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Minireview

Gap Junctions in the Ovaries[1]

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INTRODUCTION

The mammalian gap junction is a cellular structure between adjacent cells, wherein the apposed cellular membranes are separated by an apparent gap of approximately 3 nm (Fig. 1). Gap junctions are formed by connexin proteins [1, 2]. The distribution of connexins is tissue- and cell-specific [1–3]. A gap junctional channel may be composed of one or more types of connexin protein [4]. The combination of connexins determines the characteristics of the gap junctional channel, such as conductance, permeability, or gating [4–7]. Gap junctional proteins are rapidly turned over in the cell, having relatively short half-lives ranging from 1 to 3 h [8]. Gap junctions are important for cellular interactions and signal transduction because they allow for nonspecific transfer of low-molecular-weight molecules [4, 5, 7–9]. Gap junctions are believed to be critical in regulating growth and development of organs and tissues in normal and pathological conditions [4, 5, 7, 8].

Gap junctions and several connexins have been identified within ovarian tissues including follicles, corpora lutea (CL), ovarian blood vessels, and stroma of several species [10–24]. Ovarian follicles or CL represent adult organs that exhibit periodic growth, differentiation, and regression during each estrous cycle [25–28]. The rate of growth of ovarian structures is relatively high, and tightly regulated and coordinated [26, 29, 30], resembling the rate of growth of embryonic or postnatally growing tissues [31]. Therefore, the presence of gap junctions in the ovaries may be important for control of growth. In addition, gap junctional communication among ovarian cells is probably involved in control of steroid hormone production, signal transduction, and luteolysis [15, 32–34].

The aim of this review is to describe the current concepts concerning the presence and possible roles of gap junctions in the ovaries.

GAP JUNCTION STRUCTURE AND FUNCTION

The mammalian gap junction (junction communicans, nexus) is a junction of communication or electrical coupling between adjacent cells that can be open or closed (i.e., a "gated" channel [2, 4, 8, 35–38]). When open, mammalian gap junctions permit exchange of nutrients, ions, and regulatory molecules of less than about 1 kDa (e.g., calcium ions, cAMP, inositol 1,4,5-triphosphate) between contacting, communication-competent cells [4, 8, 39, 40].

Gap junctions are ubiquitous in multicellular organisms. They are present in almost all mammalian tissues except circulating blood cells and adult skeletal muscles [8]. Gap junctions are composed of two symmetrical structures that create an intracellular channel that allows passage of ions and small molecules from cell to cell. Each cell of the pair contributes a structure termed a connexon, and two connexons form one intracellular channel (Fig. 1). Connexons float laterally in the plasma membrane until a match is made with a connexon of an adjacent cell [2]. Connexons are composed predominantly of gap junctional proteins termed connexins. The connexin (Cx) family of 13 proteins includes Cx26, Cx30, Cx30.3, Cx31, Cx31.1, Cx32, Cx33, Cx37, Cx40, Cx43, Cx45, Cx46, and Cx50, which, in general, are named on the basis of their molecular size [1–3, 41–45]. Connexins Cx26, Cx30.3, Cx32, Cx40, Cx43, and Cx45 have been identified in the ovaries of several species [10–24]. Connexins have been shown to be specific gap junctional proteins [1, 2]; therefore, the localization of connexins is widely used for identification of gap junctions in a variety of tissues [11, 16, 46–51].

Gap junctions often aggregate to form gap junctional plaques at a particular locus on the plasma membrane. The number of plaques between adjacent cells is thought to be proportional to the rate of metabolic cooperation among these cells. Thus, fewer plaques may indicate a reduced ability to communicate, which in turn may suggest that the cells act more independently of each other [2]. It has been shown that about 20% of the surface of ovarian granulosa cells is occupied by gap junctions [52]. In contrast, luteal cells appear to have fewer gap junctions arranged in smaller plaques [15, 53].

Gap junctions have been implicated in the regulation and coordination of cellular metabolism and function during growth and differentiation of organs and tissues [2, 4–6, 8, 40, 54, 55]. For example, it has been shown that the development of adrenal cortical gap junctions corresponds with steroidogenic output, just as the onset of steroidogenic capacity in rat luteal cells parallels development of gap junctions [52, 56]. After ovulation, a gradual increase in the size of gap junctions conjoining luteal cells parallels differentiation of the CL in the rat [56].

Abnormal function of gap junctions may lead to developmental anomalies and abnormal cellular growth [2, 3, 55, 57–59]. For example, in transformed cell lines, there is an inverse relationship between cell growth and gap junctional intercellular communication; i.e., induction of gap junction-

