strongly diminished the response to GnRH. A weaker effect was seen when SF-1 sites were mutated. Mutation of both Egr-1 and SF-1 sites abolished GnRH induction of LHβ gene. We also found that GnRH stimulated the expression of EGR-1 mRNA in a time-dependent manner. We have also detected a strong interaction between SF-1 and other members of the EGR family, including EGR-2 (krox-20), EGR-3 and EGR4 (NGFI-C).

Conclusion: GnRH stimulation of LHβ requires the synergistic interaction of the orphan nuclear steroid receptor SF-1 and the early growth response element EGR-1. These transcription factors are likely play a significant role in regulation of LHβ gene in gonadotrophs.

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Embryology 1

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O-070. Involvement of connexin43, a gap junction protein, in oocyte maturation

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Introduction: Granulosa cells (GC) play a key role in oocyte development. They provide nutritive factors for the growing oocyte, and control the nuclear and cytoplasmic maturation of the oocyte selected for ovulation. It has been postulated that intercellular coupling mediated by gap junction channels present between the GC themselves and between the GC and the oocyte have an impact on final oocyte maturation. Indeed, it has been shown that an elevated intra-ocyte concentration of cAMP negatively regulates oocyte meiosis and that this cyclic nucleotide could be provided to the oocyte by GC via gap junctions. Therefore, meiosis resumption could either be caused by a quantitative reduction of an inhibitory substance, like cAMP, via disruption of the gap junctional network, or by the transfer of a positive signal from GC to the oocyte that could stimulate germinal vesicle breakdown in spite of the continued presence of an inhibitory substance. Gap junctions are formed by an interconnection of hemichannels consisting of hexameric proteins called connexins (Cx). The connexins comprise a multigene family and in rodents more than 14 homologous connexin sequences have been cloned and characterized to date. In bovine cumulus–oocyte complexes (COC), Cx43 has been identified as the most abundant connexin. We are studying the role of gap junction channels on in-vitro oocyte maturation using recombinant adenoviruses to inhibit Cx43 expression. We have thus constructed recombinant adenoviruses expressing the complete cDNA of rat Cx43 in the antisense orientation fused (Ad-asCx43-GFP) or not (AdasCx43) to the green fluorescent protein (GFP) cDNA. As control, we have used an adenovirus expressing GFP only (Ad-GFP). We have then analysed the effect of Ad-asCx43, Ad-asCx43-GFP and Ad-GFP on oocyte maturation of infected bovine COC.

Materials and methods: COC were cultured in M199-HCO3 supplemented with 10% synthetic serum substitute (Irvine Scientific, CA, USA), 1 IU/ml of recombinant FSH (Gonal F®), 1 IU/ml of LH (LHADI®; Serono, Geneva, Switzerland) and 1 μg/ml oestradiol. To determine the oocyte maturational stage, its chromosomes were stained with Hoechst 33342 after cell denudation in 80 IU/ml hyaluronidase. For reverse transcriptase–polymerase chain reaction (RT–PCR), total RNA from infected COC was extracted. The RNA was then reverse-transcribed and an aliquot was used for PCR using specific primers located in the Cx43 sequence and in the viral DNA. The PCR data showed that the complete cDNA fragment of rat Cx43 was efficiently transcribed in Ad-asCx43 or Ad-asCx43-GFP infected COC. Moreover, the efficacy of infection was >95% as evaluated by GFP fluorescence in GC. All oocytes (n = 32) recovered from freshly isolated bovine immature COC were in the germinal vesicle stage.

Results: After 24 h of in-vitro culture, 85% (28/33) of oocytes from intact COC matured to the metaphase II stage. In contrast, only 53% (49/92) of oocytes released from their surrounding GC before in-vitro culture were able to resume meiosis. Furthermore, only 36% (40/112) of oocytes present in COC infected either with Ad-asCx43 or with Ad-asCx43-GFP matured to the metaphase II stage. In contrast, 76% (45/59) of the oocytes from COC infected with the control recombinant adenovirus were recovered in the metaphase II stage.

Conclusion: The present data show that COC can be infected with a recombinant adenovirus and express the transgene. The adenovirus carrying the complete cDNA fragment of rat Cx43 inserted in the antisense orientation, fused or not to GFP, strongly (P = 0.0001) inhibited in-vitro maturation of intact bovine COC. These data are in agreement with our previous experiments using n-alkanols as blocking agents of gap junction channels. These experiments suggest that the absence of cell-to-cell communication within COC prevents oocyte maturation. Therefore, open gap junction channels seem to be important for the transfer of signalling molecules involved in oocyte maturation.