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New Submission

1 message

erma safitri <erma-s@fkh.unair.ac.id>
To: Ind Vet Journal <ivj83@yahoo.com>

Mon, Mar 4, 2019 at 7:30 AM

Dear Editor Indian Veterinary Journal

I hereby send our manuscript research article with the tittle :

human Chorionic Gonadotropin (hCG) from urine pregnant women to manipulate invivo ovulation and pregnanation of Madura cows

Keywords: Madura Cows, hCG, Pregnant Women Urine, Ovulation, Pregnancy

The article we sent has not been previously submitted and has not been submitted for evaluation in other journals.

Kindly considation our article for published in Indian Veterinary Journal

Thank you

Best Regard,
Corresponding Author,
Dr. Safitri E., DVM, M.Si
Veterinary Medicine Faculty of Universitas Airlangga
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Acknowledgement Letter # 77/19

1 message

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To: erma-s@fkh.unair.ac.id

Thu, Mar 7, 2019 at 12:45 PM

ACKNOWLEDGEMENT

Reg. No: 77/19

Dated : 7/03/2019

Dear Dr. Erma Safitri,

We acknowledge the receipt of the following articles entitled "humanChorionic Gonadotropin (hCG) from urine pregnant women to manipulate invivo ovulation and pregnation of Madura cows" (Erma Safitri, et al.).

For any further correspondence, please always quote the Registration Number of the Article.

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human Chorionic Gonadotropin (hCG) from urine of pregnant women to manipulate in vivo ovulation and pregnancy of Madura cows

Herry A. Hermadi, R.T.S. Adikara, Sunaryo H. Warsito, Erma Safitri*
Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, 60115

Abstract

The purpose of study was to determine biological potential of hCG for ovulation and pregnancy in Madura cows. The research was done using 45 Madura cows no pregnant and no reproductive disorders divide three groups: 15th cows were i/m injected with 500 IU of hCG from patent product (Chorulon, Intervet Holland) (T1), 15th cows were i/m injected with 500 IU of hCG from isolate of pregnant women urine (T2), 15th cows untreated synchronized group as control group (C). The results showed that there was no significant difference $p > 0.05$ for ovulation and pregnancy, between hCG from patent product and from pregnant women urine.

Keywords : Madura cows, hCG, Pregnant women urine, Ovulation, Pregnancy.

hCG is a gonadotropin hormone which is extracted from pregnant women urine at gestational 1.5 to 3.5 months (Hermadi *et al.*, 2018) and biologically it was known as LH like (Nwabuobi *et al.*, 2017). hCG have a therapeutic effect on receptive women. The use of hCG for in vitro process of cattle oocyte maturation has been done (Hermadi *et al.*, loc cit), but in vivo biological potential of hCG for ovulation and pregnancy has not been able to explain.

Materials and Methods

Urine collection of pregnant women 1.5 to 3.5 months, using charcoal for eliminating of steroid hormones and separating of hCG with sephadex columns chromatography G-100, Characterization of hCG isolates with SDS-PAGE and Western Blotting and purification was used Elusi method, Biological potential test of hCG for ovulation and pregnancy in Madura cows. 45th Madura cows no pregnant and no reproductive disorders, 2-3 years age with a minimum body score of 2 previously treated with feed concentrate a protein 15-17%, 3 kg/day/cow for 1 month. The cows were randomly divided : 15th cows were i/m injected with 500 IU of hCG from patent product (Chorulon, Intervet Holland) (T1), 15th cows were i/m injected with 500 IU of hCG from isolate of pregnant women urine (T2), 15th cows untreated synchronized group as control group (C). Before hCG treatment for manipulating ovulation and pregnancy were used PGF2 (15/gr) for estrus synchronized to Madura cows and then AI twice.

Results and Discussion

Pregnancy tests, quantitative blood tests and the most sensitive urine tests in humans usually detect hCG between 6 and 12 days after ovulation (Fricke *et al.*, 1993) However, it must be taken into account, however, that the level of total hCG can vary in a very wide range in the first 4 weeks of pregnancy, which causes incorrect results during this period (Butler *et al.*, 2001). An increase of 35% for 48 hours is proposed as a minimal increase consistent with an adequate intrauterine pregnancy (Santos *et al.*, 2001).

