

## INDUCED ABORTION AT MIDGESTATION OF SHEEP BY STRESS

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### ABSTRAK

*Stres dan abortus adalah awal dan ujung dari suatu peristiwa. Tujuan eksperimen ini memperlihatkan bahwa stres mendorong tubuh menghasilkan kortisol untuk mengatasi stres, tetapi sekaligus menyebabkan abortus. Bertolak dari tujuan tersebut, ada hipotesis yaitu kortisol mendorong terjadinya ekspresi reseptor progesteron A dan reseptor estrogen- $\gamma$  di dalam otot uterus, sehingga terjadi kontraksi uterus yang mengakibatkan peristiwa abortus. Untuk menguji hipotesis ini, dipakai kortisol sintetik yaitu deksametason sebagai stressor. Dilakukan random allocation pada 21 ekor domba bunting sehat usia 1 tahun, terbagi atas tiga kelompok masing-masing 7 ekor dengan usia kebuntingan 70-90 hari. Metoda, randomized preposttest control group design. Kelompok kontrol disuntik NaCl 0,9% intramuskular, kelompok perlakuan disuntik deksametason dosis tinggi dan rendah intramuskular. Darah tepi domba diambil untuk pemeriksaan kadar estrogen dan progesteron dengan metoda radioimmunoassay (RIA), otot rahim untuk pemeriksaan ekspresi RPA, RPB dan RE $\gamma$  dengan metoda imunohistokimia. Ekspresi RPA dan RE $\gamma$  pada kelompok perlakuan, sebaliknya ekspresi RPB menurun; kadar progesteron dan estrogen mengalami penurunan. Manfaat, jelas stres menyebabkan abortus pada domba, melalui mekanisme ekspresi RPA(FMI 2013;49:12-15).*

**Kata kunci:** stres, deksametason, PR-A, ER $\gamma$ , abortus.

### ABSTRACT

*Stress and abortion were the beginning and end of an event i.e abortion. The aim of this experiment was to show that stress enforced body to produce cortisol in order to overcome the stress itself while will caused abortion in pregnant ewe. Based on this aim, the hypothesis was cortisol will push the expression progesterone receptor A (PRA) and receptor estrogen- $\gamma$  (RE- $\gamma$ ) in uterine myometrium which produced uterine contraction and abortion. In order to test the hypothesis, I'll use the synthetical cortisol, dexamethasone as a stressor. Of 21 healthy pregnant sheep were grouping by random allocation into three groups each consist of 7 animal at the pregnant age 70-90 days. The control group were inject with normal saline 0,9% intramuscular (i.m), while treatment group divide into 2 group for intramuscular (i.m) injection of low and high dose of dexamethasone 4 mg and 8 mg. Peripheral blood were taken for estrogen and progesterone analysis by radioimmunoassay and uterine specimen for progesterone receptor A, as well as estrogen receptor  $\gamma$  expression by using immunohistochemistry preparation. High significantly were obtained among the treatment and control group, conversely the concentration of estrogen and progesterone were decline. In conclusion, it seems like that stress through cortisol production had encouraged abortion among the midpregnant sheep through the progesterone receptor A expression. (FMI 2013;49:12-15)*

**Keywords:** stress, dexamethasone, PR-A, ER $\gamma$ , abortion.

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### INTRODUCTION

Knowledge has been observed long ago that uterine contraction has happened if there was a decreased in plasma progesterone level followed by estrogen level increased at term. However, currently results revealed that reduced of progesterone level and conversely the increase of estrogen level never happened in humans and primate. If so, what caused of the uterine contraction? The answer is uterine muscle contraction responsiveness to the progesterone hormone (Pieber et al 2001). Then another reported says that the uterine

responsive expression circumstances is a hallmark of progesterone receptor A (PR-A) expression. At the same time it will followed by uterine responsive to the estrogen through estrogen receptor  $\alpha$  (ER- $\alpha$ ) (Mesiano et al 2002).

The question has emerged whether abortion could be caused by stress in sheep or preterm birth in woman through the induction of progesterone receptor A (RP-A) expression. Actually, stress phenomena which happens to humans or animals could be end at abortion

or preterm birth caused by cortisol production and uterine contraction (Mesiano 2004).

Why do the abortion or preterm birth caused by stress are important? The preterm birth in humans has an impact to morbidity and mortality as well to mother and child. The incidens rate of preterm birth about 8-10% of all birth. This incidens has reduced a little in recently 40 years (Creasy 1991). The causes of preterm birth commomly by pregnancy complications such as preeclampsia, premature rupture of the membrane with or without infection (30-40%) and idiopathic (40-50%) (Meis et al 1998).Lastly, the socio-economics impact bot in near or far future (Hannah et al 1995).

