Polyamines on the Reproductive Landscape

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The polyamines are ubiquitous polycationic compounds. Over the past 40 yr, investigation has shown that some of these, namely spermine, spermidine, and putrescine, are essential to male and female reproductive processes and to embryo/fetal development. Indeed, their absence is characterized by infertility and arrest in embryogenesis. Mammals synthesize polyamines *de novo* from amino acids or import these compounds from the diet. Information collected recently has shown that polyamines are essential regulators of cell growth and gene expression, and they have been implicated in both mitosis and meiosis. In male reproduction, polyamine expression correlates with stages of spermatogenesis, and polyamines appear to function in promoting sperm motility. There is evidence for polyamine involvement in ovarian follicle development and ovulation in female mammals, and polyamine synthesis is required for steroidogenesis in the ovary. Studies of the embryo indicate a polyamine requirement that can be met from maternal sources before implantation, whereas elimination of polyamine synthesis abrogates embryo development at gastrulation. Polyamines play roles in embryo implantation, in decidualization, and in placental formation and function, and polyamine privation during gestation results in intrauterine growth retardation. Emerging information implicates dietary arginine and dietary polyamines as nutritional regulators of fertility. The mechanisms by which polyamines regulate these multiple and diverse processes are not yet well explored; thus, there is fertile ground for further productive investigation. **(*Endocrine Reviews* 32: 694–712, 2011)**

I. Introduction

The polyamines, namely spermine and spermidine, were discovered in human semen. Remarkably, although they were first isolated by van Leeuwenhoek (1), they were not synthesized until 1927 (2–4). Polyamines are now known to be essential for the success of diverse mammalian reproductive functions. They play an important role in spermatogenesis and are actively implicated in oogenesis, embryogenesis, implantation, placentation, and, to a great extent, in parturition, lactation, and postnatal development. Although numerous studies on the occurrence and function of polyamines in mammalian pregnancy have been published over the past 40 yr, the single review dealing with the significance of polyamines to reproduction appeared more than 30 yr ago (5). Since then, extensive progress has been made in dissecting the multiple, essential functions of polyamines across the spectrum of reproductive biology. We believe that an up-to-date synthesis highlighting the involvement of the polyamines in mammalian reproductive events is merited. Although a number of molecular functions have been ascribed to polyamines, the mechanisms by which they modulate the reproductive process are mostly unknown, rendering mechanistic interpretations speculative. After a brief summary of general cellular and molecular functions of polyamines, we examine the effects of polyamines in gametogenesis,

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Abbreviations: AZI, Antizyme; AZIN, AZI inhibitor; DFMO, α-difluoromethylornithine; eCG, equine chorionic gonadotropin; eIF5A, eukaryotic translation initiation factor 5A; hCG, human chorionic gonadotropin; IUGR, intrauterine growth retardation; ODC1, ornithine decarboxylase 1; SAMDC, S-adenosylmethionine decarboxylase; SAT1, spermidine/spermine N_1-acetyltransferase.

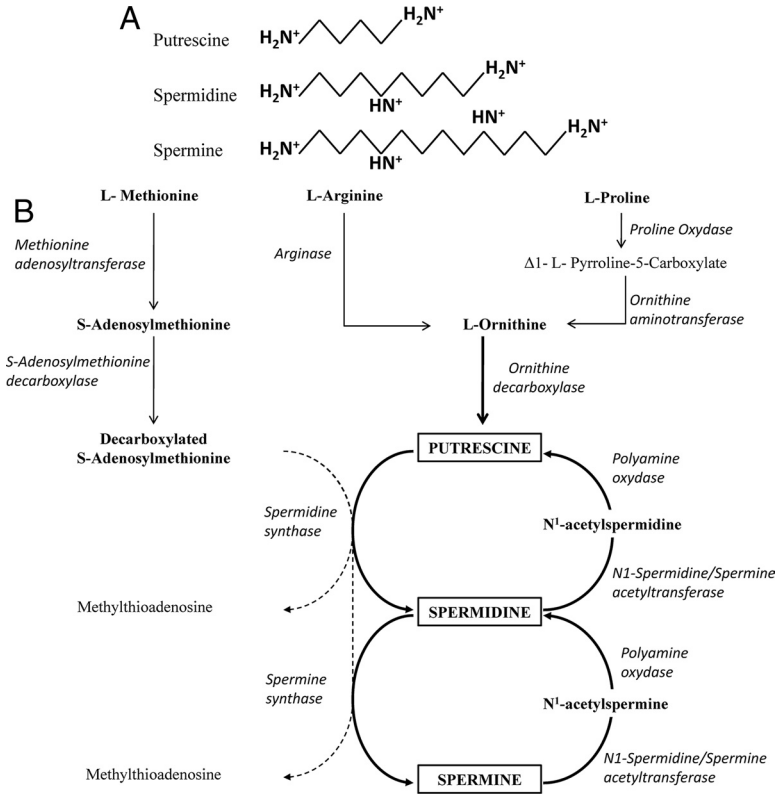

FIG. 1. A, Molecular structure of the major polyamines—putrescine, spermidine, and spermine. B, *De novo* polyamine biosynthesis. Polyamines are synthesized from L-arginine or L-proline through L-ornithine (157) and L-methionine via decarboxylated S-adenosylmethionine, the decarboxylated product formed by *SAMDC* (8). Ornithine decarboxylase catalyzes the decarboxylation of L-ornithine to yield putrescine. Putrescine combined with decarboxylated S-adenosylmethionine is then transformed into spermidine and spermine via spermidine synthase and spermine synthase, respectively, and through the formation of methylthioadenosine. Spermidine can be converted back into putrescine and spermine into spermidine by the combination of SAT1 and the polyamine oxidase (16). This back conversion leads to intermediate acetylated polyamines, namely N_1-acetylspermidine and N_1-acetylspermine.

both spermatogenesis and oogenesis, in embryonic development, in implantation and postimplantation formation of the placenta.

A. Polyamines: structure and diversity

Polyamines are low molecular weight aliphatic compounds composed of carbon chains of variable length with two or more primary amino groups (6). Their chemical structures are depicted in Fig. 1A. They are ubiquitous in living species. In mammals, four different polyamine molecules have been identified: spermine, spermidine, putrescine, and cadaverine (6, 7). Spermine and spermidine were initially discovered in human semen as the volatile compounds responsible for the typical odor of semen, whereas putrescine and cadaverine can result from bacterial decomposition and emit the odor of putrefying flesh of cadavers (7). In mammalian cells, polyamines are synthesized *de novo* from amino acids such as arginine, proline, and methionine (6, 8, 9), directly imported from the diet (10, 11), or produced by the intestinal microflora (12). After intestinal absorption, polyamines are released into blood circulation (13) and are known to reach peripheral tissues such as the intestine, thymus, and liver (10). In addition, both *de novo* synthesized and imported putrescine, spermidine, and spermine can be interconverted by interdependent enzyme reactions (Fig. 1B).

