

Biocompatibility of Low Dose of Human Menopause Gonadotropin(hMG) and Deglycosilation of Human Menopause Gonadotropine (dGhMG) Against Bovine Eggs

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ABSTRACT

No any researcher yet reported by adding gonadotropin mainly either hMG or dGhMG less than 1 ug/ml into media succesfully obtain proportional amount fertilized eggs in invitro fertilization technique (IVF), moreover this dosis twice and four times decreased from 1000 ng. The main purpose of this study was to evaluate biocompatibility of low dosis of hMG and dGhMG as additive in media maturation during in-vitro maturation (IVM) to amount of fertilized egg obtained following 48 hrs of in-vitro insemination (IVI). All those inclusive criteria oocytes that collected in laboratorium invitro Lab. obstetrics Department of Reproduction, Vet medicine, Unair randomized into sub-group (1, 2, 3, and 4) and also control group. Sub-group 1, 2, 3, 4 and control group Containing with TC199 + 10% bovine calf serum. Sub-group 1 added with hMG 250 ng/ml and 500 ng/ml for sub-group 2. Sub-group 3 and 4 added with 250 ng/ml and 500ng/ml dGhMG respectively. all these immature oocytes were transfer into those each drop 50ul media in 35 mm disposable petridisk and contained each drop 5–7 oocytes then covered in with mineral oil and polyesterene plastic petridisk cover then incubated for 24 hrs in 5% CO₂ incubator. Next morning after oocytes washed in warm Media oocyte washing solution (OWS) and once using Earle's balanced salt solution (EBSS) all predicted mature oocytes transfered into centripetal side of Rosset patern made using EBSS media 10ul each, that connected each other to centre of centripetal side 50ul. This centre side of centripetal was transfered with 30 ul semen have been followed and washed twice then swim-up process then kept in 5% CO₂, 38.5° C for 24 hrs. Observation performed 2 × 24 hrs after all oocytes transfer into maturation media 24 hrs following IVI. All sub-group showed increasing amount of fertilized eggs higher than 75% and much higher compared to control group that only 38.1%, but among subgroup was not much differed, however the other hand found highest fertilized eggs/embryo was 87.5% in sub-group 4. Wishing to reach more fertilized eggs in IVF, gonatropine hormone absolutely should be added in basically maturation media.

Key words: hMG and dGhMG in bovine eggs of IVF

INTRODUCTION

Physiologically gonadotropine hormone as well as follicle stimulating hormone (FSH) and luteinizing hormone (LH) in human and animal produced in anterior lobus of hypophysis. Released into blood stream follow general body circulation from vein organ producer to heart then follow organ artery target act and stimulate development of follicle preantral to antral follicle until reach de Graaf follicle or tertiary follicle. Later on releasing of LH occure after estrogen produced by theca and granulosa cell in surrounded oocytes that in turning develop to be a Graffian follicle. The negative feed back of FSH regulated by progesterone hormone and estrogen stimulated and released of LH and then this hormone act in ovulation process direct or indirectly through GnRH stimulation that produced by hypothalamus (**Greenspan and Gardner, 2004**). Another gonadotropine also produced by human

placenta starting 10 days of conception detectable in blood serum, but detectable in the urine after more than 30 days of conception, is known as human chorionic gonadotropine (hCG) and the concentration gradually elevate synchronize with gestation period, and reach a peak concentration 500,000 to 1000,000 iU during 60–80 days of gestation and gradually decrease the production and disappear in urine after 7 days of delivery. In-vivo hCG act same as LH to local environment favourable for the conceptus nidation and embryonal growth (**Jones, 1982**). In-vitro this hCG won't be smoothly act lonely to form follicle growth however should be initiated FSH priorly. Differ to human menopause gonadotropine (hMG) that have been known belong two action as FSH and also LH with proportion action moreless 60:40 respetively. This hormone exsecreted via urine postmenopause women can be identified and be able purified as a hormonal substance. To prepare and