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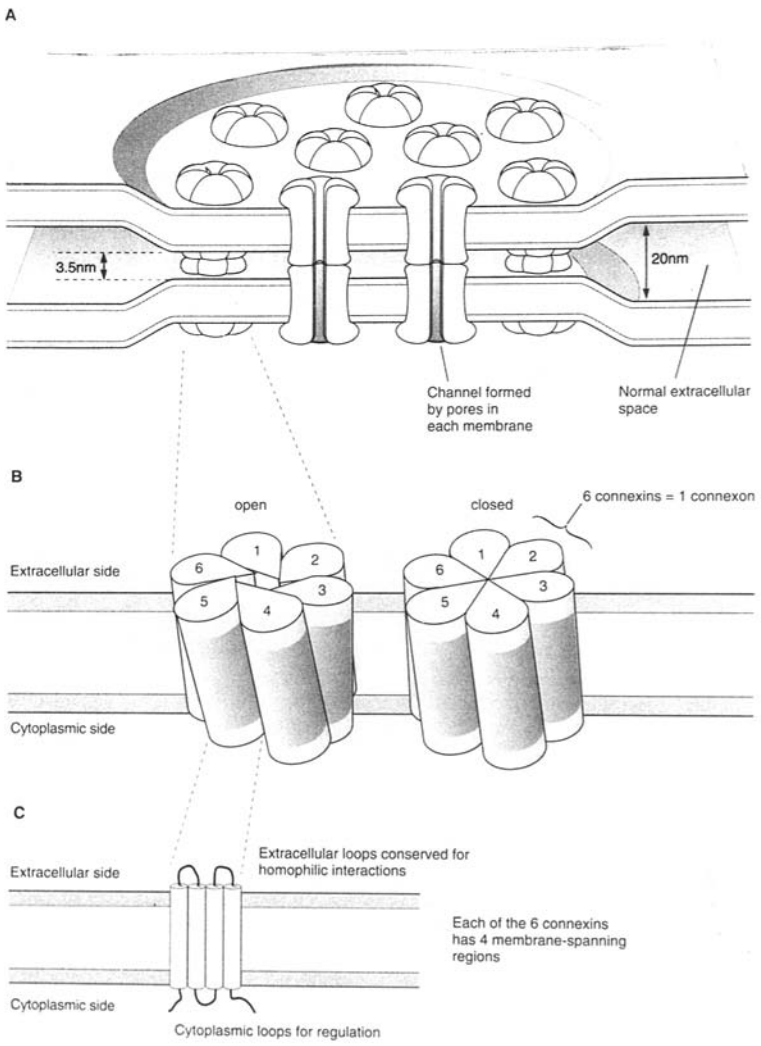

FIG. 1. A schematic view of the gap junctional channel. **A**) Model of intercellular channels, each formed by two connexons located in the cytoplasmic membrane of two adjacent cells; **B**) model of a single connexon in the open and closed states; **C**) model of a single membrane-spanning connexin. Reproduced with minor modifications from Kandel et al. [38] by copyright permission of Appleton & Lange, Norwalk, CT.

al intercellular communication leads to growth inhibition, whereas a blockade of gap junctional intercellular communication leads to uninhibited cell growth [60, 61]. Moreover, suppression of gap junctions by injection of a connexin antibody in growing embryonic tissues or cultured cells resulted in inhibition of dye transfer, electrical coupling, and/or gap junction assembly, which in the embryo causes specific developmental defects [58, 62–64]. The lower number of gap junctions in cancerous cells suggests that loss of gap junctions occurs during abnormal tissue development, accompanied by a disturbance in coordination of cell function and a subsequent loss of control of tissue growth [2, 39]. Data concerning the presence or function of gap junctions in abnormal ovarian growth (e.g., follicular or luteal cysts) or ovarian carcinoma are rudimentary at present. For rats, it has been demonstrated that gap junctions and maculae adherens are present between granulosa cells of follicles destined to become cysts. These junctions were lost during cyst formation, except those between mural granulosa cells [65]. This suggests that in pathological processes the number of gap junctions diminishes, presumably affecting tissue integrity.

During follicular development, gap junctions are involved in regulation of meiotic differentiation and maturation of the cumulus-oocyte complex [13, 66, 67]. During growth, differentiation, and regression of the CL, gap junctions mediate cellular interactions between steroidogenic cells and between steroidogenic and nonsteroidogenic cells, which may be important for normal luteal function and luteal regression [15, 34, 68–71]. In addition, in the granulosa layer and developing CL, gap junctions may be necessary for transfer of nutrients, since these tissues are avascular or poorly vascularized, respectively [16, 25, 29, 72, 73]. Moreover, granulosa cells are ionicly coupled through gap junctions [74], but luteal cells seem to be not coupled electrically [75]. Several other cell types have been shown to be nonelectrically coupled although they possess functional gap junctions [76].

However, the precise role of gap junctions in the ovaries has not been defined yet. It seems that Cx32 is not critical for ovarian function since Cx32-deficient mice are fertile and normal [77]. In contrast, Cx26- or Cx43-deficient mice die at the early stages of embryonic or perinatal development, respectively, because of several abnormalities [5, 78]. In addition, on the basis of gene knockout studies, Cx37 appears to be critical for normal follicular development and CL formation in mice [79].

PRESENCE OF GAP JUNCTIONS IN THE OVARIES

Numerous studies have demonstrated the presence of gap junctions and/or gap junctional proteins in ovarian follicles of human and other primates [80–82], rats [10, 13, 80, 83–88], mice [11, 67, 80], rabbits [89, 90], cows [12,

TABLE 1. Localization of the gap junctional proteins connexin (Cx) 26, Cx32, and Cx43 in ovine ovaries.*

Ovarian compartment	Protein
Surface epithelium	Cx26
Follicles	
Primordial and primary	Cx26
Secondary	Cx43
Antral	
Healthy	Cx26 in T**
	Cx43 in T and G†
Atretic	Cx43 in T
Corpora lutea	
Connective tissue	Cx26 and Cx32
Blood vessels	Cx26 and Cx32
Parenchyma	Cx26, Cx32, and Cx43
Ovarian blood vessels	Cx26 and Cx32
Connective tissue	Cx26 and Cx32

* Taken from Grazul-Bilska et al. [15, 17, 92].
** T, theca cells.
† G, granulosa cells.

20–22], and sheep [17, 91, 92]. For elegant micrographs of gap junctions in ovarian follicles evaluated by electron microscopy or freeze-fracture preparation, the reader is referred to other papers [11, 52, 66, 76, 80–82, 85, 86, 88, 89].