B. Polyamine synthesis and regulation of the intracellular polyamine pool

Endogenously produced polyamines are synthesized from the amino acids L-arginine or L-proline through L-ornithine (14) and/or L-methionine, via decarboxylated S-adenosylmethionine (15) (Fig. 1B). Ornithine decarboxylase (ODC1), the rate-limiting enzyme of polyamine biosynthesis and among the most highly regulated of eukaryotic enzymes, catalyzes the decarboxylation of L-ornithine to yield putrescine. Putrescine combines with decarboxylated S-adenosylmethionine and is then transformed into spermidine and spermine by spermidine synthase and spermine synthase, respectively. Polyamine catabolism principally functions by back conversion mechanisms. Spermine can be back-converted into spermidine and, spermidine into putrescine by the combination of spermidine/spermine N_1-acetyltransferase (SAT1) and polyamine oxidase (16). This reversal leads to intermediate acetylated polyamines, namely N_1-acetylspermidine and N_1-acetylspermine. Spermine oxidase induces a direct reconversion of spermine into spermidine (17).

Polyamine levels have been associated with cell proliferation and tissue growth; thus, it is not unexpected that intracellular polyamine homeostasis is under dynamic regulation (7). In addition, toxic metabolites result from deamination of polyamines (18), rendering intracellular regulation of polyamine abundance essential. Polyamine homeostasis is achieved by a combination of balance between synthesis and catabolism and by transport of polyamines between intra- and extracellular environments employing multiple, complementary mechanisms (19) (Fig. 2). Synthesis is regulated by the expression of, and consequently the activity of ODC1, modulated by the presence of antizymes (AZI) and the AZI inhibitors (AZIN) (20) (Fig. 3). The AZI family, which comprises

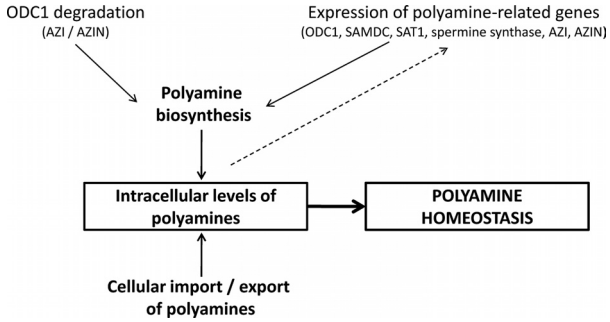

FIG. 2. Regulation of intracellular polyamine homeostasis. The intracellular pool of polyamines is tightly regulated at different levels, such as polyamine biosynthesis and polyamine transport across the plasma membrane. Degradation of the rate-limiting enzyme of polyamine biosynthesis, ornithine decarboxylase 1 (ODC1), is controlled by the AZI-AZIN tandem. Expression of genes encoding for enzymes implicated in polyamine biosynthesis, such as ODC1, SAMDC, SAT1, AZI, and AZIN, may also be induced or repressed by polyamines themselves in an autoregulatory mechanism. Equilibrium between polyamine biosynthesis and polyamine transport controls intracellular levels of polyamines and, therefore, polyamine homeostasis, which is essential for cell proliferation and survival.

three paralogs, AZI1, AZI2, and AZI3, bind and direct ODC1 to degradation by the 26S proteasome without ubiquitination (20). Although AZI1 and AZI2 are ubiquitously expressed, AZI3 is testis-specific (20, 21). The AZIN that prevent ODC1 degradation are ODC1-related proteins that lack decarboxylase activity. They interact with AZI with higher affinity than ODC1 and consequently rescue ODC1 from degradation (20). Two forms of AZIN have been identified, AZIN1 and AZIN2, and each binds to all three forms of AZI with the same affinity (20). Specificity is achieved by the cellular site of expression, *e.g.*, AZIN2 is specifically expressed in brain and testis (22). This multifaceted control of degradation of ODC1 protein by the complex AZI-AZIN results in a rapid turnover and short half-life (10–30 min) of ODC1, with the consequence that ODC1 is the rate-limiting enzyme for polyamine biosynthesis (20). In addition, polyamines can moderate their own synthesis because negative feedback by polyamines reduces translation of the polyamine-related enzymes ODC1, S-adenosylmethionine decarboxylase (SAMDC), spermine synthase, SAT1, and AZIN (23). Promoter sequences of the polyamine-regulatory genes (ODC1, AZI, and AZIN) are polyamine-responsive, and polyamines stimulate AZI translation (23), indicating that mechanisms of synthesis and degradation are complemented by autoregulation of polyamines at the transcriptional and translational levels.

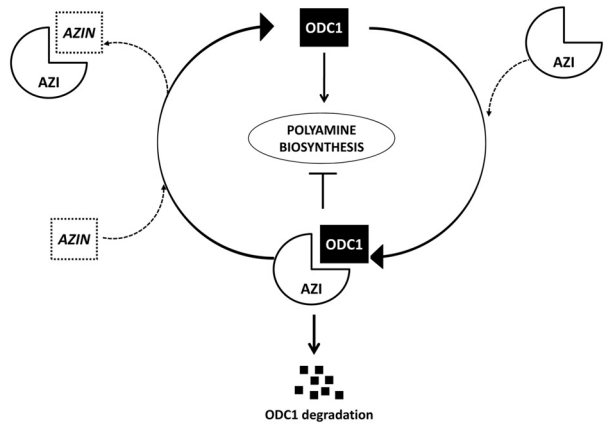

FIG. 3. Regulation of ODC1 activity by the AZI/AZIN tandem. Degradation of the rate-limiting enzyme of polyamine biosynthesis, ODC1 protein, is closely regulated by the AZI/AZIN tandem. The AZI binds and directs ODC1 to degradation, therefore reducing the rate of polyamine biosynthesis, whereas the AZIN, which is highly homologous to ODC1, may replace ODC1 and prevents ODC1 degradation, thus maintaining or increasing the rate of polyamine biosynthesis.

As noted above, import-export between the intra- and extracellular environments is an important means of maintenance of appropriate intracellular polyamine pools (19). Uptake and release do not share common membrane transport systems (24). Two mechanisms have been proposed for importation, one is based on the binding properties of polyamines, specifically spermine, to heparan sulfate on glypican-1 molecules, which are then subjected to receptor-mediated endocytosis (25). The latter process involves a caveolar-dependent endocytic mechanism and results in polyamine sequestration into secretory vesicles (25, 26). This process is time-, temperature-, and concentration-dependent; requires energy; and is saturable (27). The second mechanism proposed is based on passage through a postulated membrane transporter or channel that requires an electronegative membrane potential (26). Exportation of N_1-acetylpolyamines and putrescine from cytoplasm substantially contributes to regulation of the intracellular pool of free polyamines (28, 29). One mechanism that has been proposed is via the export solute carrier SLC3A2, which facilitates putrescine export (30). Alternatively, or concurrently, passive transport via a plasma membrane exporter may contribute to movement of polyamines from inside to outside of the cells (26).

Interestingly, regulation of polyamine transport has been reported to be dependent on the equilibrium between AZI-AZIN (20). AZI decrease polyamine importation (31), whereas AZIN stimulate polyamine uptake by counteracting the negative effects of AZI (20, 22). Although mechanisms by which AZI-induced inhibition of polyamine uptake is achieved are obscure, a recent study implicates AZIN2 in the regulation of intracellular endocytic absorption of polyamines mechanism (32).

