Open Access

Hasanuddin J. Anim. Sci.
Vol. 2, No. 2:83-90 November 2020
pISSN 2621-9182 eISSN 2621-9190

HAJAS HASANUDDIN JOURNAL OF ANIMAL SCIENCE

Effect of FSH and PMSG Additions on Maturation and Fertilization Rates of Bali Cattle Oocytes

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Abstract

The process of in vitro oocyte maturation requires stimulation of gonadotropin hormones, as well as in vivo. There are two gonadotropin hormones that are available in the market and can be used in the process, Follicle Stimulating Hormone (FSH) and Pregnant Mare Serum Gonadotropin (PMSG). This study aimed to determine the effect of the addition of FSH and PMSG hormones in the maturation medium on the in vitro maturation and fertilization rates of Bali cattle oocytes. This study had two treatments, the addition of FSH and PMSG, each of which was carried out 4 times. Oocytes matured in basic medium, M-199 plus Bovine Serum Albumin (BSA) 10 IU/ml, and 50 µg/ml Gentamycin. Furthermore, the matured oocytes fertilized in 80 µl drop of sperms. The data were analyzed by Chi Square test to determine the differences in the level of maturation and fertilization of Bali cattle oocytes in vitro. The results of this study indicated that there was no significant difference ($P>0.05$) between the addition of FSH and PMSG hormones in the maturation medium to the nuclear maturation rate (81.4 ± 2.2 vs 81.2 ± 2.3), and the fertilization rate (42.2 ± 4.3 vs 24.6 ± 2.7). It can be concluded that in vitro maturation and fertilization rate of Bali cattle oocytes with the addition of FSH and PMSG hormones to the maturation medium are similar, but showed a tendency for higher fertilization rate in the medium supplemented with FSH.

Keywords : FSH, PMSG, nuclear maturation, fertilization rate, Bali cattle oocytes

INTRODUCTION

In vitro embryo production and related technologies in livestock have shown significant advances in recent years. The combination of in vitro embryo production technology with sexing spermatozoa and genome selection has been successful and is widely used in several developed countries. The main advantages offered by this technology include a higher number of embryos and pregnancy cows per unit of time, and a wider range of potential cow donors to collect oocytes and utilization of ovarian waste from slaughterhouses (Ferre *et al*., 2019).

Embryos transfer is a technique to accelerate the reproduction process with superior genetic quality. Conventional embryos obtained in vivo are expensive and the response to super ovulation varies. The alternative is to produce embryos in vitro. The rapid development of this technology is driven by the need for embryos of superior quality and low cost because it utilizes slaughterhouse waste, while spermatozoa come from superior males (Situmorang and Triwulaningsih, 2004). In vitro embryo production procedures include: oocyte collection from the ovaries, oocyte maturation, spermatozoa capacitation, and culture (Sonjaya *et al.,* 2016; Sonjaya and Hasbi, 2019). One of the main factors to increase embryo production in vitro is by giving hormone supplements to the maturation medium. Reproductive hormones play an important role in activities through the indirect work of hormones on physiological continuity, the internal environment that ensures successful growth and development of embryonic cells (Adifa *et al*., 2010). Reproductive hormones that can be used and added to maturase media include FSH and PMSG.

The addition of FSH and PMSG hormones to the maturation medium can accelerate stimulating granulosa cells and theca cells secreting estrogen, which will also stimulate oocyte maturation. The function of adding FSH in the maturation medium will stimulate and regulate the condensation of chromatin for the meiotic division process, besides that FSH will stimulate an increase in cAMP concentrations and expansion of cumulus cells (Wattimena, 2011). PMSG hormone contains high sialic acid, which results in excessive follicles and higher levels of estrogen in the blood (Madyawati *et al*., 2002). However, the addition of PMSG hormone in the maturation medium has little known impact on the rate of oocytes fertilization. Therefore, this study aimed to determine the effect of the addition of FSH and PMSG hormones in the maturation medium on the in vitro maturation and fertilization rate of Bali cattle oocytes.