The results were showed positif estrus and ovulation and diagnosis of dominant follicles in ovary of estrus cow with USG tool (Figure 1)



Figure 1. Diagnosis of dominant follicles in ovary of estrus cow with USG tool

The results of ovulation were showed significant difference $p < 0.05$ between control group (C) with hCG from patent product (T1) and from pregnant women urine (T2), but no significant difference $p > 0.05$ between T1 and T2. This result allows the ovary to provide an opportunity to develop subordinate follicles to de Graaf follicles. The all groups: C, T1 and T2 were showed 100% of estrus symptoms, but just T1 and T2 were showed 100% ovulation based on diagnosis of dominant follicle in ovary of estrus cows with USG tool. In addition, the LH or hCG receptor is also expressed in granulosa cells. The hCG or LH signaling pathways do not fully overlap, and this fact may have implications for the use of hCG in reproduction techniques (Cole, 2010). The illustrating that gonadotropin-releasing hormone (GnRH) agonists, initially presented as a substitute for hCG, have led to a new era of GnRH agonists followed by hCG which triggers ovulation (Cole, 2009).

After ovulating that on day 60th in the T1 group with hCG from Chorullon, Intervet-Holland and T2 group with hCG from pregnant woman urine group respectively were showed a positive pregnancy of 100% (15 cows) and 90% (14 cows) and diagnosis of the 60th days of pregnancy appears on an ultrasound (USG) image (Figure 2).

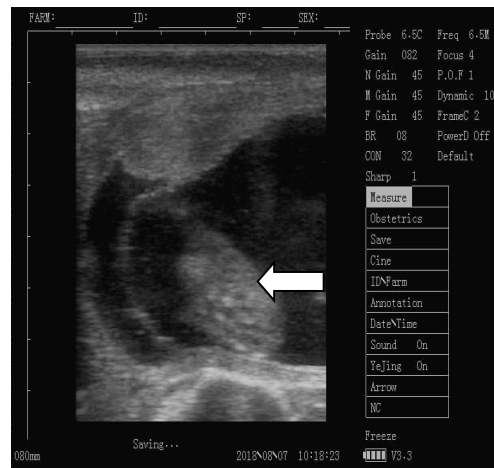


Figure 2. Diagnosis of the 60th day of pregnancy using on an ultrasound (USG)

The hCG is a glycoprotein hormone which is isolated and purified for getting hCG proteins (Nwabuobi *et al.*, loc cit). The molecular weight of hCG are 37 and 22 kDa corresponds to a 10% sds-page result. The level of hCG in urine at 1.5 months of pregnancy women was 10.567 mIU / mL. The level of hCG in urine at 3.5 months of pregnancy women was 13.444 mIU / mL (Hermadi *et al.*, loc cit).

hCG is the Gonadotropin hormone extracted from the urine of pregnant women who have the highest concentration found at 1.5 to 3.5 months of gestation or 8 to 11 weeks to 4 months or 12-15 weeks during a positive pregnancy test. Biology is known to be identical to LH and is often called LH like. hCG is a glycoprotein hormone (Hermadi *et al.*, loc cit). hCG is not of a specific species which means that even though the urine produced from a pregnant woman continues to have a therapeutic effect on receptive women or patients, even hCG may be directly involved in the IVF process (Invitro fertilization) in humans. Comparative follicles begin to occur simultaneously but many become atresia during the luteal phase of the follicular growth cycle where one of them becomes the dominant follicle. The biochemical changes in the

development of subordinate follicles to the dominant follicles when analyzed for chemical changes occur differently from the principles of FSH, LH and receptors (Daya, *et al* 1995; Roche, 1996). The use of hCG for the purpose of folliculogenesis in cattle has not been able to explain the mechanism of both *in vivo* development of follicles in the ovary and *in vitro* in the process of oocyte cattle maturation. Is the mechanism to follow endogenous FSH - LH rules or other factors. Research is needed that can help monitor ultra sonografi (ultrasound) instruments to determine follicular growth waves (Follicular waves) or oocyte maturation monitoring *in vitro*.

hCG is secreted by syncytiotrophoblast originating from diffuse and differentiated cytotrophoblast cells (Choi and Smith, 2014; Nwabuobi *et al.*, *loc cit*) for a long time, the main known role of hCG is the promotion of progesterone secretion by the corpus luteum in early pregnancy (Ng *et al.*, 2001), acting through hCG or LH receptors. However, recently, many other functions of hCG, not only in the placenta but also in the myometrium, uterus and fetus, have been described (Cole, *loc cit*; Kirk *et al.*, 2013; Guibourdenche *et al.*, 2009).

