J. King Saud Univ., Vol. 9, *Agric. Sci.* (1), pp. 87-96 (A.H. 1417/1997)

Prostaglandin F2α Alters Steroidogenic Activity but Not Insulin-like Growth Factor-I (IGF-I) Production in Brahman Ovaries

M.M. Zeitoun

Department of Animal and Fish Production, Faculty of Agriculture, El-Shatby, University of Alexandria, Alexandria, Egypt

(Received 13/1/1416; accepted for publication 30/10/1416)

Abstract. Eighteen estrous cycling Brahman cows were randomly allotted into three groups (n = 6 cows/group). All cows were subjected to daily transrectal ultrasound measurements for a complete estrous cycle plus various number of days of the consequent estrous cycle until a large dominant follicle (≥ 8 mm diameter) of the first (anovulatory) follicular wave was detected. At this time group 1 (G1) cows were ovariectomized, group 2 (G2) cows were injected (i.m.) with 50 mg Lutalyse™ and ovariectomized 24 h later, however, group 3 (G3) cows received 50 mg Lutalyse and ovariectomized 48 h later. Follicular fluids (FF) were aspirated from the dominant follicle, pool of medium (> 4-7.9 mm) and pool of small (≤ 4 mm) follicles. IGF-I concentrations in FF of the dominant follicle remained constant after PGF2α injection. Number of ovarian follicular waves within an estrous cycle has no significant effect on IGF-I concentrations.

Estradiol-17β (E2) production by granulosa cells increased by 7 (24 h) to 10 (48 h) folds after PGF2α injection as compared to control (0 hr). As follicle diameter increases, its E2 concentration increases ($p < 0.01$). However, E2 concentration in FF was not influenced by number of follicular waves. Although progesterone (P4) concentration in FF tended to decrease as time progressed after PGF2α injection, the differences were not statistically ($P > 0.05$) significant. Moreover, P4 concentration was similar in small, medium and large follicles. E2/P4 ratio was about 600 folds higher after PGF2α than in control cows. Additionally, this ratio was 50 times higher in large than in small follicle, however seven folds of increase was obtained with large than with medium follicles. Testosterone (T) production also increased by 2 times after PGF2α injection. As follicle size increases, its T-content decreases significantly, this also was confirmed by a significant negative correlation coefficient ($r = -0.70$). Injection of PGF2α at the growth phase of the dominant non ovulatory follicle could rescue its oocyte of atresia.

Introduction

In a recent study Rodes *et al.* [1] found 67% of Brahman cows have a pattern of 3 ovarian follicular waves, 26% with 2 waves and 7% with 4 waves. Each wave contains one dominant follicle in addition to several subordinate cohort of smaller size follicles. The dominant follicle is considered as non-ovulatory in the non-ovulatory wave(s) or preovulatory in the ovulatory wave.

After a single injection of prostaglandin F2α during the luteal phase, estrus occurs within 3 to 4 days in cows. During this interval, two large follicles ($\geqslant$ 6 mm diameter)/pair of ovaries are usually present [2]. One follicle is estrogen-active (E-A), the other is estrogen-inactive (E-1). The E-A follicles have higher concentrations of estradiol-17β in FF than both progesterone and androgens, whereas E-I follicles have higher concentrations of either progesterone or androgens than estradiol.

During the interval PGF2∞ injection to estrus, sizes, follicular fluid concentrations of P4, E2 and androgens and specific binding of I^{125}-human chorionic gonadotropin (hCG) to granulosal or thecal cells in E-A follicles increase, whereas specific binding of I^{125}-bovine Follicle Stimulating Hormone (b-FSH) to granulosal cells decreases [2].

The E-I follicles, although similar in size to E-A follicles, contain fewer granulosal cells, a lower concentration of E2 in FF and a reduced capacity to bind gonadotropins. It has been reported [2] that changes in growth, concentration of steroids in FF and specific binding for E-A and E-I follicles after PG-induced luteolysis were characteristic changes in ovulatory and non-ovulatory follicles, respectively.

A multitude of growth factors have been implicated in the local regulation of ovarian follicular steroidogenesis in a variety of mammalian species [3]. Of these, insulin - like growth factor-I (IGF-I) has received considerable attention [4]. Positive relationships have been shown between follicular IGF-I and estradiol concentrations in cyclic cattle [5]. Therefore, the experimental objective aimed to determine how prostaglandin F2α administered at the growth phase of the first (non-ovulatory) ovarian follicular wave do alter functions of the granulosa cells to produce steroid hormones as well as IGF-I in Brahman ovaries.

