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Effect of Two Virus Inactivation Methods: Electron Beam Irradiation and Binary Ethylenimine Treatment on Determination of Reproductive Hormones in Equine Plasma

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Kyvsgaard, N. Chr., R. Høier, I. Brück and P. Nansen: Effect of two virus inactivation methods: Electron beam irradiation and binary ethylenimine treatment on determination of reproductive hormones in equine plasma. Acta vet. scand. 1997, 38, 225-233. – Ionizing irradiation and binary ethylenimine treatment have previously been shown to be effective for *in-vitro* inactivation of virus in biological material. In the present study the 2 methods were tested for possible effects on measurable concentrations of reproductive hormones in equine plasma (luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone (P_4), and oestradiol-17β (E_2)). The inactivation methods were electron beam irradiation with a dose from 11 to 44 kGy or treatment with binary ethylenimine (BEI) in concentrations of 1 and 5 mmol/L. Generally, there was a close correlation ($r>0.8$, $p<0.001$) between pre- and post-treatment hormone levels. Thus, the different phases of the oestrous cycle could be distinguished on the basis of measured hormone concentrations of treated samples. However, both treatments significantly changed hormone concentrations of the plasma samples. For LH, FSH, and E_2 the effect of irradiation and BEI treatment was depressive and dose-dependant. For P_4 the effect of irradiation was also depressive and dose-dependant. However, the highest dose of BEI resulted in an increase of measured P_4 concentration, which may be attributed to changes in the plasma matrix due to the treatment. Although the treatments affected measured hormone concentrations, the close correlation between pre-treatment and post-treatment measurements means that the diagnostic value will remain unchanged.

luteinizing; follicle; progesterone; oestradiol.

Introduction

International research collaboration often requires the possibility for exchanging biological material e.g. for validation of test systems. If this material contains infective agents such as Foot and Mouth Disease Virus, African Horse-sickness Virus, or Equine Encephalitis Virus this might constitute a risk to the recipient country. It is therefore imperative to find methods which can safely inactivate virus in serum and plasma samples. As reproductive problems limit the success of tropical livestock, hormone analysis can be useful in improving breeding efficiency. Many developing countries lack, however, often equipment, reagents and/or expertise for these techniques, for which reason the material has to be shipped to other laboratories either regionally or overseas.

Methods to inactivate viruses in biological material have previously been developed for inactivation of virus in vaccines or for diagnostic antigens. Two of the most widespread methods are ionizing irradiation and chemical treatment with binary ethylenimine (BEI).

The virucidal effect of ionizing irradiation is due to the formation of highly reactive free radicals causing loss of aromaticity of purine and pyrimidine rings as well as hydrolysis of the RNA or DNA backbone chain (*Polatnick & Bachrach* 1968, *Latarjet et al.* 1972). Gamma irradiation has been tested against a range of viruses (*Sullivan et al.* 1971, *Sun et al.* 1978, *Thomas et al.* 1981, *Elliot et al.* 1982, *Saliki et al.* 1993). Electron beam irradiation has a lower penetration rate than γ-irradiation, but it has the advantage that the treatment lasts seconds rather than hours (*Huber* 1952).

BEI has proved to be efficient against a number of viruses (*Bahnemann* 1975, *Bahnemann* 1976, *Sun et al.* 1978, *King* 1991). BEI is prepared in solution from bromoethylamine (BEA) under alkaline conditions (*Bahnemann* 1975).

Compared to the abundance of literature on virus inactivation for vaccine preparation or for diagnostic antigens, less attention has been paid to the inactivation of virus in serum or plasma samples, which constitutes a different environment characterized by e.g. a high protein content (*Bahnemann* 1976, *House et al.* 1990, *King* 1991). Inactivation of virus in serum is expected to need higher doses of the inactivant compared to media with a lower protein content (*Sullivan et al.* 1971, *Sullivan et al.* 1973). In previous studies electron beam irradiation and chemical treatment with BEI have proved efficient for the inactivation of virus in bovine serum samples (*Preuss et al.* 1997), and their detrimental effect on antibodies in serum has been evaluated in bovine and porcine sera (*Kyvsgaard et al.* 1996). Both methods were found to have a satisfactory margin between virus inactivation and damage to antibody activity, which allows reliable interpretation of results from treated samples.