MATERIALS AND METHODS

Time and Place

This research was conducted in November 2019 - March 2020 in the In Vitro Embryos Production Laboratory, Institute for Research and Community Service, Hasanuddin University, Makassar.

Materials of the Research

The tools used in this study were a CO_2 incubator, a microscope (ZEISS, image A2: Axio Cam HRc), Syringe (10 ml), scalpel, petri dish, glass objects, caver glass, micropipettes, petri dishes and surgical scissors. The materials used were Bali cattle ovaries obtained from the Tamangapa Slaughterhouse, Makassar City, South Sulawesi Province. The others included ovarian transportation medium, maturation medium, fertilization medium, 70% alcohol, tissue, mineral oil (Sigma Chemical Co. St. Louis MO, USA), KCl 0.7%, hyaluronidase enzyme (Sigma, USA) 0.25%, FSH, and PMSG hormone.

Research Procedure

Collection and Selection of Oocytes

Ovaries were collected from the slaughterhouse and brought to the laboratory using 0.9% NaCl solution. Collection of oocytes was carried out by slicing technique using Phosphate Buffer Saline (PBS) medium. Furthermore, oocytes were selected and only oocytes with the criteria of having compact cytoplasm and cumulus cells were used in this study.

In Vitro Oocytes Maturation

The selected oocytes were washed twice then divided into two groups. The first group was matured in M-199 maturation medium added with Bovine Serum Albumin (BSA) 10 IU/ml, 50 µg/ml Gentamycin, and 10 µg/ml FSH, and the second group was added with BSA, Gentamycin, and 10 IU/ml PMSG. Maturation was carried out in the form of drop (80 µl/drop) and covered with mineral oil in a 5% CO_2 incubator, temperature 38.5 °C for 24 hours.

In Vitro Oocytes Fertilization

Frozen semen was thawed at 37 °C for 20 seconds, then put into fertilization medium then centrifuged for 5 minutes at a speed of 1800 rpm. The centrifugation process was carried out twice. After centrifugation, the supernatant was discarded, then the settled sperm was added fertilization medium with a spermatozoa concentration 1.5×10^6/ml. Furthermore, the matured oocytes washed 2 times using fertilization medium, then fertilized. Fertilization was carried out in the form of drop 80 µl and covered with mineral oil in a 5% CO_2 incubator, temperature 38.5 °C for 24 hours.

Research Parameters

Nuclear Maturation Rate

Evaluation of the level of nuclear maturation was observed by counting the number of oocytes at each stage of meiosis starting from Germinal Vesicle (GV), Germinal Vesicle Break Down (GVBD), Metaphase I (MI), and Metaphase II (MII) (Shirazi and Sadeqhi, 2007). Observations were made using 2% aceto orcein. The staining procedure was performed by removing the cumulus cells surrounding the matured oocytes with 0.25% hyaluronidase enzyme (Sigma, USA). The denuded oocytes were made preparate and then fixed in a solution containing acetic acid and ethanol (1: 3) for 3 days. After fixation, staining and observation was carried out under a microscope (Zeiss Axio Imager A2) with a Zeiss Axio cam HRc camera. The maturation rate was assessed based on the percentage of oocytes that reach the MII stage.

Fertilization Rate

Fertilization rate was assessed based on the number of oocytes capable of forming a pronucleus after fertilization. The evaluation of the fertilization rate was carried out by the same staining procedure as the evaluation of the maturation rate.

Data Analysis

Data were analyzed using the Chi Square Test with 4 replications (Steel and Torrie, 1991). The parameters in this study were nuclear maturation and fertilization rates.

RESULTS AND DISCUSSION

Nuclear Maturation Rate

Evaluation of the level of nuclear maturation was observed by counting the number of oocytes at each stage of meiotic division starting from GV, GVBD, MI and MII. The results of observing the level of nuclear maturation of Bali cattle oocytes with the addition of FSH and PMSG to the maturation medium are presented in Figure 1.

