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Review Article

ANALYSIS OF ESTRADIOL AND PROGESTERONE HORMONE LEVELS AGAINST VARIOUS CELL CULTURE IN TCM- 199 MEDIUMFOR CATTLE *IN VITRO*

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ABSTRACT

This research was aimed to obtained data base reproductive hormonal profile of the hormones estradiol and progesterone levels in various cell cultures. Culture cells used are cells fallopian tubes, ampulla, isthmus and follicle cells, whereas the culture period used were 0, 2 and 4 days. Analysis of the hormones estradiol and progesterone levels in various cell culture used ELISA method. Data results obtained are the estradiol hormone levels in various cell cultures and periods of different cultures in TCM-199 medium ie cell treatment Fallopian tubes in culture period 0, 2 and 4 days (9.07; 13.14; 9.00 pg/ml), cell culture period ampulla at 0, 2 and 4 days (9.00; 9.29; 14.39 pg/ml), cell isthmus (9.00; 12.08; 9.00 pg/ml) whereas follicular cells in culture period 0, 2 and 4 days (415.04; 476.67; 376.93 pg/ml). The highest levels of the hormone estradiol on cell cultures, namely follicle cells on the second day culture period (476.67 pg/ml), whereas the lowest in cell cultures, namely follicle cells on the fourth day culture period (376.93 pg/ml). Progesterone levels obtained in the treatment of Fallopian tube cells in culture period 0. 2 and 4 days (24.107; 24.644; 24.474 ng/ml), cell culture period ampulla at 0, 2 and 4 days (24.187; 23.753; 24.254 ng/ml), cell isthmus (24.071; 24.083; 24.034 ng/ml) whereas follicular cells in culture period 0, 2 and 4 days (26.671; 27.610; 24.034 ng/ml). For progesterone levels in various cell culture and the culture that the treatment period follicle cell culture high on the second day culture period (27.610 ng/ml) and low progesterone levels in cell culture ampulla on the second day culture period (23.753 ng/ml).

Keywords: Hormones, Cell Culture and Medium TCM-199

INTRODUCTION

The cattle is one type of livestock that contribute greatly to meet the animal protein Indonesian society. It is estimated that demand for meat and milk in the future is increasing as a result of the growth of public awareness to consume animal protein.

The needs of people in Indonesia animal protein/ meat increased. In 2000, meat consumption is 1.72 kg/ capita/year by 2010, rising meat consumption of 2.72 kg/ capita/year or during the last 10 years people's needs for meat increased by 1.0 kg/capita/year (Victorbuana, 2010).

Beef cattle population data in Indonesia in 2011 amounted to 14,824,373 tail, whereas in 2015 the cattle population increased to 15,494,288 tail. Over the last 4 years the cattle population in Indonesia increased by 4.32% (Directorate General of Livestock, 2015). In response to the low increase in the cattle population it needs attention in the breeding of cattle.

Prospects of development of in vitro cell culture system is very large. This technique will be a lot to overcome fertility problems in humans and animals are facing the problem of infertility. During this time some of the technology used as *in vitro*maturasioocytes, in vitro fertilisasiand embryo transfer are based on in vitro cell culture systems, has been developed and successfully applied with satisfactory results. Various culture systems have been developed in some species such as mice (Bishonga*et al*., 2001) and domestic animals (Miyano, 2005). Gordon (2003) suggests that the co-embryo culture supplemented several cell line like the Fallopian tube cells, cumulus and others can provide substances or growth factors necessary for embryonic development.

Reproductive process related to the mechanism of hormonal system, namely the relationship between hormones hypothalamic pituitary namely Gonadotrophin Releasing Hormone (GnRH), Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), a hormone-ovarian hormones (estrogen danprogesteron) and hormone uterus (prostaglandins) (Hafez and Hafez 2000). Ovarian hormones that have a major role on reproduction are estrogen and progesterone.

Estrogen is a steroid hormone produced by the granulosa cells and theca cells of de Graaf follicles in the ovary. The main function is to stimulate the hormones estrogen estrus, stimulate the emergence of secondary sex characteristics, maintain a system of channels and the growth of female udder udders (Wodzicka-Tomaszewska *et al*., 1991).

According to Hafez and Hafez, (2000) that progesterone is one of the important hormones related to reproduction that is secreted by cells of Corpus Luteum(CL). CL is an endocrine organ that is responsible for producing the hormone progesterone. Blood serum progesterone concentration can determine the state of the animal is in a state of infertility, normal, lust, and bunting so that it can be used for detection of estrus, pregnancy examinations and knowing other pathological conditions.

