can undergo fertilization (Fig. 2).

Design and statistical analysis

Data oinidentification,isolation, purification and measurement of hCG concentration from the urine of pregnant women at 1.5 to 3.5 months were processed descriptively. T-test (Steel and Torrie,) was used to determine difference on oocyte maturation rate between patent hCG(Chorulon, Intervet, Holland)and hCG from urine of pregnant women.

RESULTS AND DISCUSSION

HCG isolation, identification and purification

SDS-PAGE results showed that hCG from the urine of pregnant women had molecular sizes of 37kDa and 22 kDa(Fig. 1). Gam and Latiff (2005) detected other bands from non-reduced fractionally desialylated-hCG sample: 43.5, 38.5, 29.45, and 20.85 kDa, which, after being reduced, resulted to an uncompounded pledge at 35.2 kDa.

Calculation of hCG concentration

The total level samples hCG age 1.5 and 3.5 month with 10 times collected of urine human pregnancies ammonium sulphate precipitated concentration of hCG in this research was analyzed by average level hCG 27,333 mIU/ml (1.5 month) 105,66 (3.5 months). and measurement of hCG provides a specific test for pregnancy.Lyophilized hCG, although stable at room temperature for 3 weeks, should be stored at 2-8°C (Suthar, 2008). It is advised to reconstitute it in sterile 18M-cm H_2O at a concentration of 1000 IU/ml, which can be further diluted to other aqueous solutions.

***In vitro* maturation of cattle oocytes**

Oocytes that matured *in vitro* are capable of resuming meiosis, extruding a first polar body, and can undergo fertilization(Fig. 2). In this research, the rate of maturation of cow oocytes in TCM 199 + 0.5 IU PMSG + 0.5 IU hCG (Chorulon, Intervet, Holland)was $91.15^{a}\pm 8.57$, while maturation ratein TCM 199 + 0.5IU PMSG + 0.5 IU hCG from pregnant women was $90.54^{a}\pm 12.86$.T-test showed no significant difference between groups ($P>0.05$) (Table 1), suggesting that hCG from pregnant woman can be used for maturation process of cow oocytes.

Observation of the oocyte collected from the suctioned follicle fluid and poured into a petri dish and examined under a stereo microscope. Furthermore the oocyte that has been mature with hCG (control and from pregnant woman), then taken with a modified pastry pipette of the same diameter as the oocytes, and placed on another smaller petri dish which is smaller contains washing medium. The oocytes are then examined again under a microscope to determine its quality (Wang *et al.*, 2011).Study of suggeststhattransvaginal cocytes recovery (TVOR) and succeeding IVEP in eight HF x Sahiwal crossbred oxen is what?. TCM-199 + eCG + hCG, m-TALP and m-SOF for*in vitro* maturation, fecundation

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and educate media?, were used. Maturation rate, fertilization rate, and intermammary sulcus rate were 94%, 64%, and XX%, respectively.

The dairy cattle industry has perfected the application of the first reproductive biotechnology, *i.e.*, artificial insemination (AI), a success story which made use of embryo transfer technology (ETT). In addition, emerging researchers have taken interest in the field of transvaginal oocyte recovery (TVOR) and *in vitro* embryo production (IVEP). IVF has paved the starting point for the production of generative material for many sophisticated reproduction techniques, like sperm microinjection into mature oocytes. In several countries, commercial IVF facilities are already being serviced by cattle ET operators. Also, various research groups have reported on the modification of TVOR (Suthar and Shah, 2009).

This study tested the use of hCG from urine of pregnant women in inducing maturation of cattle oocytes. Recorded molecular weight of hCG via SDS-PAGE was 37 and 22 kDa. HCG concentrations via ELISA assay had 10 times the deposition?, with average level of 27,333 m IU/l at 1.5 months and 105,667 m IU/l at 3.5 months. T-test revealed no significant difference ($P>0.05$) between groups. This study illustrates that hCG extracted from urine of pregnant women at 1.5 to 3.5 months can be used to induce *in vitro* maturation in Madura cattle oocytes.

