# Transcriptome profiling of reproductive tissues characterize genetic basis of the prolificacy traits in sheep (*Ovis aries*)

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## BACKGROUND

- Prolific domestic sheep breeds (*Ovis aries*) are valuable genetic resource for global sheep industry
- Data = two pure breeds showing high (Finnsheep) and low (Texel) litter sizes and their F1-crossbred ewes
- Prolificacy traits = ovulation rate (phase 1) and litter size (phase 2)
- Feeding experiment = flushing diet prior to and during the breeding season [1]
- Analysis = focus on structural and functional variations in sheep genome; differential gene expression within and between breed groups due to diet differences

## MATERIALS AND METHODS

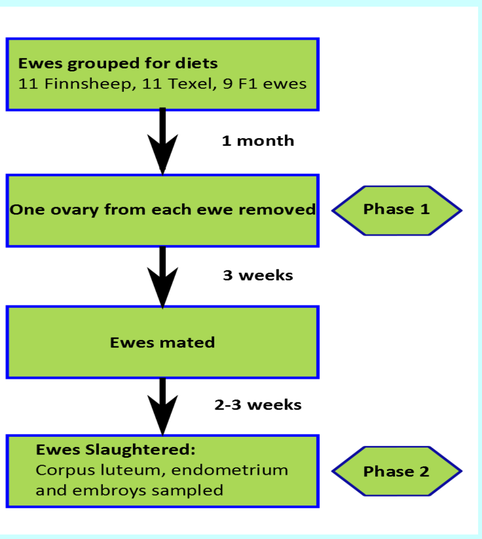


**Fig. 1 Experimental design**

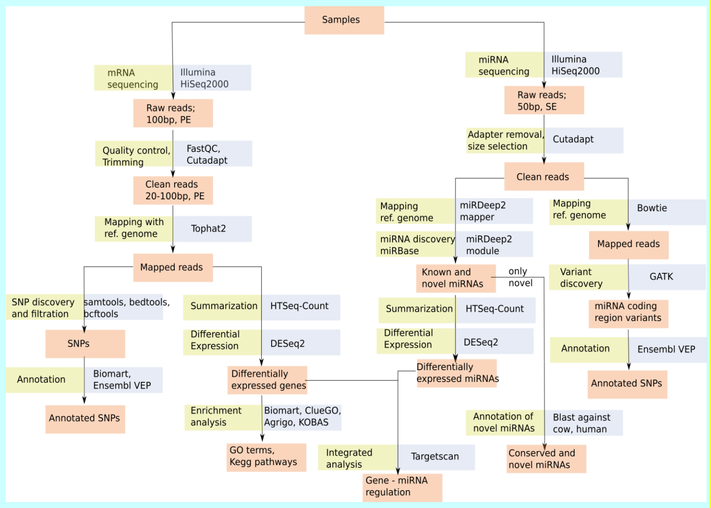


**Fig 2. Bioinformatics pipeline**

| Tissue | Finsheep | | Texel | | F1 | |
|---|---|---|---|---|---|---|
| | FLU | CON | FLU | CON | FLU | CON |
| Ovary | 6+2 | 5 | 6+2 | 5 | 5+2 | 4+2 |
| Endometrium | 3+1 | 3 | 3+1 | 3 | 3+1 | 3 |
| Corpus Luteum | 3+1 | 3 | 3+1 | 3 | 3+1 | 3 |

**Table 1. Samples under study**. FLU = Flushing diet, CON = Control diet; "+nn" indicate techincal replicates

## RESULTS

- The breed groups were clustered in SNP-genotyping, but not in the gene expression data (fig. 3).
- The number of expressed genes: ovary (16,402) > *corpus luteum* (16,067) > endometrium (15,457)
- All of the known sheep mitochondrial genes and 9 (out of 24) non-coding genes were expressed and represented 14% of the ovarian transcriptome (~5% mitochondrial transcripts in human[2]).
- Results show that F1 cross-bred ewes are closer to Finnsheep than Texel (table 2, fig. 3).
- DE genes between pure breeds in control diet: CST6, MEPE and HBB (cell proliferation and differentiation).
- DE genes between Finnsheep and Texel in flushing diet: associated with biological processes such as cardiovascular system development, cell migration, cell substrate adhesion, heart development and cellular component movement.
- Half of the Finnsheep and F1 but Texel ewes carried V371M mutation [3] in GDF9.
- The distribution of deleterious SNPs was similar in Finnsheep and Texel which shared 163,346 and 163,569 SNPs respectively, whereas F1 had 142,673 SNPs (fig. 4).
- Up to 103 known and 562 novel ovine miRNAs were expressed in the data.
- A number of gene targets of differentially expressed miRNAs were also differentially expressed between Finnsheep vs Texel with flushing diet (fig. 5).
- A high proportion of miRNAs are clustered within 194kb on chromosome 18, which is homologous to Human (Chr14), Dog (Chr8), Horse (Chr24) and other mammals. (fig. 6)
- The qRT-PCR results based on selected genes and miRNAs were consistent with the results from the sequencing.