Cellular studies of folliculogenesis begin with the GnRH hormone, prevention of a low hormonal balance of FSH-LH is corrected first, for example, feed rations are improved with good and balanced feed quality, better environment, cleaner cages, air movement good, looser, often taken out of the cage to move more. If the situation has become better followed by injecting FSH-LH preparations or likes (FSH-LH) (5). Human Chorionic Gonadotropin (hCG) does not have a specific species meaning even though it is produced from the urine of pregnant women still giving therapeutic effects to prescribed women or patients, even hCG can be involved directly in IVF (*In vitro* fertilization) in humans implantation process after embryo transfer depends on quality embryo and endometrial reception. It is estimated that fifty to seventy-five percent of pregnancies are lost due to implantation failure. There is evidence that there is an initial chorionic gonadotrophin secretion before implantation embryos, and this secretion has been associated with important functions in angiogenesis and the inflammatory response that drives the implantation process.

Summary :

The conclusion, manipulate *in vivo* by use of hCG patent and hCG pregnant woman urine was followed by estrus, ovulation and pregnancy of Madura cows. The results showed that there was no significant difference $p > 0.05$ between the treatment groups (hCG from patent product (T1) and from pregnant women urine (T2)).

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Article # 77/19 for revision & Referee comments & IVJ revised guidelines attached

2 messages

Ind Vet Journal <ivj83@yahoo.com>
Reply-To: Ind Vet Journal <ivj83@yahoo.com>
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Sun, Mar 31, 2019 at 9:54 PM

Sir / Madam,

Revise the paper according to the referees comments and corrections marked on the manuscript and resubmit the revised article as per IVJ format for further process.

Sincerely

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Fri, Apr 5, 2019 at 1:54 PM

Dear Editor Ind Vet Journal

I have revised the paper according to the referee's comments and corrections marked on the manuscript and resubmit the revised article as per IVJ format for further process.

ARTICLE # 77/19**TITLE : *human* Chorionic Gonadotropin (hCG) from urine....**

Author Answer for Editor comments

1. We have revised the title according editor sugestion.
2. We have revised introduction without sub tittle & 5 lines
3. We have revised the Materials and Methods 10 lines
4. We have delete the table.
5. .We have added short summary of 3-4 lines
6. References have revised and following IVJ format & alphabetic
7. We have revised extensively and as full research article of 5-6 pages.

Author Answer for Comments on article A-77/19

* We have revised (in abstract & Materials & Methodes): The research was done using 45 Madura cows no pregnant and no reproductive disorders devide three groups: 15th cows were i/m injected with 500 IU of hCG from patent product (Chorulon, intervet Holland) (T1), 15th cows were i/m injected with 500 IU of hCG from isolate of pregnant women urine (T2), 15th cows untreated synchronized group as control group (C).

* We have revised in Materials & Method : Before hCG treatment for manipulating ovulation and pregnancy were used PGF2 (15/gr) for estrus synchronized to Madura cows and then AI twice.

* We have completed materials & method : Urine collection of pregnant women 1.5 to 3.5 months, using charcoal for eliminating of steroid hormones and separating of hCG with sephadex coloumns chromatography G-100, Characterization of hCG isolates with SDS-PAGE and Western Blotting and purification was used Elusi method, Biological potential test of hCG for ovulation and pregnantly in Madura cows.

