PENCEMARAN MERCURY DENGAN MENENTUKAN RANTAI MAKANANNYA PADA HEWAN AKUATIK DENGAN MENGGUNAKAN MERCURY RADIO-AKTIF SEBAGAI TRASER

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SYNOPSIS

Pencemaran lingkungan yang disebabkan oleh logam-logam berat yang juga merupakan unsur langka seperti zink, timah, besi, cadmium, mercury, tembaga, arsenik, nickel, vanadium dan bryllium merupakan masalah yang serius dewasa ini.

Mercury yang merupakan salah satu unsur langka tersebut diatas, secara umum, menimbulkan pencemaran lingkungan terutama disebabkan oleh pembuangan sisa-sisa industri dengan bebas dan oleh penggunaan pesticida dan herbicida di bidang pertanian. (Dugan, 1972).

Tidak semua bentuk mercury menimbulkan kerusakan-kerusakan terhadap organisme. Cairan mercury sendiri tidak begitu beracun, tetapi unsur ini paling berbahaya bila ia berada di dalam bentuk komponen organik dan anorganik, terutama di dalam bentuk alkyl yaitu methyl dan ethyl mercury (Goldwater, 1971).

Komponen-komponen mercury organik yang mencemari lingkungan terjadi oleh dua faktor utama. Pertama akibat aktivitas bakteri aerobik dan anaerobik terhadap endapan mercury yang terdapat di dalam lumpur di dasar sungai, danau dan laut (Tonomura et al.; 1972). Kedua sebagai akibat langsung dari pembuangan sisa-sisa industri yang menggunakan mercury sebagai bahan mentah (Boureng, 1970; Goldwater, 1971).

Di lingkungan akuatik komponen mercury ini akan dikumpulkan oleh organisme akuatik dan akan terikat oleh suatu sistim kehidupan dari organisme yang bersangkutan (Giddings, 1973). Dari komponen mercury, methylmercury merupakan komponen organik yang paling tinggi dapat di kumpulkan oleh organisme akuatik (Jensen dan Jernelov, 1969).

Di alam mercury tersebar dengan konsentrasi yang sedikit sekali, meliputi lithosphere, hydrosphere, atmosphere dan biosphere (tumbuhan

dan hewan). Di dalam air laut konsentrasinya sekitar 0.1 ppb dan jumlah ini semakin bertambah dengan pertambahan dalamnya laut (Edwards, 1973; Giddings, 1973; Pryde, 1973). Konsentrasi mercury di dalam air tawar sekitar 0.1 ppb dan 6 ppb (Giddings, 1973). Dalam atmosphere mercury berada di dalam bentuk uap dan bentuk partikel-partikel (Wrobleweski et al., 1974) dengan konsentrasi kurang dari 10^{-9} gm/m^2 (Giddings, 1973). Keadaan yang berlainan berlaku didalam biosphere, di mana tumbuh-tumbuhan dan hewan mempunyai kebolehan untuk mengkonsentrasikan mercury. Organisme akuatik dapat menyerap komponen mercury dan mineral-mineral lainnya secara langsung dari lingkungan akuatik di sekelilingnya (Polikarpov, 1966; Fowler et al., 1976; Djojosoebagio, 1976).

Komponen dimethylmercury yang mempunyai ikatan kovalen akan membentuk ion methylmercury di dalam air (Giddings, 1973). Disamping itu ion mercury mempunyai ikatan yang kuat dengan ion sulfur dan membentuk ikatan Hgs. Komponen ini mempunyai ikatan yang kovalen dan ikatan ionik. Mercury sangat mudah bergabung dengan sulfur. Unsur sulfur merupakan sebahagian dari sistim kehidupan dimana ion-ion sulfur terdapat di dalam beberapa jenis protein seperti cystein. Atom-atom sulfur bergabung di dalam protein dengan cara membentuk jembatan sulfhydril dan disulfida. Mercury dapat berikatan dengan atom sulfur di dalam kumpulan sulfhydryl dan disulfida.

Di dalam organisme proses pertukaran ini dapat berlaku dengan sepenuhnya jika konsentrasi ion mercury di dalam organisme cukup tinggi dan akibatnya menimbulkan kerusakan dan kelainan pada kehidupan organisme tersebut (Giddings, 1973; Pryde, 1973).

Keracunan komponen mercury mulai diketahui pada awal tahun limapuluhan ketika nelayan-nelayan di Minamata (Jepang) bersama-sama keluarganya dan ternak mereka mengalami suatu penyakit dengan gejala-gejala rusaknya penglihatan, kurang pendengaran, lemah otot, lumpuh, coma dan menyebabkan kematian (Pryde, 1973; Schubert, 1973). Dari penelitian-penelitian yang kemudian dilakukan diketahui bahwa penyakit tersebut disebabkan oleh komponen methylmercury yang dibuang ke laut sebagai sisa-sisa pengelolaan pada sebuah industri plastik polyvinylchlorida yang terletak di tepi teluk Minamata. Ikan-ikan yang terdapat di

kawasan ini tercemari oleh methylmercury yang dibuang sebagai sampah-sampah yang tidak dapat dimanfaatkan oleh industri tersebut. Dengan cara demikian methylmercury dapat pindah ke dalam tubuh manusia dan hewan-hewan lainnya jika mereka makan ikan-ikan yang telah tercemari oleh mercury yang dibuang tanpa perhatian sama sekali (Buhler, 1971; Goldwater, 1971; Griffith, Lee, 1971; Giddings, 1973; Pryde, 1973).