ACKNOWLEDGMENT

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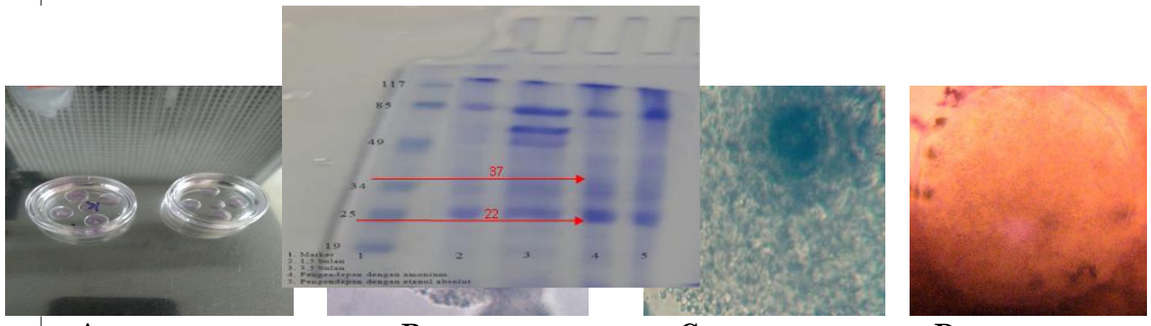
Table 1. Rate of maturation in cattle oocytes induced by different HCG sources

HCG used	*n*	Mean±SD
TCM 199 + 0.05 PMSG + 0.5 IU hCG 0.5 hCG (Chorulon, Intervet, Holland)	50	91.15[a]± 8.57
TCM 199 + 0.5 IU PMSG + 0.5 IU hCG from pregnant women	50	90.54[a]± 12.86

Fig 1.SDS-PAGEshows molecular weight of hCG at 37 and 22 kDA.



A **B** **C** **D**

Fig 2. *In vitro* maturation culture of Madura beef cattle oocytes at different stages: (A)addition of hCG to maturation culture (B) immature oocyte (C) mature oocyte (D) egg cells at metaphase stage

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RESEARCH NOTE

HUMAN CHORIONIC GONADOTROPIN FROM URINE OF PREGNANT WOMEN FOR *IN VITRO* MATURATION OF MADURA CATTLE OOCYTES

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ABSTRACT

The purpose of this study was to test whether human chorionic gonadotropin (hCG) from urine of pregnant women can be used for *in vitro* maturation of Madura cattle oocytes. Urine samples were collected from 50 healthy pregnant women gestating for 1.5 to 3.5 months. Molecular weights of hCG were 37 kDa and 22 kDa. HCG levels measured via ELISA had 10 times the deposition, with an average of 27,333 m IU/l at 1.5 months and 105,667 m IU/l at 3.5 months, respectively. T-test showed no significant difference in oocyte maturation rate between the control group with patent hCG and hCG from pregnant women at P>0.05. This study illustrates that hCG from pregnant women at 1.5 to 3.5 months can aid in *in vitro* maturationof Madura cattle oocytes.

Key words: hCG, *in vitro* maturation, Madura beef cattle, oocytes, urine of pregnant women

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INTRODUCTION

Pregnancy tests, quantitative blood tests and the most sensitive urine tests in humans usually detect hCG between 6 and 12 days after ovulation (Wilcox *et al*., 1999). However, it should be considered that the total hCG level varies a lot within the first four weeks of pregnancy, which makes tests susceptible to error (Butler *et al*., 2001). A 35% increase in hCG level over 48 h is the proposed minimum increment, consistent with an appropriate intrauterine pregnancy test (Kirk *et al*., 2013).

Human chorionic gonadotropin (hCG) is not specific-species and not limited to human hormones, *i.e.,* urine from pregnant women also holds therapeutic effects for receptive animals. In fact, Santibañez *et al*. (2014) suggested that intrauterine injection effect of hCG may be directly involved in *in vitro* fertilization (IVF) process in humans before embryo transfer. HCG is a hormone the placenta produces after implantation (Gregory and Finlay, 1999; Cole, 2009) and this can be detected in several pregnancy tests.