The mentioned methods of inactivation have not previously been evaluated for their effect on hormones. The possibility exists that the effect of inactivation varies between peptide hormones and steroid hormones, and the present study was therefore designed to cover both groups, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) representing the peptide hormones and progesterone (P_4) and oestradiol-17β (E_2) representing steroid hormones. The irradiation and BEI doses were chosen according to those considered necessary in other studies to inactivate some of the more resistant viruses in serum (*Bahnemann* 1976, *House et al.* 1990, *Preuss et al.* 1997). The dose range of irradiation was also in accordance with requirements from authorities in the European Union (EU), where irradiation with 25 kGy is mentioned as one of the methods to achieve virological safety of bovine and porcine serum (*Anon.* 1992).

Materials and Methods

Sample material

Blood samples were taken daily from the jugular vein into heparinized vials (Vacutainers®, Becton-Dickinson, USA) from a Norwegian Fjord mare during a complete oestrous cycle (22 days) in which the cyclic changes of the reproductive tract (folliculogenesis, ovulation, etc.) were monitored by echographic surveillance. The mare ovulated on day 22 of the oestrous cycle. Plasma was isolated by centrifugation, 3,000 g for 10 min and stored at –20 °C in aliquots of 1 mL until inactivating treatment.

Irradiation

Part of the aliquots was subjected to irradiation from a 10 MeV electron accelerator at Risø National Laboratory (Denmark). The tubes were

irradiated from above with 0, 11, 22, 33 or 44 kGy. To keep the samples in frozen state they were irradiated on an ice/salt mixture. In order to compensate for the low penetrative ability of the electron beam irradiation, the tubes containing plasma samples were positioned horizontally in one layer on the ice/salt and covered with a 1-cm layer of ice/salt. After irradiation the samples were stored at –20 °C until analysis.

Binary etylenimine (BEI) treatment

BEI in aqueous solution (0.1 mol/L) was freshly prepared as described by *Bahnemann* (1975): Briefly, 4% of 2-bromoethylamine hydrobromide (BEA) was incubated with an equal volume of 0.4 N NaOH; the reaction was allowed to take place at 37 °C for 1 h under agitation. This BEI-solution was then added to 2 aliquots to give final concentrations of 1 mmol/L and 5 mmol/L, respectively. (When corrected for dilution by the added volume of the BEI solution the concentrations were 0.99 and 4.76 mmol/L, respectively). The inactivation took place at 37 °C for 24 h. After BEI treatment the samples were stored at –20 °C until used.

Hormone analyses

Luteinizing hormone (LH) was determined by employing a previously described competitive radioimmunoassay (RIA) method (*Worthy et al.* 1987) as evaluated in our laboratory (*Høier* 1994).

FSH was determined with the assistance of a commercial fluoroimmunoassay FSH kit, Delfia® hFSH which was constructed for the determination of human FSH. Data on cross-reactivity (CR) from equine gonadotropins were unavailable. Recognition of equine FSH was initially investigated by analysis of different concentrations of an equine FSH reference preparation (E265B) according to the protocol supplied with the kit. The analysis for equine FSH was designed by using equine FSH for calibration: E265B was diluted to standard concentrations covering 0 to 80 μg/L with standard diluent, a 1:1 mixture of Delfia® assay buffer and bovine plasma, which did not give any response in the assay. The protocol suggested by the manufacturer was modified slightly in order to improve sensitivity. Binding of FSH in 50 μL volumes of standards and samples to the anti-FSHβ-antibody coated microtiter wells and to the tracer was performed as a two-step reaction, 180 and 45 min, respectively, and the wells were subjected to additional washing prior to addition of enhancer. CR from equine LH (E263B) and chorionic gonadotropin (Crude PMSG, UCB-bioproducts) were studied by analyzing known amounts in standard diluent. The analytical precision expressed as the intra- and inter-assay coefficients of variation (CV%), sensitivity and accurary expressed as the recovery of added known amounts of the hormone was performed as described by *Jensen et al.* (1993).