Cells Fallopian tubes play an important role in mammalian reproduction and can provide the optimal environment for oocyte maturation, capacitation of sperm, fertilization and transport of gametes and embryos, which is controlled by two sex hormone ovarian estrogen and progesterone (P4) (Leese *et al*., 2001); (Hunter, 2003).Co-cultured embryos with multiple cell line can provide substances or factors of growth necessary for the development embrio example cell oviduct, cumulus cells, etc. (Gordon, 2003); (Trilaksana and Good, 2008). Furthermore, Hunter (2003) suggests that supplementation of cells tuba fallopian can increase cultured embryonic development.

Cells of ovarian follicles and *in vitro*culture can improve the development at a later stage, including oocyte growth, maturation and ovulation (Hartshorne, 1997). In vivo maturation process takes place in the follicle. In addition to the development and maturation, cell-cell follicles also actively produce steroid hormones such as progesterone and estrogen (Gordon, 2003). Gutierrez *et al*. (2000) suggests that the culture of follicular cells has important implications for the potential of biotechnology to produce a large number of oocytes for embryo transfer and development.

To improve the efficiency of production and reproduction in cattle, it would require an animal hormonal profile information (Katongole and Gombe, 2006). Many aspects of reproductive see cows have been investigated, but the profile information of estrogen and progesterone in a variety of cell culture (cell Fallopian tubes, ampulla, isthmus and follicular cells) for cell culture *in vitro* until now have not been reported.

Based on the above, the authors are interested in reviewing the hormonal levels in various cells as a research titled: "Analysis of Estradiol and Progesterone Hormone Levels Against Various Cell Culture in TCM- 199 Medium for Cattle *In vitro".*

The purpose of this study was to establish a baseline reproductive hormonal profiles of estradiol and progesterone hormone levels in various cells in the medium of oocyte maturation and embryo production *in vitro* and has benefits to provide information and the data about the profile of the reproductive hormones (hormones estradiol and progesterone) in various cells as a reference to increase of the rate of oocyte maturation and embryo production cow in vitro in Efforts to Increase livestock numbers and assists in the rescue of germplasm and assist in providing breeding stock excels in bulk , quickly intervening and cheaply.

MATERIALS AND METHODS

Material Research

Materials used in the study is the Fallopian tubes, ovaries cow that had been cut from Slaughter House (SH) Payakumbuh, West Sumatra. While the chemicals used are physiological 0.9% NaCl, Phosphate Buffer Saline (PBS), Tissue Culture Medium-199 (TCM-199; Sigma, M-5017), 10% fetal calf serum, gentamicin 50 ug / ml, FSH (Ovagen, Sigma), trypsin, mineral oil (M-8410, Sigma), streptomicyn-pennicillin (P-3539, Sigma), aquabidest, 70% alcohol, distilled water, and Kit estradiol hormone and progesterone. Furthermore, materials for co-culture cells used are cells Fallopian tubes, cell isthmus, ampulla cells and follicular cells.

The tool used is a pasteur pipette (fisher), Millipore filter 0.22 µm (Sigma), a petri dish Φ35 and Φ60 mm, brand Nikon microscope, CO_2 incubator, refrigenerator, an analytical balance Sartorius CP brand in 2245, ovens, laminar flow, razor blades, ovarian collection flask, CO_2 gas tube, Bunsen, centrifugation, micro tube and cover glass.

Research Procedure

Procedures for implementing the study are as follows:

Ovary Retrieval of SH

Ovaries were taken from cow ovaries SH is. After the last cut of beef cattle cleared from the ovarian tissue that covers the surface, then washed with PBS medium. Furthermore, the ovaries put in place that has been filled with a medium Physiological NaCl 0.9%. To avoid contamination by microorganisms in the medium Physiological NaCl 0.9% was added streptomycine 0.1 mg / ml and penicillin 100 IU/ml and then stored in a thermos collection and ovaries were taken to the laboratory at a temperature of 30-35^{0}C.

The Oocyte Collection

Oocyte collection is done with an incision technique/ Slicing is taking oocytes from the ovary to follicle-wrenching way on the surface of the ovary in the medium collection on a petri dish. Oocytes obtained from the collection and then put in a petri dish containing media collection. Media collection consists of Phosphate Buffer Saline (PBS) which was supplemented with 10% Fetal Calf Serum and gentamicin 50 mg/ml (Sigma, G-1397) that has been filtered using Millipore filter 0.22 µm.

