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Brief Communication

Effects of Endotoxin Contaminated FSH-preparations in Heifers

Superovulation is one important step in embryo transfer. By administration of gonadotropins a multiple ovulation is induced. Several FSH-preparations for superovulation are commercially available. Most of these consist of extracts from pituitary glands from slaughtered farm animals. According to some studies *(Yagoda et al.* 1990a,b), drugs can easily be contaminated with endotoxins. Endotoxins are commonly found in the environment, for example in water and silage, and in medical preparations *(Yagoda et al.* 1990b). Endotoxins can have several effects on animals and on human beings. They can cause endotoxaemia with effects like fever, ruminal stasis, release of prostaglandins, leukopenia and decrease of the plasma levels of calcium, zinc, iron and bile acids *(Aiumlamai* 1991). The severity and range of the effects caused by endotoxin are very dose-dependent. Animals receiving a low dose of endotoxin may not show the clinical signs of endotoxaemia, but can still have subclinical changes like leukopenia, prostaglandin release, mild hypocalcaemia and decreases in the plasma levels of zinc and iron *(Yagoda et al.* 1990b, *Kindahl & Aiumlamai* 1991). For humans, 5 EU/kg bodyweight is considered to be the threshold dose for appearance of clinical effects. *(Anon.* 1987).

In this experiment the effects of 4 different commonly used FSH-preparations have been studied on 6 Swedish Red and White heifers, aged approximately 2 years. The purpose was not to cause superovulation in the heifers, but only to study possible changes in the animals due to a possible contamination of the preparations with endotoxins.

FSH-preparations and diluents were tested according to the LAL-test *(Friberger* 1985). It was found that the diluents were not contaminated, but the preparations contained the following amounts of endotoxin per injected total dose: Ovagen (Immuno-Chemical Products, New Zealand), 3500 EU; FSH-p (Schering-Plough, U.S.A.), 7 EU; Folltropin (Vetrepharm, Canada), 67 EU; Super-OV (AUSA International, U.S.A.), 26 EU. Thus the amount of endotoxin that the animals received was 7 EU/kg, 0.014 EU/kg, 0.134 EU/kg and 0.052 EU/kg, respectively. The analyses for endotoxin content were done both at Pharmacia Biotech, Analytical Control Laboratory, Uppsala and at the Central Laboratory, Apoteksbolaget, Stockholm, Sweden. Per test, 1 intramuscular injection with the full dose of FSH was given to each heifer. Two tests were done per animal and there was a period of at least 3 weeks between the tests. To avoid possible tolerance effects, the animals did not receive the same FSH-preparation twice. The tests were done in the luteal phase of the oestrous cycle; if necessary, the heifers were synchronized with cloprostenol (Estrumat vet., Pitman-Moore). Blood

Table 1. Changes seen in heifers after injection with endotoxin-contaminated FSH-preparations. Changes for the individual animals are between brackets.

	Ovagen	Fsh-p	Folltropin	Super-ov
Temp	0 (0,–,0)	$+^{+}$ (0,+,0)	0 (–,0,+)	$-^{\#}$ (–,0)
Wbc	$+^{x}$ (+,0,0)	$+^{\#}$ (0,+,+)	$+^{*}$ (0,+,+)	0 (0,0)
Pmn	$+^{x}$ (+,+,+)	$+^{+}$ (0,0,+)	$+^{*}$ (0,+,0)	0 (–,+)
Mn	0 (0,0,0)	$+^{+}$ (0,0,0)	0 (0,0,0)	0 (0,0)
Fe	$-^{x}$ (–,–,0)	$-^{x}$ (–,0,–)	$-^{*}$ (–,0,0)	0 (0,+)
Ca	0 (0,0,0)	0 (+,0,0)	0 (0,0,0)	$-^{\#}$ (–,–)
Zn	0 (0,–,0)	$+^{\#}$ (+,0,+)	0 (–,+,0)	0 (+,–)
Pg	0 (0,0,0)	0 (0,0,0)	0 (0,0,0)	$+^{\#}$(+,0)

Temp = body temperature; Wbc = total white blood cells; Pmn = polymorphonuclear cells; Mn = mononuclear cells; Fe = iron; Ca = calcium; Zn = zinc; and Pg = prostaglandin metabolite.
0 indicates no significant change; + indicates a significant increase; and - a significant decrease. Significance levels are: + = $p<0.1$; * = $p<0.05$; # = $p<0.01$; and x = $p<0.001$.

samples were obtained from the jugular vein through permanent cannulas. On day 1, blood samples were taken every 30 min from 7.30 to 10.00, then every h until 14.00 and every 2h until 20.00h. On day 2, the same sampling schedule was followed and at 8.15h the FSH-preparation was injected. On day 3, samples were taken at 7.30 and 8.00h. At every sampling rectal temperature was measured. Blood was collected in 3 types of tubes (Vacutainer, Becton-Dickinson). Blood in heparinized vacuum tubes was centrifugated at 3000 rpm for 15 min and plasma was separated for $PGF_{2\alpha}$-metabolite analysis *(Granström & Kindahl* 1982). Blood in plain vacuum tubes was kept at room temperature for 1h and centrifugated to separate serum. In serum, Ca, Fe and Zn were analyzed, according to standard methods used at the Clinical Chemistry Laboratory, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences. Both heparinized and plain tubes were used at every sampling. EDTA vacuum tubes were used every 2h to obtain fresh blood for total and differential white blood cell counts.

The changes in all parameters were evaluated by comparing the data before and after injection for each FSH-preparation. A paired t-test was used to calculate whether the differences were significant *(Colton* 1974). The results are presented in Table 1.

Body temperature increased for FSH-p and decreased for Super-OV, but was very variable from animal to animal. One cow had a fever (up to 40.1°C) on the first day (the control day) of the experiment with Super-OV. For Ovagen, FSH-p and Folltropin, a significant increase in white blood cells was seen, due to an increase in polymorphonuclear cells. The number of mononuclear cells increased only for FSH-p. The level of iron decreased for Ovagen, FSH-p and Folltropin and the level of calcium decreased for Super-OV. According to earlier findings this decrease could be due to the endotoxin contamination of the preparations. The level of zinc increased for FSH-p but was very variable from animal to animal. An increase in zinc is the opposite of what was found earlier after a low dose of endotoxin *(Kindahl & Aiumlamai* 1991). There was no significant change in prostaglandin metabolite levels except for an increase for Super-OV. The changes in the parameters were not related to the degree of contamination of the preparations, i.e. to the amount of endotoxin that the animals received.

An endotoxin contamination was found in the FSH-preparations, but of very variable degree. The amount of endotoxin was very low and may have been too low to provoke a clear response in the parameters, but still some changes were seen in the animals. It should also be pointed out that all injections were done intramuscularly. The amount of total white blood cells or polymorphonuclear cells

and the iron level in the blood seem to be the best indicators of endotoxin exposure. Endotoxin contamination of preparations should be avoided, especially when the preparations are being used in sensitive processes such as superovulation and embryo transfer.

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