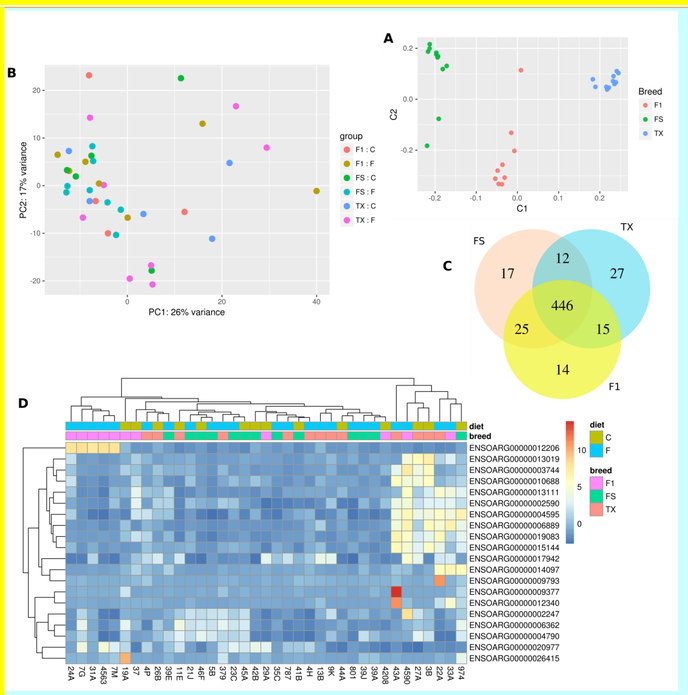


**Figure 3. Summary of sample relatedness**. (A) MDS plot based on IBS distance (SNP genotype data). (B) PCA plot of top 500 differentially expressed genes. (C) Venn diagram based on top 500 expressed genes. (D) Heatmap plot of top 20 differentially expressed genes across all samples

| | mRNA | | | miRNA | | |
|---|---|---|---|---|---|---|
| | DE | UP | Down | DE | UP | Down |
| FS – TX* | 38 | 4 | 34 | 0 | 0 | 0 |
| FS - F1* | 5 | 3 | 2 | 0 | 0 | 0 |
| TX - F1* | 68 | 44 | 24 | 0 | 0 | 0 |
| FS.F – TX.F | 600 | 113 | 487 | 8 | 4 | 4 |
| FS.F – F1.F | 47 | 18 | 29 | 0 | 0 | 0 |
| TX.F – F1.F | 305 | 249 | 56 | 0 | 0 | 0 |
| FS.C – TX.C | 3 | 1 | 2 | 3 | 2 | 1 |
| FS.C – F1.C | 2 | 1 | 1 | 0 | 0 | 0 |
| TX.C – F1.C | 57 | 9 | 48 | 0 | 0 | 0 |
| FS.F – FS.C | 0 | 0 | 0 | 0 | 0 | 0 |
| TX.F – TX.C | 118 | 71 | 47 | 0 | 0 | 0 |
| F1.F – F1.C | 25 | 4 | 21 | 0 | 0 | 0 |

**Table 3. Differentially expressed genes and miRNAs in the ovarian transcriptome**. DE – Differentially expressed; UP – Upregulated; Down – downregulated, FS – Finnsheep, TX – Texel, F1 – F1-cross of Finnsheep and Texel, F – Flushing diet , C – Control diet; * comparison that included diet as a second factor