* We have deleted Table 1 & 2

* We have listed completely & as per format of IVJ

* We have revised of English language

Thank you for your considered and chance for me to revise our article

Corresponding Author

Erma Safitri

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Comments on Article A-77/19

"human Chorionic Gonadotropin (hCG) from urine of pregnant women to manipulate in vivo ovulation and pregnancy in Madura cows"

By Herry A. Hermadi *et al.* from Surabaya, Indonesia

1. The manuscript describes the comparative utility of patented hCG (Chorulon) and hCG isolates from pregnant women urine, 500 IU i/m at AI, for manipulating ovulation and pregnancy in PGF2 α synchronized Madura cows (15/gr), and concludes that there was no significant difference between two groups for ovulation (100%) and pregnancy (100% vs 95%). The idea is novel but raises following queries for the readers.
2. Here the question is whether 100% ovulation was spontaneous or due to hCG can't be claimed in absence of untreated synchronized group. Further 100% pregnancy with single FTAI is also practically unexpected, since in routine course we get only 50-60% conception through first AI/breeding.
3. In materials and method complete protocol of extraction/isolation, purification and standardization of hCG from pregnant women urine should be described. In R&D, how one can confirm 100% pregnancy by day 16 post-ovulation by CL or even USG? There is need to revisit this statement.
4. Table 1 and 2 showing result of individual animals are not required as the sum is 100% in both the tables/groups. Reference listing should be complete and as per the format of Journal.
5. The English language throughout the manuscript need to be corrected by an English proficient researcher/teacher. Some of the local terms cited are not understandable, some statements are not clear. These are pointed out in the manuscript
6. The revised improved justified manuscript may be considered to publish in the IVJ.

English need to be improved throughout the manuscript.

RR 25/3/19

human Chorionic Gonadotropin (hCG) from urine of pregnant women to manipulate in vivo ovulation and pregnancy of Madura cows

Herry A. Hermadi, R.T.S., Adikara, Sunaryo H. Warsito, and Erma Safitri*

Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

postal code no.?

Abstract

The study purpose was to determine of biological potential of hCG for ovulation and pregnancy in Madura cows. The research was using 30 Madura female cows no pregnant and no reproductive disorders with two groups. The control groups (T1) 15 cows were intramuscular injected with 500 IU of hCG from patent product (Chorulon, Intervet Holland). The treatment groups (T2) 15 cows were intramuscular injected with 500 IU of hCG from isolate of pregnant women urine. The research was using t test in statistical analysis. The results showed that there was no significant difference $p > 0.05$ for ovulation and pregnancy, between hCG from patent product (T1) and from pregnant women urine (T2).

Keywords : Madura Cows, hCG, Pregnant Women Urine, Ovulation, Pregnancy.

human Chorionic Gonadotropin (hCG) is a Gonadotropin hormone which is extracted from the urine of pregnant women who have the highest concentration found at gestational ages 1.5 to 3.5 months (Hermadi et al., 2018). The hCG is a glycoprotein hormone and biologically it is known to be identical to LH and then is often called LH like (Nwabuobi et al., 2017). hCG is not of a specific species which means that even though the urine produced from a pregnant woman continues to have a therapeutic effect on receptive women or patients, even hCG may be directly involved in the IVF (In vitro fertilization) process in humans. The use of hCG for the purpose in vitro in the process of oocyte cattle maturation has been done (Hermadi et al., 2018) but in vivo has not been able to explain. The study purpose was to determine of biological potential of hCG for ovulation and pregnancy in Madura cows.

Materials and Methodes

Three stages of the research: 1. Urine Collection of pregnant women 1.5 to 3.5 months extra-healthy charcoal to eliminate steroid hormones, cool sterilization and separation of hCG with sephadex columns chromatography G-100; 2. Characterization of hCG isolates with SDS-PAGE, Elusi techniques and Western Blotting with nitrocellulose membranes (Hybond-C pure.

Corresponding Author, Email : erma-s@fkh.unair.ac.id

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of this study

Give detailed protocol of hCG isolation/purification from women urine.
Corr. author email ID: ?

nitrocellulosa membrane, Amersham Life Science-England); 3. Biological potential test of hCG for keberhasilan ovulation and pregnant in Madura Cows.

