RINGKASAN

Kadar Prostaglandin F_2 α Dari Cairan Vesikula Seminalis, Produk Sel Monolayer Vesikula Seminalis Dan Endometrium Sapi Bali Serta Bioaktivitasnya

Prostaglandin F_2 α ($PGF_2α$) merupakan agen luteolitik yang menyebabkan regresi korpus luteum dan kontraksi otot polos. Secara alami $PGF_2α$ berfungsi mengontrol siklus estrus, transport ovum, transport spermatozoa dan partus pada mamalia serta secara luas telah digunakan untuk sinkronisasi estrus baik pada ternak kecil maupun ternak besar, untuk penanganan beberapa kasus reproduksi khususnya kasus anestrus postpartum yang disebabkan oleh karena tidak beregresinya korpus luteum atau korpus luteum persisten (CLP).

Endometrium dan vesikula seminalis pada umumnya merupakan sumber produksi prostaglandin dan keduanya dapat dikembangkan melalui sel monolayer. Produksi PGF_2 α pada endometrium sangat tergantung dari spesies hewan, hal ini berkaitan dengan panjang pendeknya siklus estrus dan pada umumnya PGF_2 α disekresikan pada pertengahan siklus estrus atau kadar tertinggi PGF_2 α dari endometrium sapi dilaporkan antara hari ke -16 sampai hari ke -17 siklus estrus. Sedangkan dugaan bahwa vesikula seminalis juga memproduksi PGF_2 α, karena pada semen ditemukan sejumlah prostaglandin termasuk PGF_2 α yang diperkirakan berasal dari vesikula seminalis. Selain itu, pada penelitian pendahuluan yang dilakukan pada kuda betina fase luteal, pemberian ekstrak cairan vesikula seminalis sapi Bali dapat meregresi korpus luteum yang dicerminkan dari penurunan hingga 74 % kadar hormon progesteron serum dalam kurun waktu 24 jam. Hal ini menandakan bahwa vesikula seminalis menghasilkan PGF_2 α.

Berdasarkan uraian tersebut, maka telah dilakukan penelitian dengan tujuan untuk mengukur kadar PGF_2 α pada cairan vesikula seminalis, produk sel *monolayer* vesikula seminalis dan sel *monolayer* endometrium

untuk memproduksi PGF_2 α dengan memanfaatkan limbah rumah potong hewan berupa vesikula seminalis dan endometrium sapi Bali

Penelitian ini dilakukan secara bertahap, tahap I adalah menentukan kadar PGF_2 α dari cairan vesikula seminalis, produk sel *monolayer* vesikula seminalis dan endometrium. Cairan vesikula seminalis diambil secara aspirasi, sedangkan sel epithel vesikula seminalis dan sel epithel endometrium dibiakkan pada *tissue culture medium* (TCM 199) + *fetal calf serum* (FCS) 10% dan *Estrus Mare Serum* (EMS)10%. Konsentrasi sel kultur adalah 1,9 x 10^6 dan setiap kultur ditambahkan hipotaurin dengan konsentrasi 0, 4 dan 8 mM sebagai antioksidan, kemudian diinkubasikan pada temperatur 38,5 ^{0} C dengan 5% CO_2 dan masa inkubasi 6 hari dan 12 hari. Konsentrasi PGF_2 α baik dalam cairan vesikula seminalis maupun dalam produk sel *monolayer*, diukur dengan teknik *radioimmunoassay* (RIA). Tahap selanjutnya menguji bioaktivitas PGF_2 α produk penelitian secara *in vitro* dilakukan pada sel monolayer luteal yang dibagi menjadi 2 kelompok yaitu kalompok I ditambahkan 10% ekstrak produk penelitian/flask dan kelompok II ditambahkan 1,25 mg PGF_2 α produk paten/ml media/flask masing-masing pada hari ke -9 masa inkubasi. Pengukuran kadar hormon progesteron dilakukan sebelum perlakuan dan 2 hari setelah perlakuan. Sedangkan uji *in vivo* dilakukan pada kuda fase luteal yang dibagi menjadi 2 kelompok perlakuan yaitu kelompok perlakuan I diberikan 20 cc ekstrak produk penelitian/ekor secara intra uterin dan kelompok perlakuan II diberikan 4 mg PGF_2 α produk paten/ekor secara intra uterin. Pengukuran kadar hormon progesteron dilakukan sebelum perlakuan (0 jam) dan 24, 48 dan 72 jam setelah perlakuan. Kadar hormon progesteron diukur dengan menggunakan teknik RIA.