The result of changing era, since Csapo& Pinto-Dantas (1965), followed by Liggins et al (1977), then Challis et al (2000), confirmed that giving birth in any other species such as sheep, begins from the fall of progesterone level in blood plasma followed by raised up of estrogen. It means that birth processes are actively inhibited by progesterone. In thi case the roles of progesterone should be withdrawal preceded the parturition.

Based on those facts, an idea has emerged by creating a stress conditions to the pregnant sheep model by using the synthetic cortisol i.e. dexamethasone. Then we analyzed whether any effects of dexamethasone to the progesterone recepto A (PR-A) expression in the stromal cells of uterine muscle of the sheep at midgestation. We choose the midgestation sheep due to the similarities between hypothalamo-pituitary-adrenal (HPA) axis to humans (Challis et al2001). Dexamethasone injection was done at the treatment sheep at midgestation of day 70 to 100 (gestational duration (140-145). The significances of migestation of sheep is that there were no expression of PR-A. These expressions were examined by immunohistochemistry methods.

## MATERIAL AND METHODS

This study was performed in pregnant ewes at midgestation with accordance and was approved by the Animal Care and Use Committee of University of Airlangga, Veterinary Faculty for ethical clearance. Using singleton pregnancies of local ewes, randomly allocated were done into two groups : the experimental groups and treatment at day 70 – 100 of gestation. Each ewe was housed individually under natural condition of their daily maintenance requirements throughout their pregnancies. Ewes in the control group (n = 7) receive normal saline (NaCl 0,9%) via intramuscular injection (i.m); while treatment group (n = 7) were allocated to

receive dexamethasone injection 8 mg intramuscular (i.m).

The research design was Randomized posttest Control Group Design for examining the presence of PR-A in myometrial tissues. Pregnant myometrial tissues were obtained from each ewe of control group, and the treatment group which either has abortion immediately or without abortion at the day 2. The myometrial biopsies were obtained from the myometrial fundal. The myometrial tissues were immediately snap frozen and stored at minus (-) 70 until use. Data reported were expressed as means  $\pm$  SD. Statistical significance of differences between groups was tested using Student's t-test or one-way Anova. A level of  $P < 0.05$  was considered to be statistically significant. The myometrial tissues were fixed with formalin buffer for histology and immunohistochemistry examination in pathology laboratory of Veterinary Faculty of Airlangga University. Immunohistochemical staining of PR-A: All staining was carried out according to the manufacturer's instructions for paraffin section. For progesterone receptor A : was used mouse monoclonal antibody for progesterone receptor A antigen, species reactivity for sheep, immunogen from human endometrial carcinoma (EnCa 101) grown in athymic mice, made by Lab Vision Co, Warms Springs Blvd CA 94539 USA. These products are intended for research use only. Every stained were evaluated in cell with details as follows : negative stain (-), weak (+), moderate (++), densed (+++). Cells number were count by visual such as 0%, 25%, 50%, or 75%. Each observation were performed at 400 magnitude.

## RESULTS

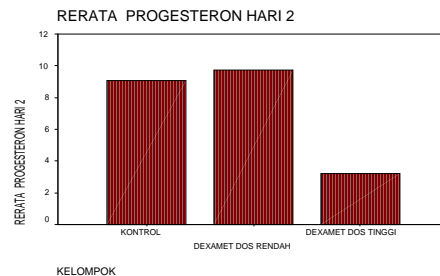


Figure 1. The means concentration of progesterone at day 2nd in control and dexamethasone treatment high and low dose.

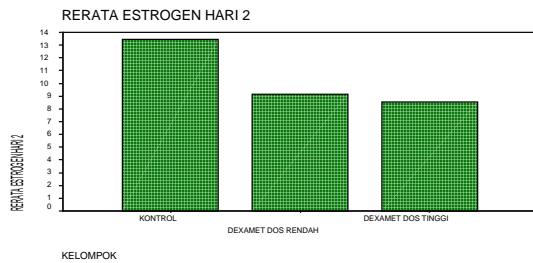
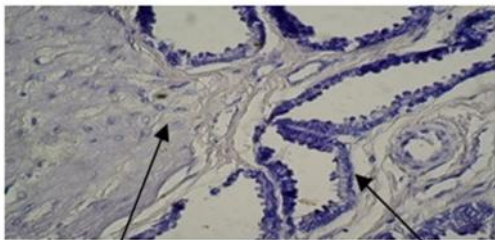
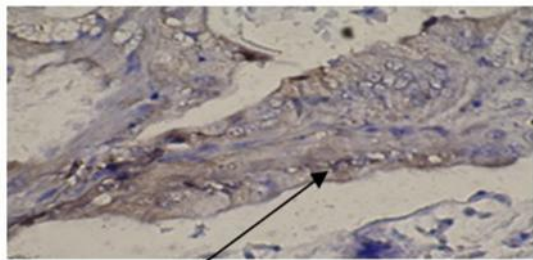


Figure 2. The means concentration of 17β-estradiol at day 2nd in control and dexamethasone treatment high and low dose.