Gap junctions or Cx43 were detected as early as the primordial or primary follicles in rats and cows [14, 18, 22, 86]. In addition, Cx26 was detected in the oocytes of primordial or primary follicles in cows and sheep ([17, 22]; Table 1), and Cx32 was present in mouse and bovine oocytes [12, 67]. As follicular development progresses, an increase in the number and size of gap junctions and/or the expression of Cx26 or Cx43 has been observed in the granulosa and theca of small, medium, and large antral follicles and in the area of contact between the oocyte and cumulus cells of several species ([10, 11, 17–19, 21, 22, 24, 74, 92]; Table 1). However, the number of gap junctions, or the expression of Cx43 or Cx45, has been shown to decline in rat preovulatory follicles probably due to disintegration or retraction of granulosa and cumulus cells, removal of gap junctions or parts of them from the cell surface by endocytotic processes, and/or changes in phosphorylation state of connexin(s) [24, 74, 76, 93, 94]. The decline is most likely caused by the preovulatory LH surge [11, 24, 66, 76, 93, 94]. On the other hand, the expression of Cx30.3 in granulosa cells of large follicles was greater than in granulosa cells of the small follicles in rats [19].

In atretic follicles of rats or sheep, expression of Cx26, Cx32, or Cx43 was low or not detectable in the granulosa layer, but Cx43 was present in a theca layer ([14, 17, 18, 92]; Table 1). In contrast, in cows, Cx32 was detected in the granulosa layer of every atretic follicle, whereas Cx43 was present in the granulosa and/or theca layers of some atretic follicles [22]. In fact, in cows Cx32 seems to be present only in atretic follicles and may serve as a marker of atresia [22].

After ovulation, the CL is formed primarily by hyperplasia and functional differentiation of the cells of the ovulated follicle [26, 29, 30, 91, 95]. Whereas the number of gap junctions declines in preovulatory follicles (as described above), after ovulation gap junctions develop as differentiation of the CL progresses [56], and Cx43 expression in developing CL is high [16]. By electron microscopy, gap junctions have been demonstrated in luteal tissues of humans and other primates ([23, 81, 96–98]; Fig.

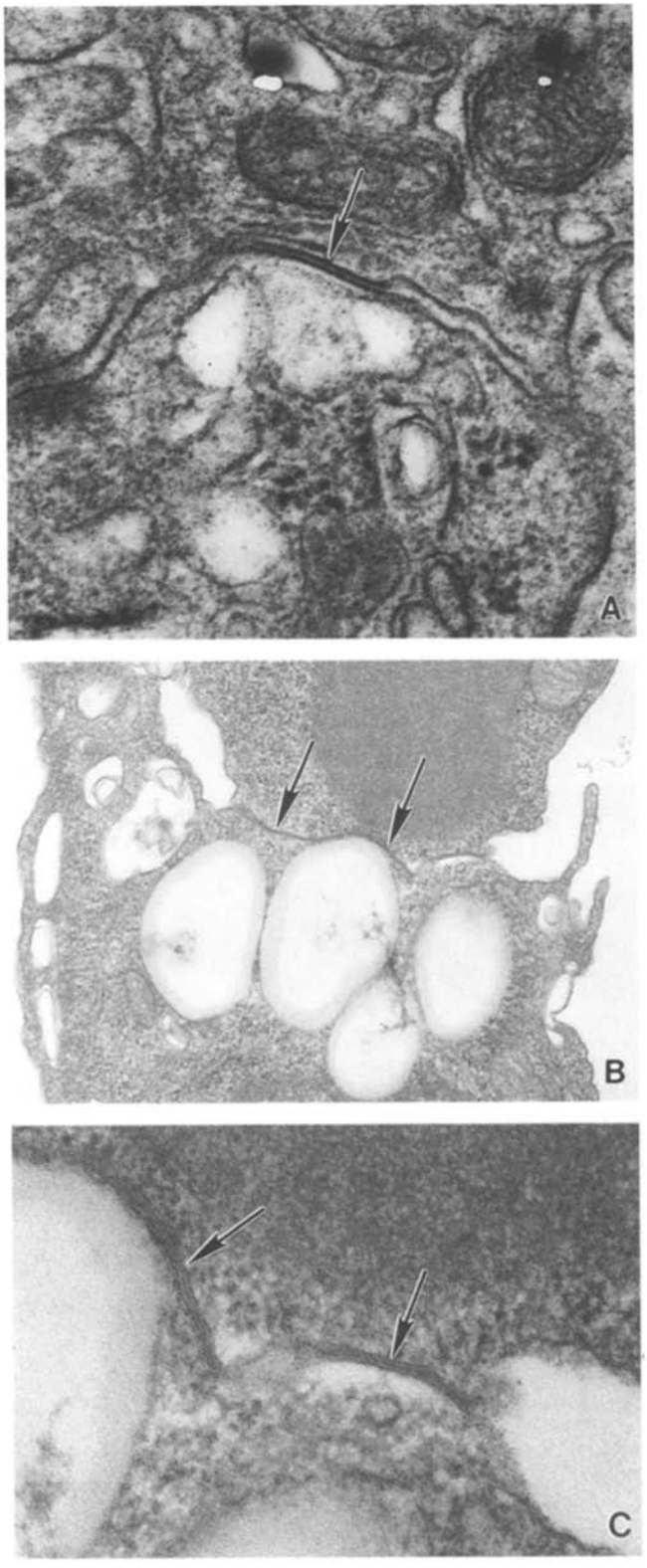

FIG. 2. Electron micrographs of the gap junctions (arrows) in **A**) baboon CL from the midluteal phase of the estrous cycle (magnification ×30 000); **B**) bovine cultured luteal cells from the midluteal phase of the estrous cycle (magnification ×24 600); **C**) a greater magnification (×82 000) of junctional complexes from **B**. **A**) Reproduced from Khan-Dawood et al. [23] and **B** and **C**) reproduced from Redmer et al. [53] by copyright permission of The Endocrine Society, Baltimore, MD.