P_4 was measured using a competitive enzyme-linked immunosorbent assay (ELISA) as previously described by *Høier* (1989) with the exceptions that equine progesterone-free plasma was used for construction of standards, and the analysis was performed as a 3-h incubation at room temperature. These modifications did not affect characteristics of the analysis. The main cross-reacting steroid was 11 α-hydroxyprogesterone 5%, and other steroids tested cross-reacted below 0.2%; intra- and inter-assay coefficients of variation were 3%-10% and 7%-15%, respectively, depending on position on the standard curve.

E_2 was quantified by use of a double antibody RIA kit designed to measure E_2 in human serum or plasma (Diagnostic Products Corporation, Los Angeles, California, USA). The anti-E_2 antibody was reported to carry the following CR's: E_2 100%, oestrone 12.5%, 17β-

oestradiol-3β-D-glucuronide 6.0%, d-equilenin 4.2%, other steroids tested <3.5%. The protocol suggested by the manufacturer was followed with some modifications: In order to approach matrix identity between standards and samples, the kit's standards were diluted 1:1 with plasma from a gelding, and the mare samples were diluted 1:1 with the kits 0-calibrator. Diluted standards and samples were added anti-E_2-antiserum and incubated for 3 h at 4 °C. Following addition of tracer, the incubation was continued overnight at 4 °C. Intra- and inter-assay coefficients of variations (CV%) were 4.8% and 7.1%, respectively, in the range from 92 to 367 pmol/L, thus being comparable to the analytical variation stated by the manufacturer, intra-assay CV% 4%-8% and inter-assay CV% 3.5%-5.5%. Analysis of samples made by addition of different, known concentrations of E_2 confirmed a straight-line recovery not different from 100% in the range from 0-184 pmol/L of added E_2.

Statistical methods

Routine statistical procedures like determination of mean, standard deviation (SD), and coefficient of variation (CV%), computation of pooled-variance estimates, lack-of-fit testing (LOF), T-statistics, and processing of linear regression models were according to standard procedures (*Box et al.* 1978). Sensitivity was calculated as the least detectable concentration (LDC), which could not be distinguished from zero value at a probability of 5%. The slope (β_1) for each comparison between control and treatment groups was tested for the hypothesis $\beta_1 = 1$, i.e. that the measured posttreatment concentration was equal to pretreatment activity.

Results

Effect of irradiation on hormone analyses

Plasma samples from a mare throughout a complete oestrous cycle were subjected to increasing doses of electron beam irradiation, and ensuing this, the contents of LH, FSH, P_4 and E_2 were measured. The resulting hormone concentrations were correlated to the concentrations prior to treatment by linear regressions (Fig. 1), the results of which are summarized in Table 1. It can be seen that for all 4 hormones the degree of irradiation was reflected in the measured hormone concentrations so that increasing doses were accompanied by a decrease in hormone concentrations. The regression lines all fitted straight-line models. The intercept with the y-axis (β_0 in Table 1) was only significantly different from 0 in 2 of the 16 combinations of irradiation dose and hormone. Investigations of regression models revealed no familiarity between the different peptide or steroid hormones (data not shown). For the purpose of comparing pre- and post-treatment profiles Fig. 2 shows hormone immunoactivity during the complete oestrous cycle determined on untreated and maximally irradiated samples, respectively.

Effect of ethylenimine treatment on hormone analyses

Plasma samples from a cycling mare were subjected to 2 doses of BEI, and ensuing this, the content of LH, FSH, P_4, and E_2 were measured. The result of comparing measured hormone concentrations with the concentrations found prior to treatment with ethylenimine is outlined as regression analyses in Table 1. It can be seen that 1 mmol/L of BEI did not affect hormone measurements. Five mmol/L, in contrast, resulted in a significant decrease in E_2 and FSH concentrations, LH concentrations remained unchanged, and P_4 concentrations increased significantly. Fig. 2 shows hormone immunoactivity during the complete oestrous cycle determined on untreated and BEI treated samples, respectively.

