N- and E-cadherin expression in human ovarian and urogenital duct development

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Objective: To investigate expression of N- and E-cadherin in the developing human ovary.
Design: The expression of N- and E-cadherin was analyzed in 18 human fetal ovaries between 8 and 20 weeks' gestation using immunohistochemistry. Fetal human male and rat urogenital tracts were used for comparison of expression.
Setting: Academic research institute.
Patient(s): Women undergoing termination of pregnancy.
Intervention(s): Immunofluorescent analysis of cadherin expression.
Result(s): In fetal ovary, N- and E-cadherins were expressed at all gestations with overlapping but not identical patterns. Expression was associated with germ cells and adjacent somatic cells, including within newly formed primordial follicles, but neither cadherin was expressed in the somatic cell cords. The epithelia of the müllerian and wolffian ducts expressed only N- and E-cadherin, respectively, in a mutually exclusive fashion. This pattern of cadherin expression was found to be conserved between human and rat fetuses of both genders.
Conclusion(s): The demonstration of N- and E-cadherin expression in the human fetal ovary indicates likely roles in gonadal development from germ cell proliferation to primordial follicle formation, as well as in the development of the urogenital ducts of both genders. This is consistent with animal studies identifying cadherins as key regulators of early germ cell development. (Fertil Steril® 2010;93:2348–53. ©2010 by American Society for Reproductive Medicine.)

Key Words: Ovary, cadherin, primordial follicle, ovarian development

The urogenital systems in the male and female are initially indistinguishable (1, 2). In the male, activation of the *Sry* gene and downstream regulatory pathways induces functional differentiation of the fetal gonads into testes (3–6). The testes then initiate masculinization of the reproductive tract by secreting antimüllerian hormone (AMH), which causes the müllerian duct to degenerate, and testosterone, which rescues the wolffian duct and supports its differentiation into the epididymis, vas deferens, and seminal vesicles (7, 8). Conversely, it is generally accepted that in the female, the major changes to the developing reproductive tract occur because of the absence of both AMH and testosterone. There is, however, increasing evidence that female reproductive development does not occur purely by default but involves activation of specific genes (9, 10).

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In both genders primordial germ cells proliferate and migrate via the embryonic endoderm, reaching the gonadal ridges at about the fifth week of pregnancy in the human, where they become known as gonocytes. The first overt signs of sexual dimorphism between the male and female gonads are detectable at around 6 weeks of development (11, 12). The gonocytes of both genders continue to expand by mitosis for a further 3 weeks, increasing tenfold in number by 9 weeks after fertilization (13–15). In the ovary, the entry of the first gonocytes into meiosis is detectable from approximately 11 weeks' development (14) and is characterized by the loss of expression of the pluripotency marker OCT-3/4 (16, 17). At this stage, the germ cells are arranged in syncitial clusters or nests interspersed with some somatic cells and surrounded by a meshwork of somatic tissue (18, 19). From approximately 18 weeks' gestation these nests break down, primordial follicles are assembled, and oocytes arrest in meiosis. Female reproductive potential is thus characterized by the presence of a finite number of oocytes within primordial follicles, whose number is determined during fetal life. The regulatory factors involved in this sequence of events, however, are incompletely understood.

The interaction between germ and somatic cells is central for all stages of germ cell development. The cadherins are a large family of intercellular adhesion molecules (20) whose appropriate expression is vital to maintain tissue integrity,

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because they also play a role in generating signals at the cell surface that can influence cell function (20). The balance of expression of different cadherins can also control cell differentiation, migration and invasion, and survival in a range of cellular environments (21). E-Cadherin may be involved in primordial germ cell specification (22), and germ cell–expressed cadherins have been demonstrated to be involved in germ cell migration, aggregation, and survival (23–25). E-Cadherin expression has been reported in the human fetal ovary after 15 weeks' gestation (17), but no data are available regarding E-cadherin or N-cadherin expression at earlier developmental stages.

We therefore investigated the expression of N- and E-cadherin in the developing human ovary, providing evidence for roles in germ cell development. We also present a striking pattern of differential expression of these two proteins in the müllerian and wolffian ducts.