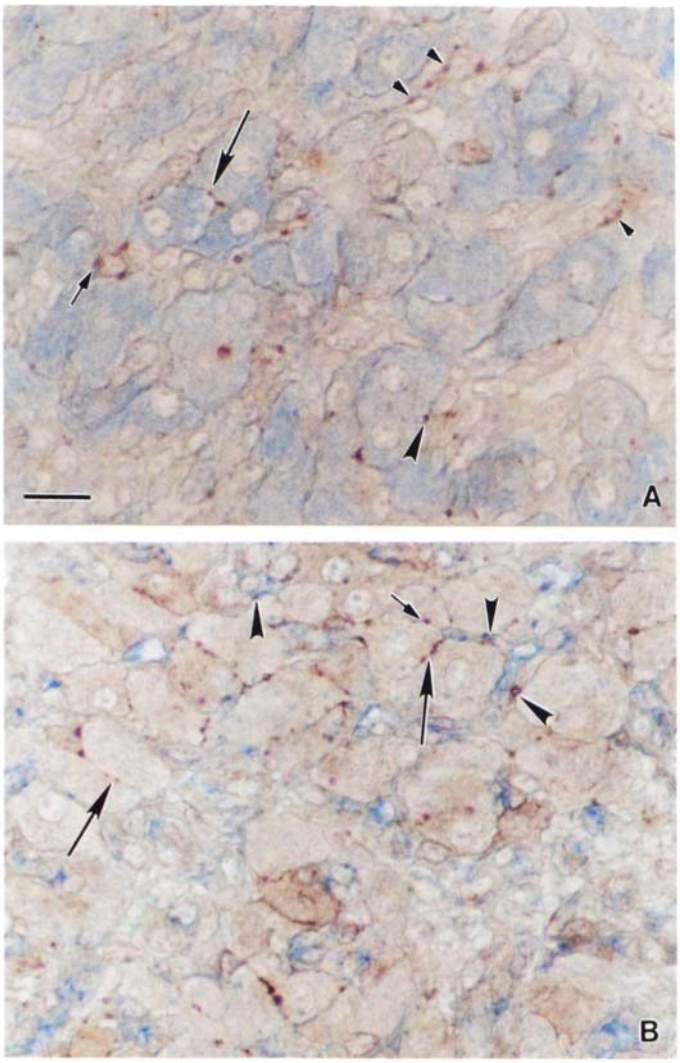

FIG. 3. Dual immunohistochemical staining for the presence of **A**) Cx43 (dark brown points) and 3β-hydroxysteroid dehydrogenase (3β-HSD; light blue cytoplasmic staining of steroidogenic luteal cells) and **B**) Cx43 (dark brown points) and lectin BS-1 (light blue staining of endothelial cells) in a section of an ovine CL from Day 5 of the estrous cycle. Control sections did not exhibit any positive staining (data not shown). In **A**, note the presence of Cx43 on the borders between small steroidogenic cells (small arrow), between large steroidogenic cells (large arrow), between steroidogenic and nonsteroidogenic cells (large arrowhead), and between nonsteroidogenic cells (small arrowheads). In **B**, note the presence of Cx43 on the borders between small and large steroidogenic cells (small arrow), between large steroidogenic cells (large arrows), and between steroidogenic and endothelial cells (large arrowheads). Bar = 20 μm; all micrographs are of the same magnification. CL were fixed in Bouin's solution, paraffin-embedded, sectioned, and then incubated with antibodies against Cx43 (gift from Drs. E.M. Hendrix and W.J. Larsen (Univ. Of Cincinnati, [47]) and then with antibody against 3β-HSD [29] or lectin (from Bandeiraea Simplicifolia, BS-1, 20 μg/ml; Sigma). Primary antibodies were detected by using a biotinylated secondary antibody (goat antirabbit; Zymed, San Francisco, CA). The color reaction was developed by using the avidin-biotin complex (ABC) system (Vectastatin; Vector Labs., Burlingame, CA) for Cx43 and alkaline phosphatase substrate kit III (Vector Blue; Vectors Labs.) for 3β-HSD and lectin (Grazul-Bilska et al., unpublished results).

2), rats, mice, and rabbits [56, 99, 100], and dogs [101]. In addition, gap junctional structures have been shown in cultured bovine luteal cells ([53]; Fig. 2), but, at present, there are no reports demonstrating the presence of gap junctions by using conventional electron microscopy in CL of domestic ruminants. However, several other techniques, including immunohistochemistry, Western immunoblot, and dye transfer in conjunction with laser cytometry, indicate that gap junctions are present in CL of cows and ewes [15–17, 20–23, 33, 53, 68, 92, 102].

Recent studies have shown the presence of Cx26, Cx32, and/or Cx43 in luteal tissues and/or cultured luteal cells of rats, monkeys, cows, and sheep ([15–18, 23, 24, 92]; Table 1). Patterns of expression of connexins change throughout the estrous cycle [16, 17, 23, 92]. Staining for connexins appears punctate, localized mostly to the cellular borders, but Cx26 and Cx36 were also detected in the cytoplasm of some steroidogenic or nonsteroidogenic luteal cells [16, 17, 92]. Early in luteal development in sheep, Cx26 is present only within connective tissue tracts and in association with the larger blood vessels, but in mature CL Cx26 is detected within the parenchyma, mostly in connective tissue and blood vessels, but also in the cytoplasm and on the borders of some luteal cells [17]. In contrast, Cx32 is present within the parenchymal lobules and in luteal connective tissue during the early luteal phase in sheep, but as the estrous cycle progresses, Cx32 is present primarily in connective tissue and blood vessels and only occasionally in the cytoplasm of the parenchymal cells [17]. In addition, the distribution of Cx26 and Cx32 is heterogeneous, with stronger staining in the periphery of the CL [17]. The expression of Cx43 in luteal tissues is greatest during the early and midluteal phases and is decreased during the late luteal phase of the estrous cycle [16, 23]. Cx43 is present on the borders of steroidogenic luteal cells in vivo and in vitro [16, 92, 102]. For bovine luteal cells in vitro, Cx43 is present on the borders between small luteal cells and between small and large luteal cells but is only rarely observed on the borders between large luteal cells [16]. For ovine luteal cells in vitro, Cx43 is present on the borders among all steroidogenic cell types [92].