Making Cell Line for Cell Culture

Fallopian tube tissue used were obtained from cows Fallopian tube that has been cut in the slaughterhouse. According Senger (1999) that the network started from the end of the Fallopian tube Fallopian tube that attaches to the cornua until the end that attaches to the infundibulum. Fallopian tube network isolation is done by inserting a medium D-PBS containing 0.25 % trypsin into the Fallopian tubes so that the cells of the Fallopian tubes fall out. Furthermore, the isolated cell is inserted into a petri dish, then isolated centrifuged for 10 minutes at a speed of 1800 rpm, 2 times. The precipitate obtained after setrifugation diluted with TCM-199 medium to a concentration of 10x10^6 cells/ml. Furthermore Fallopian tube cells cultured in petri dishes in TCM-199 medium supplemented with FSH is at 10 µ/ml and gentamicin 50 g/ml and then incubated in a 5% CO_2 incubator at a temperature 38,5^{o}C up on the base of the petri dish to form a layer of cells line (*monolayer*).

For the treatment of isthmus tissue , obtained by cutting the Fallopian tubes cow on the isthmus. Senger (1999) reported that the isthmus network starting from the end of the Fallopian tube that attaches cornua up with part of the Fallopian tubes which began to swell (ampulla). Isthmus network isolation is done by inserting a medium D-PBS containing 0.25 % trypsin into the cells of the isthmus loss. Furthermore, the isolated cell is inserted into a petri dish and then centrifuged for 10 minutes at a speed of 1800 rpm, 2 times. The precipitate obtained after setrifugation then diluted with TCM-199 medium to a concentration of 10x10^6 cells/ml. Furthermore isthmus cells cultured in petri dishes in TCM-199 medium supplemented with FSH is at 10 µg/ml and gentamicin 50 µg/ml and then incubated in a 5% CO_2 incubator at a temperature 38,5^{o}C up on the base of the petri dish to form a layer of the cell line (*monolayer*) .

Senger (1999) suggested that the network started from scratch ampulla enlargement until the end that attaches to the infundibulum. For the treatment of ampulla tissue is obtained by cutting the Fallopian tubes cow in the ampulla. Ampulla network isolation is done by inserting a medium D-PBS containing 0.25 % trypsin into the cells ampulla of the loss. Furthermore, the isolated cell is inserted into a petri dish and then centrifuged for 10 minutes at a speed of 1800 rpm, 2 times. The precipitate obtained after setrifugation then diluted with TCM-199 medium to a concentration of 10x10^6 cells/ml. Furthermore ampulla cells cultured in a petri dish with TCM-199 medium supplemented with FSH is at 10 µg/ml and gentamicin 50 µg/ ml and then incubated in a 5% CO_2 incubator at a temperature 38,5^{o}C up on the base of the petri dish to form a layer of the cell line (*monolayer*).

Making cell line (*monolayer*) of the follicle. Isolation of follicular cells used were obtained from flake when slicing follicles in the ovary. Isolated follicle cells put into a petri dish and then centrifuged for 10 minutes at a speed of 1800 rpm,two times.The precipitate obtained from centrifugation was diluted with TCM-199 medium to a concentration of 10x10^6 cells/ml. Furthermore follicle cells were cultured in a petri dish with TCM-199 medium supplemented with FSH is at 10 µg/ml and gentamicin 50 µg/ ml and then incubated in a 5% CO_2 incubator at a temperature of 38.5 °C at the bottom of a petri dish to form a layer cell line (*monolayer*).

Analysis of Estradiol and ProgesteroneHormone Levels In Various Cells Culture

After the isolation of various cell culture treatment (cell fallopian tubes, ampulla, isthmus and follicles) such as cell isolation techniques above. The isolated cells were cultured in a petri dish treatment for 0, 2 and 4 days. Each sample cell cultures treated for measuring levels of hormones estradiol and progesetron taken with a micro pipette and put into a micro tube. Samples treated cell cultures stored in a freezer for the collection of cell cultures and in the analysis of estradiol and progesterone hormone levels using ELISA.

Variables Observed

Levels of the hormone estradiol to a variety of cell culture (cell fallopian tubes, ampulla, isthmus and follicles) and a different culture period.

