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The Effects of Prolactin and Vasopressin on the Regulation of Amniotic Fluid Volume and Its Na^+ Concentration through the Membrane Surrounding Amniotic Fluid

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=ABSTRACT=

The effects of prolactin and vasopressin on the regulation of amniotic fluid (AF) volume and its Na^+ concentration ($[Na^+]$) through the membrane surrounding the AF during increase in AF volume due to fetal urination, were studied.

About 70% of AF volume was replaced with normal isotonic saline solution. Isotonic saline solution (0.5 ml) containing Congored and LiCl was introduced into each amniotic sac. Vasopressin (25 ng/ml) or prolactin (1 mg/ml) of AF was then injected into experimental amniotic sac. The concentrations of Congored, Li^+, and Na^+ were measured at 30 and 60 min intervals after injection. AF samples with decreased Congored concentration ([CR]) during the period of 30 - 60 min were analyzed. The percentage change of $[Na^+]$ and the rate of Li^+ movement during this period were calculated, and the effects of vasopressin and prolactin on them were evaluated.

Following results were obtained:

1. The rate of reduction of [CR] in the AF was retarded by vasopressin or prolactin injection. 2. The rate of reduction of $[Li^+]$ in the AF was also retarded by vasopressin or prolactin injection. 3. The rate of reduction of $[Li^+]$ in the AF was less retarded by vasopressin than that of [CR]. 4. $[Na^+]$ changed to approach to the normal level, but this was markedly retarded by prolactin injection. 5. Direction of Li^+ movement was correlated with the change in $[Na^+]$ but it always moved out of the amniotic sac even when the $[Na^+]$ increased in vasopressin injected AF.

From the above results, it is suggested that vasopressin in the AF triggers the fetus to urinate, and then the membranes surrounding the AF regulate osmolarity by efflux of Na^+. We suggest that prolactin facilitates water outflow across the amniotic membrane during increase in AF volume, in contrast to a constant volume, whereas regulation of $[Na^+]$ is partly restricted by prolactin.

Key Words: Amniotic membrane, Amniotic fluid volume, Amniotic fluid Na^+ concentration, Prolactin, Vasopressin

INTRODUCTION

Hypotonic urination of the fetus increases the volume of amniotic fluid (AF) transiently by as

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much as 50% (Tomoda et al, 1987). Although fetal swallowing is considered to be a major compensation for this change in volume, the volume of swallowing is only one-half to one-third that of urination. Nevertheless, AF volume and its osmolarity are maintained at a constant level (Morris & Boyd, 1988; Tomoda et al, 1985).

The membrane surrounding AF has been suspected as a site controlling AF volume and its composition. *In vitro* studies suggested that water transport in chorioamniotic membranes is achieved by osmotic bulk flow and diffusion. During osmotic bulk flow NaCl moves across chorioamniotic membranes with a reflection coefficient of 0.04. (Seeds, 1970). However, *in vivo* fetal urination and swallowing, which occur at unpredictable intervals, make difficult direct study of regulation of AF volume and its composition. We injected hypertonic or hypotonic solution into the amniotic cavity of umblicus- ligated fetal rabbits to increase AF volume by 50% and followed changes in AF volume and Na^+ concentration ($[Na^+]$) to elucidate, *in vivo*, the regulation of water and Na^+ movements across the membranes surrounding AF (Kim, 1991).

The results we obtained from a dead fetus were hard to apply to the AF dynamics of living fetus because AF changes, such as a decrease in AF volume and an increase in osmolarity, follow after the death of the fetus (Canning & Boyd, 1984; Cittadini et al, 1977; Tomoda et al, 1985). The reports on the amniotic-to-maternal water flux across the membranes surrounding AF from living fetus are as follows. Maternal osmotic challenge increased AF osmolarity secondary to a shift of free water to the maternal compartment (Ross et al, 1983; Ervin et al, 1986). Injection of warm distilled water in the AF resulted in a significant increase of fetal blood volume and induced hemolysis even in the fetuses with ligated esophagi (Gilbert & Brace, 1989). AF volume in the fetuses with ligated esophagi was also partly regulated after intraamniotic infusion of saline (Ross et al, 1991).