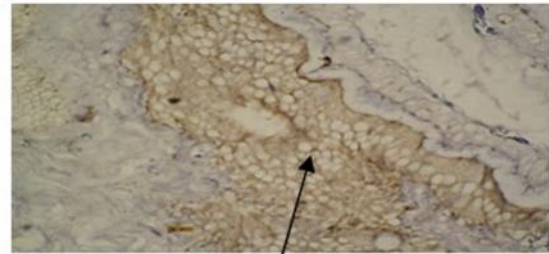
Negative (-) PRA, ERα Stroma endometrium  
 Negative (-) PRA, ERα Stroma endometrium + luminal

Figure 3. The PRA and ERα expression in uterine stromal cells in control group were absent (negative). Immunohistochemistry staining at magnitude 400 X.



Positive (+), PRA in endometrial Stromal cells.

Figure 4. The expression of PRA in endometrial stromal cells in dexamethasone treatment were present (positive). Immunohistochemistry staining at magnitude 400 X.



Positive (+), ERα in uterine stromal cells. Immunohistochemistry staining at magnitude 400 X.

Figure 5. The expression of ERα in endometrial stromal cells in dexamethasone treatment were present (positive). Immunohistochemistry staining at magnitude 400 X.

**DISCUSSION**

In this study, after a dexamethasone injection of the pregnant sheep at the midgestation followed by abortion. This event is the respons to the cortisol production by adrenal gland. However, there emerged a question how the mwechanism of action was? The answer were: First, there was a similarities between the pathway of stress from hipotalamo-hipophyse-adrenal gland which produces cortisol. The functions of cortisol are for maintaining homeostatis of the organism in a dynamic condition. That pathway is similar between humans and sheep. However in humans there is no cytochrome C17 enzyme which will change pregnenolone to estrogen and progesterone (Brown et al 2004). For those reason, in humans in uterine contractily before birth, there were no decline in progesterone concentration while raised in estrogen concentration. This event aimed for regionalitized of uterine contraction and relaxation uterine cervical.

Second, to facilitate this study by using the midgestation sheep as a model i.e. pregnant age of 70 to 90 days of age duration of sheep is 145 days. What does it means. As we believed that there were no progesterone receptor A (PRA) expression, due to the presence of progesterone receptor B (PRB) whose act as a dominant relaxation factor for uterine muscle. There was a need to withdrawal of PRB and also changed to the expression of estrogen receptor α. The effect of this changed is uterine contraction. It is clearly by administration of dexamethasone it could happened to the stress design to the sheep at midgestation. In fact there was an abortion of sheep dexamethasone administration at the midgestation (Poli 2007).

Third, in fact at the treatment group of dexamethasone of low dose (4 mg) by intramuscular injection, abortion has occurred in 3 of 7 sheep. Whilst in high dose of dexamethasone (8 mg i.m) there were abortion process in 5 of 7 sheep. These data has strongly support the data even the control group and treatment group actually there were no raised or decreased of estrogen and progesterone. So I concluded the presence of progesterone receptor A and estrogen receptor  $\alpha$  has a role in abortion process at midgestation of the sheep. Fourth, at the microscopy examination of the uterine stroma muscle by using immunohistochemistry methods which could visualised the progesterone receptors (PRs) of PRA, PRB, and ER $\alpha$  in uterine stromal cells.

The significances effect of low and high dose of dexamethasone on the expression of PRA (direction) that is the higher the dose the higher the expression, its means a positive effect. It is believe the lift up of PRA parallel to the dose. Conversely, no abortion in control group, and also there were no expression of PRA. So, in the present study I have documented for the first time in the sheep that the expression of PRA in the uterine stromal cells at the midgestation were induced by dexamethasone. I consider with the presence of PRA merely not only an evident to receive the research hypothesis but also acknowledge us that stress treatment which represent by dexamethasone i.m at day 70 and 100 of sheep gestation. Its means that the presence of PRA are no needs due to its role to stimulate the uterine contraction.

## CONCLUSIONS

Stress is the parts of individual life, either it is eustress or distress. Stress is the keyword of the upstream of problem and the end is abortion at the downstream of the sheep in the midgestation. It is clear for us the involvement of protein such as PRA. A clearly mechanism in this study that not by the change of progesterone and estrogen concentrations for the abortion process but by the presence and roles of PRA. The function of this study at last give us a new insight in order to find and effort to prevent abortion and also preterm birth in humans by using PRA inhibitor.

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