Materials and Methods

Animals

This experiment was conducted in May 1994 in the Texas A&M agricultural experimental station at Overton, TX. Eighteen mature estrous-cycling non lactating Brahman cows (3-5 yrs.) were randomly allotted into 3 groups (6 cows/group). Out of these cows, 6 expressed 2 ovarian waves, 6 with 3 waves and 6 with 4 waves. Two cows of each follicular wave category were randomly allocated into one of the three experimental groups. Cows grazed on pasture and were fed on a concentrate mixture of 6:1 corn : soybean (2.25 kg/head/day) and coastal bermudagrass free choice. Estrous behavior was visually observed 3 time daily by the aid of vasectomized chin ball-marked bulls.

Ultrasound measurements, PGF2α administration and ovarectomy

A daily (7:00 AM) transrectal ultrasound (Aloka 210, linear array 5mhz transducer, Japan) measurement was performed for each ovary for a complete estrous cycle. Each ovary was monitored for different sizes of follicles and a corpus luteum from two different angles and two images were recorded (Sony UP 850 Graphics printer, Japan). Cows were sorted according to the number of follicle waves they have expressed with their ovaries during an estrous cycle. The daily ultrasound scannings were continued in the subsequent estrous cycle until a dominant follicle ($\geq$ 8 mm diameter) of the first wave was detected. At the growth phase of this follicle wave, *i.e.* when the diameter of the dominant follicle increases daily, the G1 cows were exposed to a midflank laporotomy with local anaesthesia (20 ml 5% Lidocaiene). Only the ovary which contains the dominant follicle was removed (unilateral OVX). On the other hand, G2 cows were injected i.m. (at the growth phase of the dominant follicle) with 50 mg Lutalyse (UpJohn, Kalamazoo, MI) and 24 h later cows were subjected to ovarectomy. However, G3 cows were ovarectomized 48 h after PGF2α injection cows were kept in a restricted area without access to food or water 24 h before surgery.

Ovarian and follicular fluids collection

Ovary which contains the largest follicle was excised, submerged in a 50 ml chilled sterile media (M199, GIBCO, Grand Island, NY). Follicular fluids of small ($\leq$ 4 mm, pool) and medium ($>$ 4-7.9 mm, pool) follicles were aspirated by 1cc, 25 gauge-syring. Fluids of the largest follicle were aspirated separately. All fluids were frozen (−20°C) until P4, E2, T and IGF-I were assayed.

Radioimmunoassays of steroids and IGF-I

Progesterone [6], estradiol - 17β [7] and testosterone [8] were measured in the follicular fluids of all size follicles by radioimmunoassays using procedures validated in R.D. Randel's lab. [9]. All steroid antisera were purchased from G.D. Nisewinder (Colorado State University, Fort Collins, CO).

The E2 antibody (GDN 244) cross-reacts 2.6% with estrone and 0.02% with testosterone. The P4 antibody (GDN 337) cross-reacts 1.1% with pregnenolone, 0.3% with estrone, 0.3% with estradiol, 0.2% with testosterone and 0.2% with androstenedione. The intraassay coefficients of variation for the estradiol, testosterone and progesterone were 3.8, 6 and 9.6%, respectively.

IGF-I was only measured in the largest follicle follicular fluid by using extraction RIA kits (# 40-2100, Nichols Diagnostics Inc., Los Angeles, CA). The extraction procedure was performed by C-18 Sep-Pak® (Waters Associates, Milford, MA) chromatography. Six levels (0,0.3,0.6,1.2,2.4 and 4.8 ng/ml) of standard were established. A radiolabelled tracer (I^{125}-IGF-I) was used and samples were measured in gamma counter. All samples were analysed in duplicates. The intra-assay coefficient of variation was 2.8% and the assay sensitivity was 0.06 ng/ml.

Granulosa cells (GCs) collection and viability

Granulosa cells were collected from the largest follicle by dissecting the follicle after aspirating its FF, scraping the mural GCs. The luminal GCs were collected by centrifuging (300 × g/30 min) the FF. Both mural and luminal GCs were combined and resuspended in 1 ml of M199 medium. Ten microliters of the GCs were taken out in a microcentrifuge tube and mixed thoroughly with 10 ul Trypan blue (5%, in 2X distilled water). A drop of the GC-Trypan blue mixture was mounted on a haemocytometer and the non-stained cells (non-opaque) were counted as viable. Total number of GCs and number of viable cells in the dominant follicle was also estimated, taking in consideration that the dilution rate was 1 Gcs: 1 Trypan blue.

