Treatment of Cumulus-Oocyte-Complexes with Age-Associated FSH Glycoforms Results in Differential Modulation of Meiotic Resumption

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Differences in N-glycosylation of the β-subunit of follicle-stimulating hormone (FSH) results in macroheterogeneity. Of interest, FSH glycoform abundance changes throughout female reproductive aging. The hypoglycosylated form of FSH (FSH^{21}), which is dominant in women <35 years of age when reproductive potential is high, exhibits increased FSHR binding and bioactivity compared to fully glycosylated FSH (FSH^{24}) which is the major glycoform present in women >35 years of age. Thus, there is a shift from FSH^{21} to FSH^{24} with age that correlates with reduced bioactivity. We previously demonstrated that FSH^{21} better supports folliculogenesis compared to FSH^{24} due to enhanced cellular communication. Given that FSH is also important for later stages of oocyte development, we sought to determine whether FSH glycoforms mediate differential effects on cumulus cell expansion and meiotic progression during *in vitro* maturation (IVM). To do this, we collected intact cumulus-oocyte-complexes (COCs) containing fully grown oocytes obtained from hyperstimulated adult mice and matured them in the presence or absence of 10 ng/ml FSH^{21} and FSH^{24}. IVM was performed using an EmbryoScope+ platform with images taken every 10 min at 11 focal planes for a total of 16 hrs which enabled analysis of morphokinetic variables of meiotic progression and cumulus expansion. Both the FSH glycoforms promoted cumulus cell expansion, whereas no expansion was observed in the control without FSH. The velocity of cumulus cell expansion was similar between glycoform treatments. Oocytes within FSH^{21}-treated COCs underwent germinal vesicle breakdown (GVBD) at 0.90 ± 0.18 hrs compared to FSH^{24}-treated COCs which showed a significant delay in GVBD at 1.8 ± 1.23 hrs (n=16 oocytes; p=0.0061). Polar body extrusion (PBE) showed a delayed trend in the FSH^{24}-treated group compared to FSH^{21} with PBE occurring at 9.30 ± 1.01 and 8.72 ± 0.62 hrs, respectively (n=15-16 oocytes, p=0.08). The difference in meiotic progression between FSH^{21} and FSH^{24} treatments was not observed in oocytes that were denuded of their cumulus cells prior to IVM (n=16 oocytes, p=0.74), indicating that the effects are mediated through direct actions of FSH on the cumulus cells. Examination of metaphase-II eggs and subsequent *in vitro* fertilization will provide insight into whether these differences in maturation kinetics result in differences in egg quality and fertilization potential. This work demonstrates that the effect of age-associated FSH glycoforms is relevant beyond follicle growth, with glycoform-specific modulation of oocyte meiotic resumption.