Hasil penelitian menunjukkan bahwa rataan kadar PGF_2 α tertinggi diperoleh pada produk sel *monolayer* vesikula seminalis dan secara statistik menunjukkan perbedaan yang nyata ($P < 0,05$) dengan kadar PGF_2 α pada produk sel *monolayer* endometrium. Penambahan hipotaurin pada media kultur sel vesikula seminalis dengan konsentrasi 4 mM dan 8

mM, menyebabkan penurunan kadar PGF_2 α Sebaliknya penambahan hipotaurin pada media kultur sel epithel endometrium dengan konsentrasi 4 mM dan 8 mM, dapat meningkatkan kadar PGF_2 α dan secara statistik menunjukkan perbedaan yang nyata ($P < 0,05$) dengan konsentrasi 0 (tanpa penambahan hipotaurin). Masa inkubasi juga berpengaruh nyata ($P < 0,05$) antara masa inkubasi 6 hari dengan masa inkubasi 12 hari, sedangkan kadar PGF_2 α tertinggi diperoleh pada masa inkubasi 6 hari.

Rataan kadar PGF_2 α pada cairan vesikula seminalis dengan produk sel *monolayer* vesikula seminalis menunjukkan perbedaan yang nyata ($P < 0,05$), demikian juga rataan kadar PGF_2 α setelah cairan vesikula seminalis dan produk sel *monolayer* vesikula seminalis diekstraksi menunjukkan perbedaan yang nyata ($P < 0,05$) dan kadar PGF_2 α tertinggi diperoleh pada cairan vesikula seminalis yang diekstraksi. Uji bioaktivitas baik secara *in vitro* maupun *in vivo,* penurunan kadar hormon progesteron tidak menunjukkan perbedaan yang nyata ($P > 0,05$) antara perlakuan PGF_2 α produk penelitian dengan produk paten (*Dinoprost*). Demikian pula terhadap munculnya estrus, tidak terdapat perbedaan yang nyata ($P > 0,05$) antara PGF_2 α produk penelitian dengan produk paten.

Dari hasil penelitian dapat disimpulkan bahwa PGF_2 α dapat diproduksi melalui biakan sel *monolayer* vesikula seminalis dan endometrium sapi Bali. PGF_2 α yang diekstrak dari cairan vesikula seminalis memilikki daya efektivitas yang sama dengan PGF_2 α produk paten (*Dinoprost*) terhadap penurunan kadar hormon progesteron baik pada sel *monolayer* luteal maupun pada kuda fase luteal, demikian pula terhadap munculnya estrus.

SUMMARY

Concentrations of Prostaglandin F_2 α which Isolated from Seminal Vesicle Fluid and Product of Seminal Vesicle and Endometrium Monolayer Cells of Bali Cattle and Its Bioactivity

Prostaglandin F_2 α (PGF_2 α) is a luteolytic agent which causes the regression of the corpus luteum and the contraction of smooth muscle. Its natural function is to control oestrus cycle, ovum and sperm transport, and parturition. PGF_2 α has been widely used to treat of reproductive disorders such as anoestrus caused by corpus luteum persistent.

The main sources of PGF_2 α are endometrium and seminal vesicle. In our preliminary study in mare, the methanol extraction of seminal vesicle fluid originated from Bali cattle exhibited an ability to regress corpus luteum reflected by a sharp reduction (up to 70%) in level of progesterone at 24 hours post treatment. This result showed a clear indication that high level of PGF_2 α is present in the seminal vesicle gland and a study was therefore conducted to produce PGF_2 α by utilizing the slaughterhouse wastes i.e. seminal vesicle and endometrium derived from Bali cattle.

This study was carried out in several phases. The first phase was to determine the PGF_2 α concentration from seminal vesicle fluid and from seminal vesicle and endometrium monolayer cell cultures of Bali cattle. The seminal vesicle fluid was aspirated and the epithelial cells of the seminal vesicles and endometrium were cultured in TCM 199 growth medium with Fetal Calf Serum (FCS) 10% and Estrus Mare Serum (EMS) 10%. The cells were cultured at a density of 1.9×10^6 per ml medium with or without the presence of 4 mM and 8 mM hypotaurine as an antioxidant. Following incubation at 38.5^0 C in 5% CO_2 atmosphere for 6 and 12 days, the levels of PGF_2 α of seminal vesicle fluid and the product of cell culture were determined by radioimmunoassay techniques (RIA). The next phase was to determine the biological activity of PGF_2 α research product, both in vitro by using luteal monolayer cell cultures and in vivo in the mare with in the luteal phase of oestrus cycle. The luteal monolayer cell cultures at 9 days of incubation were divided into two groups. Group I was added with 10%

extract of cell product and group II was added with 1.25 mg dinoprost / ml medium. The level of progesterone was measured at the day of treatment and 2 days later by RIA technique. To determine the biological activity of PGF_2 α research product, 20 cc extract of the reseach product / head compared with 4 mg dinoprost/head as a patent product were administered intra uterine into the mares with in luteal phase of oestrus cycle. The level of progesterone was measured on the day of treament and at 24, 48, 72 hours after treament.