The movement of monovalent ions including Na^+ across the membranes surrounding AF has rarely been studied. Lim et al. (1994) reported that there is a massive efflux of AF and electrolytes through the surrounding membranes by filtrative bulk flow, in which the rate of Na^+ efflux is linearly related to that of water efflux, and this was assumed to have taken place through the enlarged intercellular space during AF volume increase.

A considerable amount of vasopressin and prolactin, which coordinate the metabolism of water and electrolytes in the kidney, is known to be contained in fetal blood and AF. These hormones have been suspected to participate in the metabolism of water and electrolytes in the fetus and the AF and the maternal compartment. The osmotic elevation in the fetus stimulates the vasopressin secretion. Fetal-to-maternal water transfer is diminished (Leake et al, 1985; Wood, 1986) and the amount of fetal urination is decreased along with an increase in urine osmolarity (Gilbert et al, 1991), and urine excretion of solutes is increased by fetal vasopressin (Ervin et al, 1986; Fujino et al, 1991). Although the vasopressin injected into the AF is degraded to desglycinamide vasopressin by a trypsin-like enzyme (Uyehara & Claybaugh, 1988; Uyehara et al, 1989), a considerable amount moves to the fetal side across the membranes surrounding the AF in 30 min and shows the direct action of vasopressin (Ervin et al, 1986; Gilbert et al, 1991). Consequently, fetal urination in the presence of vasopressin may induce changes in AF volume and composition, and these affect the regulation of AF composition by movement of solutes across the membranes surrounding AF.

Although the concentration of prolactin in human AF (approximately 400 ng/ml at term) is twice as high as that in the maternal compartment,

its function remains unclear (Leake et al, 1985; McCoshen, 1989). Prolactin has been reported to promote the osmotic equilibrium in AF in response to changes of maternal plasma osmolarity (Ross et al, 1983; Ervin et al, 1986). A close correlation between the prolactin concentration in the umbilical cord and $[Na^+]$ in AF has also been reported (Demir et al, 1992). Accordingly, prolactin has been suspected to participate in the control of AF volume and its composition, however, definite evidence has not been reported yet.

In this study we have observed the effects of vasopressin and prolactin on the AF volume regulation during volume increase by fetal urination and tried to elucidate the origin of AF volume regulation between fetal and maternal compartments, as well as the permeability of the AF membrane, by observing the change in AF $[Na^+]$ and Li^+ movement across the membranes surrounding the AF.

METHODS

Animal preparation

Late stage pregnant rabbits were anesthetized in supine position by injecting pentobarbital sodium (Nembutal) through the marginal ear vein at a dose of 30 mg/kg. The carotid artery was catheterized and connected via a 3 way stop cock to a physiograph (Device Mx 6) to monitor the blood pressure. The jugular vein was also catheterized and infused with 150 mM NaCl solution using a constant speed infusion pump.

Victims were incised in the abdomen; the uterus was exposed and the membranes surrounding the AF of the middle fetus were incised to collect AF. Right and left fetuses were paired as control and vasopressin- or prolactin-treated fetus. In each fetus 70% of the AF was replaced with physiological saline to remove hormones. After 30 min a solution (0.5 ml) containing Congored (0.5 g/L), LiCl (15 mM), NaCl (135 mM) was injected into the AF of the experimental fetus and 1 ml of physiological saline of was injected into the control AF. In the vasopressin group 25 ng of vasopressin per ml total AF, which is the sum of initial AF and injected solution, was injected. Prolactin (1 mg per ml of total AF) was injected in the prolactin group. At 30 minutes after the injection, AF from the first fetus of each group was collected and at 60 minutes after injection, AF from the second fetus was collected.

Measurement of Congored, Li^+ and Na^+ concentrations

The concentration of Congored ([CR]) was determined from 1 ml of fluid extraction with a colorimeter (Corning 253), using a saline containing 30% of normal rabbit plasma as diluent. $[Na^+]$ and $[Li^+]$ were determined in the remaining AF using the IL 943 Automatic Flame Photometer (Allied Instrumentation Laboratory).