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perform this world's hormone Gonadotropin as known as Menotropine, the urine send to American Medical Factory in USA mainly contributed from China, Europe USA and Latin america , meanwhile In Indonesia this urine not yet occupied well (**Lunenfeld**, 2004). Compared to hCG this HMG more worldwide can be applied is due to bephasic action as FSH an LH there are physiologically needed either in-vivo and in-vitro of follicle or oocyte development, to outcome infertility problem either human and animal. In Animal by cutting relationship between fructosa chain and sialic acid chain in molecular hMG , folliculogenesis to be develop faster (**Anderson et al.**, 2000). The same study also have been reported in -vivo in human by using deglycosilation of FSH molecule affected folliculogenesis also faster occurred (**Zambrano et al.**, 2007). This hormone has also been injected in-vivo in women for ovum pick up and followed with in-vitro fertilization found 85% conception rate (**Argawal et al.**, 2000). By cutting this carbohydrate chain of hMG then this hormone called as dGhMG realized could also belong the same action to FSH that also had been cutted the carbohydrate molecule could stimulate and to develop granulosa , theca and Germinal Visicle Breakdown faster in turn of folliculogenesis process (**Wendy et al.**, 2001). Succesfully to perform in-vitro fertilization is depend on 3 factors as well as, media maturation+ it's additive hormones, oocytes and sperm quality. This oocytes quality and it's development is depend on what kind and dose of Gonadotropin added as additive in maturation process (**Mahaputra**, 2007). The objective of this study was to evaluate the bovine cleavage obtained eventhough edded in low dose hMG and dGhMG.

MATERIALS AND METHODS

All selected oocytes that used and involved as inclusive criteria in this experiment was randomized into all sub-group (1, 2, 3, and 4) and control group containing 5–7 oocytes only each and replicated 3 times inwhich totally there were 94 cows oocytes immature with structure cummulus-oocyte-complex (COC), meanwhile denuded oocyte excluded. Bovine ovaries were picked up from abattoir trasported and carried with thermos containing phosphat buffer saline Dulbeccos to to laboratory IVF during 30–45 minutes. All those oocytes collected by aspiration process using 18 G needle connected with 5 ml syringe inserted into connective tissue around 2–5 mm follicle, and after aspirated all follicle content first follicle, the tip needle was pull it out a little bit but still lay inside of ovary tissue then directed to another immature

follicle to perform the same procedure and repeated 3–5 times depend on immature follicle available on the surface of ovary. Each drop 50 ul Media Tc199 + 10% Bovine calf serum used as media in control group, sub-group 1, each drop 50 ul containing TC199+10% bovine calf serum +250 ng/ml hMG, sub-group 2, containing each drop 50 ul with TC199+10% bovine calf serum +500 nghMG. Meanwhile subgroup3 containing each drop 50ul with TC199+10% bovine calf serum +250 ng dGhMG and sub-group 4 containing each drop 50 ul with TC199+10% bovin calf Serum +500 ng dGhMG. Into this each drop put in 5–7 immature oocytes inside of 35 mm diameter dispossable petridisk (Valcon, Sweden) and pawed with mineral oil until all drop covered in. This invitro maturation (IVM) priod all those petridisk that containing oocytes incubated for 24 hrs in incubator 5% CO₂ 38.5° C with 95% humidity.

Following next morning to be performed in-vitro insemination (IVI), all oocytes after observation and desided having good development in cummulus structure then washed twice in warmed media Earle's balanced salt solution (EBSS) and transfered 4–7 oocytes to each centripetal side of ROSSET pattern developed from also EBSS media in 35 mm dispossable petridisk (**Mahaputra**, 2007). Coinciding of this oocytes transfer process, one frozen mini straw containing bovine semen was picked up from the container and thaw in warm water 38° C for 30 second and washed twice with Media EBSS. Sperm Swim-up acted by layed down the sperm pellet in gradual scale tube an then fill in with 3 ml EBSS then kept in room temperature for 20 minutes. Following this 20 minutes over, picked up 30 ul EBSS media on the top tube should be containing motil sperm at least 1×10^4 and transfer into centre sentripetal of the ROSSET pattern that containing matured oocytes in centripetal sides that kept for 24 hrs in 5% incubator CO₂. Next morning all these oocytes washed in warmed TC199++ while repipetted 3–4 times due to most of cummulus complex fall down and transfered back to maturation media drop then retuned back kept for 12 hours to evaluate first cleavage stad.

Evaluation of the cleavage by observation quantitatively blastomer cell in side of the fertilized egg, and the data transform into descriptive statistic percentage.