Human chorionic gonadotropin is a glycoprotein composed of 237 amino acids. The molecular mass of intact, α(alpha) and ß(beta) hCG are 36.7, 14.5 and 22.2 kDa, respectively (Gam and Latiff, 2005). It is heterodimeric with alpha subunits identical to luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and beta subunit unique to hCG. The α subunit contains 92 amino acids, while ß subunit contains 145 amino acids, encoded by six high homologous genes composed of tandems and pairs on chromosome 19q13.3-CGB (Steel and Torrie, 1960; Canfield *et al*.; 1987; Agrawal *et al*., 2000).

HCG treatment, in terms of embryonic

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development and oocyte collection, has been shown to be a more promising treatment to female infertility compared with recombinant human FSH. In the study by Ng *et al*. (2001), 85% of the oocytes reached metaphase at IVF treatment 2; consequently, it was tested whether there would be an additional benefit to administering FSH on top of hCG in priming *in vitro* maturation (IVM). Third to fifth cycles were treated with 75 IU rFSH for 6 days, while 33 cycles were given 10,000 IU hCG 36 h before oocyte retrieval and fertilization via IVF and ICSI. The resulting embryo was replaced on day 2 or 3. A total of 1528 immature oocytes were found. The overall maturation and fertilization rates were 74.2 and 72.8%, respectively. After embryo transfer, 23 pregnancies (33.8%).

The use of hCG in animals, specifically on follicular growth and development *in vivo* or *in vitro*, has been investigated. Due to its similarity to LH, hCG may also be used clinically to induce ovulation in the ovaries. For this reason, some organizations collect urine from pregnant women to extract hCG. Using hCG, comparative follicles begin to occur simultaneously, but many cases become atretic. During the luteal phase of the follicular growth cycle, one of the follicles becomes dominant. Notably, biochemical changes in the development of subordinate follicles to dominant follicles, when analyzed for chemical changes, are different from those in FSH, LH and receptor (Zemitis *et al*., 2015).

In the study of molecular hCG, folliculogenesis is highly dependent on the influence of gonadotropin hormone; antral folliculi is responsible for FSH-LH. Inhibin, activin, insulin-like growth factor I (IGF I) and protein walls (bonds with proteins) directly affect granulosa cells and techa cells that stimulate follicular growth and steroidogenesis (Gardner *et al*., 2004). Injection of 1,000 IU hCG exogenously within 24 h of sow postpartum farrowing induced ovulation in 41%-75% of the population at 7-10 days after injection (Armstrong *et al*., 1999; Kirkwood *et al*., 1999). Postpartum sow ovaries have potentially estrogenic medium-sized follicles (4 to 5 mm), and some sows have exhibited estrous behavior (De Rensis *et al*., 1993; Sesti and Britt, 1993; Sesti and Britt, 1994; Langendijk *et al*., 2007). But the postpartum estrous behavior observed at 2=4 days postpartum farrowing is anovulatory, which is likely due to the inability to generate a pre-ovulatory LH surge (Sesti and Britt, 1993).

Medical experts up to this day have been relying on LH activity-dependent alone in inducing final oocyte maturation, which overlooks the redundancy of the natural waves of mid-cycle FSH surge. Thus, this needs to be resolved. Evidence from clinical research suggests that in an undetermined subset of patients, the LH and FSH waves are doubly advantageous compared to the LH spike in the form of a human chorionic gonadotropin (hCG) trigger. Double waves can be triggered by gonadotropin-releasing hormones, agonist boluses, causing endogenous LH and FSH flare-ups and resembling natural waves of concomitant hCG to ensure adequate exposure to LH activity (Kol and Humaidan, 2010). Further research is needed to characterize hCG from urine of pregnant women, which can then be potentially used for *in vitro* maturation of Madura cattle oocytes.

The purpose of this research was to extract hCG from urine of pregnant women gestating at 1.5 to 3.5 months, characterize and determine the molecular weight of the hCG protein and perform a biological potential test to determine its effect on the maturation level of Madura cattle oocytes.