C. Molecular mechanisms of polyamine action on cellular processes

Because polyamines are fully protonated at physiological pH, they are considered to be supercations that have

TABLE 1. Summary of binding sites of polyamines and molecular mechanisms and processes in which polyamines are involved

Binding site	Molecular mechanisms	Molecular process
DNA	DNA binding (200) Polyamine acetylation (43, 201–205) Histone acetylation (43, 203–205)	DNA replication (206) DNA transcription (23) DNA stabilization (200) DNA protection (207–209)
RNA	Ribosome binding and ribosomal frame shifting (23)	RNA translation (210) Initiation of mRNA translation (211, 212)
Protein	Posttranslational protein modifications, protein phosphorylation (213), binding to cytoskeleton (37)	Cell cycle (206) Signaling (214, 215) Translation (133, 210) Mitosis (38)

the capacity to strongly interact with polyanionic macromolecules such as nucleic acids, proteins, and phospholipids (4). They therefore play multiple and diverse roles in cellular function (Table 1). It has been reported that a large proportion of cellular polyamine content (>90%) is bound to RNA and DNA in cardiac tissue (33). Through these molecular interactions, polyamines are known to stabilize DNA structure, induce chromatin remodeling, and thereby regulate gene expression (4, 34). A recent study has provided the novel hypothesis that polyamines bind to charged sites on protein interfaces, thereby affecting electrostatic protein-protein interactions (35). A series of investigations revealed that, by this mechanism, polyamines potentiate neurotransmitter receptors, specifically the glutamate receptor, N-methyl-D-aspartate receptor (36). By binding to protein interfaces, polyamines are believed to affect the polyinositol phosphate signal transduction system (37). In the polyinositol phosphate context, it is hypothesized that both spermine and spermidine modulate ion channel functions and affect actin cytoskeleton reorganization (37).

Polyamines appear to play essential roles in cell proliferation, with intracellular increases occurring during the G_0/G_1 and G_2/M transitions (38). Polyamines are also implicated in programmed cell death (39) and in epigenetic modifications of chromatin (40–43). These multiple and varied roles indicate that precise regulation of intracellular polyamine homeostasis by mechanisms described above is essential to cell survival. Indeed, dysregulation of polyamine biosynthesis leading to increased intracellular concentrations of polyamines has been correlated unequivocally with the development of forms of cancer in several cell types (44, 45). For this reason, targeting of polyamine metabolism has been proposed as a strategy for antiproliferative therapy. The polyamine analog α-difluoromethylornithine (DFMO), a potent irreversible inhibitor of ODC1 (46), has been studied as a potential chemotherapeutic agent (47). DFMO has also been employed widely to evaluate the roles of polyamine in reproductive function.

II. Polyamines and Gametogenesis

A. Polyamines in male reproduction

1. Polyamines in somatic cells of the testis

Polyamines are present in and synthesized by germ, Sertoli, and Leydig cells of the testis. Polyamine concentrations increase in both Sertoli and germ cells through puberty in the rat (48). ODC1 is abundantly expressed in proliferating Sertoli cells as well as in interstitial Leydig cells in immature rats, and its activity is substantially higher in adults (49, 50). The most abundant expression of both ODC1 activity and AZIN2 is in Leydig cells (50, 51). Gonadotropins modulate polyamine synthesis in Leydig cells (50, 51), and it has been shown that injections of FSH and LH increase testicular ODC1 activity in Sertoli cells of immature and hypophysectomized rats (49, 52). ODC1 activity is enhanced by FSH supplementation to the culture medium in decapsulated testes from immature rats *in vitro* (52). Cultured Sertoli cells from immature rat and bovine testes treated with FSH displayed increased ODC1 activity, along with elevations in spermine and putrescine content (53, 54). There is evidence to indicate that the classic protein kinase A pathway is an effector of the gonadotropin signal because cAMP elevates ODC1 activity in Sertoli cells, mimicking the response to FSH (54). One study has shown that treatment with testosterone decreased ODC1 activity and its expression in Sertoli cells *in vitro* (55, 56), indicating a potential paracrine regulation complementing the gonadotropin effects.

Polyamines appear to be necessary for the function of Leydig and Sertoli cells. It has been shown that inhibition of ODC1 reduces steroid synthesis in rodent testis by an as yet undetermined pathway, suggesting a polyamine requirement for steroidogenesis (51). Treatment of hamster Sertoli cells *in vitro* with spermine or FSH increases concentrations of lactate and metabolic enzymes, suggesting a role of polyamines in the function of this cell type (57).

2. Polyamines and spermatogenesis

Evidence for the occurrence of polyamines and for polyamine action indicates that they may play an important role in testicular development and spermatogenesis. Expression of *Odc1* is detectable in, and specific to, the spermatogonia of prepubertal mice (58) and rats (59, 60).

Its expression and activity follow a well-defined temporal and spatial pattern in which there is substantial elevation of *Odc1* during the first wave of spermatogenesis, principally in pachytene spermatocytes during meiotic prophase and in round spermatids and residual bodies (58–60). ODC1 protein can be localized to the cytoplasm of freshly isolated epididymal spermatozoa and to the acrosomal region of round spermatids (50). Polyamines, or at least spermine, are also essential to completion of spermatogenesis because transgenic mice with an inactivating mutation of spermine synthase are infertile, with an arrest of spermatogenesis at the spermatogonia and primary spermatocyte stages, as revealed by reduction of meiotic and postmeiotic cell number (61, 62). This is consistent with a role in the cell division, given the two peaks of ODC activity during the cell cycle (63). Interference with synthesis in cells in the G_0/G_1-phase delays or prevents the S-phase of the cycle (38). It is therefore reasonable to speculate that ODC1 activity is essential for the first meiotic division in primary spermatocytes. Together, these findings argue for a role for polyamines in meiosis, and, although definitive confirmation in the testis is lacking, information supporting this concept has been derived from studies of the frog oocyte (see *Section II.B.3*).

Although polyamines are essential to spermatogenesis, an excess is detrimental to the process. Transgenic mice overexpressing the human *ODC1* gene display a 20- to 80-fold increase in testicular ODC1 activity and a consequent increase in putrescine abundance (64, 65). Relative to wild-type controls, males from the first generation display reduced reproductive performance and exhibit an increase in spermatogonial DNA synthesis and a reduction in DNA synthesis in spermatocytes during spermatogenesis (65). Second-generation male offspring are infertile and have extremely high ODC1 activity in the testis, hypoplastic germinal epithelium, and no spermatogenesis (64, 66).

Temporal patterns of changes in testicular ODC1 activity and gene expression are inversely correlated. Although ODC1 transcript abundance increases during spermatogenesis and reaches its maximum during spermiogenesis, maximal testicular ODC1 activity is restricted to the prepubertal period in the rat (67). This effect appears to be under paracrine control because coincubation of testicular cytosolic extracts from prepubertal rats with extracts from mature males resulted in inhibition of ODC1 activity in prepubertal samples (67).

AZI3, which inactivates ODC1 activity and inhibits intracellular polyamine uptake, is specifically expressed in spermatids and spermatozoa in the human and rodent testis from early spermiogenesis to the late spermatid phase (21, 68). Because *AZI3* expression follows the *ODC1* gene expression pattern, it has been hypothesized that AZI3 abolishes ODC1 activity to avoid detrimental effects of putrescine overproduction during spermiogenesis (22). Recently, *AZI3* knockout mice were found to have disrupted spermatogenesis, characterized by aberrant spermatozoa, with consequent loss of fertilizing capacity (69). Furthermore, the testis-specific AZIN2, which abrogates the inhibitory effects of the AZI on ODC1 activity and polyamine uptake, is abundant in haploid germ cells in human and mouse testis and following the temporal and spatial expression patterns of *AZI3* and *ODC1* (70–74). The *ODC1* gene is expressed during spermiogenesis, but its activity, along with polyamine transport and intracellular polyamine levels, appears to be regulated by the equilibrium between AZI3 and AZIN2 in haploid germ cells. A role for AZIN2 in the process of intracellular vesicle trafficking underlying polyamine transport was recently reported (32) and may indicate a potential involvement of AZIN2 in intracellular redistribution of polyamines during spermiogenesis.

