(OR), and named FecL. The influence of the prolific allele $FecL^L$ on OR is additive with one copy increasing OR by about 1.5 and two copies by about 3.0. Previous work on genetic mapping localized FecL on sheep chromosome 11 within an interval of 1.1 megabases encompassing 20 genes. With the aim to identify the FecL gene, we developed a high throughput sequencing strategy (Roche 454 GS FLX) of long-range PCR fragments spanning the 1.1 Mb interval to identify potential informative markers from an heterozygous animal. This approach led to define a 190 kb minimal interval for the FecL locus containing only two genes, insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1) and beta-1,4-*N*-acetyl-galactosaminyl transferase 2 (B4GALNT2). The full sequencing of the 190 kb interval of a homozygous $FecL^L/FecL^L$ ewe and a non-carrier ewe identified only one SNP in the intron 7 of B4GALNT2 in complete linkage disequilibrium with $FecL^L$. Moreover, the $FecL^L$ mutation is associated with the ectopic expression of B4GALNT2 mRNA in the ovarian follicles of $FecL^L/FecL^L$ Lacaune ewes (1000-fold increased compared to wild-type). Thus, B4GALNT2 appears as the best positional and expressional candidate for FecL. Since it is implicated in glycosylation pathway, we localized the B4GALNT2 activity by specific lectin-histochemistry in granulosa cells of ovarian follicles only in hyperprolific $FecL^L$ carrier ewes. This was confirmed by the results of lectin-precipitation of follicular fluid and subsequent western blot experiments. The identification of the lectin-labeled proteins from $FecL^L$ and wild-type follicular fluids by mass spectrometry revealed around 30 proteins suspected to be glycosylated by B4GALNT2 only in $FecL^L$ ovaries. Among those proteins, the versican proteoglycan, inhibin alpha and betaA subunits, and bone morphogenetic protein 1 are good candidates for further studies to understand the physiological pathway used to increase ovulation rate. The identification of the FecL gene as B4GALNT2 implicated in glyscosylation leads to the discovery of a new pathway involved in folliculogenesis and the regulation of ovulation rate. Now, the question is, does the altered expression of B4GALNT2 affect the transforming growth factor beta/bone morphogenetic protein pathways, known to be affected in other ovine prolific breeds?

Key Words: Ovine, major gene, ovulation rate, B4GALNT2, glycosylation

1409

Determination of anti-Müllerian hormone plasma concentration can help to select donor sheep for laparoscopic ovum pick-up

B Lahoz*[1], JL Alabart[1], D Monniaux[2], S Fabre[2], P Jarrier[2], MJ Cocero[3], José Folch[1]

[1]*Centro de Investigación y Tecnología Agroalimentaria (CITA) de Aragón, Zaragoza, Spain;* [2]*INRA, UMR85 Physiologie de la Reproduction et des Comportements, CNRS UMR 7247, Université de Tours, Nouzilly, France;* [3]*Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain*

Anti-Müllerian hormone (AMH) is produced by granulosa cells of small antral follicles able to respond to gonadotropins. Recent results indicate that AMH represents a very interesting endocrine predictor of the ovarian response to hormonal treatments. In sheep, besides the limited efficiency of the LOPU and IVP of embryos, the expensive cost and variable individual response to FSH treatments remain important limits to the development of embryo biotechnologies. The aim of the present study was to determine AMH plasma concentrations in sheep and to relate them with the number of follicles (≥3 mm) available for oocyte puncture after FSH treatment in a LOPU-IVP program. With this objective, 16 adult Rasa aragonesa ewes were subjected to two consecutive LOPU sessions 1 week apart. Ewes received 32 mg of porcine FSH administered in decreasing doses: 8 mg (60 h), 8 mg (48 h), 6 mg (36 h), 6 mg (24 h) and 4 mg (12 h) prior to each session, and a blood sample was taken from each ewe coinciding with the first injection of each session. Plasma concentrations of AMH were determined using AMH GENII ELISA Kit (Beckman Coulter). AMH plasma concentration was positively correlated with the number of punctured follicles ($r = 0.65$; $p < 0.0001$), the number of recovered cumulus-oocyte complexes (COCs, $r = 0.41$; $p < 0.05$), and the number of oocytes suitable for *in vitro* maturation (IVM, $r = 0.39$; $p < 0.05$) at each LOPU session. AMH and the number of follicles at the first session were highly correlated with AMH and follicle number found at the second session, respectively ($r = 0.75$; $p < 0.005$ and $r = 0.55$; $p < 0.05$). AMH plasma concentration was about two fold higher in ewes with a high follicular response to FSH compared with low-responding ewes (Table 1). In conclusion, AMH plasma concentrations could be used to improve the output of the LOPU-IVP technique in sheep. AMH measurement in a single blood sample taken before FSH treatment can predict the number of follicles available for puncture per donor ewe, with a low variability between sessions. Thus circulating AMH measurement is a useful tool to select the best oocyte donors. Financed by INIA (RTA2011-128 project and fellowship for B. Lahoz) and INRA (PREDICTOV project).