Batas maksimum konsentrasi mercury yang diperkenankan di dalam makanan oleh FDA adalah sekitar 0.5 ppm (Clark, 1971). Konsentrasi methylmercury di dalam ikan dari perairan Minemata ada sekitar 5-20 ppm dan konsentrasi air lautnya ada di sekitar 1,6 - 3.6 ppb (Pryde, 1973). Pencemaran methylmercury telah pula dilaporkan di Iraq, Pakistan Barat, Guatemala, Amerika Utara dan Swedia (Arighi, 1971; Klein, 1971; Goldwater, 1971; Giddings, 1973; Pryde, 1973).

Methylmercury menyerap masuk ke dalam hewan vertebrata dengan cara melalui makanan dengan paruh umur biologis ($t^1/2$ biologis) 70 hari (Dunlap, 1971; Goldwater, 1971; Pryde, 1973). Mercury dapat masuk ke dalam otak melalui "bloodbrain-barrier" dan merusak tenunan otak. Ia juga dapat melintasi plasenta dan masuk ke dalam fetus melalui darah sehingga anak yang dilahirkan dapat juga mengalami keracunan mercury (Kurland, 1971; Giddings, 1973). Unsur ini dapat pula berikatan dengan atom sulfur di dalam membran sel dan dapat mengakibatkan perubahan distribusi ion-ion, voltase listrik dan pergerakan cairan pada membran sel. Di dalam sel unsur ini akan menggganggu fungsi dan struktur organela seperti mitokondria dan lysosome (Goldwater, 1971).

Industri-industri yang menggunakan mercury diantaranya ialah industri kertas, pembuat plastik, industri chlorine dan caustic soda (Buhler et al.; 1971; Lee, 1971)

Penelitian yang dilaporkan disini ialah menentukan distribusi mercury dengan menggunakan mercury-203 sebagai traser didalam bentuk mercuric acetate dan methyl mercuric chloride.

Sebagai hewan percobaan telah dipakai ikat sepat (Trichogaster sp.) udang (Macrobranchium sp) dan kerang (Anadara sp). Tumbuhan air yang dipakai ialah Hydrilla verticillata.

Data yang dihasilkan menunjukkan bahwa methyl mercuric chloride lebih banyak dikonsentrasikan dari pada mercuric acetate di dalam

hewan-hewan akuatik dan tumbuhan akuatik.

Pengumpulan komponen-komponen tersebut di dalam hewan-hewan dapat berlangsung baik dengan jalan perpindahan secara langsung dari medium sekelilingnya maupun dengan jalan memakan tumbuhan akuatik yang telah tercemari oleh mercury.

Jika hewan-hewan akuatik tersebut yang telah tercemar dengan mercury diberikan kepada mamalia (didalam hal ini tikus), maka mercury dapat berpindah dan terkumpul di dalam hati, otak, buah pinggang, otot dan usus. Pada umumnya buah pinggang dan hati mengumpulkan mercury lebih tinggi dari pada otot, usus dan otak. Manusia yang menggunakan ikan atau organisme akuatik lainnya yang telah tercemari oleh mercury akan mengalami peristiwa pengumpulan mercury yang sama karena mempunyai proses fisiologi dan metabolisme yang sama atau hampir sama dengan mamalia yang digunakan di dalam percobaan.

Hasil dari penelitian menunjukkan bahwa konsentrasi komponen CH_3Hg-203Cl dan Hg-203 Acetat dalam ikan Trichogaster sp. berbeda-beda berdasarkan tenunannya. Kedua macam komponen didapati di dalam tenunan usus, sirip otot, dan otak ikan tersebut sesudah 24 jam. Pengumpulan paling tinggi terjadi didalam usus dan sirip. Konsentrasi CH_3Hg-203Cl menjadi lebih tinggi pada kebanyakan tenunan apabila ikan tersebut berada di dalam akuarium yang mengandung Hydrilla verticillata. Hal yang sebaliknya terjadi pada pengumpulan Hg-203 Acetat.

Macrobranchium sp. mengumpulkan CH_3Hg-203 Cl lebih banyak di dalam dagingnya sedangkan Hg-203 Acetat lebih banyak dikumpulkan di dalam kutikel.

Dalam percobaan dengan Anadara sp. di dalam medium tanpa makanan, didapati CH_3Hg-203Cl dan Hg-203 Acetat lebih banyak terkumpul di dalam dagingnya dari pada di dalam kulitnya. Bila Anadara ini diberi makanan pengumpulan mercury di dalam daging meningkat sedangkan pengumpulan di dalam kulitnya menurun.

Data dari tikus menunjukkan bahwa absorbsi Hg-203 oleh usus lebih cepat bila unsur ini diberikan bersama dengan udang atau kerang.

Hydrilla verticillata mengumpulkan CH_3Hg-203Cl lebih tinggi dari pada pengumpulan Hg-203 Acetat (60.6 kali dibandingkan dengan 4.57 kali). Pencemaran methylmercury di dalam lingkungan dapat dihapus dengan menggu-

nakan DDT karena DDT dapat menghambat enzim-enzim bakteria yang melakukan synthesis methylmercury di dalam air (mrpses methylation) (Dunlop, 1972). Namun masalah pencemaran DDT akan timbul karenanya. Dengan kebolehan Hydrilla verticillata untuk menyerap CH3Hg-203Cl begitu tinggi maka tumbuhan air ini di dalam prakteknya akan dapat dipakai sebagai pembersih (biological decontamination) sumber-sumber air yang dicemari mercury.