3. Polyamines, reproductive fluids, sperm motility, and fertilization

As noted in *Section I*, spermine and spermidine are the oldest known organic constituents of human prostatic secretions (1), and they appear essential to male gamete function. Human and rat seminal plasma contains higher levels of spermine than any other body fluid or tissue (75). As with the testis, polyamine synthesis is hormone-dependent in the accessory glands; in this case, it is regulated by androgens. In the rat, castration induces a significant reduction in both ODC1 and SAMDC activity and a concomitant decrease in concentrations of polyamines in the ventral prostate (60, 76, 77), epididymis (78), and seminal vesicles (60, 77). The repression of polyamine production in response to castration correlates with a reduction in total RNA and DNA synthesis in the seminal vesicle and the ventral prostate (77). Testosterone treatment reverses castration-induced inhibitory effects on ODC1 activity in all three of the accessory glands (60, 76, 77, 79). Further evidence for the importance of androgens comes from studies where androgen effects were counteracted by DFMO in the ventral prostate and the seminal vesicles (77, 80).

Levels of spermidine and spermine are markedly lower in seminal plasma of infertile men compared with their normospermic counterparts (81, 82). Treatment of men with oligospermia with S-adenosylmethionine increases polyamine content and enhances sperm count and motility (83). A positive correlation between concentrations of spermidine and spermine and motility of ejaculated spermatozoa has also been demonstrated in rams (84). The

effect appears direct because addition of polyamines or L-arginine to human spermatozoa with reduced or no motility (asthenozoospermia) increased sperm motility (85). Indeed, inception of motility in immotile mouse, rat, guinea pig, and rabbit spermatozoa from the vas deferens, which have not yet been in contact with polyamines from the prostatic fluid, can be induced by spermine (6). The effects transcend motility alone because higher frequency of success in *in vitro* fertilization was obtained when epididymal spermatozoa employed for fertilization were preincubated with spermine (86). Spermine and spermidine concentrations in *in vitro* fertilization supernatants also associate with improvement in pregnancy success in mice (87) and humans (88).

The mechanisms by which polyamines affect motility are unknown, but *in vitro* assays reveal a number of possibilities related to intracellular signaling and energy metabolism. The presence of spermidine, spermine, or putrescine in the culture medium of mature spermatozoa collected from the rat epididymis enhances glycolysis (89), stimulates adenylate cyclase activity in bovine and human spermatozoa (90, 91), and reduces the activity of phosphodiesterase, an inhibitor of the cAMP signaling pathway in human spermatozoa (90).

Progress has been made in understanding the effects of polyamines on fertilizing capability. In the ram, spermine localizes mainly in the acrosome of the spermatozoid (92), and spermine dissociation from spermatozoa is facilitated by heparin, an oviductal factor known to be implicated in sperm capacitation and the acrosome reaction in the female reproductive tract (93). The acrosome reaction can be induced in capacitated bovine spermatozoa by exposure to low concentrations of spermine, whereas higher concentrations inhibited this effect (94). Thus, spermine appears to be a decapacitating factor in seminal fluid that may prevent both premature capacitation and the acrosome reaction.

B. Polyamines in ovarian function

1. Polyamines, onset of puberty, and folliculogenesis

Parallels exist between ovary and the testis, in that polyamine synthesis, under endocrine influence, appears necessary for function and differentiation of the somatic cell component of the gonad. ODC1 activity is elevated during the prepubertal period in rabbit and mouse ovaries (95, 96). In the immature rat ovary, ODC1 activity is induced by both FSH and LH and by the placental gonadotropins equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG), which have the same respective biological effects (97–99). Similar observations have been made in hamster (100) and mouse ovaries (96). The ovarian distribution of ODC1 is interesting because the protein localizes to the theca interna of preantral and antral follicles (101, 102), a pattern of expression similar to the LH/choriogonadotropin receptor. In the prepubertal rat ovary, the stimulation of ODC1 by hCG is specific to the thecal layer of follicles and to the interstitial glands (103). Stimulation with eCG (and presumably FSH) induces *ODC1* mRNA and protein expression and ODC1 activity in granulosa cells (104, 105). Increases in ODC1 activity specific to granulosa cells of antral follicles follow administration of ovulatory doses of LH or hCG to adult female mice (106), hamsters (100), and rats (101, 107, 108). These findings are in keeping with the known distribution of LH and FSH receptors in theca and granulosa cells, in follicles, and the follicle stage-dependent responses of these tissues to gonadotropins (109).

The DFMO paradigm has been employed to investigate the overall significance of polyamines to the ovary. Treatment of immature female mice with DFMO inhibits ovarian growth, antral follicle formation, and the onset of puberty (96). The overall responses can be attributed, at least in part, to effects on the central nervous system. Treatment of rat females with DFMO during the first 10 d of life interferes with normal brain development, resulting in aberrant, prolonged high FSH serum levels and a delay in the onset of puberty without affecting later fertility (110). In addition, there is evidence for direct effects on the ovary because acute treatment with DFMO counteracts the stimulatory effects of eCG and hCG on follicle development in both adult and immature mice (96).

As with the testis, concentrations of specific polyamines are essential for follicular development, and excesses are detrimental. Transgenic overexpression of *SAT1* in the mouse results in distortion of cellular polyamine pools, including large accumulation of putrescine, accompanied by decreases in intracellular spermine and spermidine (111). The consequence is an infertile phenotype characterized by an arrest of folliculogenesis at the secondary follicle stage (111). Whether the excess of putrescine or lack of spermine/spermidine is responsible for these effects is unclear at this time. Because folliculogenesis is essentially a somatic cell event (109), the sum of the results argues for a local requirement for polyamines in appropriate quantities for theca and granulosa cell replication and differentiation.

2. Polyamines in ovulation and luteinization

In the adult cycling mouse (96), hamster (102), and rat (101, 107), ovarian ODC1 activity is elevated during late proestrus, the time when the LH surge initiates the ovulatory process. Because LH administration was more effective in inducing ovarian ODC1 activity than FSH (96), it was hypothesized that polyamines are necessary for peri-

ovulatory maturation of the follicle. In female rats, administration of DFMO immediately before proestrus was associated with a decrease in plasma LH and in prolactin levels, a reduction of polyamine content in the pituitary gland, and a decline in the number of ovulated oocytes and in circulating progesterone concentrations (112, 113). Because pituitary gonadotropin contents were unaffected by DFMO treatment, it was suggested that polyamine deprivation was not disrupting gonadotropin production but was rather affecting its release (113). Similar effects induced by DFMO were noted in ovariectomized rats treated with progesterone and estradiol, thus showing that the reduced plasma LH concentration is not a consequence of a disrupted ovarian feedback on the pituitary gland, but rather of interference with gonadotropin secretion (113).