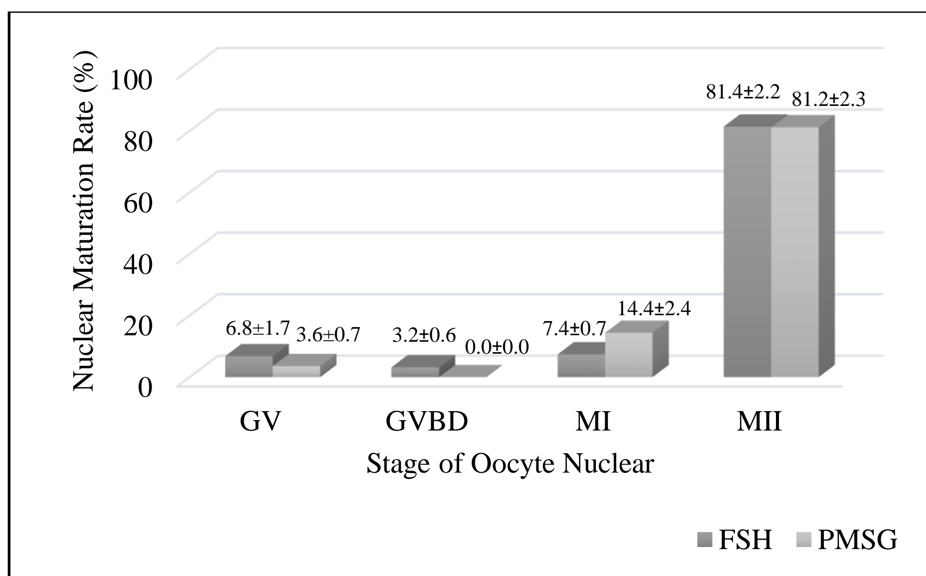

Figure 1. Nuclear Maturation Rate of Bali Cattle Oocytes

Chi Square test showed that there was no significant difference (P>0.05) between the addition of FSH and PMSG in the maturation medium to the maturation rate of Bali cattle oocyte. It showed that adding FSH to the maturation medium has same effect as PMSG. The effect of FSH in oocytes maturation process is achieved via secondary messengers such as cyclic adenosine 3'-5'-monophosphate (cAMP). FSH initially induces cumulus cells to produce cAMP, which is transferred to the oocyte via gap junctions. However, at a later stage, the cAMP concentration decreased (Salustri *et al.,* 1985). Furthermore Andersen *et al.* (2001) explained that FSH induces maximum cAMP accumulation after 30 minutes, after which the cAMP concentration decreases progressively, reaching the lowest concentration at 120 minutes later.

There is no difference in the addition of FSH and PMSG in the maturation medium, possibly due to PMSG hormone which can function as a substitute for FSH. Administration of commercial PMSG at a dose of 40-50 IU/ml is effectively used to replace the pure FSH for buffalo oocyte maturation and makes it more cost efficient for in vitro studies (Gupta *et al.,* 2001). Eyestone and Boer (1993) explained that FSH functions to stimulate follicular growth in

the ovaries, oocyte maturation process and early embryonic development, but it does not play a role in further development.

The oocytes used in this study were oocytes that have compact cumulus cells and have a homogeneous cytoplasm as described by Abdoon *et al*. (2014). The presence of cumulus cells supports the maturation of oocytes in vitro until the MII stage and is associated with cytoplasmic maturation (Lapathihis *et al*., 2002). Furthermore, Schroeter and Meinecke (1995) reported that during in vivo maturation, the cumulus cells play a role in providing nutrients for the oocyte and assisting protein synthesis to form the zona pellucida at the prophase stage. Oocytes without cumulus lose a lot of protein after maturation; while oocytes with intact cumulus have the fixed protein. During in vitro maturation of bovine oocytes the presence of cumulus cells is very helpful until the development of blastocysts (Boediono and Suzuki, 1996).

Fertilization Rate of Bali Cattle Oocytes

Fertilization rate was assessed based on the number of oocytes that have 2 pronuclei (2PN) or more after fertilization. The number of Bali cattle oocytes that can form 2 pronuclei or more with the addition of FSH and PMSG to the maturation medium are shown in Figure 2.