Lokalisasi dan distribusi mercury di dalam tenunan tidak ditentukan di dalam penelitian ini. Penelitian selanjutnya akan dilakukan dengan menggunakan kaedah mikroautoradiografi untuk menentukan penyebaran mercury pada taraf seluler.

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USE OF TRACER TECHNIQUES IN STUDIES OF UTERO-OVARIAN-PITUITARY RELATIONSHIPS IN CATTLE*

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The recent establishment of competitive protein binding and radioimmunoassay techniques for the measurement of small amounts of hormone in the peripheral blood circulation has greatly increased our present knowledge of reproductive physiology and pathology of domestic animals.

Hormonal factors involved in the development and regression of the corpus luteum during the estrous cycle are of fundamental interest in all domestic species. A better understanding of this physiology may result in improved breeding procedures such as: heat synchronization, heat detection etc. This report deal with some studies undertaken within the present IAEA-project. The studies are focused around luteolysis during the estrous cycle of the cow.

Role of the Uterus

The importance of the uterus for the control of corpus luteum life-span in the non-pregnant cow has been established through hysterectomy (Wiltbank & Casida 1956). Anderson et al. (1962) found that corpus luteum function was maintained for up to 270 days after hysterectomy. In the sheep. Moor and Rowson (1966) removed one ovary and parts of the uterus and found normal estrus cycle lengths when the retained uterine horn was adjacent to the corpus luteum. However, when the retained uterine horn was opposite to the ovary bearing the corpus luteum, the estrous cycle length was prolonged. The same unilateral effect of the uterus on the corpus luteum has been demonstrated in heifers (Ginther et al. 1967). Maintenance of corpus luteum function has also been reported in the cow

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and in the ewe with congenitally absent uterine horn (Bland 1970; McCracken & Caldwell 1969, respectively). Goding and coworkers (1967 a,b) separated the ovary and the uterus in the ewe by either auto-transplanting the ovary or the uterus to the neck. In these cases corpus luteum persisted. If both the uterus and the ovary are auto-transplanted normal cyclic corpus luteum activity is maintained (Harrison et al., 1968). In the cow and the ewe experimental data thus indicate that the uterine luteolytic effect is local and dependent on the presence of a uterine horn ipsilateral to the corpus luteum.

Nature of the Uterine Luteolysin

Pharris and Wyngarden (1969) proposed that since prostaglandin F_{2a} is an abundant uterine prostaglandin and since it has a pronounced venoconstricting effect, it is potentially a substance which might control luteal function. These authors also showed that prostaglandin F_{2a} (PGF_{2a}) administered subcutaneously caused a shortening of pseudopregnancy in the rat. Thereafter exogenous PGF_{2a} has been shown to be luteolytic in many species: in the quinea-pig (Blatchley & Donovan 1969; Chaichareon et al. 1974), hamster (Johnston & Hunter 1970; Labhsetwar 1971), rabbit (Gutknecht et al. 1969; Pharris 1970), gerbil (Chaichareon et al. 1974), sheep (McCracken et al. 1970; Barrett et al. 1971), goat (Currie & Thorburn 1973), swine (Gleeson 1974; Hallford et al.,1974), horse (Douglas & Ginther 1972; Noden et al. 1974) and cattle (Rowson et al. 1972; Lauderdale 1972).

McCracken et al. (1972) demonstrated high levels of PGF_{2a} in the utero-ovarian vein of the auto-transplanted ovary and uterus in the ewe. The high levels of PGF_{2a} occurred before estrus and coincided with the decrease in the progesterone levels. Similar findings have been reported by others using blood samples obtained either from the utero-ovarian vein in situ (Bland et al. 1971; Thorburn et al. 1972, 1973) or from the caudal caval vein (Fitzpatrick & Sharma 1973). Nancarrow et al. (1973) reported surges of PGF_{2a} production coinciding with decreses in progesterone concentrations in the utero-ovarian vein in one cow. Shemesh and Hansel (1975) reported increased levels of PGF_{2a} in the uterine vein during day 15-20 of the bovine estrous cycle.

PGF_{2a} has a very short half-life in the peripheral circulation (<30 sec.) due to its rapid metabolism. The measurement of PGF_{2a} concentrations is thus difficult because of the necessity of surgical cannulation of the utero-ovarian blood vessels either *in situ* or after auto-transplantation of the uterus and ovaries with intact vascular connections. To avoid these difficulties, attempts have been made to determine PGF_{2a} in peripheral blood plasma. However, this method is doubtful, because of the rapid conversion of PGF_{2a} into 15-keto-13,14-dihydro-PGF_{2a} (Granstrom 1972). The most serious drawback of this method is that measured peripheral plasma levels of PGF_{2a} do not reflect the synthesis of PGF_{2a} in the body (Samuelsson 1973). Since the metabolite of PGF_{2a}, 15-keto-13,14-dihydro-PGF_{2a}, has a considerably longer half-life in the circulation (Granstrom & Samuelsson 1972). With this method available it is possible to study the release of PGF_{2a} as reflected in the concentration of 15-keto-13,14-dihydro-PGF_{2a} in peripheral blood. This allows frequent blood sampling from a peripheral vein without the necessity of extensive surgery.