30 Madura female cows no pregnant and no reproductive disorders, aged 2-3 years with a minimum body score of 2 previously treated with feed milk concentrate a protein 15-17% (Charoen PhokPhand, Indonesia) 3 kg/day/cow for 1 month. The groups randomly was divided into 2 groups, the control groups (T1) = 15 cows were intra muscular injected with 500 IU of hCG from patent product (Chorullon, Intervet-Holland) and the treatment groups (T2) = 15 cows were intra muscular injected with 500 IU of hCG from isolate of pregnant women urine. The research was using t test in statistical analysis. Before treatment, the cows were synchronized of estrus with PGF2 α (Glandin, Intervet-Holand) 25 mg, pola 2 kali twice. The second injection was carried out at 11-days interval. 72 hours later injection of hCG is carried out of artificial insemination, after estrus has appeared

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Results and Discussion

The results were showed positif estrus and ovulation (Table 1) and diagnosis of dominant follicles in ovary of estrus cow with USG tool (Figure 1)

Table 1. Estrus Synchronization with PGF2 α and Combined with hCG for Ovulation

Positif Estrus and Ovulation			
hCG(Chorullon, Intervet-Holland) (T1 Group)	Result	hCG(Pregnant Women Urine) (T2 Group)	Result
C.1	+	U.1	+
C.2	+	U.2	+
C.3	+	U.3	+
C.4	+	U.4	+
C.5	+	U.5	+
C.6	+	U.6	+
C.7	+	U.7	+
C.8	+	U.8	+
C.9	+	U.9	+
C.10	+	U.10	+
C.11	+	U.11	+
C.12	+	U.12	+
C.13	+	U.13	+
C.14	+	U.14	+
C.15	+	U.15	+
Total	+ 15		+ 15

- Note required some research running statement

100%
 → The formulation & pregnancy was due to hCG (patent or urine) can not be proved unless a synchronized non-hCG treated group is kept and compared. The present almost-similar results in both the groups could be due to spontaneous ovulation from synchronized estrus even without effect of hCG.

100% pregnancy through single AI is also practically impossible.

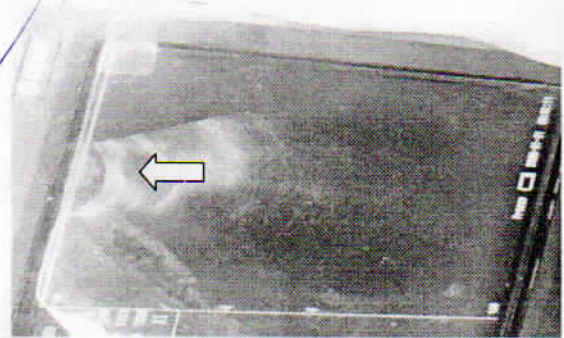


Figure 1. Diagnosis of dominant follicles in ovary of estrus cow with USG tool

The results of estrus and ovulation were showed no significant difference with the T test > 0.05. This result ^{treatm} allows the ovary to provide an opportunity to develop subordinate follicles to de Graaf follicles. The both groups T1 and T2 were showed 100% of estrus symptoms. In addition, the LH or hCG receptor is also expressed in granulosa cells. The hCG or LH signaling pathways do not fully overlap, and this fact may have implications for the use of hCG in reproduction techniques Cole (2010). The illustrating that gonadotropin-releasing hormone (GnRH) agonists, initially presented as a substitute for hCG, have led to a new era of GnRH agonists followed by hCG which triggers ovulation (Cole, 2009; Cole, 2010).

After ovulating that on day 16 everything in the T1 group with hCG from Chorullon, Intervet-Holland and T2 group with hCG from pregnant woman urine group were showed a positive pregnancy of 100% (15 cows) and 90% (14 cows) berturut-turut (Table 2) and diagnosis of the 60th days of pregnancy appears on an ultrasound (USG) image (Figure 2).

The hCG is a glycoprotein hormone which is isolated and purified for getting hCG proteins (Nwabuobi et al., 2017). The molecular weight of hCG are 37 and 22 Kda corresponds to a 10% sds-page result. The level of hCG urine at 1.5 months of pregnancy women was 10.567 mIU / mL. The level of hCG urine at 3.5 months of pregnancy woman was 13.444 mIU / mL (Hermadi et al., 2018).