RESULTS AND DISCUSION

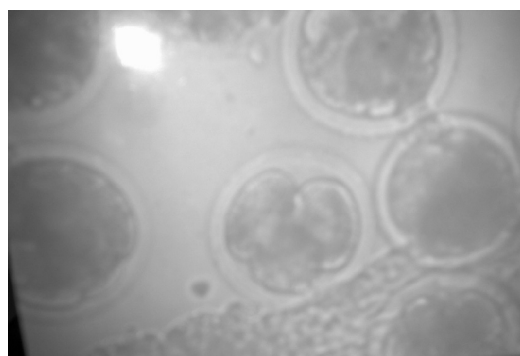
Totally 94 immature cows oocytes in 3 replicates each subgroup were used in this study appeal that by adding 250 ng hMG/ml media TC199+ showed that the fertilized oocyte/embryos developed folowing 48 hrs in this-sub

group1 was 78.9% (15/19) . During this 48 hrs following invitro insemination (IVI) commonly fertilized eggs found were in 2–8 cell stadium for all groups (*Picture 1*). Still in this group by adding 500ng hMG/ml media TC199+ in this sub-group 2 showed that fertilized eggs/embryos developed following 48 hours IVI were 80% (16/20), that mean this finding were not much different to previously subgroup inwhich were added with 250 ng hMG/ ml Media TC199+ during maturation. Meanwhile in the other group dGhMG, mainly in sub-group 3 that added with 250 ng GdhMG/ml media TC199+ found 77.8% fertilized aggs and unfertilized were 22.2%. Compared to sub-group 4 in which that added with 500 ng GdhMG/ml media TC199+ revealed 87.5% fertilized eggs and 12.5% unfertilized eggs. Appearily only this sub-group 4 that added with 500 ng dGhMG/ ml media TC199 showed fertilized eggs extremely higher compared to sub-group 3, 2 or sub-group 1. This finding of sub-group 4 with 87.5% (14/16) fertilized eggs also very extremely higher compared to control group inwhich without added hMG nor dGhMG to the media during maturation process in which found only 38.1% fertilized eggs. This finding 38.1% fertilized eggs in control group also extremely lower compared to sub-group 1, 2 and sub-group 3 (*Table 1*). The other hand previous study showed that only found 73% fertilized eggs by added 10% estrus mare serum or bovine estrus serum (**Mahaputra and Simorangkir** 1999). Differ Incubation period during maturation process that added 4ug dGhMG found mean embryos cleaved 70.2 for 20 hours and 76.5 for incubation 24 hours (**Hermadi et al.**, 2011). Talking about estrus serum that used as additive maturation media that mean by addition either hMG or dGhMG even those dosis were low, but still obtained much higher embryo cleaved compared with estrus serum. Although without any additive gonadotropine hormone either hMG or dGhMG the immature oocyte still be able to develop is due to in TC199 already added with 10% Foal mare serum in which containing more less 300–350 ng IGF/ml serum (**Mahaputra**, 2007). That mean to reach much more fertilized eggs, hormonal gonadotropine additive absolutely should be added in the media maturation during in-vitro maturation process. All those oocytes used on this study having Cummulus-oocyte-complex (COC), meanwhile denuded oocyte excluded, that why the maturation process quite smoothly occurred is due to hMG and dGhMG having molecularly FSH-LH like that added in the media reacted and bound in cummulus mainly in granulosa cell membrane that known having FSH-LH

receptor (**Simoni et al.**, 1997) to stimulate intracellular development of oocyte through cummulus cell to be performed mature spindle, pronucleus and polar body (**Greenspand and Gardner**, 2004).

Table 1. Fertilized and non fertilized oocyte following treated with two main groups hMG

	Sum of Oocytes	Fertilized (%)	Non-Fertilized (%)
Group 1: hMG			
Sub-G 1, 250 ng/ml	19	15 (78.9)	4 (21.1)
Sub-G2, 500 ng/ml	20	16 (80)	4 (20)
Group 2: dGhMG			
Sub-G 3, 250 ng/ml	18	14 (77.8)	4 (22.2)
Sub-G 4, 500 ng/ml	16	14 (87.5)	2 (12.5)
Without hMG /Control	21	8 (38.1)	13 (61.9)
Total	94	67 (71.3)	27 (28.7)



Picture 1. Bovine Fertilized Eggs/ Embryos Following 48 hours of IVI.

CONCLUSION

1. Gonadotropin hormone as well as hMG or dGhMG is needed as additive in media maturation to obtain more fertilized eggs in IVM process, was due to without gonadotropin fertilized egg found only 38.1%.
2. Even low dose there was 250 ng/ml hMG still obtained fertilized eggs quite high enough 78.9% and was not differ to higher dosis 500 ng/ml.
3. The highest fertilized egg /Embryo found 87.5% in sub-group 4, in which media maturation edded with 500 ng/ml of dGhMG.

4. dGhMG could not act to stimulate in increasing mature eggs mainly in sub-group 3 compared to subgroup 1 and 2, but showed differed result obtained between sub-group 3 and 4.

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