Some contradictory evidence exists because another study has revealed that inhibition of ODC1 activity by DFMO treatment on the day of proestrus in the rat resulted in a near doubling in the number of ovulated eggs (114). Perhaps this may be attributed to the finding that DFMO treatment increases FSH levels (113), thereby increasing recruitment of preovulatory follicles. The short half-life of DFMO in circulation (115) may have allowed sufficient clearance of the inhibitor to permit normal preovulatory LH secretion, and thus ovulation. These results are puzzling because local polyamine synthesis in ovarian somatic cells is necessary for ovulation and luteinization. Indeed, acute DFMO treatment interdicts gonadotropin-induced expression of genes essential to steroidogenesis, thus indicating that polyamines are locally necessary for steroid hormone synthesis and, consequently, establishment of the plasma progesterone levels associated with luteinization (96).

Other studies have shown that exogenous LH, the combination of LH and FSH, but not FSH alone, induce ovarian ODC1 activity (96). ODC1 localizes to the bovine corpus luteum during pregnancy (116). Recently, AZIN2, which prevents ODC1 degradation directed by AZI, was localized in the theca lutein cells of the human corpus luteum (51). Although no evidence is available to indicate hormonal regulation of this inhibitor, its occurrence in the theca-derived component of the corpus luteum suggests a role in polyamine regulation at the ovarian cellular level. Mechanisms have not yet been explored in detail. Clearly, cAMP signaling and transcription of steroidogenic genes are candidates for investigation.

3. Polyamines, oogenesis, and oocyte meiotic maturation

There appears to be little information on the role of polyamines in oogenesis in mammals. Correlative studies have revealed that an increase in ODC1 activity in neonatal rabbit ovaries is associated with the onset of meiotic prophase (95). Furthermore, increases in ODC1 immunoreactivity in the cytoplasm of oocytes of antral follicles have been observed after the preovulatory resumption of meiosis in adult mouse ovaries (96).

More detailed information linking polyamine synthesis to meiosis in oocytes comes from investigations of the amphibian, *Xenopus laevis* (117). In this species, induction of meiotic maturation *in vitro* either in follicles containing oocytes or in follicle cell-free oocytes can be induced by progesterone or hCG treatment (118, 119). This maturation is both preceded by and accompanied by increases in ODC1 activity in oocytes (118, 119). Up-regulation of ODC1 activity occurs before germinal vesicle breakdown and the extrusion of the first polar body, a temporal sequence that suggests cause and effect. DFMO treatment results in the blockage of meiotic maturation of oocytes contained in mature follicles and in *in vitro* culture of frog ovarian fragments (119). In the latter case, exogenous replacement with putrescine counteracts DFMO inhibition, and meiotic arrest can be overcome. In contrast, a single study indicates that germinal vesicle breakdown in follicle cell-free oocytes is not disrupted by DFMO treatment (118). This contradiction is puzzling, given that frog oocytes are not released from meiotic arrest by liberation from the follicle, as are mammalian oocytes. It suggests that ODC1 activity and polyamine biosynthesis act on follicular cells in support of meiotic maturation rather than on the oocyte itself. Nonetheless, changes in Ca^{2+} transients associated with resumption of meiotic maturation in oocytes (120) are consistent with effects of polyamines on ion channels (37). Thus, a mechanism exists for direct regulation of meiosis in the oocyte by polyamines.

The physiological role of the increase in expression of ODC1 in *Xenopus* oocytes during maturation was recently investigated using an antisense morpholino oligonucleotide strategy to inhibit *ODC1* translation (63). Although ODC1 antisense-injected oocytes appeared to undergo complete meiotic maturation, the metaphase II oocytes exhibited high levels of reactive oxygen species and were apoptotic. When transferred to host frogs and subsequently ovulated, these eggs could be fertilized but exhibited embryo fragmentation and did not survive. This novel investigation indicates that polyamines are essential for cytoplasmic maturation of the oocyte and to protect it at the metaphase II stage from reactive oxygen species-induced apoptosis.

III. Polyamines in Embryogenesis and Gestation

A. The polyamine requirement for early embryogenesis

Evidence for a role for polyamines in early embryogenesis comes primarily from the detection of increases in

transcription of *Odc1* in mouse two-cell embryos and in embryonic expression that increases through the blastocyst stage in this species (121). ODC1 activity in the embryo likewise increases between the two-cell and early blastocyst stages in the mouse (122, 123). Similar increases in pig (124) and *Xenopus* (117, 125) embryos have been recorded. If there is a requirement for polyamines in the very early embryo, it appears to be met by maternal sources because null mutation of *Odc1* is not lethal until the gastrulation stage of embryogenesis (126). There is a requirement for later embryogenesis because embryos of the *Odc1*−/− genotype collected during the late morula/early blastocyst stage cannot survive *in vitro* due to apoptotic cell loss in the inner cell mass (126). Provision of putrescine in drinking water of the dam can rescue embryo development to the early implantation stage, but not beyond in this mouse model (126). Other investigations have shown a requirement for spermine and spermidine in early embryogenesis in the mouse because transgenic deletion of SAMDC is followed by demise of embryos between d 3.5 and 6.5 post coitum (127). In addition, incubation of eight-cell embryos with an inhibitor of SAMDC, methylglyoxal-bis(guanylhydrazone) inhibits blastocyst formation *in vitro*, an effect that could be reversed by adding either spermine or spermidine to culture media (128).

Polyamines appear to enhance embryo development and survival *in vitro*. In the mouse, *in vitro*-fertilized oocytes incubated with polyamines showed a higher rate of development to blastocysts and the more frequent occurrence of trophoblast outgrowths relative to untreated embryos (129). Similar increases in developmental success were observed in studies of pig parthenotes generated *in vitro* and incubated with polyamines (124).

A potential mechanism for spermidine and spermine effects on normal embryo proliferation can be found in the cellular requirement for these polyamines in synthesis of hypusine, a modified lysine that is unique to the eukaryotic translation initiation factor 5A (eIF5A) (130, 131). This factor regulates cell proliferation (132). Deletion of an isoform of *elF5A1* in mice was accompanied by infertility (so far unexplored) (133), adding support to the hypothesis. This notwithstanding, there is evidence from a mammary carcinoma cell line to indicate that EIF5A and polyamines have independent effects on proliferation (134).

B. Polyamines and embryonic diapause

Delayed implantation, also known as embryonic diapause, is a reversible arrest in embryo development at the blastocyst stage before implantation (135, 136). It can be obligate (present in each gestation) or facultative (induced by lactational or other stress) (135). In the mink, a species that exhibits obligate delayed implantation as a normal aspect of its embryo development, polyamine-related genes, such as *ODC1*, *SAT1*, and *AZI*, and uterine polyamine contents are significantly up-regulated in the uterus during the early stages of embryo reactivation (137, 138). ODC1 protein was also found to be up-regulated in the uterine luminal and glandular epithelium at the time of embryo reactivation (138). Inhibition of polyamine biosynthesis by DFMO during embryo reactivation returned the mink blastocyst to a diapause-like state by repressing cell proliferation. This treatment further delayed implantation, without any detrimental effect on pregnancy (138).