The result showed that the level PGF_2 α in the monolayer culture of seminal vesicle was significantly higher ($P < 0.05$) than in the monolayer culture of endometrium. The presence of 4 mM and 8 mM hypotaurine significantly ($P < 0.05$) reduced the level of PGF_2 α produced by the monolayer culture of seminal vesicle. In contrast, the presence of 4 mM and 8 mM hypotaurine increased the level of PGF_2 α in the monolayer cell culture of endometrium. The level of PGF_2 α was significantly higher ($P < 0.05$) in cell cultures incubated for 6 days than incubated for 12 days. The level of PGF_2 α detected in the monolayer culture of seminal vesicles was significantly lower ($P < 0.05$) than that originated from seminal vesicle fluid. Extraction of seminal vesicle monolayer cells culture significantly ($P < 0.05$) decreased the level of PGF_2 α, where as extraction of seminal vesicle fluid significantly ($P < 0.05$) increased the level of PGF_2 α. The highest level of PGF_2 α was found on the extract of seminal vesicle fluid. Biological activity test in vitro and in vivo, indicated that the PGF_2 α research product was as effective as dinoprost to decrease the level of progesterone and synchronised the onset of oestrus

In conclusion, the PGF_2 α can be isolated from seminal vesicle fluid and produced by monolayer cell cultures of seminal vesicle and endometrial of Bali cattle. PGF_2 α extract from seminal vesicle fluid had effectivity similar to dinoprost to reduce the level of progesterone in the mare with luteal phase of oestrus cycle and synchronised the onset of oestrus.

ABSTRACT

Concentrations of Prostaglandin F_2 α which Isolated from Seminal Vesicle Fluid and Product of Seminal Vesicle and Endometrium Monolayer Cells of Bali Cattle and Its Bioactivity

A study was conducted to produce PGF_2 α by utilizing the slaughterhouse wastes i.e. seminal vesicle and endometrium derived of Bali cattle.

The study was carried out in several phases.The first phase was to determine the PGF_2 α concentration from seminal vesicle fluid and from seminal vesicle and endometrium monolayer cell cultures of Bali cattle. The seminal vesicle fluid was aspirated and the epithelial cells of the seminal vesicles and endometrial were cultured in TCM 199 growth medium with fetal calf serum (FCS) 10% and 10% Estrus Mare Serum (EMS). The cells were cultured to a density of 1.9 x 10^6 per ml medium with or without the presence of 4 mM and 8 mM hypotaurine as antioxidant. Following incubation at 38,5^0 C in 5% CO_2 atmosphere for 6 and 12 days, the level of PGF_2 α in the original seminal vesicle fluid and in the medium of cell culture were determined by radioimmunoassay techniques (RIA). The next phase was to determine the biological activity of PGF_2 α research product, both in vitro by using luteal monolayer cells culture and in vivo in the mare with luteal phase of oestrus cycle. The luteal monolayer cell cultures at 9 days of incubation were divided into two groups. Group I was added with 10% extraction of cell product and group II was added with 1.25 mg dinoprost/ml medium. The level of progesterone was measured on the day of treatment and 2 days later by RIA techniques. To determine the biological activity of PGF_2 α 20 cc extract of research product / head compared with 4 mg dinoprost/head were administered intra uterine into the mare with luteal phase of oestrus cycle. The level of progesterone was measured on the day of treatment and at 24, 48, 72 hours after treatment.

The result showed that the level PGF_2 α in the monolayer culture of seminal vesicle was significantly higher ($P < 0.05$) than that detected in the monolayer culture of endometrium. The presence of 4 mM and 8 mM hypotaurine in the cell culture medium significantly ($P<0.05$) reduced the level of PGF_2 α produced by the monolayer culture of seminal vesicle. In contrast, the presence of 4 mM and 8 mM hypotaurine increased the PGF_2 α level in the monolayer cell culture of endometrium. cell culture incubated for 6 days than incubated for 12 days. The level of PGF_2 α detected in the monolayer culture of seminal vesicles was significantly ($P < 0.05$) reduced compared that originated from seminal vesicle fluid. Extraction of seminal vesicle monolayer cell cultures significantly ($P < 0.05$) decreased the level of PGF_2 α, where as extraction of seminal vesicle fluid significantly ($P < 0,05$) increased the level of PGF_2 α. The highest level of PGF_2 α was found in the extract of seminal vesicle fluid. Biological activity test in vitro and in vivo, indicated that the PGF_2α research product was as effective as dinoprost to decreased the level of progesterone and synchronised the onset of oestrus.

Keywords: PGF_2 α, Hypotaurine, Seminal Vesicle, Endometrium