With the aid of dual staining techniques, we have been able to localize Cx43 on the borders between steroidogenic luteal cells and endothelial cells (Fig. 3). This suggests that steroidogenic and endothelial cells may be connected by gap junctional channels. Such a possibility may exist during the early luteal phase, when newly created capillaries in the CL are devoid of basement membranes [29, 72]. It has been demonstrated for several tissues that endothelial cells may make direct contact with subjacent cells by traversing the capillary basement membrane [103, 104]. However, similar data are not available for luteal tissues. In the CL, endothelial cells comprise more than 50% of the total cells [105, 106], and the CL is extensively vascularized [26, 72, 102, 107, 108]. The capillary network is so dense, in fact, that the majority of steroidogenic cells are in direct contact with at least one capillary vessel [26, 72, 107]. This suggests that endothelial cells are critical to support luteal function. For luteal tissues, several studies have demonstrated interactions between parenchymal and endothelial cells in cows and sheep [26, 34, 102, 109, 110]. However, whether functional gap junctional intercellular communication (GJIC) occurs between luteal steroidogenic and endothelial cells remains to be determined.

Functional gap junctions in a variety of tissues may be demonstrated by using 1) laser cytometry to evaluate the rate of recovery of fluorescence after specific photobleaching of a fluorescent dye in selected cells [15, 33, 53] or the transfer of fluorescent Lucifer Yellow dye after microinjection of selected cells [111–113], or 2) electrophysiological techniques like current or voltage clamps to evaluate junc-