Four heifers of the Swedish Red and White Breed received either 58, 108, 240 or 510 ug prostaglandin F_{2a} each during 60 minutes in the jugular vein. Blood samples were drawn from the contralateral jugular vein, several times during and after the infusion, and resulting plasma levels of 15-keto-13,14-dihydro-PGF_{2a} were measured. This experiment was carried out in order to obtain information on the conversion of PGF_{2a} into 15-keto-13,14-dihydro-PGF_{2a}.

The plasma levels of 15-keto-13,14-dihydro-PGF_{2a} during infusion of PGF_{2a} at various rates are shown in Fig., 1. Pre-infusion levels of the prostaglandin metabolite ranged from 45 to 65 pg/ml. Infusion of PGF_{2a} at a rate of 970 ng/min (58 ug/h) resulted in an increase to a mean level of about 120 pg/ml (Fig. 1). The corresponding mean levels of the metabolite for the higher infusion rates 1800 ng/min (108 ug/h), 4000 ng/min (240 ug/h) and 8500 ng/min (510 uh/h) were 210, 540 and 1200 pg/ml, respectively. After infusion the levels of the metabolite rapidly decreased to pre-infusion levels. The half-life of the metabolite in the peripheral circulation was calculated to be between 7 and 8 min for the two heifers receiving the highest dose.

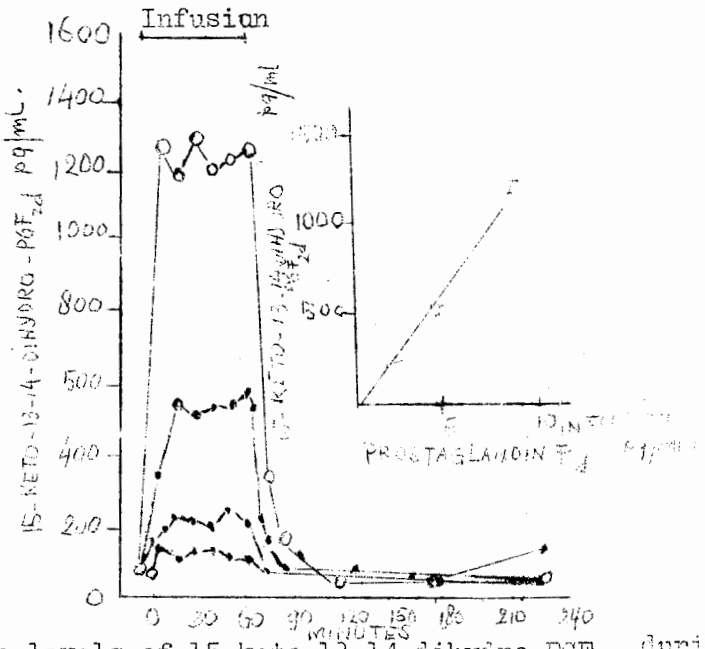

Figure 1: Plasma levels of 15-keto-13,14-dihydro-PGF_{2a} during intravenous infusion of PGF_{2a} in four heifers. The animals received 970 (▲), 1800 (△), 4000 (●) and 8500 (o) ng/min, respectively, during 60 minutes. From Kindahl et al. 1976 a.

The precision for the prostaglandin metabolite assay has been calculated from duplicate determinations. The coefficient of variation was 11.7% (n=57) for determinations in the range 10-50 pg/ml; 8.9% (n=138) in the range 51-100 pg/ml; 8.0 % (n=54) in the range 101-200 pg/ml; 8.1 % (n=28) in the range 201-300 pg/ml; 6.6 % (n=24) in the range 301-400 pg/ml; 7.3% (n=17) in the range 401-500 pg/ml and finally 9.7% (n=20) in the range 501-800 pg/ml. The accuracy of the method was determined from two different experiments: a) addition of known amounts of the prostaglandin metabolite (range 25-300 pg) to bovine plasma (Y=1.0x + 63); b) comparison between analyses by the radioimmunoassay and the mass spectrometric method developed for this metablite (Samuelsson & Green 1974) in human and bovine plasma. The values between the tow methods agree well (Granström & Kindahl 1976).

Prostaglandin Release During the Bovine Estrous Cycle.

The peripheral blood plasma levels of 15-keto-13,14-dihydro-PGF_{2a} have been determined during the normal estrous cycle in the cow (Kindahl et al. 1976 a,b). When employing a sampling schedule with bleedings every three hours during the period of luteolysis well defined and marked pulsatile increases of the metabolite was obtained (Fig. 2). However, each of the

peaks recorded consisted of only one elevated level. Considering the relative short half life of the metabolite in the cow and the extremely short half life of the primary prostaglandin in the same species a more precise study was undertaken in order to study the temporal relation - ship between prostaglandins and progesterone. For this study two heifers of the Swedish Red and White Breed were followed with hourly samples through the expected time of luteolysis (Fig. 3).

