A HOMOLOGOUS RADIOIMMUNOASSAY FOR QUANTIFICATION OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3 IN BLOOD FROM CATTLE

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Keywords. Cattle, insulin-like growth factor-binding-protein-3, radioimmunoassay.

Insulin-like growth factor-I and -II (IGF-I, IGF-II) circulate in biological fluids bounds to at least six different IGF-binding proteins that regulate IGF bioactivity. The IGF-binding protein-3 is regulated by growth hormone, and its concentration depends on nutrition and physiological state. Unavailable from commercial societies, bovine IGFBP-3 has been previously purified from precolostrum collected 3–5 days before parturition. This preparation has been used to produce specific antibodies, and IGFBP-3 levels in bovine blood samples were quantified by radioimmunoassay. Parallel displacement curves showed strong cross-reactivity with bovine and ovine plasma and no cross-reactivity with porcine, rat, dromedary or chicken plasma. Addition of IGF-I to a control pool of bovine plasma did not significantly alter control IGFBP-3 values in a radioimmunoassay. Nycthemeral periods, determined for three young bulls bled on two occasions were stable throughout the day; two or three samples were sufficient to characterize the animal. Heifers treated with recombinant bovine somatotropin (bST) had significantly higher serum levels of IGF-binding protein-3 than did control cows. Likewise, plasma IGFBP-3 concentrations were decreased in growing bulls treated with clenbuterol (a β-agonist) while these concentrations increased after corticoid (dexamethasone) injection. Furthermore, IGFBP-3 levels were dramatically increased at the onset of puberty and decreased during the first postpartum weeks. This radioimmunoassay for bovine IGFBP-3, which enables quantitative assessment of IGFBP-3 concentration in cattle, confirmed the previous observations using the less precise Western ligand blotting method.
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DOES ANNUAL REPETITION OF OESTRUS INDUCTION INFLUENCE THE FERTILITY OF GOATS AFTER A.I. AT A FIXED TIME ?

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Keywords. Goat, fertility, oestrus, induction.

To verify the effects on various herd parameters of repeated gonadotropic treatments we followed throughout four consecutive years two herds of goats (H1=31; H2=67) annually subjected to AI after oestrus induction including progestagen administration for 11days followed by prostaglandins and eCG. Various reproductive parameters such as occurrence of oestrus, LH surge outcoming, number of ovulations, pregnancy rates, kidding rate and prolificacy after AI at a predetermined time were recorded. Blood samples were collected before and after each treatment. These samples were screened for eCG-binding activity (BR). Results were analyzed taking the herd, number of treatment, female within the herd, eCG binding rate before treatment and eCG binding rate after treatment as independent variables. The binding rates were correlated with other parameters: herd, delay for LH surge, time of oestrus, number of ovulation, number of kids, prolificacy and fertility.

Table 1. Oestrus and repetition of treatments in goats.

Parameter	Herd	Treatment			
		1st	2nd	3rd	4th
Interval sponge	H1	24±3.9	26±2.0	22±6.5	26±8.8
Removal–oestrus (hours ± SD).	H2	17±2.0	21±3.9	20±6.7	21±7.3

Table 2. Kidding rate in goats, antibodies level and repetition of treatment.

Parameter	Herd	Treatment			
		1st	2nd	3rd	4th
Fertility (%)	H1	68.0	56.0	37.0	40.0
	H2	57.0	65.0	71.0	51.0
BR before treatment	H1	–	5.6	8.8	11.8
	H2	1.57	3.0	6.9	11.3
BR after treatment	H1	0	4.1	26.0	27.0
	H2	0	5.1	20.0	26.0

Statistical analysis of the results established a significant effect of female and treatments on eCG-binding but no effect of the herd neither of the age. We found that the antibodies significantly influenced the time of oestrus outcoming as well as the time of LH surge. No influence of the age on the time of oestrus or the time of LH surge was found. The antibodies after treatment significantly influenced the ovulation rate and the kidding rate whatever the age of the female. Finally, a significant effect of antibodies on fertility was found but no effect of antibodies on prolificacy.

The originality of this experiment resides in the fact that it was performed over 4 years on the same animals, which did not receive eCG injection before the experiment. We found a linear liaison between eCG antibodies and delay for oestrus outcoming what allowed us to confirm the variability of the onset of oestrus in dairy goats after synchronization with a progestagen/ eCG treatment described by Baril *et al.* (1993) and Freitas (1997) and to reasonably postulate that the significant decrease in fertility induced by late oestrus, was to rely to the immune response against eCG. In herd 1, we observed that the repetition of treatments was associated with both increases in eCG binding and in the percentage of females that did not present LH surge. As dairy goats are inseminated at a predetermined time after the end of the progestagen administration, it is highly probable that the decrease in fertility observed after repeated treatments is due to the consequences of the reaction against eCG by increasing delay between sponge removal and oestrus/ovulation time. Baril *et al.* (1996) already suggested that the late oestrus be related to the appearance of anti-eCG antibodies with its frequency being dependent on the number of treatments the females had received previously. The same proposed a diminution of the biological activity of eCG with subsequent delayed oestrus and ovulation. We support this hypothesis by our experiment with a highly significant effect of the eCG binding on the time of apparition of oestrus (herds considered together).

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EFFECTS OF FSH, GH, GROWTH FACTORS AND FOLLICULAR FLUID ON OESTRADIOL-17β PRODUCTION BY OVINE OOCYTES DURING *IN VITRO* MATURATION

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Keywords. Sheep, oocyte, maturation, Oestradiol-17β (E2), oestrogens, steriods.

1. Introduction

The oestradiol production during *in vitro* maturation has been measured in order to correlate the cumulus-oocyte-complexe (COC) secretory ability and the success of the maturation.

2. Materials and methods

During various experiments on the control of *in vitro* maturation of ovine oocytes, samples of the medium have been collected before and after the treatment and assayed for steroids (P4, Δ4 and E2). The difference in steroid concentration was expressed in term of production by COC.

Some of these experiment's goal was to compare the effects of FSH (100 ng/ml) and/or GH (50 ng/ml) in TCM 199 with 10% follicular fluid (FF). Other's goal was to replace FF by various growth factors (IGF-1, IGF-2, FGF-2, EGF).

3. Results and discussion

The hormones and growth factors have no effect on P4 and D4 production. The results show that FF is necessary to maintain the COC ability of E2 production (**Figure 1**). FSH and GH significantly

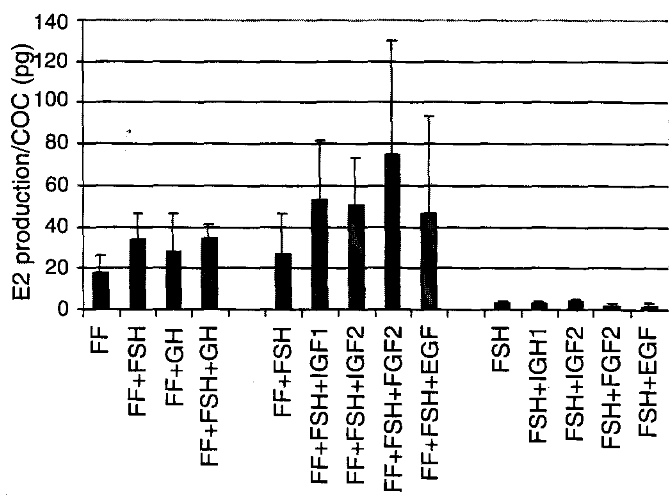

Figure 1. Composition of IVM medium.