MATERIALS AND METHODS

HCG isolation, identification and purification

Urine samples were taken from fifty healthy pregnant women gestating for 1.5 to 3.5 months. Samples were obtained by a midwife from a clinic in Surabaya, East Java, Indonesia. In the morning, 100 ml of urine was collected from each pregnant woman and these were centrifuged at 3,000 rpm for 15 min at 4°C to separate the metabolite cells. Thirty mg/100 ml activated charcoal powder was added, and the mixture was centrifuged at 3,000 rpm at 4°C for 20 min (Green and Leake, 1987) until mixture was homogeneous. Charcoal removes toxic substances, dyes and steroid hormones; it also absorbs and inactivates organic chemicals. Supernatant was filtered through an Erlenmeyer tube using a filter paper, producing a 50 ml supernatant. This was poured into Sephadex G-100 filtration device and poured into a chamber with running buffer

to carry out electrophoresis (Sakakibara *et al*., 1987). Furthermore, hCG extract obtained from urine extract was processed through SDS-PAGE and ELISA (Gam and Latiff, 2005). A running gel was inserted through the wall roughly less than the upper limit. Around 1 ml of butanol was added, and the gel was left for 25 min until it has solidified. Butanol was discarded, and the gel was cleansed with PBS and dried with Whatman paper. Comb was removed and remnants of the gel with buffer were cleaned.

HCG concentration

HCG concentration was determined through ELISA based on the sandwich principle (Mahaputra and Mustafa, 2003). The microtiter wells were coated with a monoclonal antibody directed towards a unique antigenic site of the hCG molecule. An aliquot of sample containing endogenous hCG was incubated in the coated well. After washing, a second incubation followed with an enzyme conjugate, an anti-hCG antibody conjugated with horseradish peroxidase. After incubation, the unbound conjugate was washed off. The amount of bound peroxidase was proportional to the concentration of hCG in the sample. Having added the substrate solution, color intensity developed was proportional to the concentration of hCG in the sample (Kariman *et al*., 2011).

***In vitro* maturation of cattle oocytes**

Extracted hCG was used to investigate its potential in inducing *in vitro* maturation in Madura beef cattle oocytes. There were two setups: the control group was given TCM 199 + 0.5 IU pregnant mare serum gonadotrophin (PMSG) + 0.5 IU hCG (Chorulon, Intervet, Holland), while the experimental group received the same treatment but with 0.5 IU hCG from gestating women.

Ovaries from slaughterhouses were washed 2 to 3 times with physiological saline solution, placed in a glass above water bath at 37°C, taken one by one with sterile tweezers and dried with sterile tissue paper. Using a 10 ml sterile disposable syringe, samples were washed with 1.5 ml of oocyte washing medium. Suction was done by stabbing the adjacent part of the ovary parenchyma in the next follicle bubble and directing the needle tip to the follicle (approximately 5 mm in diameter) near the point of needling (without removing the needle first). After each follicle fluid was siphoned off (about 3-4 ml), this was carefully transferred into test tubes to avoid mechanical damage to the oocyte and placed in a water bath. After the oocyte had dropped to the bottom of the tube, precipitate was evaluated by placing it on a large petri dish and examining under a stereo microscope (Mahaputra and Mustofa, 2003).

Once an oocyte was found, this was collected with a modified pastry pipette (of the same diameter as the oocyte), placed on a smaller petri dish with washing medium and examined again under a microscope to determine its quality (Mahaputra and Mustafa, 2003). Afterwards, all oocytes were washed 3-4 times with washing medium and was finally washed with 2.5-3 ml of tissue culture medium 199 (TCM 199). Oocytes with no cumulus debris were transferred into the maturation medium, dropping 100 µl each in a petri dish. A total of 10 oocytes were used in this study, prepared 2 h earlier in incubator with 5% CO_2. Four 100 µl drops of maturation medium were placed in each 35 mm sterile petri dish. Each drop of medium can culture as much as 5 oocytes. Media were then covered with mineral oil and placed in an incubator containing 5% CO_2 set at 39°C and humidity of 95-100% for 24 h. One percent aceto-orcein stain was added to determine maturity level of egg cell (Chen *et al*., 2013).