Levels of the hormone progesterone to a variety of cell culture (cell fallopian tubes, ampulla, isthmus and follicles) and a different culture period.

Data Analysis

Analysis of the data of the measured parameters presented descriptively displayed in the form of tables and figures.

RESULTS AND DISCUSSION

Hormone Levels of Estradiol

Results of research estradiol hormone levels obtained in the treatment of a variety of cell culture supplementation and culture period in medium TCM- 199 are presented in Table 1.

Table 1. Hormone Estradiol Levels In Different Cell Culture andCulture Period (pg/ ml)

No	Cells Culture	CulturePeriod (days)		
		0	2	4
1.	Fallopiian Tube	9,07	13,14	9,00
2.	Ampulla	9,00	9,29	14,39
3.	Isthmus	9,00	12,08	9,00
4.	Folicle	415,04	476,67	376,93

Table 1 showed that the levels of the hormone estradiol in various cell culture and different culture period in medium TCM-199 is the highest Fallopian tube cell treatment on the second day culture period (13.14 pg/ml) followed by declines in 0 days (9,07 pg/ml) and the fourth day showed the lowest levels of the hormone estradiol (9.00 pg/ml). Levels of the hormone estradiol on cell cultures ampulla and culture that the treatment period ampulla cell culture high on the fourth day culture period (14.39 pg/ml) was followed by a decrease in the second day (9.29 pg/ml) and at 0 days showed levels of the hormone estradiol the lowest (9.00 pg/ml). Levels of the hormone estradiol on cell cultures isthmus and culture that the treatment period isthmus cell culture high on the second day culture period (12.08 pg/ml) followed by declines in 0 days and the fourth day (9.00 pg/ml). Levels of the hormone estradiol in follicular cell culture that the treatment of follicular cell culture high on the second day culture period (476.67 pg/ml) followed by declines in 0 days (415.04 pg/ml) and the fourth day showed the lowest levels of the hormone estradiol (376,93 pg/ml).

The research results are obtained as shown in Figure 1 thatthe highest levels of the hormone estradiol follicle cell culture on the second day culture period (476.67 pg/ml) compared to the treatment of other cell culture, while the lowest estradiol levels in cell culture Fallopian tubes on the fourth day culture period (9.00 pg/ml), followed by cell culture ampulla of the culture period on day 0 (9.00 pg/ml) and in cell culture isthmus at the culture period on day 0 and day four (9.00 pg/ml). This is due to the close ties of the follicular phase estradiol levels. According to Ganong (2003), estrogen will increase along with the development of follicles in the ovaries. Fluctuations in hormones estradiol in line with the development of follicles in the ovaries. When the development of follicles (follicular phase) of this hormone increases gradually, as the development of primary follicles into tertiary follicles. Estradiol hormone secretion peak occurs before ovulation occurs. Formed after ovulation and corpus luteum of the ovary (luteal phase), this hormone has decreased gradually until the end of the luteal phase.

High estradiol levels were predicted to be in the follicular phase, while cows that have lower estradiol content is predicted to be in the luteal phase. This is supported by Toelihere (1985) argues that the hormones progesterone blood blood is very high in the luteal phase and thus the activity of ovarian follicles in the growth of diminishing returns and as a result of the hormones estradiol further be low. In contrast to the follicular phase occurs around proestrus and estrus in estrous cycles showed that levels of the hormone estradiol in the blood high enough.

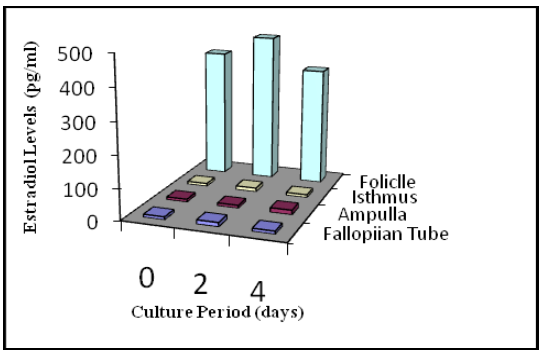

Fig. 1: Hormones Estradiol Levels In Different Cell Culture andCulture Period

The research result was better than the results Saili *et al.* (2014), levels of the hormone estradiol in Bali cattle that ranged from 6.4 pg/ml up to 392 pg/ml. This is due to the levels of the hormones estradiol obtained from TCM-199 medium which was cultured in a variety of cultured cells. The follicle has a better percentage growth. Sirard and Coenen (1995) states that the culture medium, serum types that interact in the medium affects the growth and formation of groups of antral follicles *in vitro*.