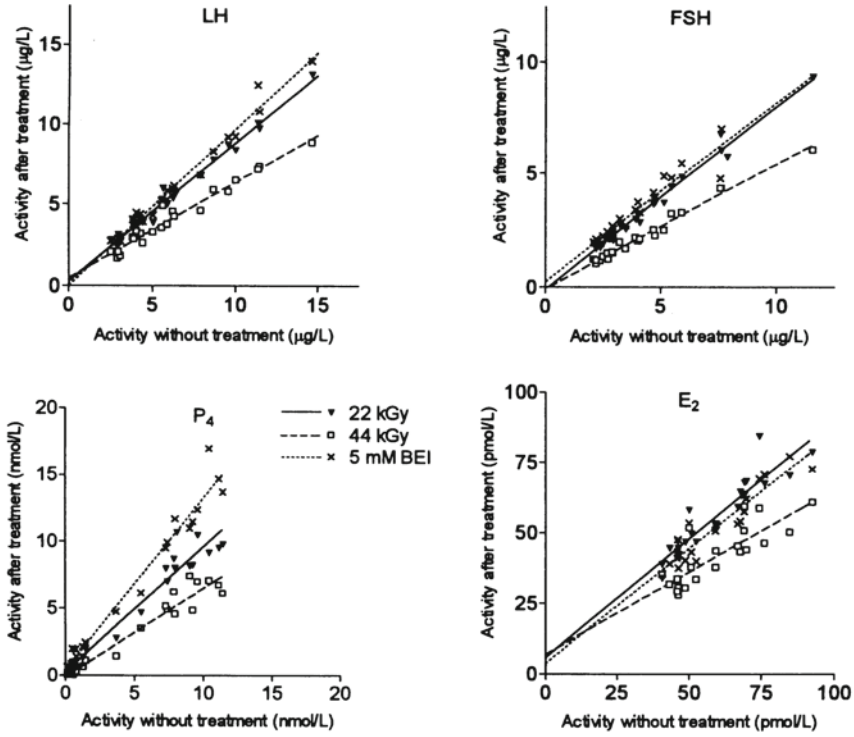

Figure 1. Measured plasma concentrations of reproductive hormones plotted against the activity of untreated samples. Values and regression lines are shown for 22 kGy and 44 kGy irradiation as well as for treatment with 5 mmol/L BEI.

Validation of the FSH analysis

Initial investigations showed that equine FSH responded significantly in the Delfia® hFSH kit. The result is summarized in the regression equation (X,Y) = (Added equine FSH, measured human FSH; µg/L): Y = –1.048 + 0.145•X, n = 16, r = 0.989, p<0.0001. Equine LH and CG responded slightly, but CR's were less than 1% as compared to equine FSH. Intra-assay CV% for low and high FSH concentrations was calculated to be 7.1% (n = 90, mean = 2.12 µg/L, SD = 0.15 µg/L) and 3.7% (n = 101, mean = 6.53 µg/L, SD = 0.24 µg/L), respectively, according to pooled-variance estimates. Inter-assay CV% was 10.6% when calculated as the assay-to-assay variation of different samples (n = 15, mean = 3.65 µg/L, SD = 0.39 µg/L). LDC was 0.19 µg/L (SD = 0.093 µg/L). The recovery of 10 µg/L equine FSH (E265B) added to various samples was 100% (n = 15, recovered = 9.81 g/L, p = 0.23 in a paired T-test).

Discussion

Effects of irradiation and BEI treatment on hormone analyses

Both irradiation and ethylenimine treatment of mare plasma samples influenced on the result of hormone analyses. Irradiation resulted in a dose dependent response where increased dose was reflected in a lower slope in regression analysis of post-treatment vs. pre-treatment

Table 1. Correlation between measured concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone (P_4), and oestradiol-17β (E_2) in mare plasma samples exposed to 4 different doses of electron beam irradiation or 2 doses of binary ethylenimine (BEI) versus non-treated samples.

Dose	11 kGy	22 kGy	33 kGy	44 kGy	1 mmol/L	5 mmol/L
N	23	23	22	22	13	21
LH:						
r	0.994	0.992	0.986	0.981	0.956	0.990
β_0	ns0.128	ns0.292	*0.472	*0.495	ns–0.060	ns0.032
β_1	*0.947	***0.852	***0.743	***0.587	ns1.00	ns0.963
FSH:						
r	0.993	0.991	0.986	0.991	0.985	0.947
β_0	ns–0.057	ns–0.090	ns–0.063	ns–0.070	ns0.297	ns0.263
β_1	**0.889	***0.808	***0.724	***0.550	ns1.025	**0.791
P_4:						
r	0.976	0.974	0.977	0.973	0.998	0.985
β_0	ns0.202	ns0.287	ns0.152	ns–0.092	ns0.285	ns0.429
β_1	ns1.085	ns0.934	***0.833	***0.660	ns1.024	***1.283
E_2:						
r	0.953	0.925	0.902	0.826	0.940	0.940
β_0	ns5.70	ns5.91	ns5.89	ns6.93	ns0.607	ns3.82
β_1	ns0.910	*0.842	*0.774	**0.584	ns0.994	*0.813