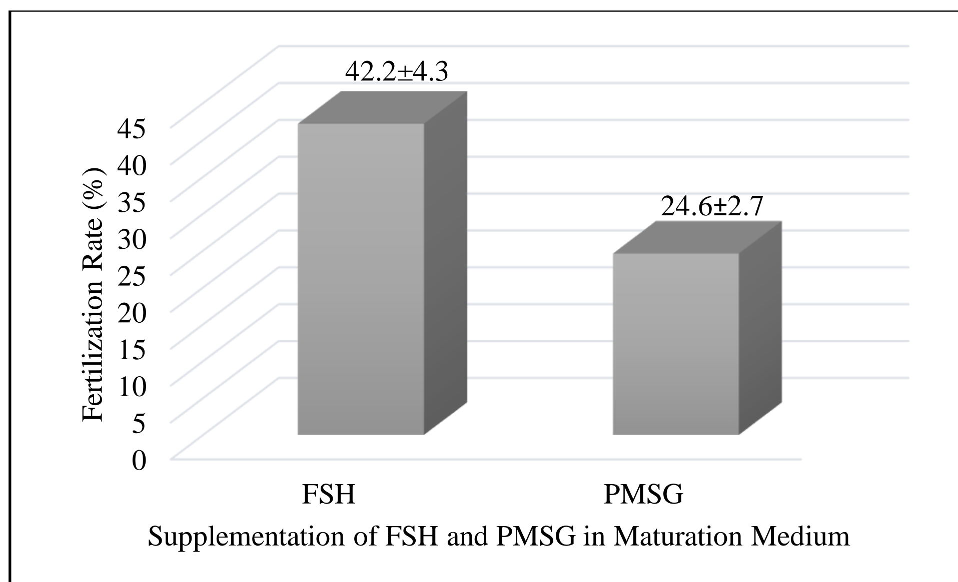

Figure 2. Fertilization Rate of Bali Cattle Oocytes

Chi Square test showed that there was no significant difference (P>0.05) between the addition of FSH and PMSG to fertilization rate of Bali cattle oocytes. This means that adding FSH and PMSG hormone to the maturation medium was similar (42.2 ± 4.3 and 24.6 ± 2.7% respectively). According to Licht *et al.* (1979) that PMSG have specific activity for FSH in bioassays. Based on the results of this study, although there was no significant difference, it showed a tendency for the addition of FSH to be better than PMSG. Hurk and Zhao (2005) reported that during the maturation process, oocytes will undergo two main changes, maturation of the nucleus and the cytoplasm. Cytoplasmic maturation is needed by oocytes to be able to prevent polyspermi, spermatozoa decondensation, and assist pronucleus formation after fertilization. Eyestone and Boer (1993) explained that FSH functions to stimulate the growth of

follicles in the ovaries and the maturation process of oocytes. Addition of FSH in vitro can improve both nuclear and cytoplasmic maturation, so that it can be fertilized by sperm (Assidi *et al*., 2013).

Fertilization failure is influenced by several factors, including imperfect maturation of the nucleus and cytoplasm, inadequate capacitation and acrosome reactions so that the ability of spermatozoa to fertilize is less than optimal, and failure of male pronucleus formation caused by failure of spermatozoa to experience condensation in the cytoplasm of the oocyte (Boediono *et al*., 2000). Another factor that can also affect the ability of in vitro fertilization is the production of reactive oxygen species (ROS). Reactive oxygen species can cause changes in mitochondria, DNA fragmentation, and cell death (Guerin *et al*., 2001; Fahruddin *et al*., 2002).

Fertilization is a process that involves penetration of the ovum by spermatozoa, activation of the ovum, the formation of male and female pronuclei and the linking of maternal and paternal chromosomes to form the genome (Elder and Dale, 2011).

CONCLUSION

The addition of FSH and PMSG did not affect the nuclear maturation and fertilization rate of Bali cattle oocytes; however the fertilization rate showed a tendency to be higher with the addition of FSH.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Research, Technology and Higher Education for financing the research through the Higher Education Leading Basic Research program with contract number T/140/E3/RA.00/2019.

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