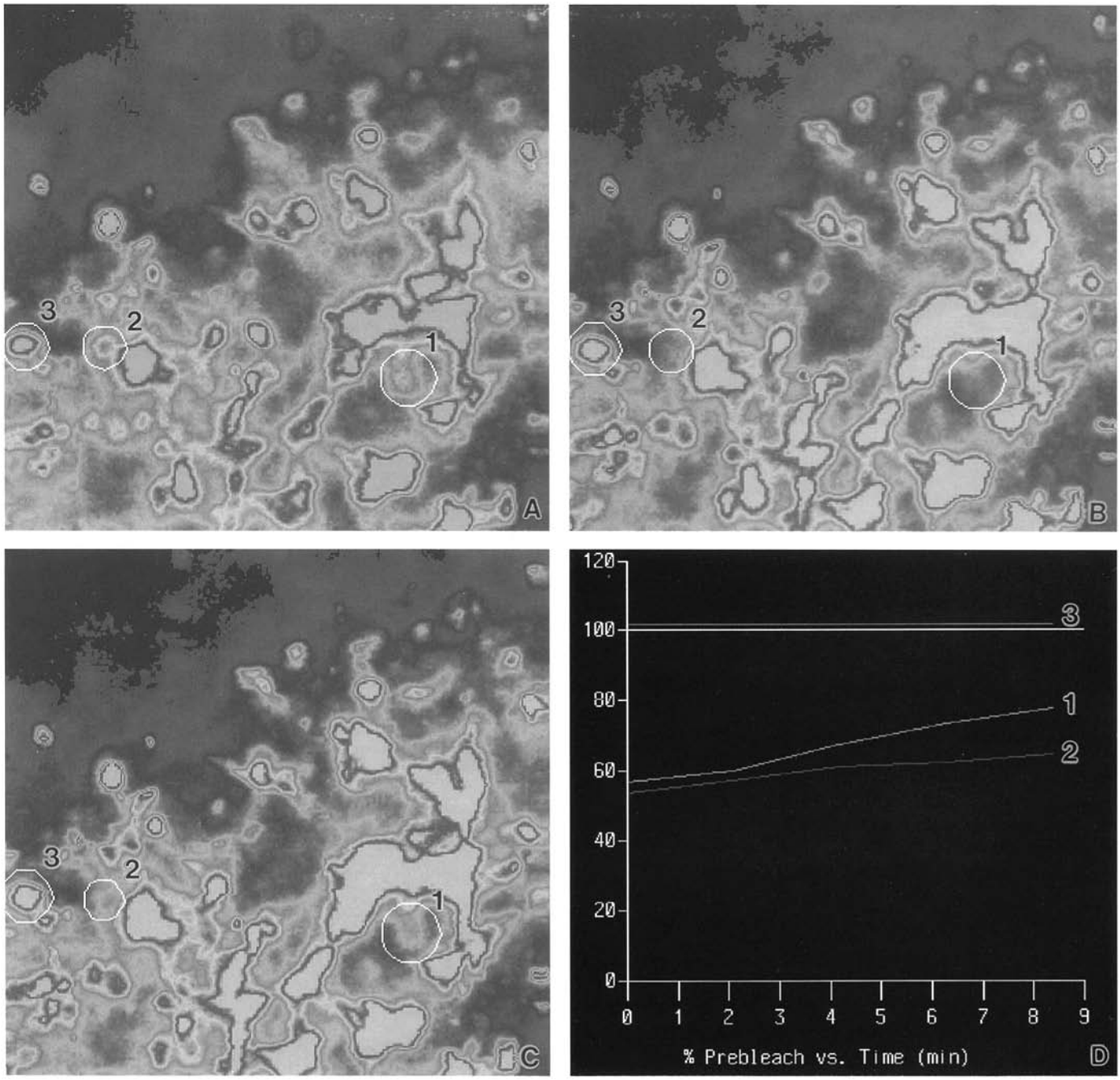

Fig. 4. Fluorescence recovery after photobleaching (FRAP) analysis of ovine luteal cells in situ (luteal tissue slice). **A**) Two fluorescently labeled cells (#1 and #2) were selected for laser photobleaching with a third cell (#3) serving as an unbleached control. **B**) Immediately after photobleaching, cells #1 and #2 lost about 45% of initial fluorescence. **C**) Eight min after photobleaching, cells #1 and #2 recovered a portion of their initial fluorescence from contiguous unbleached cells, indicating functional gap junctions. **D**) An increase in fluorescence recovery over the 8-min scanning period was monitored in the selected cells. The rate of FRAP for cell #1 was 2.6% and for cell #2 was 1.3% per minute. Total recovery after the 8-min period for cell #1 was 21%, and for cell #2 was 10%, which is comparable to that of cultured luteal cells [15, 33, 53, 68, 117]. CL were obtained from superovulated ewes (n = 3; [33]) on Day 10 of the estrous cycle, cut into approximately 2-mm cubes, covered with a low melting temperature agarose (FMC BioProducts, Rockland, ME), and cut into slices approximatley 50 μm thick by using a Vibratome (Technical Products International, St. Louis, MO). Poly-L-lysine coated dishes (0.25 mg/ml double distilled H_2O; Sigma, St. Louis, MO) were used to hold tissue slices in place during 30- to 40-min incubation in serum-free medium [33, 53], which was followed by laser cytometry [15, 33, 53]. These data are from an unpublished experiment (Grazul-Bilska et al.).

tional conductance [114–116]. Cell-to-cell communication among granulosa, theca, or luteal cell types has been demonstrated by using laser cytometry methods in our laboratory under in vitro conditions [15, 20, 33, 53, 68, 117]. More recently, we have also demonstrated GJIC among luteal cells in situ (Fig. 4). These data demonstrate for the first time that GJIC exists in intact, living luteal tissues, and, furthermore, corroborates our previous work utilizing in vitro techniques to study functional gap junctions. In addition, intercellular coupling has been demonstrated between ovine cumulus cells and oocytes by using intracellular markers derived from ^{3}H-labeled choline, uridine, and inositol [118], and among porcine granulosa cells by using dual-electrode whole-cell clamping and dye transfer [111]. These studies have provided evidence that functional gap junctions exist among ovarian cells.

TABLE 2. Effects of LH and $PGF_{2\alpha}$ on GJIC of bovine and ovine luteal cell types throughout the estrous cycle.*

Species	Stages of the estrous cycle	Cell types**	Treatment†		
			LH	$PGF_{2\alpha}$	LH+$PGF_{2\alpha}$
Cow	Early-luteal	S-S	NE‡	NE	NE
		L-S	NE	NE	NE
	Mid-luteal	S-S	↑	↑	NE
		L-S	NE	NE	NE
	Late-luteal	S-S	↑	NE	NE§
		L-S	NE	NE	NE
Ewe	Early-luteal	S-S	↑	↓	↑§
		L-S	↑	NE	NE
		L-L	NE	NE	NE
	Mid-luteal	S-S	↑	↓	↑§
		L-S	↑	NE	↑
		L-L	NE	↑	↑
	Late-luteal	S-S	NE	NE	NE
		L-S	↑	NE	↑
		L-L	NE	↑	↑

* Data taken from Redmer et al. [53] and Grazul-Bilska et al. [68, 117] for cow; and Grazul-Bilska et al. [33] for ewe.
** Cell types: S-S, small luteal cells in contact only with small luteal cells; L-S, large luteal cells in contact only with small luteal cells; L-L, large luteal cells in contact only with large luteal cells.
† Cells were incubated with or without hormones, LH (100 ng/ml; bovine LH B-5 or ovine NIADDK-oLH-25), $PGF_{2\alpha}$ (1 μM; Upjohn Corp., Kalamazoo, MI), and LH plus $PGF_{2\alpha}$ for 16–24 h before analysis of GJIC.
‡ NE, no effects; ↑, stimulatory effects ($p < 0.05$); ↓, inhibitory effects ($p < 0.05$) compared with control (no treatment).
§ Inhibitory effects ($p < 0.05$) for LH + $PGF_{2\alpha}$ compared with respective LH-treatment.

REGULATION OF GAP JUNCTIONS IN OVARIAN CELLS

In a variety of tissues, the structure and function of gap junctions are regulated by numerous factors, including hormones, growth factors, and intracellular regulators [32, 41, 43, 52, 55, 57, 85, 88, 111–113, 119–123]. Unfortunately, data concerning the regulation of gap junction function in the ovary are sparse. For cells from ovarian structures, we and others have demonstrated that the stage of follicular or luteal development as well as hormones and second messengers affects the number of gap junctions, connexin expression, or GJIC.

The stages of follicular or luteal development affect expression of Cx43 and GJIC. During preantral follicular development, Cx43 was present only in the granulosa layer, but in antral follicles Cx43 was detected in the granulosa and theca layers, and the intensity of staining appeared to be greater in large than in small or medium antral follicles of sheep [17, 92]. In addition, large and medium follicles expressed more Cx43 than did small follicles of cows [21]. For luteal tissues, expression of Cx43 was greater during the early and midluteal phases compared with the late luteal phase of the estrous cycle [16, 23]. In addition, the rate of GJIC between bovine small luteal cells or between small and large luteal cells from the early and midluteal phases were significantly greater than for those from the late luteal phase in cows [68]. These data indicate that when luteal cells are in the rapid growth (proliferative) or differentiation phases of luteal development, Cx43 expression and GJIC are greater than during luteal regression. Although we do not yet know their specific roles, these dramatic changes in structural and functional gap junctions indicate an important role in follicular and luteal growth, differentiation, and regression.

