protamination in condensing chromatin, facilitates DNA breakage. It is suggested that a reduction in the level of deoxyribonucleic acid protection, render the DNA molecule more sensitive to external damaging agents.

P375
GnRH-a induced steroid hormone receptor regulation in bovine endometrium

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Introduction Ovarian steroids consistently influence the endometrium and maintain its cyclicity by acting through their corresponding receptors. Estrogen receptors(ER α and ER β) and progesterone receptors (PR) are present in bovine endometrium in follicular and luteal phases of the estrous cycle, bovine ovaries and placentomes. We, most recently demonstrated the presence of GnRH receptors (GnRH-R) in bovine endometrium at both mRNA and protein level and localized these receptors to endometrial epithelial cells in both the phases of the estrous cycle. Additionally GnRH-R mRNA is also present in normal and carcinogenic human endometrium and endometriosis, where GnRH acts in an apoptotic and antiproliferative manner. GnRH is widely used in the bovine reproductive management including estrous and ovulation synchronization, induction of ovulation, post partum cyclicity, treatment of cystic ovarian disease, to overcome early embryonic mortality, and increase pregnancy rates; but there is clear lack of information on its local modulatory role in the endometrium. We find the co-existence of GnRH-R and steroid hormone receptors as interesting and there are prior reports about ligand independent activation of steroid hormone receptors. Whether GnRH through its receptors could regulate these receptors in normal endometrium is still not known and this study, for the first time examined the GnRH induced regulation of ER α and ER β and PR in bovine endometrium.
Materials and Methods Uteri belonging to follicular and luteal phases of the estrous cycle were collected from the local abattoir, transported to lab within one hour. One hundred mg of endometrial explants were cultured at 37^0C, 5% CO_2 in humidified atmosphere. After 20 h incubation**,** explants were treated with different doses of GnRH agonist – buserelin (0, 200, 500, 1000 ng/mL respectively), GnRH antagonist – antide (500 ng/mL) and a combination of antide (500ng/mL) and buserelin (200ng/mL) for 6 h. Two µg of total RNA extracted from each treatment was reverse transcribed using commercially available kit and the mRNA levels of ER α, ER β and PR were assessed by semi-quantitative RT-PCR and using the gene specific primers G3PDH was used as an internal control in the experiments. Optical intensity of individual bands was analyzed by Scion imaging beta and statistically analyzed by comparing to control and using student t test.
Results This study revealed that GnRH (200ng/mL) upregulated ER α mRNA in both follicular and luteal phases of the estrous cycle and it this effect was more pronounced ($P \leq 0.05$) in the luteal phase; whereas mRNA levels of ER β and PR were not altered.
Conclusions GnRH induced upregulation of ER α could have potential implications on reproductive process such as gamete transport, fertilization, cellular proliferation, uterine receptivity, implantation and maintenance of normal physiological status of the uterus and increases our understandings of the molecular basis of the reproduction at the endometrial level.

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Effect of pre-incubation of male and female gametes with fibronectin prior to fertilization *in vitro* in cattle

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Carbohydrates and glycoproteins modulate various adhesion and binding events in reproductive processes, including sperm-oviduct adhesion, sperm-egg interaction and embryo implantation. When fibronectin (Fn) – an extracellular matrix glycoprotein – is supplemented to the fertilization medium, a substantial inhibition of sperm penetration during bovine *in vitro* fertilization (IVF) was observed. To identify whether Fn interacts with either male or female gametes, 2 experiments were conducted incubating either sperm cells or cumulus oocyte complexes (COCs) with Fn prior to IVF.
To evaluate the effect of Fn on sperm, 2 groups of *in vitro* matured bovine COCs were fertilized in standard medium. One group was inseminated with spermatozoa ($1x10^6$ sp/ml) previously incubated with 500 nM Fn for 30 min. The second group was fertilized with spermatozoa (same ejaculate) incubated with standard medium. Two extra experiments – where the sperm cells were incubated for 2 h resp 4 h – were performed to evaluate effect of time of sperm pre-incubation on inhibition of sperm penetration. To assess the effect of Fn on the female gamete, *in vitro* matured COCs were divided into 2 groups. The first group was fertilized under standard conditions, the second one was treated with 500 nM Fn for 30 min prior to IVF. Subsequently, a similar setup was applied on zona-free oocytes. Twenty hours after insemination, all presumed zygotes were fixed, stained with Hoechst 33342 and evaluated by fluorescence microscopy for sperm penetration and fertilization. Differences in fertilization and penetration percentage were analyzed by binary logistic regression (SPSS 15.0).
Pre-incubation of sperm cells with Fn significantly decreased the sperm penetration compared to that of the control (75.2% vs 87.0%) resulting in an inhibition of penetration of 13.6%. The same tendency was observed for fertilization with or without Fn pre-incubated sperm (68.6 % vs 78.2 %). Prolonging the duration of sperm pre-incubation caused more prominent inhibition of penetration (22.2% after 2 h; 42.8% after 4 h). When pre-incubating COCs with Fn, penetration was not significantly reduced (76.2% vs 83.0 %) compared to that of the control, nor was the fertilization rate (67.3% vs 75.4%). Furthermore, Fn pre-incubation of zona-free oocytes did not affect sperm penetration (42.0% vs 46.9%) nor fertilization (37.1% vs 37.0%).
In conclusion, Fn inhibits sperm penetration in bovine COCs through interaction with the sperm cell. To elucidate the underlying mechanism, identification of receptors for Fn on bovine sperm is required.