N = number of observations. β_0 and β_1 = intercept and slope of regression; r = coefficient of correlation. ns, *, **, *** = statistical significance for slope $\neq 1$, corresponding to nonsignificant, $p<0.05$, $p<0.01$, $p<0.001$, respectively.

measured activity. The slopes of the regression lines for the highest dose (44 kGy) were β_1 = 0.59, 0.55, 0.66, 0.58 for LH, FSH, P_4 and E_2, respectively. When comparing the slopes of the posttreatment vs. pretreatment regression lines (Table 1) dose-effect correlations were found which were independent of both molecular size and type of hormone, steroid hormone versus peptide hormone since each hormone had its own dose-decay curve. Treatment of plasma samples with BEI resulted in decreasing hormone concentrations, with the exception that P_4 concentrations were elevated.

Generally, changes in measured hormone concentrations after treatment can be caused by two main mechanisms: Firstly, the treatment(s) could change the hormone molecules such that it is not recognized by the antibody (-ies). Concerning irradiation this effect can be either through direct hit by the incoming electron or, which is more likely for small molecules, through reactions with free radicals formed by the irradiation (*Thomas et al.* 1981). Secondly, a general detriment of proteins and other plasma components might well affect the binding between antibody and hormone, i. e. change the antibody affinity. Thus, the apparently higher P_4 concentration could be caused by either a changed binding between carrier proteins and P_4 or it might be due to changes in the plasma matrix which could influence the immunoassay. In this study it is difficult to judge which effect is the most dominating in the various hormone assays.

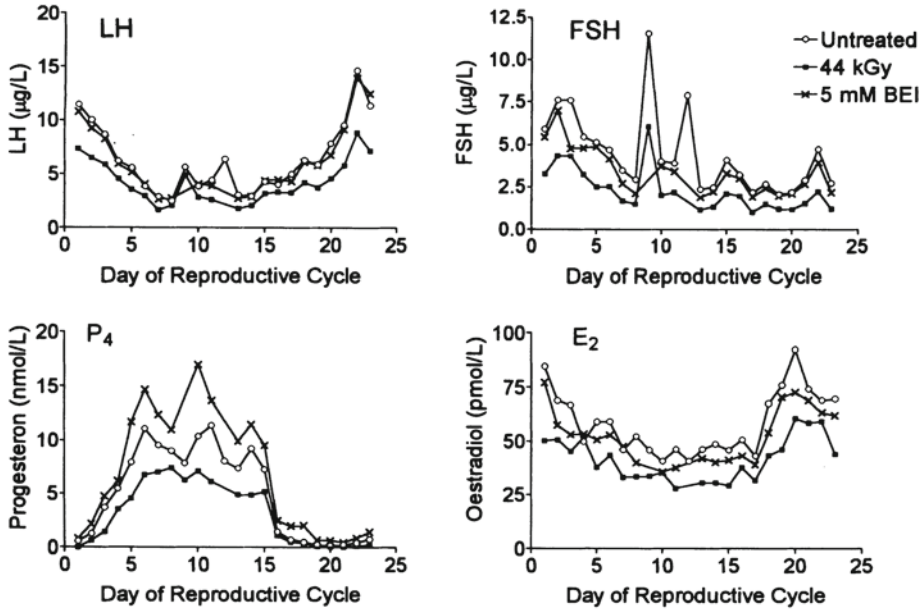

Figure 2. Measured plasma concentrations of reproductive hormones during an oestrous cycle for untreated samples, samples irradiated with 44 kGy and samples treated with 5 mmol/L BEI for 24 h. Values are missing for the 44 kGy irradiated samples at day 12 and for the BEI treated samples at day 9 and day 12.