Statistical analysis

Data were analyzed by the analysis of variance procedures and treatment differences determined by preplanned mean comparisons using Student's test [10].

Results

Average number of viable GCs in the dominant follicle was not significantly ($P > 0.10$) different between treatments (9.0 ± 7.8, 5.1 ± 2.4 and $6.4 \pm 5.5 \times 10^6$ cells

for 0, 24 and 48 h, respectively). Although there was a slight decrease in ovarian weight after PGF2α injection, the differences were not statistically significant ($P > 0.10$). PGF2α injection increased ($P < 0.01$) testosterone and estradiol-17β concentrations in follicular fluid, however, no significant effect was found on progesterone or IGF-I concentrations (Table 1). IGF-I was only measured in the largest follicles.

Table 1. Steroid hormone (ng/ml) in follicular fluids of all size follicles and IGF-I concentrations (ng/ml) in large follicles of Brahman cows at 0, 24 and 48 h after PGF2α injection (Mean ± sem)

	Time after PGF2α(h)		
Steroid of IGF-I	**0**	**24**	**48**
Estradiol-17β (E2)	30.4 ± 86.3[a]	306.7 ± 79.0[b]	210.1 ± 91.3[b]
Progesterone (P4)	95.5 ± 29.9	73.2 ± 27.4	52.5 ± 31.7
Testosterone (T)	16.2 ± 5.1[a]	30.0 ± 4.7[b]	32.5 ± 5.4[b]
IGF-I	163.3 ± 21.5	172.2 ± 18.3	177.9 ± 22.7
E2/P4	0.008 ± 2.3[a]	5.2 ± 2.1[b]	4.2 ± 2.5[b]

Values in the same row with different superscripts differ at $P < 0.01$

Similarly, when steroid hormones data were analyzed based on the amounts secreted by 1×10^6 viable GCs, there were 18 (24 h) and 10 (48 h) folds increase in E2 concentrations as compared to that secreted in control group. Moreover, PGF2α treatment caused a sharp increase in the E2/P4 ratio (0.008 ± 2.3, 5.2 ± 2.1 and 4.2 ± 2.5 for 0, 24 and 48 h respectively).

Regardless of the treatment effect, there were significant effects ($P < 0.01$) due to follicle size (Table 2) on the testosterone and estradiol-17β but not on proges-

Table 2. Steroid hormone concentrations (ng/ml) in follicular fluids of small, medium and large follicles (Mean ± sem)

	Follicle size		
Steroid	**Small**	**Medium**	**Large**
Estradiol-17β	29.6 ± 77.3[a]	97.2 ± 103.0[a]	420.5 ± 77.3
Progesterone	66.0 ± 26.8	101.3 ± 35.7	53.9 ± 26.8
Testosterone	43.2 ± 4.6[a]	25.4 ± 6.1[a]	10.1 ± 4.6[c]
E2/P4	0.2 ± 2.1[a]	1.3 ± 2.8[a]	8.3 ± 2.1[b]

Values in the same row with different superscripts differ at $P < 0.01$

terone concentrations in follicular fluids. Follicular fluids of small follicles have 4 times as much testosterone/ml FF as these found in large follicles. Contrariwise, E2 concentration in large follicles approached 14.5 folds as much as these determined in small follicles. Evidently, E2/P4 ratio was highest in large (8.3) and lowest in small (0.2) follicles.

A positive correlation coefficient ($r = 0.90$) was found between ovarian weight and total volume of follicular fluids. The PGF2α injection did not affect this relationship ($P < 0.10$). Likewise, a positive correlation coefficient was obtained between size of dominant follicle and its content of follicular fluids. This relationship was highest at 0 h ($r = 0.98$) and decreased after PGF2α injection ($r = 0.39$ and 0.76 at 24 and 48 h, respectively).

Discussion

Concentrations of steroids in FF have been shown to reflect capacity of a follicle to produce and secrete steroids [11, p. 230; 12,13]. Thus, changes in the relationship of concentrations of E2 to other steroids within a follicle during an estrous cycle may reflect alterations in capacity of a follicle to synthesize E2. Consequently, when concentrations of P4 or androgens in FF were higher than E2, such a follicle has probably lost the capacity to produce E2 or may have not approached enough development or may became atretic [2,14,15]. This is in agreement with the present finding (Table 2) that the large follicle posses as much 40-folds E2/P4 ratio as that in small follicles.