Data analysis

The cases in which [CR] in AF at 60 min was lower than that at 30 min were selected to calculate % change in Congored, Li^+ and Na^+.

$$\%\text{change} = \{([X]_{60\text{min}} - [X]_{30\text{min}}) / [X]_{30\text{min}}\} \times 100$$

To calculate the Li^+ movement rate the concentration at 60 min was normalized against 30 min value in each fetus as the initial [CR] and $[Li^+]$ at 30 min were variable between fetuses. Thus the equation for Li^+ movement rate was $\{1 - (r[Li^+] / r[CR])\} \times 100$, where r is the rate of relative decrease. A positive value indicates Li^+ influx to amniotic cavity and a negative value Li^+ efflux.

Statistics

The difference in the rate of decrease of [CR]

and $[Li^+]$ between control and hormone-treated fetus was evaluated using the Student's t-test (paired comparison). Changes in $[Na^+]$ were plotted against initial $[Na^+]$ and changes in Li^+ movement to explore the correlation. Effects of hormones on the slope of the regression line were evaluated by t-test. If a linear regression could not be made, scatter distribution on four quadrants was analyzed using the χ^2 test.

RESULTS

Changes in [CR], $[Li^+]$, and $[Na^+]$ during the period, 30~60 min after Congored injection are presented in table 1. The rate of decrease in [CR] was 38.3±18.83% in control, 17.0±14.68% in vasopressin-treated, and 16.8±8.64% in prolactin-treated AF. Although the variation was large, the rate of change of [CR] in vasopressin- or prolactin-treated AF was distinctly lower than that in control AF ($p<0.005$). The rate of decrease of $[Li^+]$ was 34.4±15.59% in control, 24.3±13.09% in vasopressin-treated, and 11.7±6.98% in prolactin-treated AF. In spite of their large variation, the rate of decrease in hormone-treated AF was significantly lower than that in control ($p<0.005$). In vasopressin-treated AF, the degree of reduction was greater in $[Li^+]$ than in [CR] ($p<0.005$). The initial $[Na^+]$ was comparable in all groups and $[Na^+]$ change between 30 min and 60 min after hormone treatment was not significantly different between groups.

The Li^+ movement rate which was calculated from the rates of reduction in [CR] and $[Li^+]$ is presented in Fig. 1. As shown in table 1, the individual variation between [CR] and $[Li^+]$ in control AF was so large that we followed the Li^+ movement rate individually. The pattern of Li^+ movement showed both net influx and net efflux in control and prolactin-treated AF, but showed only net efflux in vasopressin-treated AF.

The percent change of $[Na^+]$ between 30 and 60 min in relation to initial $[Na^+]$ at 30 min ($[Na^+]_{30min}$) is presented in Fig. 2. The inverse

Table 1. The effects of vasopressin and prolactin on the percentage change of sodium and lithium ion concentrations during decrease in Congored concentration in amniotic fluid of rabbits

	$100(\Delta[CR]_{60-30}/[CR]_{30})$ (n)	$100(\Delta[Li^+]_{60-30}/[LI^+]_{30})$ (n)	$100(\Delta[Na^+]_{60-30}/[Na^+]_{30})$ (n)	$[Na^+]_{initial}$(mEq/L) (n)
control	−38.3±18.83 (8)	−34.4±15.59 (8)	0.03±16.64 (6)	137.4±15.21 (6)
vasopressin	−17.0±14.68* (10)	−24.3±13.09*† (10)	6.3±7.14 (7)	133.3±50.64 (7)
prolactin	−16.8±8.64* (7)	−11.7±6.98* (7)	4.1±1.79 (7)	135.5±47.15 (7)

About 70% of the amniotic fluid was replaced with normal saline. 0.5 mL of isotonic saline solution containing 15 mM LiCl and 0.5 gm/L Congored was introduced into each amniotic sac. Vasopressin (25 ng/mL) or prolactin (1 mg/mL) of amniotic fluid was injected into each experimental amniotic sac. Among total experiments, amniotic fluids with decreased Congored concentration ([CR]) during the period of 30-60 minutes were selected for analysis. Initial Na^+ concentration ($[Na^+]_{initial}$) was measured at 30 minutes after mixed solution injection. The percentage changes $100(\Delta[X]_{60-30}/[X]_{30})$ were calculated by $100\{([X]_{60min}-[X]_{30min}) / [Na^+]_{30min}\}$. Asterisks indicate * $p<0.005$ which differ significantly from control and † $p<0.005$ from $\%\Delta$[CR].