Key Words: AMH, COCs, ewe, follicle, IVP

Table 1. Anti-Müllerian hormone plasma concentration before FSH treatment and numbers of follicles, recovered COCs, and COCs suitable for IVM per ewe and session (Mean ± SE), for low or high-responding groups of ewes

	n	AMH (pg/ml)	Follicle number	Recovered COCs	IVM_COCs
<20 follicles	18	97.5 ± 12.2[a]	14.8 ± 0.7[a]	9.2 ± 0.7[a]	8.2 ± 0.8[a]
≥20 follicles	14	176.7 ± 21.0[b]	23.9 ± 1.0[b]	14.4 ± 1.0[b]	13.0 ± 0.9[b]

Within a column, means differ at [a,b]$p < 0.0001$.

1410

Screening of functional shRNA molecules to knock down the expression of ovine BMPR-1B gene

J Lin[1,2], X Jin[1], L Wang[1], J Bai[2], C Liu[2], W Li[1,2], M Liu[1,2], J Huang*[1,2]

[1]*The Key Lab of Animal Biotechnology of Xinjiang, Urumqi, China;* [2]*The Key Lab of Livestock Reproduction & Biotechnology of MOA, Xinjiang Academy of Animal Science, Urumqi, China*

Bone morphogenetic protein 1B receptor (BMPR-1B) is one of the most important genes in regulating ovine ovulation rate and litter size. The $FecB^B$, which is Q249R mutation in BMPR-1B and leads to a higher ovulation rate, has also been supposed as a partial 'loss of function' of BMPR-IB (Fabre, 2006). Thus, we hypothesized that a decreased BMPR-IB expression leads to an increasing in ovine ovulation rate. In this study, we attempt to screen the functional shRNA molecules to knock down the expression of ovine BMPR-1B gene. Based on the cDNA sequence of ovine BMPR-1B (NM_001009431), Thermo's web siRNA design software (http://www.dharmacon.com/designcenter/DesignCenterPage.aspx) was used to select suitable siRNA targeting sites at the whole cDNA of ovine BMPR-1B (sh749 in transforming growth factor beta type I GS-motif, sh803 and sh1306 in catalytic domain of protein kinases, sh1475 in protein kinase domain, sh1685, sh2567 and sh2745 in 3′UTR). In experiment 1, BMPR-1B gene, which was derived from ovine ovarian tissue containing 1515 bp coding region sequence, was overexpressed in HEK293 cells and the HEK293 cells stably transfected with BMPR-1B were used in the following experiment. Then these 7 shRNAs were transferred to the HEK293 cells, respectively. All of seven shRNAs can reduce the expression level of exogenous ovine BMPR-1B gene in HEK293 cells from 68.3% to 99.86% (sh749/68.3%, $p < 0.05$; sh803/71.2%, $p < 0.05$; sh2745/80.5%, $p < 0.05$; sh1685/94.37%, $p < 0.01$; sh2567/96.41%, $p < 0.01$ sh1475/99.75%, $p < 0.01$ and sh1306/99.86%, $p < 0.01$) detected by qRT-PCR, and this result was confirmed by Western blot. In experiment 2, these seven shRNA molecules were then verified to knock down the expression of endogenous BMPR-1B gene in ovine granulosa cells which were cultured in DMEM/F12 containing 10% FCS. After transfected, the new DMEM/F12 containing 1% FCS was added. The result showed that all of 7 shRNAs can decrease the mRNA level of BMPR-IB gene from 54.21% to 98.14% (sh1475/54.21%, $p > 0.05$; sh2745/63.31%, $p > 0.05$; sh2567/76.78%, $p < 0.05$; sh749/80.2%, $p < 0.05$; sh1685/90.23%, $p < 0.01$; sh803/96.69%, $p < 0.01$ and sh1306/98.14%, $p < 0.01$) in ovine granulosa cells by qRT-PCR. In summary, both experiments showed that the sh1306 produced the most efficient inhibition among 7 shRNA molecules to knock down the expression of

Reproduction in Domestic Animals

Vol. 47 · Supplement 4 · August 2012

Proceedings of the 17th International Congress on Animal Reproduction (ICAR)

Vancouver, Canada
29 July – 2 August 2012

WILEY-
BLACKWELL