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Effect of replacer of fetal calf serum in the development and gene expression in bovine embryos *in vitro* cultured

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Introduction The period of post fertilization embryo culture is the most critical affecting blastocyst quality. Knockout SR (Gibco Labs., Grand Island, NY) is a serum replacer optimized to support embryonic stem cells in culture and can also be used to replace serum during culture of bovine embryos. The expression of genes associated to stress response, such as heat shock proteins (Hsp), can be affected by *in vitro* culture conditions, including culture medium components. The aim of this study was to evaluate the effect of Knockout™ SR on

the development, total number cells and relative abundance of Hsp70.1 of *in vitro* fertilized bovine embryos.
Materials and methods COCs collected in slaughterhouse were matured and *in vitro* fertilized. The presumptive zygotes were randomly distributed in three groups of medium culture CR2aa supplemented with 10% of fetal calf serum (FCS); 10% knockout serum replacer (KSR) and 3 mg/ml of polyvinyl alcohol (PVA). Cleavage rate and blastocyst rate were determined respectively 72 and 168 hours post-fertilization (hpf). The total number of cells were determined at 192 hpf. Pools of ten embryos obtained at 192 hpf were frozen for RNA extraction and real time RT-PCR methodology was used to obtain quantitative data of Hsp 70.1 transcripts. Expression of GAPDH gene was used as endogenous reference. Calculations of relative quantification were performed by Comparative Ct method, using the value found in PVA group as calibrator. Data of cleavage and blastocyst rate were analyzed by the Kruskal Wallis test and the total number of cell by variance analyses. Means were compared by Student Newman Keuls test.
Results and Discussion No significant difference (P>0.05) was found among FCS (57,8±4,6), KSR (62,2±4,5) and PVA (60,4±4,4) on cleavage rate. However, blastocyst rate (12,2±2,1 and 18,6±3,0) and total number of cells (105,9±5,9 and 109,4±6,1) were similar (P>0.05) for KSR and FCS, and higher (P<0.01) when these supplements were compared to PVA (4,2±1,0 and 79,6±8,4). Expression levels for FCS and KSR group were 1.2±0.06 and 1.4±0.08 fold different relative to PVA group without differences (P>0.05) between FCS and KSR groups. These data show that bovine embryos cultured in medium supplemented with KSR has similar patterns of development, quality and Hsp70.1 expression than those cultured in presence of the serum. In conclusion, KSR is able to support development of *in vitro* fertilized bovine embryos and it can be an alternative when serum-free culture medium is recommended.
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Melatonin treatment and undernutrition affect expression of uterine estrogen and progesterone receptors in ewes during the reproductive and the anestrous seasons