Pulses of E2 are secreted in response to pulses of LH during the luteal and periovulatory phases of the estrous cycle in cows [16,17].

The largest follicle generally regresses following an injection of PGF2α on day 8 or 9 of the estrous cycle of the cow [18]. This would explain the large variation between cows within and between treatments with regard to the secretion pattern of E2 as time progresses after PGF2α injection. Increased concentrations of E2 (Table 1) after PGF2α injection have been previously reported [19]. Louis *et al.* [20] have observed increased basal concentrations of luteinizing hormone (LH) between 6 and 12 h after injection of PGF2α in heifers. Secretion of E2 by the dominant follicle in rats is dependent on LH to stimulate the conversion of cholesterol to testosterone in thecal cells [21].

Therefore, some basal concentration of LH is required to provide androgen precursors from thecal to granulosal cells for follicle stimulating hormone (FSH) - directed synthesis of E2 [21]. In the present study both E2 and T were elevated in FF,

however, P4 concentration remained similar after PGF2α injection (Table 1). It was proposed that after luteal regression, due to PGF2α injection, LH pulse frequency increased, thus allowing the dominant follicle to increase its production of estradiol and androgens. Low levels of progesterone were required for maintenance of dominant follicle and increased LH pulse frequency, whereas high levels of progesterone were associated with follicular turnover and lower LH pulse frequency [22].

Likewise, E2 linearally increased with the increase of follicle size (Table 2). The E2 concentration in large follicles was 14.5 folds greater than those in small follicles. On the contrary, a linear decrease in T concentrations in FF was obtained as follicle enlarges in diameter. Follicle growth involves hormonally induced proliferation and differentiation of both theca and granulosa cells, leading ultimately to the increased ability of follicles to produce E2 and to respond to gonadotropins [23; p.121].

Ratio of concentration of E2 to P4 and androgens in FF has been shown [24] to be a reliable method of separating healthy (E-A) from atretic (E-1) antral follicles in heifers.

Insulin-like growth factors were found to be secreted by granulosa cells in FF. There is an increasing acceptance that these growth factors being autocrine/paracrine amplifers of gonadotropin action on follicular development and actions. However, IGF-I does not modulate FSH binding. Effects of IGF-I on FSH-mediated responses include increased aromatase activity and estrogen production [4]. The positive relationship ($r = 0.43$) found in the present study between IGF-I and E2 concentrations in FF has been confirmed elsewhere [25].

In conclusion, the ultrasound-time based injection of PGF2α at the growth phase of the non ovulatory dominant follicle could rescue this follicle of atresia and enhance an ovulatory LH surge resulting in a normally ovulated oocyte.

Acknowledgement. The author acknowledges the unlimited help and advise of Dr. R.D. Randel, the financial support of the Fulbright Commission and the technical help of A. Lewis.

References

[1] Rhodes, F.M., Fitzpatrick, L.A., De'ath, G., and Entwistle, K.W. "The Effects of Follicle Wave Numbers Per Estrous Cycle on the Ovulatory Follicle in *Bos indicus heifers.*" (Abstr.) *Theriogenology,* 41 (1994), 284.

[2] Ireland, J.J. and Roche, J.F. "Development of Antral Follicles in Cattle after Prostaglandin-induced Luteolysis: Changes in Serum Hormones, Steroids in Follicular Fluid and Gonadotropin Receptors." *Endocrinology,* 111 (1982), 2077-2086.

[3] Hsueh, A.J.W., Adashi, E.Y., Jones, P.B.C., and Welsh, T.H., Jr. "Horomne Regulation of Granulosa Cell Differentiation." *Endocr. Rev.* 5 (1984), 76-127.

[4] Adashi, E.Y., Resnick, C.E., Brodie, A.M.H., Svoboda, M.E., and Van Wyke, J.J. "Somatomedin-C-mediated Potentiation of Follicle Stimulating Hormone-induced Aromatase Activity of Cultured Rat Granulosa Cells." *Endocrinology,* 117 (1985), 2313-2320.