METHODS

Tissue Samples

Human fetal ovaries at up to 20 weeks' gestation were obtained after medical termination of pregnancy, with written consent obtained in accordance with national guidelines (26) and approval of the Lothian Ethics Committee. Termination of pregnancy was induced by treatment with mifepristone (200 mg orally), followed by misoprostol (200 mg administered once per vaginam for first-trimester terminations or repeated every 3 hours for second-trimester terminations; Pharmacia, Milton Keynes, U.K.). After delivery, fetuses were transfered rapidly to the laboratory. All specimens used were in good structural condition. Gestational age was determined by ultrasound before termination and was confirmed by direct measurement of foot length in second-trimester specimens. The gender of first-trimester fetuses was determined by detection of *SRY* by polymerase chain reaction (16). Ovaries from 18 fetuses were used in this study (eight from first trimester, ten from second trimester). Four first-trimester male specimens were also used for immunofluorescent detection of E- and N-cadherins.

Wistar rats were bred and maintained under standard conditions according to U.K. Home Office guidelines. Dams were killed by inhalation of carbon dioxide and subsequent cervical dislocation at embryonic day (e) 15.5–21.5; fetuses were recovered, decapitated, and placed in ice-cold phosphate-buffered saline (PBS).

Tissues were fixed in Bouin solution, then transfered to 70% ethanol before being mounted in paraffin using standard procedures.

Immunofluorescence

Paraffin embedded gonads and ducts were cut into 5-μm sections and mounted onto electrostatically charged microscope slides (VWR, Lutterworth, U.K.). Immunohistochemistry was performed as previously described (27) and outlined here. The slides were dewaxed and rehydrated, then pressure cooked for 5 minutes in 0.01 mol/L citrate buffer and left to stand for 20 minutes before cooling in water. Sections were blocked with 3% hydrogen peroxide in methanol for 30 minutes, followed by 20% normal goat serum (NGS) and 5% bovine serum albumin (BSA) in PBS for 30 minutes. Primary antibodies used were directed against N-cadherin (mouse monoclonal, 1:7,000 dilution; Zymed, Paisley, U.K.) and E-cadherin (mouse monoclonal, 1:10,000 dilution; BD Transduction Lab, San Jose, CA). The primary antibody, in 20% NGS, 5% BSA, and PBS, was applied to sections and incubated overnight at 40 °C. Bound antibody was detected using a 1:200 dilution of polyclonal goat antimouse peroxidase (Pink Sheed 0516) incubated at room temperature for 30 minutes, followed by a tyramide amplification step with fluorescein (Perkin Elmer Life Sciences). For negative control samples, nonimmune mouse IgG replaced primary antiserum and gave no signal. Propidium iodide was used to counterstain all sections. Mounted slides were visualized using a LSM510 laser scanning confocal microscope (Zeiss).

RESULTS

Overlapping but Distinct Patterns of N -Cadherin and E -Cadherin in Human Fetal Ovary

Both N- and E-cadherin were detected in both first- and second-trimester ovaries (Fig. 1). In the first trimester, N-cadherin was predominantly expressed by germ cells, including regions of germ cell–germ cell contact (Figs. 1A and 1B). E-Cadherin expression was low in first-trimester ovary and was mostly expressed by somatic cells; however, some staining was apparent in regions of contact both between somatic cells and germ cells and between germ cells (Figs. 1 G and 1H).

N-Cadherin was strongly expressed in the second-trimester ovary (Figs. 1C–1F). There was a clear differential distribution, with expression in the germ cell clusters at both germ cell–germ cell and germ cell–somatic cell points of contact. The cords of somatic cells between the germ cell clusters did not express N-cadherin, thus highlighting the arrangement of these two main structural components of the developing ovary (Figs. 1C and 1D). In regions of primordial follicle formation, the expression of N-cadherin was confined to the pregranulosa cells surrounding the oocyte (Fig. 1F), with other adjacent somatic cells not expressing N-cadherin. Strong expression in adjacent germ cell clusters persisted at this stage however (data not shown, but similar to Fig. 1D).

The pattern of expression of E-cadherin in the second trimester was generally similar to that of N-cadherin, with expression in the germ cell clusters but not in the somatic cell cords (Figs. 1I–1K). It was clear that the germ cells were the main site of expression, with clear immunofluorescence all around their perimeter (Figs. 1J and 1K). Expression by the smaller somatic cells intermingled within the germ cells cords was less clear: although there was expression at sites of contact between these somatic cells and germ cells, regions of the same cells not adjacent to germ