According to McDowall *et al*. (2004) that the medium TCM-199 is often used as the basic medium for oocyte maturation *in vitro* because it contains elements of biochemical role in oocyte maturation. The addition of serum as a source of protein in TCM-199 is needed to support the process of oocyte growth. Fetal Bovine Serum (FBS) one supplement *in vitro* oocyte maturation medium used to stimulate the growth of a large number of cell cultures.

Levels of the hormone estradiol in various cell culture and the culture period is shown in Figure 1Showed that the treatment of follicular cell culture high on the second day culture period (476.67 pg/ml) and low estradiol levels in cell culture ampulla of the culture period on day 0 (9.00 pg/ml) in cell culture followed isthmus at the culture period on day 0 and day four (9.00 pg/ml). Lopez-Barbella *et al*. (1979) reported that the strong correlation between the concentration of estrogen at the time captivated by the number of CL.

According to Dewi (2015), the ovaries secrete several reproductive hormones estradiol one of them is. Estradiol is a steroid hormone that plays an important role in reproductive status, while the most common components of hormone estradiol. Hormone estradiol serves to indicate the status of reproduction like effect on the reproductive system of female secondary sex characteristic and behavior of female lust. Follicular estradiol levels in small and large sizes are the same ranging from 1873.27 to 2012pg/ml.

Progesterone Hormone Levels

The results of the study progesterone levels obtained in the treatment of a variety of cell culture supplementation and culture period in medium TCM- 199 are presented in Table 2.

Table 2 showed that the levels of the hormone progesterone in a variety of cell culture and different culture period in medium TCM-199 is the highest fallopian tube cell treatment on the second day culture period (24.644 ng/ml) was followed by a decrease in the fourth day (24.474 ng/ml) and day to zero indicate low progesterone levels (24.107 ng/ml). Progesterone levels in cell culture ampulla and the period of the culture that the treatment of cell culture ampulla highest culture period fourth day (24.254 ng/ml) was followed by a decrease in days to zero (24.187 ng/ml), and the second day showed progesterone levels low (23.753 ng/ml). Progesterone levels in cell culture isthmus and the period of the culture that the treatment of cell culture isthmus highest culture

period the second day (24.083 ng/ml) followed by declines in 0 days (24.071 ng/ml) and the fourth day showed progesterone levels low (24.034 ng/ml).

Table 2. Progesterone Hormone Levels In Different Cell Culture and Culture Period (ng/ml)

No	Cells Culture	Culture Period (days)		
		0	2	4
1.	Fallopiian Tube	24,107	24,644	24,474
2.	Ampulla	24,187	23,753	24,254
3.	Isthmus	24,071	24,083	24,034
4.	Folicl1e	26,671	27,610	24,034

From the research results that progesterone levels in various cell culture and the culture period showed follicular development have increased and decreased. Levels of the hormone progesterone in follicular cell culture high on the second day culture period (27.610 ng/ml) was followed by a decrease to zero day (26.671 ng/ml) and the fourth day showed the lowest levels of the hormone progesterone (24.034 ng/ml). The increase in the hormone progesterone in the follicle cells occurs due to the LH peak that indicates the role of progesterone, while a decrease may occur due to follicles is already approaching the peak period of growth.

According Duria (2011) that progesterone is a hormone produced CL. Added by Aparicio *et al*. (2011) found increased levels of progesterone in cultured two days due to the formation of CL occur after the follicles release an egg, so that the cows produce more CL and progesterone. The increase in the hormone progesterone in follicular cells occurred with short and quick, which is caused by the LH peak that indicates the role of progesterone.

Pahl*et al*. (2004) states that the large follicular growth rate is lower because of the antrum is fully formed so that the response to the lower medium. Follicles with small size are more likely to experience growth due to the size of small follicles (2 - <4mm) in its infancy whereas large follicles almost reached the peak stage of growth (follicle *De graff*). *De graff* follicle growth rate is lower because of the antrum is fully formed so that the response to the lower medium.

From the research that increased levels of progesterone occurs on the second day, this is due to CL. Confirmed by Cor (2014) points out that increased CL will increase production of the hormone progesterone. Siregar (2002) adds that the concentration of progesterone during the establishment period associated with the number of CL. Results of research Telfer *et al*. (2008) that the establishment of antral follicles were cultured happen quickly on a two-day culture.

