Formation of Ovarian Follicular Fluid

Thesis submitted for the degree Doctor of Philosophy

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Abstract

The formation of a fluid filled antrum is a key feature of folliculogenesis. The oocyte is expelled in the fluid in the event of ovulation, making follicular fluid necessary for unassisted reproduction. The mechanism(s) by which follicular fluid is formed is not understood. The studies described in this thesis were the basis of a project designed to investigate the hypothesis that an osmotic gradient exists between follicular fluid and serum. This osmotic gradient drives the recruitment of fluid from the vascularised theca layer surrounding the follicle. The aim of this study was to identify potential osmotically active molecules within follicular fluid of bovine follicles and advance existing data in this area.

The osmotic potential created by follicular fluid changes during folliculogenesis and that the constitution of the fluid was subtly different between healthy and atretic follicles. By specific enzyme digestion of chondroitin sulphate, dermatan sulphate, heparan sulphate, keratan sulphate and hyaluronan glycosaminoglycan chains, proteins and collagen the classes of molecule responsible for the potential were distinguished.

Using a combined physical, immunological and molecular approach hyaluronan, and the chondroitin sulphate proteoglycans versican and inter-alpha trypsin inhibitor (ITI) were identified as potential contributors to the colloid osmotic pressure of the fluid and that of these hyaluronan was the most osmotically active. Both hyaluronan and versican exist in high molecular weight forms and could contribute to colloid osmotic pressure as discrete molecules. However, ITI may contribute via its potential to form very large molecular weight aggregates with hyaluronan and versican. It is proposed that a fluid matrix consisting of these molecules provides versatility of the fluid osmotic potential, in addition to the variations in permeability of the follicular basal lamina and vasculature, and hence the follicles ability to recruit fluid in to the antrum from the follicles vascular surrounds.

To examine the hypothesis further three specific enzymes synthesise hyaluronan, hyaluronan synthases 1, 2 and 3 (HAS 1, HAS 2 and HAS 3) were made. RT PCR, Northern analysis and in situ hybridisation identified which cells were expressing these enzymes in the follicle during devlopment. Expression of HAS 1 and HAS 3 could not be correlated with antrum expansion but may contribute in a secondary manner to fluid osmotic potential. Expression of HAS 2 by the mural granulosa cells could be temporally linked to the visualisation of a follicular antrum and hence could be responsible in part for antrum formation. The regulation of transcription of HAS 2 is probably tightly controlled by differential signalling or receptor activity of any number of growth factors and gonadotrophins and that the turnover of hyaluronan in the fluid at any time is tempered by

differential expression of HAS enzymes and the hyaluronidases responsible for its degradation.

Macromolecules have been identified in follicular fluid, which have an osmotic effect as hyaluronan and the chondroitin sulphate proteoglycans versican and inter-alpha trypsin inhibitor. Hyaluronan was the most osmotically active of these molecules and its synthesis by the HAS 2 enzyme could be correlated with the timing of antrum formation.