How can we say confirm pregnancy on day 16?

not required

Table.2 Diagnosis of Pregnancy 60 Days with Ultrasound (USG)

hCG (Chorullon, Intervet-Holland) (T1 Group)	Result	hCG (Pregnant Women Urine) (T2 Group)	Result

C.1	+	U.1	+
C.2	+	U.2	+
C.3	+	U.3	+
C.4	+	U.4	+
C.5	+	U.5	+
C.6	+	U.6	+
C.7	+	U.7	+
C.8	+	U.8	+
C.9	+	U.9	+
C.10	+	U.10	-
C.11	+	U.11	+
C.12	+	U.12	+
C.13	+	U.13	+
C.14	+	U.14	+
C.15	+	U.15	+
Total	+ 15		+14

hCG is secreted by syncytiotrophoblast originating from diffuse and differentiated cytotrophoblast cells (Choi and Smith, 2014; Nwabuobi *et al.*, 2017) for a long time, the main known role of hCG is the promotion of progesterone secretion by the corpus luteum in early pregnancy (Hung *et al.*, 2010), acting through hCG or LH receptors. However, recently, many other functions of hCG, not only in the placenta but also in the myometrium, uterus and fetus, have been described (Cole *et al.*, 2010; Kirk *et al.*, 2013; Guibourdenche *et al.*, 2009).

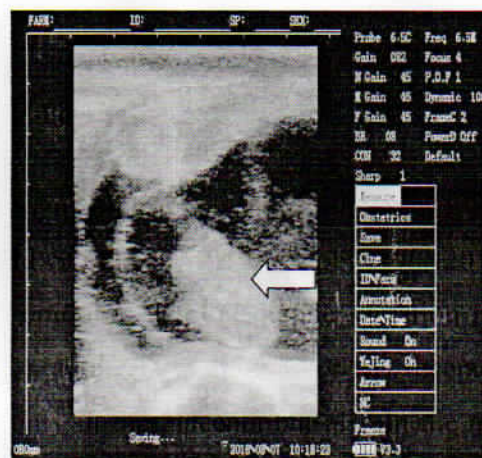


Figure 2. Diagnosis of the 60th day of pregnancy appears on an ultrasound (USG) image in the ultrasound image, the oblong round head and the hyper ecogenic spine show bone white and the hypoecogenic appearance of the middle is amniotic fluid.

Summary:

The conclusion, manipulate ⁱⁿ vivo by used of hCG patent and hCG pregnant woman urine was followed by estrus, ovulation and pregnancy of Madura cows. The results showed that there were no significant differences $p > 0.05$ between the both treatment groups.

References

Alphabetical order?

or 2010?

✓ Hung, Y.E., Estella, Y.L.L., William, S.B.Y. and Ho, P.C. (2000). hCG is as good as recombinant human FSH in term of oocyte and embryo quality : a prospective randomized trial Dept of obstetrics and gynaecology, Queen Mary hospital, the University of Hongkong. ?

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Fri, Apr 5, 2019 at 1:54 PM

Dear Editor Ind Vet Journal

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ARTICLE # 77/19**TITLE : *human Chorionic Gonadotropin (hCG) from urine....***

Author Answer for Editor comments

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Author Answer for Comments on article A-77/19

* We have revised (in abstract & Materials & Methodes): The research was done using 45 Madura cows no pregnant and no reproductive disorders devide three groups: 15th cows were i/m injected with 500 IU of hCG from patent product (Chorulon, intervet Holland) (T1), 15th cows were i/m injected with 500 IU of hCG from isolate of pregnant women urine (T2), 15th cows untreated synchronized group as control group (C).

* We have revised in Materials & Method : Before hCG treatment for manipulating ovulation and pregnancy were used PGF2 (15/gr) for estrus synchronized to Madura cows and then AI twice.

* We have completed materials & method : Urine collection of pregnant women 1.5 to 3.5 months, using charcoal for eliminating of steroid hormones and separating of hCG with sephadex coloumns chromatography G-100, Characterization of hCG isolates with SDS-PAGE and Western Blotting and purification was used Elusi method, Biological potential test of hCG for ovulation and pregnantly in Madura cows.

* We have deleted Table 1 & 2

* We have listed completely & as per format of IVJ

* We have revised of English language

Thank you for your considered and chance for me to revise our article

Corresponding Author

Erma Safitri

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human Chorionic Gonadotropin (hCG) from urine of pregnant women to manipulate in vivo ovulation and pregnancy of Madura cows

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Abstract

The purpose of study was to determine biological potential of hCG for ovulation and pregnancy in Madura cows. The research was done using 45 Madura cows no pregnant and no reproductive disorders divide three groups: 15th cows were i/m injected with 500 IU of hCG from patent product (Chorulon, Intervet Holland) (T1), 15th cows were i/m injected with 500 IU of hCG from isolate of pregnant women urine (T2), 15th cows untreated synchronized group as control group (C). The results showed that there was no significant difference $p > 0.05$ for ovulation and pregnancy, between hCG from patent product and from pregnant women urine.