[5] Spicer, L.H. and Enright, W.J. "Concentrations of Insulin-like Growth Factor I and Steroids in Follicular Fluid of Preovulatory Bovine Follicles: Effects of Daily Injection of a Growth Hormone-releasing Factor Analog and (or) Thyrotropin-releasing Hormone." *J. Anim. Sci.*, 69 (1991), 1133-1139.

[6] Abraham, G.E., Swerdloff, R, Tulchinsky, D., and Odell, W.D. "Radioimmunoassay of Plasma Progesterone." *J. Clin. Endocr. Metab,* 32 (1971), 619-624.

[7] Korenman, S.G., Stevens, R.H., Carpenter, L.A., Robb, M., Niswender, G.D., and Sherman, B.M. "Estradiol Radioimmunoassay without Chromatography: Procedure, Validation and Normal Values." *J. Clin. Endocr. Metab.* 38 (1974), 718-720.

[8] Murdoch, W.J. and Dunn, T.G. "Alteration in Follicular Steroid Hormones during the Preovulatory Peroid in the Ewe." *Biol. Reprod.*, 27 (1982), 300-307.

[9] Harrison, L.M., Randel, R.D., Forrest, D.W., Betts, J.G., Humphery, W.D., and Hardin, D.R. "Cloprostenol Induced Luteal Regression in the Cow Beef. I. Ovarian Response and Endocrine Changes." *Theriogenology,* 23 (1985), 511-521.

[10] *SAS, Statistical Analysis System.* SAS User's Guide, Cary, NC, 1986.

[11] McNatty, K.P. "Follicular Fluid." In: R.E. Jones (Ed.) *The Vertebrate Ovary.* New York: Plenum Press, 1978.

[12] England, B.G., Webb, R., and Dahmer, M.K. "Follicular Steroidogenesis and Gonadotropin Binding to Ovine Follicles during the Estrous Cycle." *Endocrinology,* 109 (1981), 881-887.

[13] Hillier, S.G., Reichert, L.E., Jr., and Van Hall, E.V. "Control of Preovulatory Follicular Estrogen Biosynthesis in the Human Ovary." *J. Clin. Endocrinol. Metab.*, 52 (1981), 847-856.

[14] Moor, R.M., Hay, M.F., Dott, H.M., and Cran, D.G. "Microscopic Identification and Steroidogenic Function of Atretic Follicles in Sheep." *J. Endocr.*, 74 (1978), 309-317.

[15] Hay, M.F., Moor, R.M., Cran, D.G., and Dott, H.M. "Regeneration of Atretic Sheep Ovarian Follicles *in vitro*." *J. Reprod. Fertil.*, 55 (1979), 195-207.

[16] Walters, D.L., Schams, D., and Schallenberger, E. "Pulsatile Secretion of Gonadotrophins, Ovarian Steroids and Ovarian Oxytocin during the Luteal Phase of the Estrous Cycle in the Cow. *J. Reprod. Fert.*, 71 (1984), 479-491.

[17] Walters, D.L. and Schallenberger, E. "Pulsatile Secretion of Gonadotrophins, Ovarian Steroids and Ovarian Oxytocin during the Periovulatory Phase of the Estrous Cycle in the Cow." *J. Reprod. Fert.*, 71 (1984), 503-512.

[18] Schallenberger, E., Schams, D., Bullermann, B., and Walters, D.L. "Pulsatile Secretion of Gonadotrophins, Ovarian Steroids and Ovarian Oxytocin during Prostaglandin-induced Regression of the Corpus Luteum in the Cow." *J. Reprod. Fert.*, 71 (1984), 493-501.

[19] Hixon, J.E., Nadaraja, R., Schecter, R.J., and Hansel, W. "Prostaglandin F2α-induced Stimulation of Estrone and Estradiol-17β Secretion in Cattle." *Prostaglandins,* 4 (1973), 679-689.

[20] Louis, T.M., Stellflug, J.N., Tucker, H.A., and Hafs, H.D. "Plasma Prolactin, Growth Hormone, Luteinizing Hormone and Glucocorticoids after Prostaglandin F2α in Heifers." *Proc. Soc. Exp. Biol. Med.*, 147 (1974), 128-133.

[21] Fortune, J.E. "Effects of Follicle-stimulating Hormone (FSH) and Luteinizing Hormone (LH) on Androgen Production by Cell Preparations Isolated from Rat Ovarian Follicles." *Ninth Ann. Meeting Soc. Study Reprod (Abstr.).* (Held on July 20-24, 1976, Ann Arbor, MI.