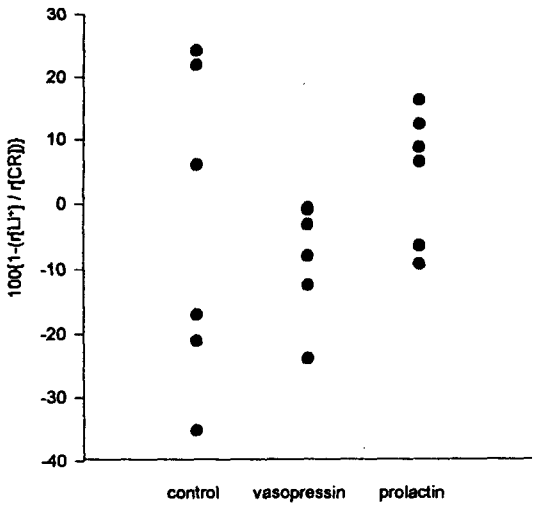

Fig. 1. Li^+ movement into or out of amniotic sac during decrease in Congored concentration in control, vasopressin- and prolactin-injected amniotic fluid(AF). The rate of Li^+ movement during the period of 30-60 minutes after injection of 0.5 mL of isotonic saline solution containing 15 mM LiCl and 0.5 gm/L Congored was calculated by the equation 100{1-(r[Li+]/r[CR])}. Li^+ and Congored concentration were normalized against the 30 minute value, with proportionate adjustments(r) of all other values at 60 minutes.

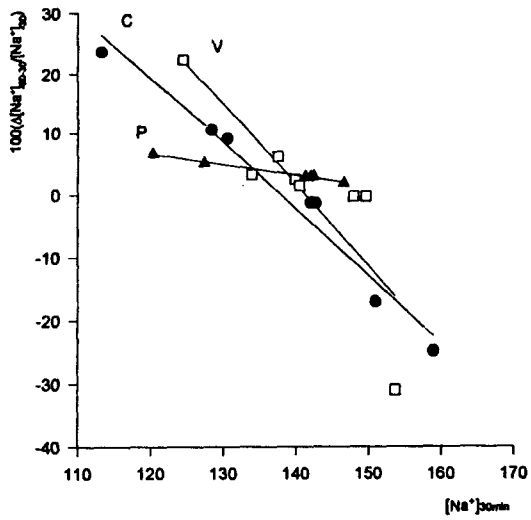

Fig. 2. The percentage change of Na^+ concentration in relation to initial concentration ($[Na^+]_{30min}$) during decrease in [CR] in control, vasopressin- and prolactin-injected AF. The percentage change of $[Na^+]$ during the period of 30-60 min $[100\{([Na^+]_{60min} - [Na^+]_{30min}) / [Na^+]_{30min}\}]$ was plotted against initial $[Na^+]$. Linear regression for control (C) $y=148-1.07x$ $r=0.98$, vasopressin(V) $y=185-1.31x$ $r=0.84$ and prolactin(P) $y=27.33-0.17x$ $r=0.99$. Slope of P Vs. C; $t=7.45$, $p<0.005$.

correlation between the initial $[Na^+]$ and the change in $[Na^+]$, shown in control and vasopressin-injected AF, implied that the $[Na^+]$ change tended to return to the normal range of $[Na^+]$ (in each AF, $r>0.84$). With the confidence interval set at 95%, the regression line shown in vasopressin-injected AF was similar to that of control (-1.07 ± 0.11). But, the interval shown in prolactin injected AF was -0.17 ± 0.02 which differs significantly from that of control ($p<0.005$). This indicated that the change in $[Na^+]$ towards normal range is retarded by prolactin.

A scatter diagram for Li^+ movement relative to the change in $[Na^+]$ is shown in Fig. 3. All points from control AF were located in the first and third quadrants. This positive value formed a linear regression ($y=1.24x-3.58$, $r=0.92$). Li^+ moved into the amniotic sac when $[Na^+]$ increased and Li^+ moved out of the amniotic sac when $[Na^+]$ decreased. This suggests that monovalent ions move towards the normal $[Na^+]$ during AF volume increment following fetal urination. Points of vasopressin or prolactin injected AF were located not only in the first or third quadrant but also in the fourth quadrant. Because of these negative values in the fourth quadrant, it was difficult to evaluate their linear regression. Therefore, using the χ^2 test, hormone dependency was evaluated. It was shown in the scatter diagram that vasopressin significantly triggers Li^+ to move out of amniotic sac whenever $[Na^+]$ decreases or increases ($p<0.05$).

