



## Localization of angiotensin receptor type 2 in fetal bovine ovaries



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### ABSTRACT

In the ovary, angiotensin II (ANGII) acts through the type 2 receptor (AGTR2) to induce ovulation and may play a role in follicle atresia. In this study, we determined the expression of AGTR2 mRNA and protein during follicle formation in the bovine ovary. Female fetuses at different gestational ages (60, 75, 90, 120, 150 and 210 days) were used for immunolocalization of AGTR2. At day 60, AGTR2 was localized to the cytoplasm of oogonia; from days 75 to 150, during follicle formation and development to secondary stage, AGTR2 immunostaining was weak and irregular, but from day 210 staining became evident in granulosa cells of preantral follicles and in both granulosa and theca cells of small antral follicles. These data differ from those in pigs, in which AGTR2 protein is detected in preantral follicles throughout gestation. Abundance of AGTR2 mRNA in whole ovaries did not change with fetal age. In conclusion, AGTR2 protein is expressed in ovigerous cords in fetal bovine ovaries but not in preantral follicles until the formation of antral follicles. These data suggest important species-specific differences in the expression of AGTR2 in fetal ovaries from polyovulatory and monovulatory animals.

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### 1. Introduction

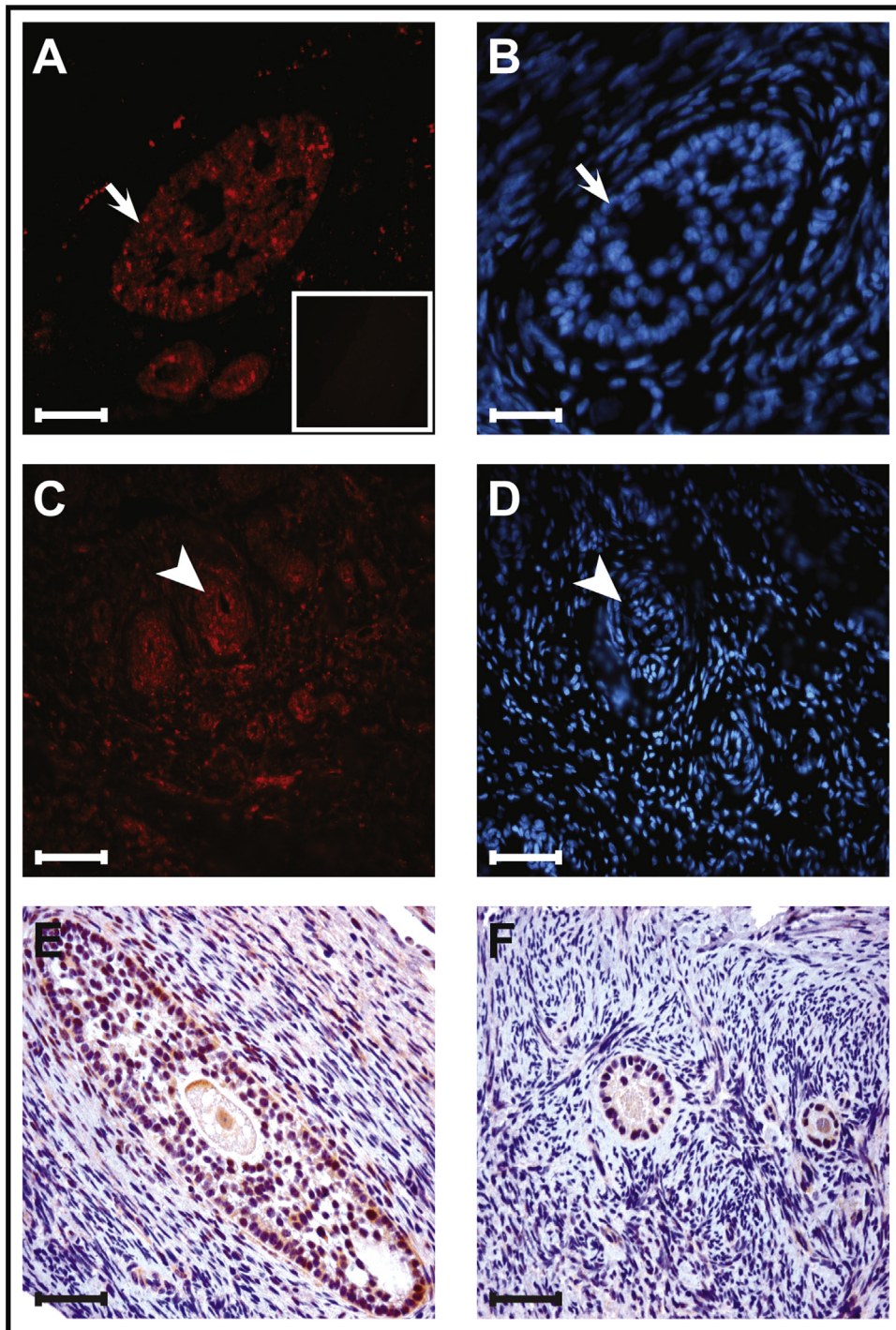
In cattle, ovarian folliculogenesis begins during fetal development (Tanaka et al., 2001). Primordial follicles, composed of an oocyte surrounded by squamous pregranulosa cells, are observed from approximately day 75 of fetal life in *Bos indicus* cattle. By day 90, primary follicles develop, characterized by a single layer of cuboidal granulosa cells. The development of a second layer of granulosa cells marks the formation of secondary follicles, which occurs from day 150 of fetal life. Antral follicles are observed from day 210

of fetal development (Castilho et al., 2014). The regulation of preantral follicle development is poorly understood, although several paracrine signaling molecules have been implicated. These include members of the transforming growth factor  $\beta$  family, the fibroblast growth factor family and kit ligand (Buratini and Price, 2011).

Another potential signaling molecule is angiotensin II (ANGII), which acts through two transmembrane receptors. The effects of ANGII on blood pressure are via the type 1 receptor (AT1) whereas other actions are believed to be via the type 2 receptor (AGTR2) (Paul et al., 2006). Within the ovarian follicle, AGTR2 protein is generally found in the granulosa cell layer, although there appear to be species differences in the precise pattern of localization. In rabbits, AGTR2 is expressed in granulosa cells of preovulatory follicles, consistent with the role of ANGII in ovulation (Kuji

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**Fig. 1.** Localization of AGTR2 protein in preantral follicles of adult ovaries. A and B, immunofluorescent detection of AGTR2 in primary and secondary follicles (A) and DAPI staining in the same section (B). The inset in A is an adjacent section processed in the absence of AGTR2 antibody and shows weak background fluorescence. (C), ovarian blood vessels stained for AGTR2, and the same field stained for DAPI (D). (E) and (F), colorimetric immunostaining of secondary and primary follicles confirms the localization observed by immunofluorescence. Photomicrographs were taken at  $\times 400$  magnification; bars =  $40 \mu\text{m}$ .

et al., 1996), whereas in rats and pigs AGTR2 was located to granulosa cells but only in atretic follicles (Daud et al., 1988; Pucell et al., 1991; Shuttleworth et al., 2002; de Gooyer et al., 2004). Cattle appear to be different, as AGTR2 has

been localized to theca cells in some studies (Brunswig-Spickenheier and Mukhopadhyay, 1992; Acosta et al., 1999; Schauer et al., 2001) and to both granulosa and theca cells in another study (Portela et al., 2008). We found that the

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