Validation of hormone analyses

In this study it was shown possible to determine equine FSH in plasma samples with a commercially available fluoroimmunoassay (Delfia®) designed for the measurement of human FSH. This finding was not unexpected since heterologous assays have previously proven valid for the evaluation of follicle-stimulating activity in mares (*Alexander et al.* 1987, *Hines et al.* 1991, *Høier* 1994), thereby stating a high degree of interspecies homology. It can be concluded that the Delfia® hFSH kit is suitable for monitoring of the cyclic fluctuation in mares, especially since the low CR from LH and CG will not confound the FSH results. It cannot be excluded that at least part of the measured CR's are caused by amounts of FSH in the hormone preparations. Assay precision and accuracy appeared acceptable, and the least detectable concentration in the assay was well below the lowest FSH concentrations measured.

Applications of virus-inactivated plasma hormone concentrations

EU regulations for biological materials mention irradiation with 25 kGy as one possible method for virus-inactivation in sera (*Anon.* 1992). It has been concluded above that irradiation of mare plasma samples with this or higher doses or exposure to BEI affects the outcome of subsequent hormone analyses. This fact will, however, not necessarily declare virus-inactivated material to be un-useful for hormone measurements, since the concentrations of LH, FSH, P_4, and E_2 in the treated material are proportional and highly correlated to the concentrations prior to virus-inactivation. Hormone profiles of treated samples will thereby be parallel to pro-

files of untreated material (Fig. 2). The profiles will not reflect the actual hormone concentrations, but still the diagnostic value of hormone measurements will remain unchanged.

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References

Alexander SL, Irvine CHG, Turner JE: Comparison by three different radioimmunoassay systems of the polymorphism of plasma FSH in mares in various reproductive states. J. Reprod. Fert., 1987, *Suppl.* 35, 9-18.

Anonymous: Council Directive 92/118/EEC of 17 December 1992 laying down animal health and public health requirements governing trade in and imports into the Community of products not subject to the said requirements laid down in specific Community rules referred to in Annex A (I) to Directive 89/662/EEC and, as regards pathogens, to Directive 90/425/EEC.

Bahnemann HG: Binary Ethylenimine as an Inactivant for Foot-and-Mouth Disease Virus and Its Apllication for Vaccine Production. Arch. Virol. 1975, *47,* 47-56.

Bahnemann HG: Inactivation of Viruses in Serum with Binary Ethyleneimine. J. Clin. Microbiol. 1976, *3,* 209-210.

Box GEP, Hunter WG, Hunter JS: Statistics for Experimenters, New York, U.S.A., John Wiley & Sons, Inc., 1978, 653pp.

Elliot LH, McCormick JB, Johnson KM: Inactivation of Lassa Marburg, and Ebola Viruses by Gamma Irradiation. J. Clin. Microbiol. 1982, *16,* 704-708.

Hines KK, Affleck K J, Barrows SP, Murdoch WL, Fitzgerald BP, Loy RG: Follicle-Stimulating Hormone Pulse Amplitude with the Onset of the Breeding Season in the Mare. Biol. Reprod. 1991, *44,* 516-521.

House C, House JA, Yedloutschnig RJ: Inactivation of viral agents in bovine serum by gamma irradiation. Can. J. Microbiol. 1990, *36,* 737-740.

Huber W: Cold Sterilization by Electron Beam as a Possible Tool for the Inactivation of the Virus of Homologous Serum Jaundice in Plasma. Ann. N. Y. Acad. Sci. 1952, *55,* 536-542.

Høier R: Determination of Plasma Progesterone in the Blue Fox (*Alopex lagopus*) at Pro-oestrus and Oestrus by Use of a Commercial Kit. Acta Agric. Scand. 1989, *39,* 181-186.

Høier R: Determination of Equine Follicle-stimulating Hormone Assisted by A Commercially Available Enzymeimmunoassay Kit. Reprod. Domestic Anim. 1994, *29,* 289-295.

Jensen AL, Høier R, Poulsen JSD: Adaptation of a commercially available enzyme linked immunosorbent assay (ELISA) for the determination of thyroxine in canine plasma samples. *J. Vet. Med. Ser. A* 1993, *39,* 741-746.

King DJ: Evaluation of Different Methods of Inactivation of Newcastle Disease Virus and Avian Influenza Virus in Egg Fluids and Serum. Avian Dis. 1991, *35,* 505-514.