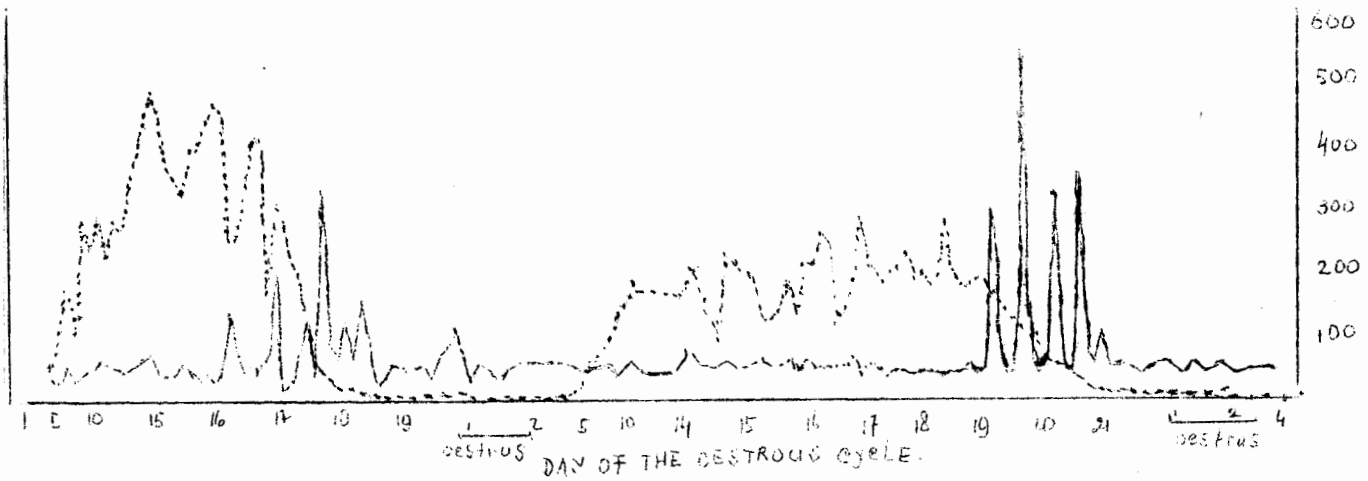

Figure 2: Peripheral plasma levels of progesterone (o———o) and 15-keto-13,14-dihydro-PGF_{2a} (●- - - - - -●) during two consecutive cycles in one heifer. Blood samples were obtained every three hour during the period of luteolysis. From Kindahl et al. 1976a.

The results of the study including hourly blood samples revealed prostaglandin metabolite peaks comprising of more than one observation (Fig. 3). Both of the animals showed the same pattern during luteolysis: three days before estrus a series of prostaglandin metabolite peaks started and the first of these peak s was almost immediately followed by a sharp drop in the progesterone concentration. The magnitude of the prostaglandin release did not decrease during the later part of luteolysis and sometimes higher levels were found even when the progesterone level was very low. The role of this latter peaks is not well understood, however, it is possible that a continued release of prostaglanding F_{2a} is necessary to complete luteolysis.

The meachanism of the post-estral bleeding is not well understood, however, the hemorrhage is associated with a breakdown of endometrial capillaries (Salisbury & Van Demark 1961), followed by a repair process. The putput of prostaglandins could be associated with either the breakdown or with the repair (Sequin et al. 1974). Heifer No. II (Fig 3) had a somewhat lower basal level of the prostaglandin metabolite at the end of the experimental period than in the beginning, which could be explained by a corresponding decrease in the packed cell volume.

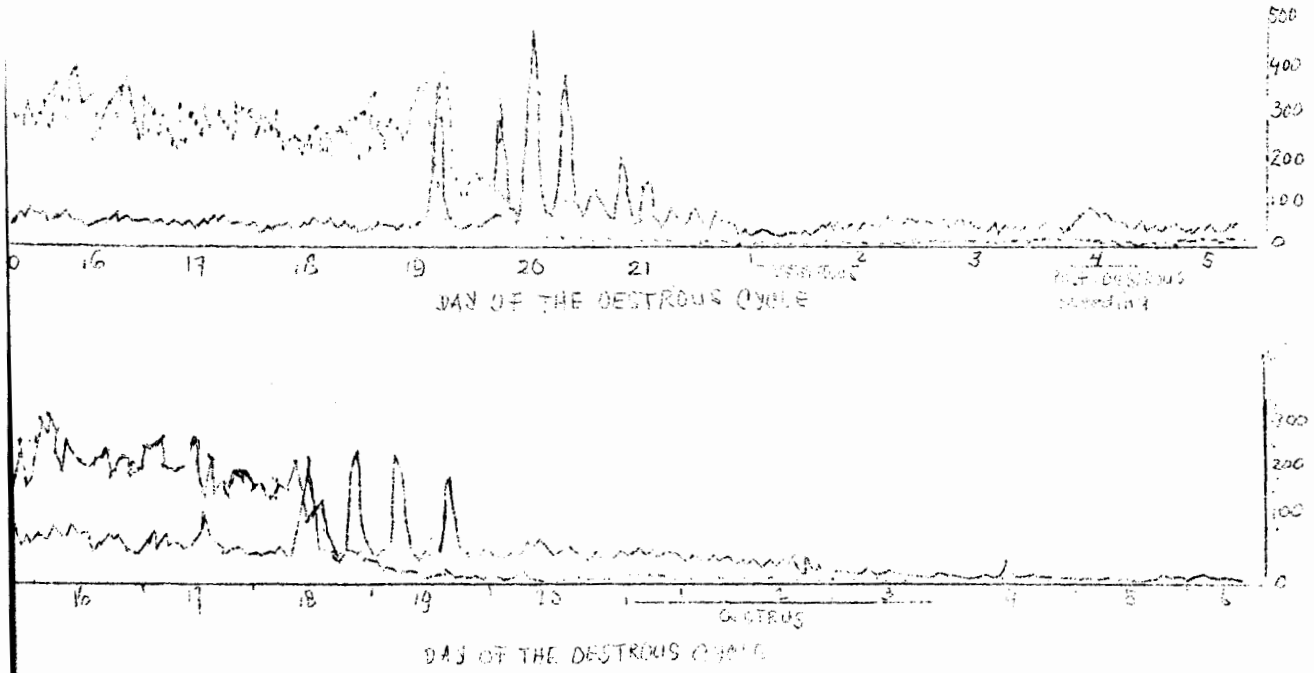

Figure 3: Peripheral plasma levels of 15-keto-13,14-dihydro-PGF_{2a} (o——o) and progesterone (●——●) during the estrous cycle in heifer No. I (upper panel) and in heifer No. II (lower panel). From Kindahl et al. 1976 b.