To collect data on egg maturity, harvested eggs were placed and covered on the glass object, then placed in a fixative solution for 48 h. These were stained with 1% aceto-orcein for 2-3 min, washed with solution and viewed under an inverted microscope. Assessment of egg maturity level was based on germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I and metaphase II criteria (de Oliviera *et al*., 2016). Mature oocytes were identified by locating those which can resume meiosis (extruding a first polar body) and can undergo fertilization (Fig. 1).

Data analysis

Data on identification, isolation, purification and measurement of hCG concentration from the urine of pregnant women at 1.5 to 3.5 months were processed descriptively. Student's t-test (Steel and Torrie, 1960) was used to determine the difference on oocyte maturation rate between patent hCG (Chorulon, Intervet, Holland) and

hCG from urine of pregnant women.

RESULTS AND DISCUSSION

HCG isolation, identification and purification

SDS-PAGE results showed that hCG from the urine of pregnant women had molecular sizes of 37 kDa and 22 kDa (Fig. 2). Gam and Latiff (2005) detected other bands from non-reduced fractionally desialylated-hCG sample: 43.5, 38.5, 29.45 and 20.85 kDa, which, after being reduced, resulted to an uncompounded pledge at 35.2 kDa.

Calculation of hCG concentration

The human pregnant urine ammonium sulphate precipitated concentrations of hCG at age 1.5 and 3.5 month were 27,333 m IU/l and 105,66 m IU/l, respectively. The measurement of hCG provides a specific test for pregnancy. Lyophilized hCG, although stable at room temperature for 3 weeks, should be stored at 2-8°C (Suthar and Shah, 2009). It is advised to reconstitute it in sterile 18 M-cm H_2O at a concentration of 1,000 IU/ml, which can be further diluted to other aqueous solutions.

In vitro maturation of cattle oocytes

Oocytes that matured *in vitro* are capable of resuming meiosis, extruding a first polar body and can undergo fertilization (Fig. 1). In this research, the rate of maturation of cow oocytes in TCM 199 + 0.5 IU PMSG + 0.5 IU hCG (Chorulon, Intervet, Holland) was 91.15± 8.57, while maturation rate in TCM 199 + 0.5IU PMSG + 0.5 IU hCG from pregnant women was 90.54±12.86. T-test showed no significant difference between groups (P>0.05) (Table), suggesting that hCG from pregnant woman can be used for maturation process of cow oocytes.

The dairy cattle industry has perfected the application of the first reproductive biotechnology, *i.e.*, artificial insemination (AI), a success story which made use of embryo transfer technology (ETT). In addition, emerging researchers have taken interest in the field of transvaginal oocyte recovery (TVOR) and *in vitro* embryo production (IVEP). IVF has paved the starting point for the production of generative material for many sophisticated reproduction techniques, like sperm microinjection into mature oocytes. In several countries, commercial IVF facilities are already being serviced by cattle ET operators. Also, various research groups have reported on the modification of TVOR (Suthar and Shah, 2009).

This study tested the use of hCG from urine of pregnant women in inducing maturation of cattle oocytes. Recorded molecular weight of hCG via SDS-PAGE was 37 and 22 kDa. HCG concentrations via ELISA assay had 10 times th[illegible]eposition, with average level of [illegible],333 m IU/l at 1.5 months and 105,667 m IU/l at 3.5 months. T-test revealed no significant difference (P>0.05) between groups. This study illustrates that hCG extracted from urine of pregnant women at 1.5 to 3.5 months can be used to induce *in vitro* maturation in Madura cattle oocytes.