Keywords : Madura cows, hCG, Pregnant women urine, Ovulation, Pregnancy.

hCG is a gonadotropin hormone which is extracted from pregnant women urine at gestational 1.5 to 3.5 months (Hermadi *et al.*, 2018) and biologically it was known as LH like (Nwabuobi *et al.*, 2017). hCG have a therapeutic effect on receptive women. The use of hCG for in vitro process of cattle oocyte maturation has been done (Hermadi *et al.*, loc cit), but in vivo biological potential of hCG for ovulation and pregnancy has not been able to explain.

Materials and Methods

Urine collection of pregnant women 1.5 to 3.5 months, using charcoal for eliminating of steroid hormones and separating of hCG with sephadex columns chromatography G-100, Characterization of hCG isolates with SDS-PAGE and Western Blotting and purification was used Elusi method, Biological potential test of hCG for ovulation and pregnancy in Madura cows. 45th Madura cows no pregnant and no reproductive disorders, 2-3 years age with a minimum body score of 2 previously treated with feed concentrate a protein 15-17%, 3 kg/day/cow for 1 month. The cows were randomly divided : 15th cows were i/m injected with 500 IU of hCG from patent product (Chorulon, Intervet Holland) (T1), 15th cows were i/m injected with 500 IU of hCG from isolate of pregnant women urine (T2), 15th cows untreated synchronized group as control group (C). Before hCG treatment for manipulating ovulation and pregnancy were used PGF2 (15/gr) for estrus synchronized to Madura cows and then AI twice.

Results and Discussion

Pregnancy tests, quantitative blood tests and the most sensitive urine tests in humans usually detect hCG between 6 and 12 days after ovulation (Fricke *et al.*, 1993) However, it must be taken into account, however, that the level of total hCG can vary in a very wide range in the first 4 weeks of pregnancy, which causes incorrect results during this period (Butler *et al.*, 2001). An increase of 35% for 48 hours is proposed as a minimal increase consistent with an adequate intrauterine pregnancy (Santos *et al.*, 2001).

The results were showed positif estrus and ovulation and diagnosis of dominant follicles in ovary of estrus cow with USG tool (Figure 1)



Figure 1. Diagnosis of dominant follicles in ovary of estrus cow with USG tool

The results of ovulation were showed significant difference $p < 0.05$ between control group (C) with hCG from patent product (T1) and from pregnant women urine (T2), but no significant difference $p > 0.05$ between T1 and T2. This result allows the ovary to provide an opportunity to develop subordinate follicles to de Graaf follicles. The all groups: C, T1 and T2 were showed 100% of estrus symptoms, but just T1 and T2 were showed 100% ovulation based on diagnosis of dominant follicle in ovary of estrus cows with USG tool. In addition, the LH or hCG receptor is also expressed in granulosa cells. The hCG or LH signaling pathways do not fully overlap, and this fact may have implications for the use of hCG in reproduction techniques (Cole, 2010). The illustrating that gonadotropin-releasing hormone (GnRH) agonists, initially presented as a substitute for hCG, have led to a new era of GnRH agonists followed by hCG which triggers ovulation (Cole, 2009).

After ovulating that on day 60th in the T1 group with hCG from Chorullon, Intervet-Holland and T2 group with hCG from pregnant woman urine group respectively were showed a positive pregnancy of 100% (15 cows) and 90% (14 cows) and diagnosis of the 60th days of pregnancy appears on an ultrasound (USG) image (Figure 2).