The physiological mechanism responsible for the release of prostaglandin at a precise time during the bovine estrous cycle is not fully known. Wlodawer et al. (1976) have shown that the bovine endometrium contains an inhibitor of prostaglandin synthesis. It is possible that this inhibitor is of physiological significance for prostaglanding synthesis and release. However, further studies of the nature of the inhibitory factor are necessary before its possible significance can be fully eva-

luated.

The mechanism by which prostaglandin is transferred from the uterine venous blood to the ovary is not known. The countercurrent theory proposed by McCracken et al. (1972) in the ewe has been subjected to some controversy (Lamond & Drost 1973; McCracken 1973; Coudert et al. 1974). The vascular anatomy of the uterus and the ovary in cattle is very similar to that in sheep as well as in other species in which the uterus has been reported to cause regression of the corpus luteum through a local pathway (Ginther & Del Campo 1974; Hixon & Hansel 1974).

The bovine corpus luteum contains receptors which are specific for PGF_{2a} (Powell et al. 1975; Rao 1975). It is likely that a significant step in initiating luteolysis in the bovine is the binding of PGF_{2a} to such receptors. The further action of prostaglandins on the function of corpus luteum in not totally evaluated.

Effect of Intrauterine Iodine on Prostaglanding Release:

Several studies have demonstrated the effect of intrauterine iodine infusions, a commonly used method for treatment of subclinical endometritis in cows, on estrus cycle length in the bovine species (Ishikhara et al. 1967, 1971, 1975; Moorow et al. 1971; Grunert et al. 1973; Seguin et al. 1974; Domeki et al. 1975). In general, uterine infusions performed early in the estrous cycle shortened the cycle length and infusions late in the cycle lengthened it, while infusions during mid-cycle appeared to have no effect. Iodine is rapidly absorbed from the uterine cavity (Ekman et al. 1965) and could influence endocrine organs; however, it has recently been suggested that the irritating effect of iodine on the endometrium is the important factor in altering the cycle length (Seguin et al. 1974). Thus, during endometrial repair, PGF_{2a} might be synthesized and induce luteolysis.

In a study undertaken to investigate prostaglandin release in relation to intrauterine iodine infusions in cows infusions were given on day 5, 11-12 and 16-20 of the estrous cycle. In fig. 4 is given the resulting peripheral plasma levels of progesterone and 15-keto-13,14-dihydro-PGF_{2a} in cows infused on day 5. As can be seen from the fig. the cows shortened

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The bovine corpus luteum contains receptors which are specific for PGF_{2a} (Powell et al. 1975; Rao 1975). It is likely that a significant step in initiating luteolysis in the bovine is the binding of PGF_{2a} to such receptors. The further action of prostaglandins on the function of corpus luteum in not totally evaluated.

Effect of Intrauterine Iodine on Prostaglanding Release:

Several studies have demonstrated the effect of intrauterine iodine infusions, a commonly used method for treatment of subclinical endometritis in cows, on estrus cycle length in the bovine species (Mukherjee et al. 1967, 1971, 1975; Moorow et al. 1971; Grunert et al. 1973; Seguin et al. 1974; Domeki et al. 1975). In general, uterine infusions performed early in the estrous cycle shortened the cycle length and infusions late in the cycle lengthened it, while infusions during mid-cycle appeared to have no effect. Iodine is rapidly absorbed from the uterine cavity (Ekman et al. 1965) and could influence endocrine organs; however, it has recently been suggested that the irritating effect of iodine on the endometrium is the important factor in altering the cycle length (Seguin et al. 1974). Thus, during endometrial repair, PGF_{2a} might be synthesized and induce luteolysis.

In a study undertaken to investigate prostaglandin release in relation to intrauterine iodine infusions in cows infusions were given on day 5, 11-12 and 16-20 of the estrous cycle. In fig. 4 is given the resulting peripheral plasma levels of progesterone and 15-keto-13,14-dihydro-PGF_{2a} in cows infused on day 5. As can be seen from the fig. the cows shortened

their estrus cycle length and showed heat 5 days after the infusion. No measureable increase of the prostaglanding metabolite was found in conjunction with the infusion of the iodine.