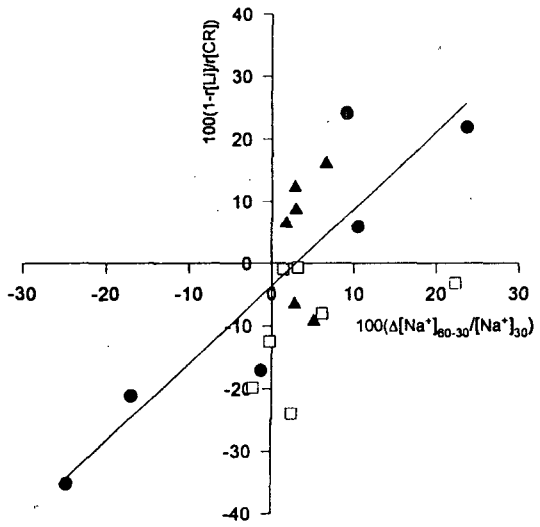

Fig. 3. Li^+ movement into or out of the amniotic sac in relation to the percentage change of $[Na^+]$ during decrease in [CR] in control, vasopressin- and prolactin-injected AF. Li^+ movement was calculated by the equation $[100\{1 - (r[Li^+]/r[CR])\}]$ and plotted against the percent change of $[Na^+]$.

DISCUSSION

Fetal urination increases AF volume by 47% maximally (Tomoda et al, 1985) and the osmolarity of fetal urine can be reduced to as low as 75 mosm/L (Lind et al, 1971). Nevertheless, the volume and osmolarity of AF are maintained at a constant level that is somewhat lower than the normal osmolarity of body fluid (Tomoda et al, 1985).

Surprisingly, we presently know very little about the mechanisms by which AF volume and composition are regulated. With respect to AF volume, fetal swallowing is as much as one-half to one-third of fetal urination (Morris & Boyd, 1988) and no explanation for the difference has yet been observed.

According to *in vitro* experiments, chorioamniotic membranes allow passage of only small molecules less than 1,000 in molecular weight during bulk flow and diffusion (Seeds, 1970). However, *in vivo* experiments have not been extensively carried out on the transport across the membranes surrounding AF.

Recently, the AF of esophagi-ligated fetuses was reported to be regulated normally and the injection of warm distilled water into AF induced rapid absorption of the water into the fetal circulation, resulting in a significant decrease in fetal osmolarity in normal as well as esophagi-ligated fetuses (Gilbert & Brace, 1989; Ross et al, 1991). Such data may indicate that water in AF can diffuse across the membranes surrounding AF.

It was reported that the reflection coefficient of Na^+ is 0.04 at chorioamniotic membranes during bulk flow *in vitro* (Seeds, 1970). In *in vivo* experiments, Li^+ appearance rate in maternal blood and Li^+ clearance rate in AF of expired fetuses has been reported to be about one half of those in living fetuses (Kim et al, 1990; Chang et al, 1993). Kim (1991) reported that Na^+ transport in hypervolemic AF across the membranes surrounding the AF is carried out by a bulk flow at an early stage and then accompanied by a diffusion in a later stage, since the rate of AF volume decrease falls when the osmolarity of AF increases while $[Na^+]$ adjusts to normal levels when AF volume decreases.

However, the AF volume of expired fetuses is smaller than that of living fetuses and $[Na^+]$ in AF of expired fetuses decreases in spite of the increase in osmolarity (Tomoda et al, 1985). It is hard to apply the data from expired fetuses, which only indicate the possibility of water and monovalent cation movement across the membranes surrounding the AF, to normal AF.

In living fetuses AF effluxes across the membranes surrounding the AF and the efflux rate in-

creases with increase in AF [Na^+] in the case of low AF osmolarity. While these phenomena become more prominent as the AF volume increases, the contribution of solvent drag to monovalent ion transport increases in proportion to AF efflux (Lim et al, 1994).