In facultative diapause in the mouse, the polyamine-related genes *Odc1*, *Sat1*, *Samdc*, *Azi*, spermidine synthase (*Sms*) and spermine oxidase (*Smox*) are substantially up-regulated in the uterine subluminal stroma at the time of estrogen-induced reactivation of the embryo from facultative diapause (139). No sign of reactivation in mouse blastocysts in diapause, as indicated by trophoblast outgrowths, occurred when embryos were cultured in the presence of DFMO and/or the SAMDC inhibitor, methylglyoxal-bis(guanylhydrazone) (140). Nonetheless, when blastocysts were washed and incubated in an inhibitor-free medium, they attached to culture dishes, and the expected trophoblastic outgrowths were observed. These findings are consistent with the report that removal of arginine, the ornithine precursor, from culture media prevents trophoblast outgrowth and attachment of mouse blastocysts (141). Embryos deprived of ornithine remained arrested in free-floating conditions up to 5 d, and reactivation was only observed when the embryos were transferred to a complete culture medium. The observations of both facultative and obligate diapause support the hypothesis that privation of polyamines is a factor in the developmental arrest that defines delayed implantation.

C. Control of polyamine biosynthesis by steroid hormones in the uterus

Polyamine abundance in intracellular pools in the reproductive tract is modulated by extracellular signals, in particular by steroid hormones. Estrogen and catecholestrogens administered to immature rats increases ODC1 activity as measured in the uterine horns (142). In the ovariectomized mouse, estrogen, rather than progesterone, stimulates uterine *Odc1* and *Azi* gene expression (139). Uterine ODC1 and SAMDC activities are elevated within a few hours in response to estradiol-17β injection in both immature and nonpregnant adult rats (143–145) and in pseudopregnant rat females undergoing artificially induced decidualization (146–149). Coadministration of cycloheximide, to inhibit protein synthesis, prevents the rise of uterine ODC1 activity induced by estradiol-17β,

indicating that ODC1 expression is the estrogen-regulated process (143). Further support for this view comes from administration of estrogen antagonists, a treatment that reduces *Odc1* gene expression in mouse and the rat uteri (139, 150, 151). The effects are not limited to rodents because ODC1 activity is substantially higher in human endometrium during the estrogen-dominated follicular phase than in the progesterone-dominated secretory phase (152). Uterine *Odc1* gene expression is up-regulated at implantation sites in the mouse uterus after estrogen induction of nidation (139). Although the above information suggests that estrogens are the principal stimulants, the response may vary between species and timing in gestation. For instance, porcine uterine *SAT1* gene expression is more responsive to progesterone than to estrogen during conceptus elongation (153). These variations notwithstanding, the sum of available information indicates that uterine polyamine-related gene expression is modulated by steroid hormones.

Paracrine or autocrine signals may also regulate polyamine synthesis in the pregnant uterus. When ODC1 activity was measured in pregnant rat uterine horns on the day before implantation, it proved to be elevated relative to activity in pseudopregnant uterine horns, despite similar circulating steroid profiles (154). The same author demonstrated that induction of decidualization resulted in increases of large magnitude in ODC1 activity. This indicates that local embryonic messages may alter polyamine synthesis. The nature of these signals remains undiscovered.

D. Polyamine production during implantation and postimplantation development

In Eutherian mammals, the consequence of embryo implantation is the formation of the placenta to provide the fetomaternal interactions essential for successful intrauterine fetal development (136). The ancestral form of the placenta is believed to be a discoid structure, endotheliochorial in invasiveness of the trophoblast and characterized by terminal differentiation of endometrial stromal cells to form the deciduum (155). The models that have been most studied are evolutionary derivations of the ancestral form with either greater or less invasiveness. In hoofed animals, the most common form is the epitheliochorial placenta where little or no trophoblast invasion occurs, whereas in rodents and most primates, the highly invasive hemochorial placental form dominates (155). Polyamine biosynthesis is a feature of both noninvasive and invasive implantation types, and observations suggest highly conserved roles for polyamines during implantation and placentation across the spectrum of mammalian diversity.

1. Polyamines and embryo attachment in species with noninvasive placentation

In ruminants, the diffuse, cotyledonary placenta with minor invasion of the maternal epithelium by bi- and trinucleate trophoblast cells is common. In pigs, the trophoblast attaches to the endometrial epithelium with no invasion taking place. In species from both phylogenetic orders, polyamines have been reported to be produced by the conceptus and the dam around the time of conceptus elongation. The principal site of synthesis appears to be the endometrium, rather than the embryo itself. In pigs, concentrations of spermidine, spermine, and putrescine in uterine luminal fluids are at their maximal levels on d 12 of pregnancy, the time when estrogen secretion from the conceptus signals the presence of the embryos (156). At this moment, uterine *SAT1* gene expression is substantially elevated in the endometrium of the pregnant pig compared with the cyclic endometrium, and the protein for this gene localizes to the luminal epithelium (156). ODC1 activity and concentrations of ornithine and polyamines are also elevated during the period of attachment of the pig trophectoderm to the luminal epithelium, between d 20 and 40 of pregnancy, coinciding with formation of the porcine placenta (157). Concentrations decreased thereafter (158). In culture of porcine uterine glandular epithelium and stroma cells, exogenous putrescine and spermidine increased the rate of DNA synthesis, whereas spermine stimulated DNA synthesis only in stromal cell lines (156). In porcine allantoic fluid, the abundance of the ornithine precursor and the polyamine products increases between d 40 and 60 of gestation, when placental development is maximal (157, 159).

Ruminants follow a similar pattern. In the ewe, *ODC1* gene and protein expression are elevated in the uterine luminal and superficial glandular epithelium before attachment of the trophoblast (160). Gao *et al.* (160) demonstrated that ovine fetal ODC1 expression localizes to the trophectoderm. Between d 30 and 60 of gestation, the rapid growth of the ovine placenta is accompanied by large increases in ornithine, arginine, and polyamine concentrations in the uterus (161). Concurrently, ODC1 and arginase activities increase to a peak at d 40 of gestation in placentomes, intercarunculary endometrium, and amniotic and allantoic fluids (161).

2. Polyamines and invasive implantation

Polyamine biosynthesis appears to take place in both the maternal and fetal compartments during the early stages of invasive implantation. Elevated uterine ODC1 and SAMDC activities are present on the day of embryo implantation (d 5) in the rat (162). Increases in activity of ODC1 and polyamine contents occur at implantation sites

on the day of implantation in the mouse (163), in the rat (148, 154, 164, 165), in the rabbit (165), and in the hamster (166, 167). At early implantation (again at d 5 post coitum), polyamine-related genes such as *Odc1*, *Sat1*, *Samdc*, *Azi*, *Smz*, and *Somx* are up-regulated in the mouse uterine subluminal stroma, specifically at implantation sites (139).

In many species with invasive placenta, the endometrium undergoes proliferation, followed by terminal differentiation of stromal cells to form the decidual tissue. In rodents, the presence of the embryo evokes this response, whereas in primates, it is a normal event of the menstrual cycle and the decidualized tissue is lost at menstruation. The process appears to be essential to successful postimplantation gestation because mouse models with absent or aberrant decidualization are unable to successfully maintain pregnancy (168).

In the decidualized uterus, both embryo and decidua contribute to polyamine biosynthesis, and no differences could be detected in the level of the ODC1 activity between maternal and embryonic tissues (169). Polyamines appear to play a role in decidualization because ODC1 activity increases dramatically as this differentiation process progresses in the rat uterus (154). Moreover, high doses of DFMO administered to hamsters on the day of implantation compromise the normal course of decidual function, resulting in failure of pregnancy (167). The mechanism of polyamine action in decidualization includes new gene transcription because treatment of nuclei of stromal cells isolated from rat decidual tissue with spermine or spermidine stimulates the rate of RNA synthesis (148). There is also evidence for polyamine induction of the S-phase of the cell cycle because ODC1 activity increases in striking parallel to total uterine synthesis of DNA and DNA at implantation sites (154). This finding is consistent with the known phenomenon of endometrial stromal proliferation that accompanies decidualization (168).