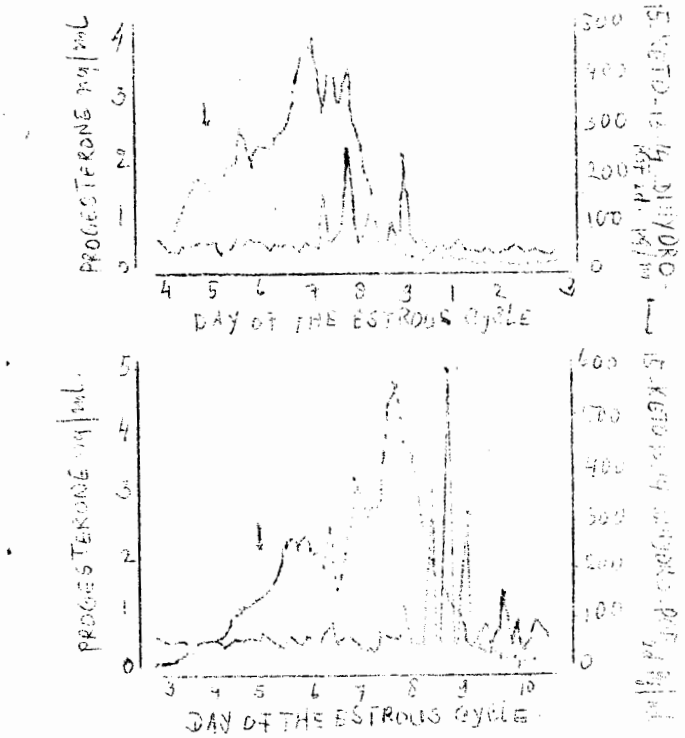

Figure 4: Peripheral plasma levels of 15-keto-13,14-dihydro-PGF_{2a} (o——o) and progesterone (●——●) in two cows subjected to intrauterine iodine infusions on day 5 of the estrous cycle. Arrows denote time of infusion. From Kindahl et al. 1977.

In fig. 5 is presented the hormonal levels measured in cows which were treated on day 11 and 12 of the estrous cycle. In this case 8 and 7 days, respectively, elapsed in between the infusion of the iodine and the occurrence of heat. However, in both cases the length of the estrous cycles tended to be shorter as compared to their untreated control cycles.

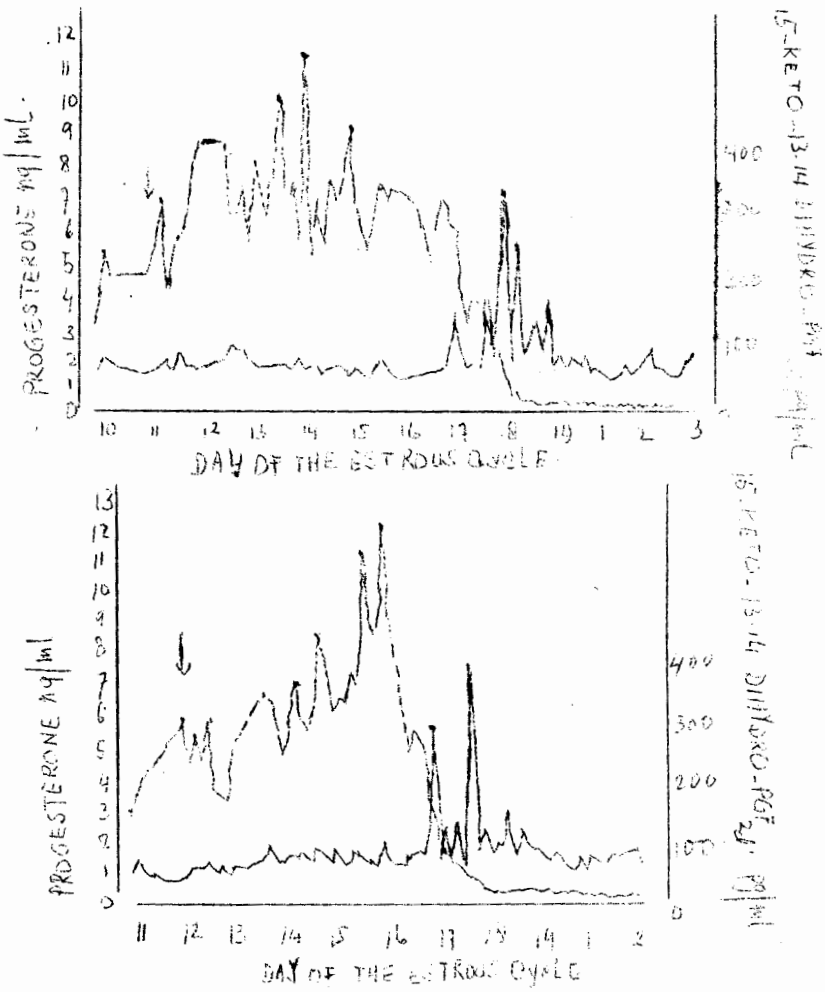

Figure 5: Peripheral plasma levels of 15-keto-13,14-dihydro-PGF_2 (o——e) and progesterone (●——●) in two cows subjected to intrauterine iodine infusion on days 11 and 12 of the estrous cycle. Arrows denote time of infusion. Day 1 = first day of estrus. From Kindahl et al. 1977.

Both in cows given intrauterine iodine on day 5 and days 11 and 12 of the estrous cycle the iodine infusion shortened estrous cycle length. In both cases were high levels of the prostaglandin metabolite recorded in conjunction with luteolysis. The reason for the delay in prostaglandin release in these cases are not known. Seguin et al. 1974 showed that intrauterine iodine infusions in cows caused a necrotizing endometritis. It is thus reasonable that the inflammatory response destroyed the source of the luteolysin (PGF_{2a}) and that the synthesis of prostaglandin probably was resumed at some point of endometrial repair. This is illustrated in fig. 6 which shows the hormonal response to intrauterina iodine given late in

the cycle. This particular cow was infused on day 20 of the cycle and luteolysis had at this time already started as illustrated by one prostaglandinmetabolite peak on day 19. Here the intrauterine iodine infusion resulted in an inhibition of prostaglandin release with subsequent pro - longed estrous cycle. Prostaglandins were released 6 days after the infusion and the cow at this time regressed the corpus luteum and showed heat after a 26 day estrous cycle. Thus after the destruction of the endometrium the normal control mechanism for prostaglandin release does not seem to function. It is possible, that during repair, a time-lag exists between the reestablishment of the prostaglandin inhibitory system as compared to the prostaglandin synthesizing system.