Kyvsgaard NC, Lind P, Preuss T, Kamstrup S, Lei JC, Bøgh HO, Nansen P: The activity of antibodies against *Salmonella dublin, Toxoplasma gondii* or *Actinobacillus pleuropneumoniae* in sera after treatment with electron beam irradiation or binary ethylenimine. Clin. Diag. Lab. Immunol. 1996, *3,* 628-634.

Latarjet R: Interaction of Radiation Energy with Nucleic Acids. Curr. Top. Radiat. Res. Q. 1972, *8,* 1-38.

Polatnick J, Bachrach HL: Ionizing Irradiation of Foot-and-Mouth Disease Virus and Its Ribonucleic Acid. Arch. gesamte Virusforsch. 1968, *23,* 96-104.

Preuss T, Kamstrup S, Kyvsgaard, NC, Lei JC, Nansen P, Miller A: Comparison of Two Different Methods for Inactivation of Viruses in Serum. Submitted for publication.

Saliki JT, Berninger ML, Torres A, House JA, Mebus CA, Dubovi EJ: Effect of Gamma Irradiation on Reactivity of Rinderpest Virus Antigen with Bovine Immune Serum in Enzyme-Linked Immunosorbent Assay and Virus Neutralization and Indirect Flourescent-Antibody Tests. J. Clin. Microbiol. 1993, *31,* 428-432.

Sullivan R, Scarpino VS, Fassolitis AC, Larkin EP,

Peeler JT: Gamma Radiation Inactivation of Coxsackievirus B-2. Appl. Microbiol. 1973, *26,* 14-17.

Sullivan R, Fassolitis AC, Larkin EP, Read RB, Peeler JT: Inactivation of Thirty Viruses by Gamma Radiation. Appl. Microbiol. 1971, *22,* 61-65.

Sun IL, Gustafson DP, Scherba G: Comparison of Pseudorabies Virus Inactivated by Bromo-Ethylene-Imine, ^{60}Co Irradiation, and Acridine Dye in Immune Assay Systems. J. Clin. Microbiol. 1978, *8,* 604-611.

Thomas FC, Davies AG, Dulac GC, Willis NG, Papp-Vid G, Girard A: Gamma Ray Inactivation of Some Animal Viruses. Can. J. comp. Med. 1981, *45,* 397-399.

Worthy K, Colquhoun K, Escreet R, Dunlop M, Renton JP, Douglas TA: Plasma Prolactin Concentrations in Non-pregnant Mares at Different Times of the Year and in Relation to Events in the Cycle. J. Reprod. Fert. 1987, *35,* 269-276.

Sammendrag

Effekt af to virus inaktiveringsmetoder (Elektronbestråling og binær etylenimin behandling) på bestemmelse af reproduktionshormoner i equin plasma.

Det er tidligere blevet påvist at bestråling og behandling med binær etylenimin er effektive metoder til inaktivering af virus i biologisk materiale. I nærværende arbejde blev de to metoder vurderet for deres mulige virkning på de målte koncentrationer af reproduktions hormoner i equin plasma (luteiniserende hormon (LH), follikel-stimulerende hormon (FSH), progesteron (P_4), og østradiol-17β (E_2)). Inaktiveringsmetoderne var elektron-bestråling med doser fra 11 til 44 kGy eller behandling med binær etylenimin (BEI) i koncentrationer på 1 og 5 mmol/L. Generelt var der en tæt korrelation ($r>0,8$, $p<0,001$) mellem præ- og post-behandlings hormon bestemmelser. Således kunne de forskellige faser af østral cyklus identificeres på basis af de målte koncentrationer i de behandlede prøver. Begge behandlinger ændrede ganske vist hormon koncentrationerne i plasma prøverne. De målte koncentrationer af LH, FSH og E_2 blev ændret i negativ retning i et omfang der var afhængig af dosis. Bestråling resulterede også i en reduktion af de målte koncentrationer af P_4, hvorimod den højeste dosis af BEI resulterede i en forøgelse af de målte P_4 koncentrationer, hvilket kan skyldes forandringer i plasma matrix som følge af behandlingen. Selv om begge behandlingsformer ændrede de målte hormon koncentrationer var der en tæt korrelation mellem præ-behandlings og post-behandlings målinger hvorved den diagnostiske værdi ikke blev affekteret.

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