It is possible to deplete intracellular polyamine pools by inhibition of downstream enzymes in polyamine metabolic pathways. One such compound, MDL-725227DA, inhibits spermine oxidase and acetylpolyamine oxidase (45). In pregnant female mice and hamsters, administration of MDL-725227DA at the time of or soon after implantation induces embryo loss, and this effect is dose-dependent (170). Histological analysis revealed complete absence of decidualization accompanied by an elevated occurrence of fetal resorption in the treated females. *In vitro*, trophoblast cells displayed consistent dose-dependent responses to the presence of MDL-725227DA in culture media, ranging from reduction of cell proliferation to degeneration (170).

Nonetheless, low doses of DFMO administered in drinking water to female mice and rats did not affect uterine decidualization because weight of the decidualized horn, as well as the rate of RNA, DNA, and protein synthesis at implantation sites were normal after this treatment (145, 163, 165, 171). Histological analyses of mouse implantation sites after polyamine deprivation indicated that embryo implantation occurs normally without apparent decidual deficiency (163). Fozard *et al.* (163) suggest that the low-dose DFMO employed eliminated the decidual increases in ODC1 activity in the mouse uterus, but it remains possible that polyamine synthesis was not abolished by this treatment.

In the mouse conceptus (including the decidua and the embryo), the amount of ODC1 protein and the rate of ODC1 activity are reported to be maximal on d 8 of gestation (172). Spermidine and polyamine oxidase concentrations are elevated on d 8 of pregnancy in the mouse uterus (170), when robust trophoblast proliferation takes place to develop the ectoplacental cone, a preplacental organelle (173). This finding can perhaps be extended to other species because concentrations of polyamine oxidase increase during human pregnancy (174), whereas low concentrations of serum polyamine oxidase are present in women that experience spontaneous abortion (175).

3. Polyamines and placentation

ODC1 activity and putrescine concentration were found to be higher in the fetal relative to the maternal compartment of placental tissue after d 15 of pregnancy in the rat and in the mouse (162, 172). Polyamines appear necessary for placental formation. In addition, ODC1 activity was lower in fetal tissues, compared with the placenta, but polyamine content was higher in the fetus and yolk sac relative to the placental compartment (172). This indicates that fetal and extraembryonic membrane requirements may be met by transfer of polyamines from the placenta.

Histological analysis revealed that polyamine deprivation induced by DFMO is associated with abnormal development of the extraembryonic structures, the yolk sac, and the placenta (169) with multiple nefarious consequences. In the yolk sac of the mouse, the first site of fetal hematopoeisis (176), DFMO treatment reduced both the number of blood islands and the expression of genes encoding for embryonic globins (172).

ODC1 protein localizes consistently and specifically to the labyrinthine zone of the placenta (169), and development of this zone and of the spongiotrophoblast layer are abnormal when embryo development is arrested by polyamine deprivation (169). This is consistent with observations in other species showing that a reduction in arginine precursors results in reduced placental angiogenesis (reviewed in Ref. 177). There appear to be direct effects of polyamine deprivation on the trophoblast because expres-

sion of markers of trophoblastic lineage, such as trophoblast specific protein-α, placental specific protein 1, reproductive homeobox 6, Cbp/p300-interacting transactivator, integrin α4, and eomesodermin, are down-regulated in response to polyamine deprivation (172). In addition, plasma progesterone is reduced in DFMO-treated females, and the expression of genes encoding for steroidogenic enzymes and proteins, steroidogenic acute regulatory protein, 3β-hydroxydehydrogenase IV, and 17-α-steroid hydrolase in the placenta is compromised. Because expression of these same steroidogenic proteins in the ovary was not affected by DFMO treatment, it was suggested that alterations in plasma progesterone concentrations resulted from a placental rather than an ovarian deficiency (169).

4. Polyamines and postimplantation embryo development

Specific inhibitors of polyamine biosynthesis have been employed to investigate polyamine function and effects during pregnancy. Administration of DFMO by multiple means (in drinking water, by gavage, or sc, intrauterine, or ip injection) during the periimplantation period unequivocally provoked embryo developmental arrest soon after implantation in the mouse (165), the rat (171, 178), the rabbit (163), the hamster (167), and the mink (138). This detrimental effect is specific to the early postimplantation period because polyamine deprivation by DFMO before and after this critical period was not correlated with abnormal pregnancy in the mouse (163, 167, 169, 178). The direct effects of polyamines in inducing cessation of postimplantation embryo development were demonstrated by reversal of DFMO-induced arrest by putrescine administration in both the hamster and the rat (167, 171). Similarly, administration of two putrescine analogs, 1,3-diaminopropane and 1,6-diaminohexane, to pregnant female mice during the time of maximal fetal ODC1 activity (d 10–14 of gestation) inhibits fetal ODC1 activity and causes a reduction of the fetal weight on d 18 of gestation (179). Clearly, an adequate level of polyamines appears to be essential for the postimplantation development of the fetus.

Detailed studies have been undertaken to evaluate the developmental effects of an increasing range of orally administered DFMO during the postimplantation period in rodents (167, 180–182). The severity of maternal and fetal detrimental effects in response to DFMO treatment is dose-dependent. Low doses provoked an increase of postimplantation losses due to embryo resorption, a reduction of fetal and placental weight, and a decrease of the rate of DNA synthesis in the placenta. Nonetheless, viable fetuses did not show any external, visceral, or skeletal abnormalities at any treatment dose (180, 181). Because polyamine deprivation induces defects on fetal development that are comparable to those observed in a case of intrauterine growth retardation (IUGR) in the rat (182), it may be that the maternal environment is compromised by low doses of DFMO to the point that it is not optimal to sustain fetal development. More drastic effects on fetal survival and maternal health can be provoked by administration of higher doses of DFMO. As suggested above, many of these effects may be the result of placental abnormality and insufficiency under conditions of reduced or absent polyamines. Nonetheless, these doses of DFMO have effects on the dam, resulting in diminished maternal food and water consumption and consequent reduction of body and uterine weights (181).

E. Regulation of polyamine homeostasis in the fetoplacental unit

Because polyamines are unequivocally required for postimplantation embryo development, the intracellular polyamine pool must be maintained, implying regulation of polyamine metabolism and catabolism and polyamine transport in both fetal and maternal compartment of implantation sites. This is achieved by multiple mechanisms. In the short term, interconversion between polyamine species provides a mechanism to maintain the levels of specific polyamines. Mouse females subjected to DFMO treatment during postimplantation displayed a decline in uterine ODC1 activity but an increase in *SAMDC* gene expression and activity and in spermine concentration at implantation sites (139, 163). The addition of DFMO to culture media of mouse uterine stromal or epithelial cells inhibits ODC1 activity but stimulates *Samdc* gene expression (139). Conversely, ODC1 gene overexpression in those cells resulted in the down-regulation of *Samdc* expression (139). Gene expression of *SAT1* can be up-regulated by exogenous supplementation of spermidine and spermine to culture media of porcine and mouse uterine stroma and epithelial cells (139, 156). These results support the concept that the compensatory mechanisms to maintain the optimal level of the intracellular polyamine pool during pregnancy are triggered when the rate of polyamine biosynthesis is experimentally manipulated.