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Melatonin treatment in ewes increases prolificacy and fertility. A reduction in PGF2α in vitro secretion by endometrial cells after melatonin addition has been reported, suggesting that melatonin could act directly on sheep endometrium. In previous studies we have shown that undernutrition affects endometrial sensitivity to estradiol and progesterone by decreasing their receptor concentration (ERα and PR, respectively) which could explain the lower pregnancy rates found in undernourished ewes. In this study we tested the hypothesis that melatonin treatment could counteract subnutrition effects, and thus ERα and PR content in different endometrial cell types were studied in undernourished ewes. Adult Rasa Aragonesa ewes were assigned to a 2 x 2 factorial design performed both in the reproductive (RS, n=25) and anestrous seasons (AS, n=24). They were treated (+MEL) or not (-MEL) with a subcutaneous implant of melatonin for 42 days (Melovine®, CEVA) and fed to provide 1.5 (Control, C) or 0.5 (Low, L) times daily maintenance requirements from synchronization day with intravaginal pessaries. Ewes were mated at oestrus (Day=0) and slaughtered on Day 5, when pieces of uterus were collected to determine PR and ERα by immunohistochemistry. There was an effect of season on the staining intensity of PR (P<0.0001), and a tendency for ERα (P=0.10); the expression was higher during the anestrous season, being more evident in the deep stroma. No effect of undernutrition or melatonin was observed during the AS in any cell type. However, differences were found during RS: C ewes had greater ERα staining than L ewes (luminal epithelium, P<0.05); PR staining was greater in C+MEL than in L+MEL (superficial stroma, P<0.05). Treatment with melatonin in undernourished ewes decreased PR expression in both superficial and deep glandular epithelia and in superficial stroma (P<0.05). This study shows that neither melatonin nor nutrition treatment had an effect on ERα and PR expression during anoestrous. Melatonin treatment could not counteract the detrimental effects of undernutrition on sex steroid receptors; moreover, it even provoked a higher decrease in PR content in undernourished ewes which was associated with lower embryo viability during reproductive season.
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Effects of E and F prostaglandin receptor agonists on luteal function in vivo in ewes

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Introduction $PGF_2\alpha$ is delivered locally from the uterus to the adjacent corpus luteum (CL)-containing ovary in ewes. However, $PGF_2\alpha$ during early pregnancy is not decreased in uterine endometrium or venous blood, ovarian venous blood, or CL, nor binding to CL membranes. Ewes become resistant to $PGF_2\alpha$[1] (C. Weems et al. 2006). PGE_1 and PGE_2 increased two fold in endometrium of day 13 pregnant ewes[2] (Wilson et al. 1972). PGE_1 or PGE_2 prevented luteolysis only when infused chronically into the uterine horn lumen adjacent to the CL-containing ovary, increase luteal progesterone (P4) secretion in vitro and in vivo, and PGE_1 in vivo increased P4 secretion longer than PGE_2[1] (C. Weems et al. 2006). Four PGE receptor subtypes (EP_1, EP_2, EP_3, and EP_4) and one $PGF_2\alpha$ (FP) receptor have been identified (Narumiya 1995).
Objective The objective of this experiment was to elucidate the effects of EP_1, EP_2, EP_3, and FP receptor agonists on CL function
Methods On day-9, ewes received a single treatment into the vascular interstitium adjacent to the CL-containing ovary of Vehicle, $PGF_2\alpha$ (100 μg) an FP receptor agonist, or 500 μg of the EP receptor agonists 17-Phenyl-Tri-Nor-PGE_2 (EP_1 and EP_3). Butaprost (EP_2), 19-(R)-OH-PGE_2 (EP_2), or Sulprostone (EP_3). Jugular venous blood was collected at 0 and every 6 hours up to 48 hours for analysis for P4 by RIA. CL were collected at 48 hours, bissected, weighed, and stored in liquid nitrogen until analysis for the mRNA:actin ratio for LH receptors and unbound and bound LH receptors. P4 in jugular venous plasma was analyzed by a Split-Plot ANOVA for repeated measures. CL weight, CL mRNA:actin ratio for LH receptors, and CL unbound and bound LH receptors were analyzed by a One Way ANOVA.
Results and conclusion PGF2α or Sulprostone reduced ($P \leq 0.05$) CL weight, circulating P4, CL mRNA:actin ratio for LH receptors, and CL unbound and bound LH receptors. 17-Phenyl-Tri-Nor-PGE_2 did not affect ($P \geq 0.05$) any parameter analyzed. Butaprost and 19-(R)-OH-PGE_2 increased ($P \leq 0.05$) circulating P4, CL mRNA:actin ratio for LH receptors, and CL unbound and bound LH receptors. Luteal mRNA for LH receptors and unbound and bound receptors for LH may be increased via the EP_2 receptor, while the EP_3 receptor may decrease CL mRNA for LH receptors and unbound and bound receptors for LH to contribute to luteolysis.
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