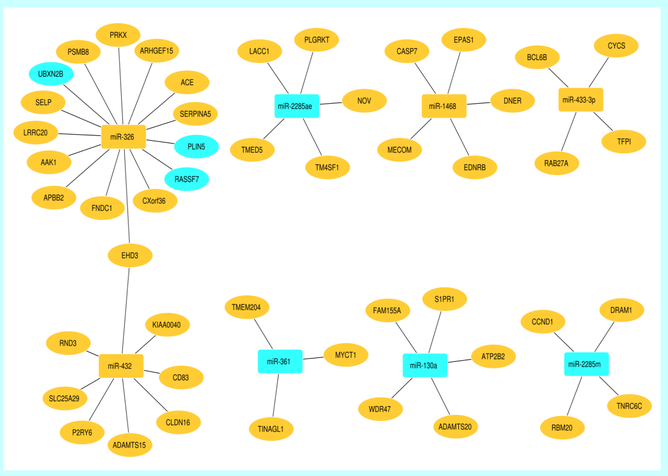


**Fig 5. Gene – miRNA network (ovary).**

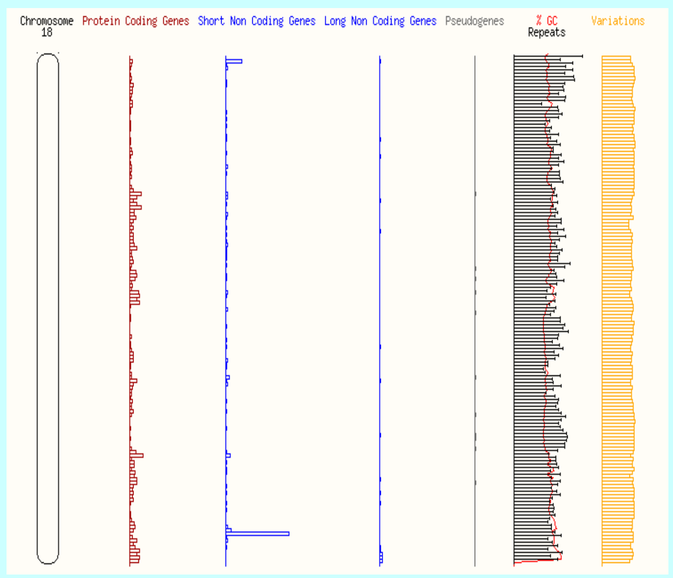


**Fig 6. Chromosomal location of miRNA cluster**. The lower peak representing short noncoding genes represent miRNA clusters

| Diet | 1st Phase | | | 2nd Phase | | |
|---|---|---|---|---|---|---|
| | Finnsheep #ovarian follicles | F1 #ovarian follicles | Texel #ovarian follicles | Finnsheep #CL | F1 #CL | Texel #CL |
| Flushing | 11 | 9.5 | 9.2 | 3.83 | 3.5 | 1.8 |
| Control | 12.8 | 7.75 | 10.2 | 4.4 | 4 | 1.6 |
| Average | 11.9 | 8.63 | 9.7 | 4.09 | 3.75 | 1.7 |

**Table 2. Phenotypic observations**.

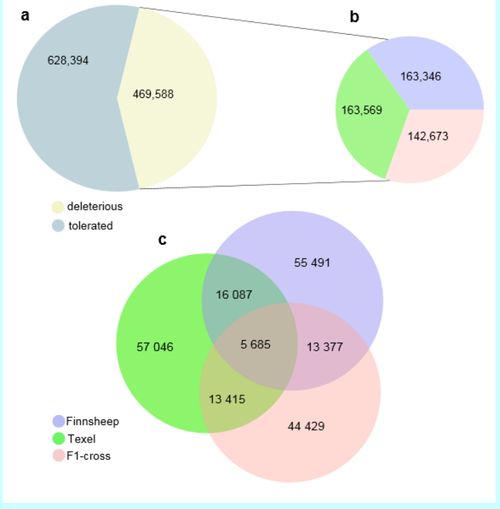


**Fig 4. Non-synonymous SNPs distribution (Ovary; RNA-Seq data).**

## CONCLUSIONS

- Finnsheep were least and Texel ewes were most responsive to the flushing diet.
- While the genotypes place F1 crosses as a true hybrid of Finnsheep and Texel, phenotypes and gene expression patterns showed that F1 individuals are closer to Finnsheep than Texel.
- The high amount of mitochondrial transcripts in the sheep ovaries indicate that they are one of the tissues with high-energy demands compared to monovulatory species like human.
- Three genes, *CST6*, *MEPE* and *HBB* that were differentially expressed between the pure breeds in control diet appeared to be candidate genes for prolificacy.

## References.

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RESEARCH DATA ALLIANCE EUROPE

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