As noted above, gene expression of *Az1* and *Azi2* is elevated at the implantation site after postimplantation in the mouse (139, 172). In the mouse conceptus, *Azin2* was significantly up-regulated in response to DFMO treatment (172). *Odc1* gene overexpression in the mouse uterine stromal and epithelial cells induces an increase in *Azi1* gene expression (139). Modulation of polyamine transport by AZI1 and AZIN2 in response to inhibition or stimulation of ODC1 activity may therefore indicate the presence of a further compensatory mechanism to ensure the maintenance of homeostasis of intracellular pools of polyamines. This autoregulatory circuit of polyamine bio-

synthesis, implicating the AZI-AZIN tandem (Fig. 3), also plays an important role during organogenesis because both ODC1 and AZI proteins follow a spatiotemporal expression pattern during the fetal development (183). Moreover, the *Azin1* knockout mouse is not viable after parturition, exhibiting abnormal liver morphology, a high rate of ODC1 degradation, and reduced putrescine and spermidine production (184). This confirms the requirement for the autoregulatory circuit of polyamine biosynthesis for organogenesis during fetal development.

1. Contribution of maternal diet to polyamine content

As indicated in *Section I*, polyamines are synthesized from arginine, proline, methionine, and/or ornithine (6, 158), and a major source is importation from the diet (10, 11). Because substrate availability has an impact on polyamine synthesis, reproductive events, and reproductive success are expected to be related to the quality and quantity of amino acid nutrition (for review, see Ref. 185). The link between maternal substrate intake, polyamine biosynthesis, placentation, and/or embryo/fetal development has been investigated primarily in large animals (186, 187). In sows, deficiency in maternal dietary protein is associated with IUGR, characterized by a decrease of placental and fetal growth and lower birth fetal weight (188, 189). In the latter case, IUGR associates with reduced ODC1 activity and with reduced concentrations of arginine, ornithine, and polyamines in the endometrium and placenta between d 40 and 60 of gestation (14, 157). In sheep, IUGR also results from maternal undernutrition (188). Nutritional restriction markedly reduces arginine and polyamine concentrations not only in maternal and fetal plasma, but also in allantoic and amniotic fluids during mid and late gestation (190). Surprisingly, the reduction in amino acids and polyamine concentrations can be reversed in all of these compartments by realimentation with sufficient diet, and this treatment prevents IUGR (190).

In the rat, dietary arginine supplementation to pregnant females during the first 7 d or throughout gestation elevates circulating ornithine and arginine concentrations and results in a 30% increase in litter size at birth (191). Similarly, arginine supplementation of pregnant gilts between d 30 and 114 of gestation increased litter size at birth by 22% and weight of live-born piglets by 24% (192). The effects are specific to the later periods of gestation because supplementation with L-arginine between d 1 and 25 of porcine pregnancy reduces fetal survival due to effects on the ovary and the formation of corpora lutea (193).

Deprivation of polyamines has pernicious effects on gestational success. It has been shown that low birth weight, delayed embryo development, and fetal or maternal death occur in pregnant females affected by diabetes mellitus (194). This delayed embryo development in diabetic rat females correlates with a reduction of embryonic ODC1 activity on d 11 of gestation and with a decline of embryo DNA and polyamine content (195). Administration of arginine or polyamines on d 5 of pregnancy in diabetic females reverses the delay in development (196).

The question that arises is whether the beneficial effect of supplementation of arginine, a treatment that clearly increases gestational success, is mediated solely by increased polyamine synthesis, or whether the amino acid acts directly or through other pathways. Arginine is known to affect angiogenesis and placentation in the pig, but it appears not to be important as a substrate for ornithine and, consequently, polyamine synthesis (158). Other effects of arginine, such as the stimulation of intracellular signaling pathways in isolated ovine trophoblast, have been reported (197, 198), and these effects may be independent of polyamine biosynthesis.

IV. Perspectives and Future Directions

When in 1678, van Leeuwenhoek depicted the presence of the "three-sided crystals" in human semen (1), he could not have suspected that he was the first witness to a phenomenon as significant to reproduction as polyamines have proven to be (Fig. 4). The polyamines are implicated in gametogenesis, where they participate in both male and female meiotic maturation of haploid germ cells and have effects on the somatic cells, including the Sertoli, Leydig, and granulosa cells, all essential to sustaining gametogenesis. Polyamines regulate ovarian steroidogenesis during

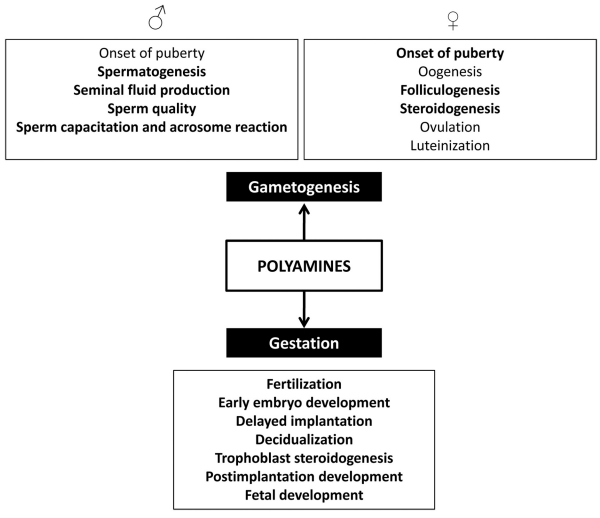

FIG. 4. Summary of polyamine implications in reproductive functions. Reproductive functions noted in *bold* are supported by experimental studies, whereas others are hypotheses resulting from descriptive analysis and remain to be validated.

the estrous cycle and pregnancy and appear to be indispensable for early embryogenesis, consequent embryo implantation, and postimplantation development. Several studies support the hypothesis that polyamines are required for development of the placenta, a critical step in pregnancy because it allows the fetomaternal communications that sustain fetal development. Moreover, polyamine actions in both invasive and noninvasive implantation types suggest that the role of polyamines in pregnancy has been highly conserved among mammalian species, which further reflects their significance to reproductive function.

The *Odc1* null mutant mouse embryo does not survive beyond the blastocyst stage; thus, the common paradigm for polyamine deprivation has not been the knockout mouse model. Rather, inhibitors of polyamine-related enzymes have been widely used to induce polyamine deprivation and to reveal major defects during gametogenesis and embryogenesis. The generalized effects of inhibition of polyamine synthesis may confound interpretation. Therefore, further experimental tools are needed to target inhibition of polyamine synthesis specifically in reproductive tissues. For instance, tissue-specific gene ablation as has been employed successfully for many other genes in mice (199) and the suppression of specific polyamine-related gene expression in reproductive organs could discriminate local effects from global effects of polyamine deprivation on the reproductive functions. Furthermore, ovary-, testis-, embryo-, or uterus-specific gene deletion will provide a better window into understanding polyamine regulation of reproduction than emerges from studies of inhibitors of polyamine synthesis. Given the plethora of actions of polyamines on molecular processes, intensive investigation is required to elucidate the mechanisms by which these unique and interesting molecules function.

In conclusion, the present review provides strong clues for the *sine qua non* requirement of polyamines in reproductive function, adds to the understanding of various aspects of the reproductive processes, and sheds light on the role of polyamines in the reproductive landscape.

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