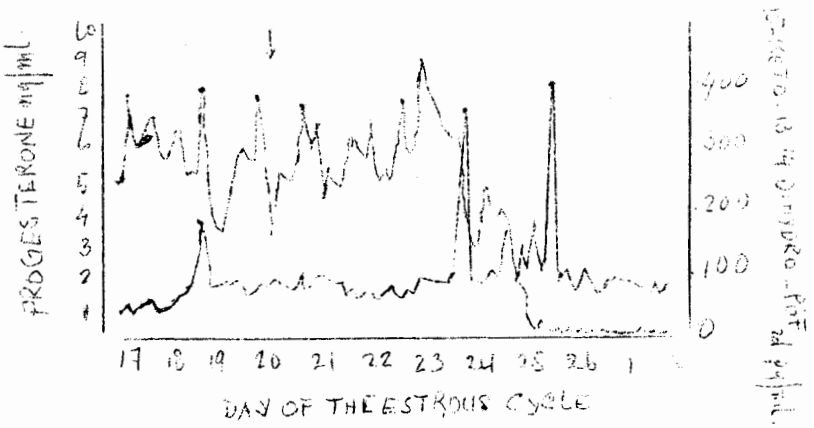

Figure 6. Peripheral plasma levels of 15-keto-13,14-dihydro-PGF_{2a} (o——o) and progesterone (•——•) in one cow subjected to intrauterine iodine infusion on day 20 of the estrous cycle. Arrows denote time of infusion. From Kindahl et al. 1977.

Treatment on Bovine Pyometra with PGF_{2a}:

The classical bovine pyometra is characterized by acumulation of pus in the uterus, persistence of the corpus luteum and anestrus. Retention of the corpus luteum is probably secondary to the pathological changes in

the endometrium which reduce or inhibit the production of PGF_{2a}. Post-partum pyometra is the most common form in the bovine and often follows peripartal disturbances such as dystocia, retained placenta or acute puerperal metritis.

Treatment of pyometra includes several different methods. Parenteral administration of various estrogens are often used. Estrogens may initiate luteolysis, stimulate uterine contractions, induce estrus and evacuation of the uterus. The risks involved in the use of repository estrogens for treating pyometra involve periovaritis, adhesions and sterility due to infection passing through the oviducts. Enucleation of the corpus luteum has been used successfully for treatment of pyometra and induction of estrus. Hemorrhage and adhesions are well known disadvantages of the corpus luteum enucleation. The availability of a therapeuticluteolytic agent free of adverse side effects would greatly assist in treatment of pyometra.

Twenty-six cows with pyometra were treated with various dosages of PGF_{2a} (Table 1).

Dosage of PGF_2	No. of cows	No. of cows emptying uterus	No. of cows inseminated	No. of cows pregnant (& pregnant of inseminated cows)
5 mg i.v.	5	4	3	2 (67)
5 mg i.m.	5	3	2	1 (50)
12.5 mg i.v.	12	11	11	8 (73)
25 mg i.m.	4	4	4	2 (50)
	26	22	20	13 (65)

Table 1: The clinical response and fertility results. From Gustafsson et al. 1976.

A total of 3 inseminations were performed and then if not pregnant the cows were culled for infertility. An average number of 2.2 inseminations were required for each pregnancy. The mean concentration of progesterone

in blood plasma before treatment was 3.1 ng/ml (range 1.4-7.5 ng/ml; n= 24). There was no significant difference in progesterone concentration between the group of cows that responded to the treatment by emptying the uterus (n=22) and the group not responding (n=4).

The number of animals in each dosage group is not large enough to allow definite conclusions about the minimum effective dose to induce estrus and evacuation of the uterus. It appears that the low dose - 5mg PGF_{2a} i.m. or i.v. - was less effective as treatment failed in 3 out of 10 cases. When the higher doses were employed failure was only 1 of 16 animals.

The pregnancy rate that was achieved in the present case must be considered rather good and promising for future use of PGF_{2a} as a therapeutic agent for bovine pyometra. Our results suggest that cows should not be inseminated to early after treatment. The best time would probably be to breed the cows at the second estrus after the induced one.

Conclusions

The use of prostaglandin opens the possibility of employing efficient breeding control by which females can be brought into estrus at a predetermined time and also to ovulate at a predetermined time. Such a breeding control would be beneficial to grazing cattle when using artificial insemination. Numerous reports are available today on the use of prostaglandins for heat synchronization (e.g. Tervit et al. 1973; Hearnshaw et al. 1974; Dobson et al. 1975; Edqvist et al. 1975). Prostaglandins can of course also be used to replace therapy which in the past has involved the manual enucleation of the corpus luteum such as in bovine pyometra (Gustafsson et al. 1976) and fetal mummification. It should, however, be emphasized that prostaglandin is not a fertility promoting substance per se. Absence of heat due to ovarian atrophy or supression of the estrous cyclicity due to malnutrition or other wasting conditions are not amenable to prostaglanding therapy.

Any breeding control system must be safe to the animals treated, to the offspring and to humans (both personnel handling of the drug, and also on consumption of animal products). It seems as prostaglandins are sub-

stances which might fullfillthese criteria and thus be available for use in bovine reproduction.

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