[22] Savio, J.D., Thatcher, W.W., Badinga, L., De La Sota, R.L., and Wolfenson, D. "Regulation of Dominant Follicle Turnover during the Oestrous Cycle in Cows." *J. Reprod. Fertil.*, 97 (1993), 197-203.

[23] Hafez, E.S.E. "Folliculogenesis, Egg Maturation and Ovulation." In: E.S.E. Hafez (6th ed.) *Reproduction in Farm Animals,* Philadelphia: Lea & Febiger, 1993.

[24] MeNatty, K.P., Smith, D.M., Osatnanondh, R., and Ryan, K.J. "The Human Autrol Pollicles: Functional Correlates of Growth and Atresia." *Ann. Biol. Anim. Biochem. Biophys,* 19 (1979), 1547-1556.

[25] Badinga, L, Driancourt, M.A., Savio, J.D., Wolfenson, D., Drost, M., De La Sota, R.L., and Thatcher, W.W. "Endocrine and Ovarian Responses Associated with the First-wave Dominant Follicle in Cattle." *Biology of Reproduction,* 47 (1992), 871-883.

البروستاجلاندين يحور النشاط التخليقي للاستيرودات وليس لإنتاج العامل المشابه للأنسيولين (IGF-I) في حويصلات مبايض ماشية البراهمان

مصطفى محمد زيتون

قسم الإنتاج الحيواني والسمكي، كلية الزراعة، الشاطبى

جامعة الإسكندرية، الإسكندرية، مصر

ملخص البحث. تم استخدام ١٨ بقرة براهمان ذي شياع منتظم حيث قسمت عشوائيًا إلى ٣ مجاميع (كل مجموعة تحتوي ٦ أبقار) وكانت كل الأبقار تتعرض لتشخيص المبايض اليومي باستخدام جهاز الموجات فوق الصوتية وذلك لمدة دورة شياع كاملة بالإضافة لعدة أيام من دورة الشياع التالية حتى ظهور حويصلة كبيرة (≥ ٨مم) سائدة في أول موجة حويصلية في كلا المبيضين. عند هذه المرحلة كانت **المجموعة الأولى (المقارنة)** تتعرض لإزالة المبيض المحتوي على الحويصلة السائدة **المجموعة الثانية** كانت تحقن بالبروستاجلاندين (٥٠مجم Lutalyse) ويزال المبيض المحتوي على الحويصلة السائدة بعد ٢٤ ساعة، **المجموعة الثالثة** كانت يزال المبيض بعد ٤٨ ساعة من الحقن بالبروستاجلاندين. تم جمع السائل الحويصلي من الحويصلات السائدة والمتوسطة (٤٢ ـ ٧٩مم) والصغيرة (≥ ٤مم) وتم قياس هرمونات البروجستيرون والتستستيرون والإستراديول ١٧ بيتًا وأيضًا تم قياس IGF-1 في سوائل الحويصلات الكبيرة فقط. ولقد وجد أن تركيز الإستراديول زاد بمقدار ٧ (عند ٢٤ ساعة) إلى ١٠ (عند ٤٨ ساعة) مرات عنه في المجموعة المقارنة وأيضًا بزيادة حجم الحويصلة كان هنا زيادة طردية في محتواها من الإستراديول. بينما لم يتأثر تركيز البروجستيرون أو IGF-1 في السائل الحويصلي سواءً بالمعاملة بالبروستاجلاندين أو بحجم الحويصلة. أما نسبة الإستراديول/البروجستيرون فزادت بمقدار ٦٠٠ ضعفا بعد الحقن بالبروستاجلاندين وهذه النسبة كانت أكبر بحوالي ٥٠ ضعف في الحويصلات الكبيرة عنها في الصغيرة و٧ أضعاف في الكبيرة عنها في المتوسطة. أما تركيز التستستيرون في السائل الحويصلي فوجد أنه زاد بمقدار الضعف بعد الحقن بالبروستاجلاندين عنه في المقارنة وكان هناك علاقة عكسية ($r = -0.70$) بين تركيز التستستيرون في السائل الحويصلي وبين حجم الحويصلة.

ويخلص البحث إلى أن حقن البروستاجلاندين أثناء نمو الحويصلة السائدة في الموجة الحويصلية الأولى أثناء دورة الشياع ربما يؤدي لإنقاذ الحويصلة من الضمور وبالتالي الحصول على بويضة قابلة للإخصاب.