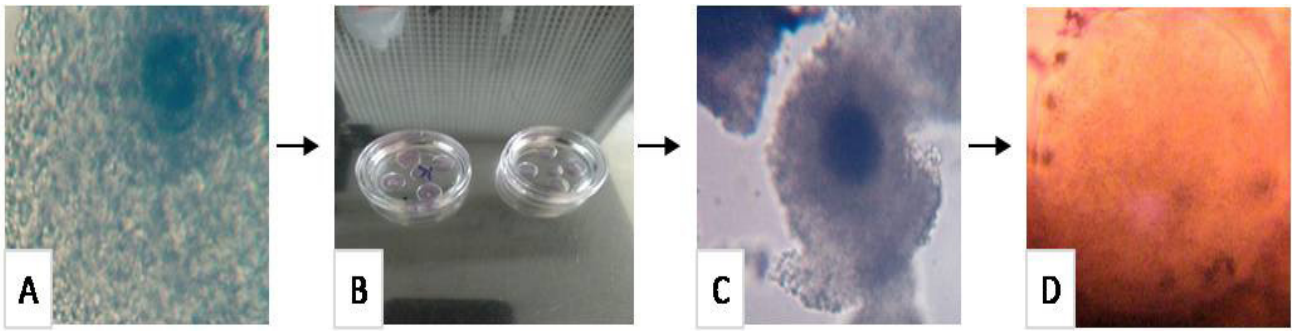

Fig. 1. *In vitro* maturation culture of Madura beef cattle oocytes at different stages. A: addition of hCG to maturation culture; B: immature oocyte; C mature oocyte; D: egg cells at metaphase stage.

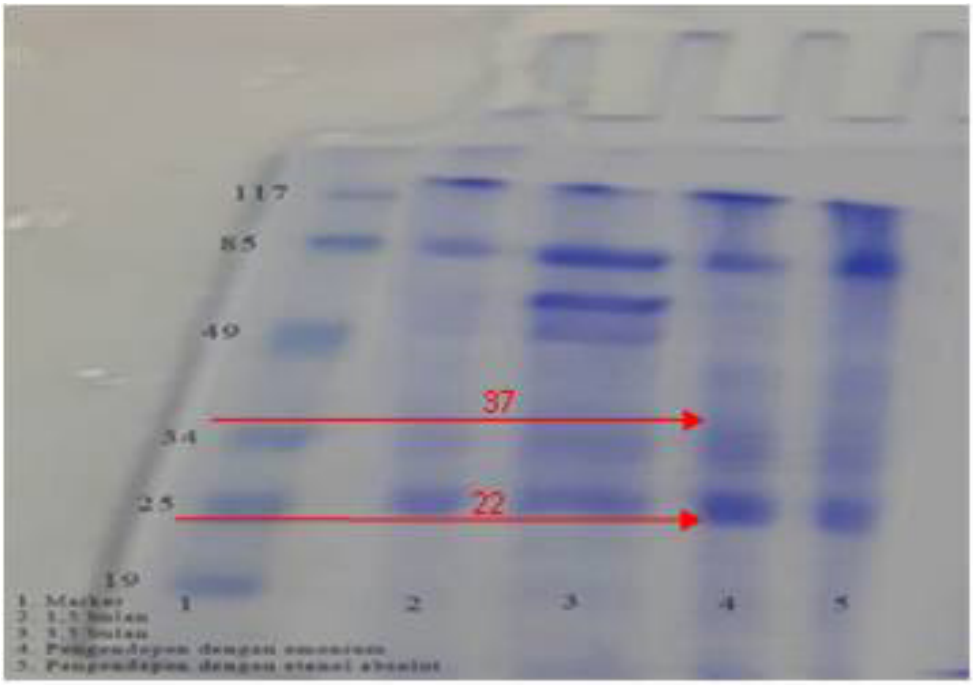

Fig. 2. SDS-PAGE shows molecular weight of hCG from urine of pregnant woman at 37 and 22 kDa.

Table. Rate of maturation in cattle oocytes induced by different HCG sources.

hCG used	*n*	Mean ±SD
TCM 199 + 0.05 PMSG + 0.5 IU hCG 0.5 hCG (Chorulon, Intervet, Holland)	50	91.15± 8.57[a]
TCM 199 + 0.5 IU PMSG + 0.5 IU hCG from pregnant women	50	90.54±12.86[a]

ACKNOWLEDGMENT

This research was partially supported by funding from the Directorate General of Higher Education (DIKTI), Ministry of National Education, Indonesia.

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