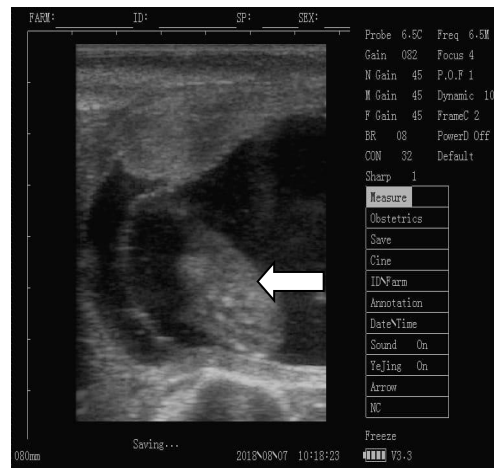


Figure 2. Diagnosis of the 60th day of pregnancy using on an ultrasound (USG)

The hCG is a glycoprotein hormone which is isolated and purified for getting hCG proteins (Nwabuobi *et al.*, loc cit). The molecular weight of hCG are 37 and 22 kDa corresponds to a 10% sds-page result. The level of hCG in urine at 1.5 months of pregnancy women was 10.567 mIU / mL. The level of hCG in urine at 3.5 months of pregnancy women was 13.444 mIU / mL (Hermadi *et al.*, loc cit).

hCG is the Gonadotropin hormone extracted from the urine of pregnant women who have the highest concentration found at 1.5 to 3.5 months of gestation or 8 to 11 weeks to 4 months or 12-15 weeks during a positive pregnancy test. Biology is known to be identical to LH and is often called LH like. hCG is a glycoprotein hormone (Hermadi *et al.*, loc cit). hCG is not of a specific species which means that even though the urine produced from a pregnant woman continues to have a therapeutic effect on receptive women or patients, even hCG may be directly involved in the IVF process (Invitro fertilization) in humans. Comparative follicles begin to occur simultaneously but many become atresia during the luteal phase of the follicular growth cycle where one of them becomes the dominant follicle. The biochemical changes in the

development of subordinate follicles to the dominant follicles when analyzed for chemical changes occur differently from the principles of FSH, LH and receptors (Daya, *et al* 1995; Roche, 1996). The use of hCG for the purpose of folliculogenesis in cattle has not been able to explain the mechanism of both *in vivo* development of follicles in the ovary and *in vitro* in the process of oocyte maturation. Is the mechanism to follow endogenous FSH - LH rules or other factors. Research is needed that can help monitor ultra sonografi (ultrasound) instruments to determine follicular growth waves (Follicular waves) or oocyte maturation monitoring *in vitro*.

hCG is secreted by syncytiotrophoblast originating from diffuse and differentiated cytotrophoblast cells (Choi and Smith, 2014; Nwabuobi *et al.*, *loc cit*) for a long time, the main known role of hCG is the promotion of progesterone secretion by the corpus luteum in early pregnancy (Ng *et al.*, 2001), acting through hCG or LH receptors. However, recently, many other functions of hCG, not only in the placenta but also in the myometrium, uterus and fetus, have been described (Cole, *loc cit*; Kirk *et al.*, 2013; Guibourdenche *et al.*, 2009).

Cellular studies of folliculogenesis begin with the GnRH hormone, prevention of a low hormonal balance of FSH-LH is corrected first, for example, feed rations are improved with good and balanced feed quality, better environment, cleaner cages, air movement good, looser, often taken out of the cage to move more. If the situation has become better followed by injecting FSH-LH preparations or likes (FSH-LH) (5). Human Chorionic Gonadotropin (hCG) does not have a specific species meaning even though it is produced from the urine of pregnant women still giving therapeutic effects to prescribed women or patients, even hCG can be involved directly in IVF (*In vitro* fertilization) in humans implantation process after embryo transfer depends on quality embryo and endometrial reception. It is estimated that fifty to seventy-five percent of pregnancies are lost due to implantation failure. There is evidence that there is an initial chorionic gonadotrophin secretion before implantation embryos, and this secretion has been associated with important functions in angiogenesis and the inflammatory response that drives the implantation process.

Summary :

The conclusion, manipulate *in vivo* by use of hCG patent and hCG pregnant woman urine was followed by estrus, ovulation and pregnancy of Madura cows. The results showed that there was no significant difference $p > 0.05$ between the treatment groups (hCG from patent product (T1) and from pregnant women urine (T2)).

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The following article has been accepted and will be published in **AUGUST, 2019** issue of Indian Veterinary Journal.

Article No.	Title	Author (s)
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