Effects of several hormones on gap junctions of ovarian follicles have been reported. Human CG, FSH, and estrogens affected the morphology of gap junctions in rat ovarian follicles [85, 86, 88]. Hypophysectomy decreased the total surface area of gap junctions in granulosa and theca cells [85]. This effect was reversed by estrogens but not by progesterone. Estrogen treatment increased the total surface area of gap junctions 5-fold above that of nontreated control rats in granulosa but not theca cells [85]. Administration of exogenous progesterone and hCG to hypophysectomized rats had no effect on the size and frequency of gap junctions in the granulosa layer, but it increased those in the theca layer [85]. In addition, FSH stimulated gap junction growth and turnover in rat granulosa cells [86]. Risek et al. [124] reported an increase in Cx43 mRNA in rat ovaries after estradiol administration.

Godwin et al. [111] reported that protein kinase A regulates GJIC of porcine granulosa cells. After injection of a protein kinase A inhibitor, granulosa cells become communication-incompetent, and this effect was reversed by injection of active C subunit from protein kinase A or by FSH. Protein kinase C had a positive effect on GJIC of granulosa cells under basal conditions but reduced GJIC when the enzyme was maximally activated [111]. In porcine granulosa cells, Godwin et al. [111] observed that the effects of protein kinase A and protein kinase C on GJIC were reversible and suggested that the amplitude of the effect was a reflection of interactions between these two enzyme systems. LH, which controls ovulation and luteal function in most mammalian species [21, 71, 108, 125], affects gap junction function in follicles and CL. Several investigators have shown that in rat follicles during the preovulatory period or after hCG injections, the area and/or number of gap junctions or Cx43 expression diminishes [13, 66, 74, 88, 94, 126], which indicates that just before ovulation LH decreases gap junction function within follicles. In contrast, LH has been demonstrated to increase GJIC among bovine and ovine luteal cells [68, 117]. For bovine luteal cells from the mid and late luteal phases, LH increased the rate of GJIC between small luteal cells but did not affect the rate of communication between small and large luteal cells ([53, 68]; Table 2). GJIC between bovine large luteal cells was negligible and was not affected by LH [53]. For ovine luteal cells from the early and midluteal phases, LH increased the rate of GJIC between small and large luteal cells and also between small luteal cells ([33]; Table 2). LH has been shown previously to increase luteal progesterone secretion, cell size, and blood flow in several species [71, 108, 125, 127]. Our data demonstrate that LH increases GJIC between luteal cell types in cows and ewes, which indicates that the luteotropic effects of LH also include control of luteal GJIC. In addition, these data agree with those from other cell systems, in which protein hormones (e.g., hCG, FSH, thyroid-stimulating hormone) have been shown to affect gap junctions in their target organs [52, 88, 112, 119, 121].

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the hormone that probably is responsible for luteal regression at the end of the estrous cycle and pregnancy in most mammalian species [71, 108]. $PGF_{2\alpha}$ increased GJIC between bovine small luteal cells from the midluteal phase of the estrous cycle ([68]; Table 2). In contrast, $PGF_{2\alpha}$ decreased GJIC between ovine small luteal cells from the early and midluteal phases ([33]; Table 2). In addition, $PGF_{2\alpha}$ increased GJIC between ovine large luteal cells from the mid and late luteal phases of the estrous cycle ([33]; Table 2). Moreover, $PGF_{2\alpha}$ diminished the stimulatory effect of LH on GJIC between bovine and

TABLE 3. Effects of second messengers on GJIC of bovine and ovine luteal cell types throughout the estrous cycle.*

Species	Stages of the estrous cycle	Cell types**	Treatment†,‡ cAMP dbcAMP	cAMP Rp-cAMPS	PKC TPA	PKC H-7	Calcium A23187	Calcium EGTA	Calcium A23187 +EGTA
Cow	Early-luteal	S-S	NE	NE	↓	NE	NE	NE	NP
		L-S	NE	NE	↓	NE	↓	NE	NP
	Mid-luteal	S-S	↑	↓	↓	NE	NE	NE	NP
		L-S	NE	↓	↓	NE	↓	NE	NP
	Late-luteal	S-S	↑	NE	↓	NE	NE	NE	NP
		L-S	↑	↓	↓	NE	↓	NE	NP
Ewe	Early-luteal	S-S	↑	NE	↓	NE	NE	NE	↓
		L-S	↑	NE	↓	NE	↓	NE	↓
		L-L	↑	↓	↓	NE	↓	NE	↓
	Mid-luteal	S-S	↑	↓	↓	NE	NE	NE	↓
		L-S	↑	↓	↓	NE	↓	NE	↓
		L-L	↑	↓	↓	NE	↓	NE	↓
	Late-luteal	S-S	↑	↓	↓	NE	NE	NE	↓
		L-S	↑	↓	↓	NE	↓	NE	↓
		L-L	↑	↓	↓	NE	↓	NE	↓

* Taken from Grazul-Bilska et al. [68, 117] for cow; and Grazul-Bilska et al. [137] for ewe.
** Cell types: S-S, small luteal cells in contact only with small luteal cells; L-S, large luteal cells in contact only with small luteal cells; L-L, large luteal cells in contact only with large luteal cells.
† Cells were incubated with or without dbcAMP (1 mM for cows and 2 mM for ewes; Sigma, St. Louis, MO), Rp-cAMPS (100 μM for cows and 300 μM for ewes (BioLog, Life Institute, LaJolla, CA), TPA (100 ng/ml, Sigma); H-7 (100 μM, Seikagaku Corp., Tokyo, Japan for cow, and Toronto Research Chemicals, Canada for ewe), A23187 (1 μM, Sigma) or EGTA (100 μM, Sigma) for 2 h before analysis of GJIC.
‡ NE, no effects; ↑, stimulatory effects ($p < 0.05$); ↓, inhibitory effects ($p < 0.05$) compared with control (no treatment); NP, not performed.