According Ratnawati (2011) that the hormone progesterone produced by CL and placenta. Progesterone levels in pregnant cow more than the cow is not pregnant. Progesterone occurs after ovulation and cause widespread development of the endometrium, uterus prepare to be ready to receive the embryo and feed. Broadly speaking, the physiological function of progesterone to the uterus of which block the effect of oxytocin on the myometrium, inhibit contraction of the myometrium and stimulate the growth of uterine glands in the endometrium.

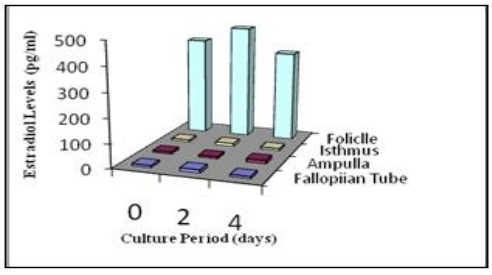

Fig. 2: Progesterone Hormone Levels In Different Cell Culture and Culture Period

The research results are obtained as shown in Figure 2 that the level of progesterone in cultured fourth day decline. This can happen because the follicle is approaching the peak of its growth and follicle cells are in the luteal phase. According Tjiptosumirat (2009), when the ovaries do not contain CL, progesterone concentration decreases. According Senger (2003) that the levels of progesterone in the eventual development of the follicle after luteolysis (without CL) and decreased levels of progesterone.

Sariubang and Nurhayu (2011), when the cattle are already in the luteal phase means it has had a CL. As a result of these hormones reacted by lysing CL formed. This causes the lysis of CL progesterone levels decrease, resulting in loss of barriers to the hormone gonadotropin, which is followed by the growth and maturation of follicles, oestrus and ovulation arise.

Profile of reproductive hormones during the cycle can describe the female ovarian function is concerned that analyzes hormone profile and its metabolites can indicate the condition of the female reproductive (Mostl and Palme, 2002). According to Siregar *et al*. (2004), the onset of estrus caused by lysis CL so that blood flow to the CL decreases dramatically. As a result, the levels of progesterone produced by CL will decrease. The decrease in progesterone levels will stimulate the anterior pituitary produces and releases FSH and LH. These hormones are responsible in the process of folliculogenesis and ovulation, resulting in the growth and maturation of follicles. Follicle eventually produce the hormone estrogen which is able to manifest the symptoms of estrus (Hafez and Hafez, 2000).

From the research that progesterone levels in various cell culture and the culture period in medium TCM-199 that the treatment culture follicular cells highest in the culture period the second day (27.610 ng/ml) and progesterone levels are lowest in cell culture ampulla of the culture period the second day (23.753 ng/ml). This is due to the development of CL during the estrous cycles that affect the levels of progesterone. CL formation has occurred after ovulation, which the hormone progesterone being produced. Conversely a decrease in progesterone levels occur after CL started to regress after estrus and began released luteolitik agent that can regressing CL. Hafez and Hafez(2000) suggested that CL regresses, causing reduced levels of the hormone progesterone.

Follicular growth can be influenced by the size of the follicles in culture, old culture and FBS. Goto *et al*. (1995) states that the culture conditions consist of the medium, the concentration of GH and use CO_2 incubator (long time culture) to support the growth of the follicle culture. Sirard and Coenen (1995) states that the culture medium, serum types that interact in the medium affects the growth and formation of groups of antral follicles *in vitro*. The length of time required for culturing follicles for 4 days, is intended to produce follicles and production of oocytes is better than the process of meiosis *in vitro*.

Adam *et al*. (2004) reported that follicle consists of several core cells are coated by the cell membrane. The core of these cells have the potential to grow and become egg maturation occurs, if follicles fully developed, but there are several follicles did not develop and die then be replaced with new follicles.

CONCLUSION

Based on the research results can be concluded is that the estradiol hormone levels in various cell culture and the culture that the treatment period follicle cell culture high on the second day culture period (476.67 pg/ml) and the lowest levels of estradiol on cell cultures in the period ampulla culture on day 0 (9.00 pg/ml) in cell culture followed isthmus at the culture period on day 0 and day four (9.00 pg/ml). Whereas progesterone levels in various cell culture and the culture that the treatment period follicle cell culture high on the second day culture period (27.610 ng/ml) and low progesterone levels in cell culture ampulla on the second day culture period (23.753 ng/ml).

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