The transport of AF and solutes across the membranes surrounding AF in living fetuses has not been clearly established. This may be mostly due to a large variation in volume and timing of fetal swallowing and urination. Decrease in injected [CR] has been used as proof of fetal urination (Nelson et al, 1954). From the [CR] change and Li^+ movement rate we attempted to characterize the Li^+ movement across the membranes surrounding the AF and delineate the transport mechanism of monovalent ions including Na^+ and its regulation by vasopressin and prolactin.

In this experiment there was no significant difference between [CR] and Li^+ movement rate increase. Their variation was large and [Na^+] approached normal from an initial low or high value. This observation is consistent with a previous study (Kim, 1991). Monovalent ions including Na^+ are considered to diffuse across the membranes surrounding AF to keep the normal level even during fetal urination, since the direction of Li^+ movement was directly correlated with the change in [Na^+].

Congored dilution and Li^+ movement rate in vasopressin-treated AF were much lower than that in control AF. The decrease in the rate of [CR] change may imply that urine volume decreased as compared with control fetus or AF moved out of amniotic sac through the membranes surrounding AF. Vasopressin, injected into amniotic cavity, is delivered to the fetus in 30 min and functions right after delivery (Ervin et al, 1986; Gilbert et al, 1991). Vasopressin inhibits the water transport from fetus to maternal blood (Wood, 1986) and reduces fetal urination without any change in Na^+ excretion rate (Gilbert et al, 1991). On that ground a much lower rate of Congored dilution in vasopressin-treated AF seems to be due to a reduction of fetal urination. When the AF was diluted by fetal urination, the Li^+ dilution rate was much higher than the Congored dilution rate in vasopressin-treated AF, while [CR] and [Li^+] decrease rates were similar to control AF. This phenomenon suggested that Li^+ effluxed across the membranes surrounding AF in large amounts. In vasopressin-treated AF [Na^+] changed to normal value as in control AF but, in contrast to control AF, Li^+ always moved out of the amniotic cavity even when [Na^+] increased. Considering that Li^+ is similar to Na^+ in size and movement in aqueous condition, Li^+ efflux in the face of increase in [Na^+] suggests that Li^+ moved out of amniotic sac along with Na^+ efflux induced by a high Na^+ concentration in the AF that resulted from fetal excretion of concentrated urine. We therefore believe that AF volume increment following fetal urination is not so extensive and monovalent ions including Na^+, supplied by concentrated fetal urine, efflux through the membranes surrounding AF in vasopressin-treated AF.

[CR] and [Li^+] decrease rates shown in prolactin-treated AF were much lower than in control AF. Prolactin is found in high concentrations in human AF (approximately 400 ng/ml at term) while maternal plasma concentration of prolactin is one- to two-thirds the AF concentration (Leake et al, 1985). However, its function remains unclear and it is only suspected to participate in the regulation of AF osmolarity (Demir et al, 1992). The osmolarity of AF is kept slightly lower than that of maternal plasma. If the osmolarity in maternal plasma is increased, the osmolarity in the AF increases by the osmotic efflux of AF across the membranes surrounding the AF. Prolactin is reported to restrict this water efflux (Ross et al, 1983). [Na^+] in AF is also reported to be

proportional to the concentration of prolactin in umbilical blood (Demir et al, 1992). According to these reports, when AF volume is normal, prolactin seems to restrict water efflux rather than solute transport across the membranes surrounding AF. But, in this experiment, the rate of decrease in [CR] in prolactin-treated AF was much lower than that in control AF. This may be due to a large water efflux across the membranes surrounding AF rather than a small fetal urination. The capacity for the regulation of $[Na^+]$ in the membranes surrounding AF is likely to be limited during the increase in water permeability by prolactin. Li^+ moved in or out of amniotic sac at a constant increase in $[Na^+]$ in all cases. Therefore, during AF volume increase prolactin is considered to facilitate water efflux from the AF and rarely participates in the regulation of electrolyte transport across the membranes surrounding the AF.

If AF volume is increased and the osmolarity of AF is decreased by some means such as fetal urination, the membranes surrounding the AF regulate the osmolarity of the AF to normal range by influx or efflux of Na^+. If vasopressin in the AF triggers fetus to urinate, the membranes surrounding the AF regulate the osmolarity of the AF easily by efflux of Na^+. However, prolactin seems to facilitate water outflow when the AF volume increases and prevent excessive increase in the AF volume by increasing the water permeability of the membranes surrounding the AF.

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