ovine small luteal cells ([33, 68; Table 2). However, GJIC between bovine and ovine small and large luteal cells was not affected by $PGF_{2\alpha}$ ([33, 68]; Table 2). Thus, the varied actions of $PGF_{2\alpha}$ that may contribute to functional and structural luteolysis now include the modulation of cell-to-cell communication among luteal cell types, in addition to a direct cytotoxic effect, reduced ovarian blood flow, uncoupling of LH receptors from adenylate cyclase, reduced steroidogenic enzyme activity and progesterone production, decreased LH receptor concentrations, changed membrane fluidity, changed luteal cell populations, and increased lysosomal enzyme activity [128–131]. The observation that $PGF_{2\alpha}$ diminished GJIC between luteal cell types suggests that inhibition of cellular interactions may be involved in the luteolytic effects of $PGF_{2\alpha}$. Others have suggested that during luteolysis $PGF_{2\alpha}$ induces a factor from large luteal cells that affects small luteal cells [108, 132]. In agreement with these data, prostaglandins have been shown to affect gap junctions and/or gap junctional communication in several other cell types [120, 133].

Cyclic AMP is a second messenger that is important for signal transduction within luteal tissues [108, 134]. Moreover, cAMP agonists stimulate progesterone production by luteal cells in several species [117, 135–137]. In our experiments with cultured bovine and ovine luteal cells, cAMP agonists increased GJIC. Forskolin and dibutyryl cAMP (dbcAMP) increased GJIC between small luteal cells, and between small and large luteal cells from the mid and/or late luteal phases of the estrous cycle in cows ([53, 117]; Table 3). Similarly, dbcAMP increased GJIC between ovine luteal cell types ([137]; Table 3). The cAMP antagonist, Rp-cAMPS, decreased the rate of communication between bovine or ovine luteal cell types ([117, 137; Table 3). These data indicate that cAMP is involved in the regulation of gap junctional communication in luteal tissues.

In sheep, even though both cell types contain functional cAMP-dependent protein kinases, increased intracellular concentrations of cAMP did not influence progesterone secretion by large luteal cells but stimulated progesterone production by small luteal cells [135, 138]. Our results suggest that another role of intracellular cAMP may be to regulate contact-dependent cellular interactions among luteal cells. In numerous other cell types, cAMP stimulates gap junctional communication and/or gap junctional conductance, and/or expression and phosphorylation of gap junction proteins [32, 41, 42, 48, 139–141].

Other intracellular regulators like protein kinase C or calcium, which are involved in the control of luteal function [71, 125, 142, 143], also affect GJIC of luteal cells. Activation of protein kinase C by using TPA (12-*O*-tetradecanoylphorbol 13-acetate) completely inhibits GJIC among bovine or ovine luteal cell types, but a protein kinase C antagonist (H-7) has little effect ([68, 137]; Table 3). In addition, a calcium ionophore (A23187) can decrease GJIC between small and large luteal cells and between large luteal cells in cows and sheep ([68, 137]; Table 3). Use of a chelator (EGTA) to maintain a low level of calcium in the culture medium augments the inhibitory effects of a calcium ionophore on GJIC among all ovine luteal cell types examined ([137]; Table 3). Activation of protein kinase C has been shown to inhibit GJIC in numerous cell types by affecting channel permeability and connexin trafficking and/or synthesis [55, 57]. In addition, increasing intracellular calcium concentrations can result in loss of GJIC in several cell types [41, 42, 144]. However, calcium does not affect gap junction function directly at physiological conditions [41].

Numerous studies demonstrated that second messengers are important regulators of gap junction function in many tissues including ovaries [15, 41, 42, 53, 117, 143, 144]. In addition, gap junctions are important for transfer of second messengers within tissues [144]. Interestingly, a reciprocal relationship exists between gap junctions and second messengers. That is, gap junctions are dynamically regulated by second messenger pathways, and the extent by which second messengers are spread from one cell to another de-

pends on the permeability and conductance of gap junctions [42].

SUMMARY AND FUTURE DIRECTIONS

Gap junctions and GJIC play an essential role in the integrated regulation of growth, differentiation, and function of organs and tissues. Ovarian follicles and CL possess structural and functional gap junctions, which are important for the coordination of cellular interactions during follicular and luteal growth, differentiation, and regression. The presence of gap junctions, expression of connexins, and rate of GJIC depend on the stage of follicular or luteal development and are affected by various regulators of ovarian function. Nonetheless, our current knowledge of the role of gap junctions in ovarian function is still limited, and future research on the regulation of cellular interactions and gap junction function during critical periods of follicular and luteal development will be needed to provide further insight into the control of growth and differentiation of normal (e.g., ovarian) as well as abnormal (e.g., tumor) tissues.

We suggest that future studies address the following questions: 1) How does alteration of gap junction function and/or structure affect growth, differentiation, or regression of follicles or CL? 2) Are differences in the rate of GJIC at the various stages of follicular or luteal development due to differences in the number or functional status (open vs. closed) of gap junctions? 3) What is the mechanism of action of extracellular and intracellular regulators on ovarian gap junction function (e.g., do these regulators affect the number of gap junctions, the functional states of gap junction [open vs. closed], or synthesis and/or trafficking of gap junction proteins)? 4) How is gene expression for the gap junctional proteins affected by stage of development or regulators of ovarian function? By understanding the role of gap junctions in ovarian growth and differentiation, we should be able to better understand and regulate ovarian function during normal as well as abnormal states.

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