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Postpartum Fertility in Relation to some Antioxidant Activity and Hormonal Profile in Buffaloes

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ABSTRACT

A total of 30 normally calved buffaloes, on day 14 postpartum, were used to study the relationship between fertility status and each of antioxidant activity, follicles (≥ 5mm in diameter) populations and hormonal profiles. The reproductive indices in terms of interval to first heat and first service, Days open and services per conception were calculated. The buffaloes were classified into two groups on the basis of Days open whether it was ≤ 90 days (G1, n=20) or from 91 to 120 (G2, n=10). Ultrasonographic (US) examination was conducted on Day 21 postpartum to check cervical and uterine gravid horn diameter as well asevery 3 days to estimate follicles populations (≥ 5m) during two intervals: from 14 to 20 day and from 21 to 29day postpartum. Blood samples were collected on the Day of the first service for estimating serum concentration of antioxidant activity, Zinc, Selenium and estradiol and on Day 10 of the same cycle to assay serum progesterone concentration. While there was an increase (P<0.01) in the follicles populations on both ipsi- and contra- lateral ovaries in G1 compared with G2 during the interval: 14-20 day, there was an increase in G2 compared with G1 during the interval 21-29. The anti-oxidant activity and Malonaldehyde as well as Zinc and Selenium serum concentrations were higher (P<0.01) in G1 compared with G2. Also serum estradiol concentration on the Day of first service and progesterone on Day 10 of the same cycle were higher in G1 compared with G2.It is concluded that estimating follicular populations beyond Day14 postpartum and antioxidant activity on the day of the first service are predictive for postpartum fertility in buffaloes

Keywords: fertility, antioxidant activity, hormonal profile, buffaloes, postpartum.

INTRODUCTION

Reproductive function is considered a major factor determining the economic importance of buffalo cows (Barile, 2005). Fertility in dairy cows is defined as the ability to ovulate a competent oocyte and provides an oviductal and uterine environment capable of fertilization and complete embryonic and fetal development (Pursley *et al*., 1997). Reproductive inefficiency in buffaloes such as delayed first service, silent heat, poor conception rates or ovarian inactivity is a major problem facing buffalo production, especially in animals kept in small holder farms (Sharma, 2003 and Ahmed, 2006). Managemental, nutritional and pathological factorsare considered the main causes of the reproductive inefficiency(Campanile *et al*., 1997). Blood profiles might be a potential aid in characterizing the problem. The blood components are mirror, which reflects the healthy condition of animals. So, the biochemical studies are very important for clinicians in the field during interpretation of their findings (Nebel and Jobst, 1998).Biochemical metabolites concentrations in the follicular fluid of the bovine ovary fluctuate considerably with the stage of cycle and follicle size (Tabatabaei and Mamoei 2010). Oxidative stress has a negative effect on animal performance which causes tissue damage and changes in the female biology (Megahed *et al*. 2002). Trace elements as selenium and zinc are among the antioxidants which can positively affect fertility in cattle.Decreased blood concentrations of these elements was associated with infertility in heifers (Baldi *et al*.2000).Nishimoto *et al*.(2009) found a highly significant positive correlation between the concentration of estradiol and follicular size in follicles of the healthy cattle. Abe *et al*. (2002)reported that highprogesterone concentration in the follicular fluid was predictive of subsequent implantation and pregnancy. Also, for thyroid hormones, Suriyasathaporn (2000) mentioned that thyroid hormones are part of the complex hormonal mechanism that regulates steroidogenesis in the ovary.

Controlled and regulated estrous cycle is done by many hormones which affected follicular growth and corpus luteum development and maintenance. Follicle stimulating hormone, luteinizing hormone, estradiol and progesterone are the hormones most commonly mentioned when discussing the estrous cycle. However, there are other substances that plays an important role in controlling the estrous cycle in either an autocrine or paracrine type within the ovary (Borromeo *et al*., 1996). Enzymes such as hydroxyl steroid dehydrogenase (3β - HSD) plays a central role in the biosynthesis of steroid hormone, including androgen inter (Penning, 1997). It catalyzes the final step in biosynthesis of progesterone (P4) in the ovary. Increasing level of P4 during the the ovulatory follicle growth is a key factor to improve fertility rate of lactating dairy cows, hence cows with greater P4 level have greater probability to be pregnant.

The goal of the present study was to test if there is a relationship between Antioxidant activity, follicle populations and hormonal profile in relation to fertility of Egyptian buffaloes.

MTERIALS AND METHODS

This study was carried out at Mehallet Mousa Experimental station belonging to Animal Production Research Institute (APRI), Agricultural Research Center, located in the North Nile Delta, Kafr el sheikh Governorate, Egypt.

Animals.

A total of 30 multiparous Egyptian lactating buffaloes (4-5 lactations), with live body weight ranged between 440 and 600 kg,were enrolled in this study. Body condition score (BCS) was recorded for all buffaloes (2.5-4.5) based on a scale from 1 (emaciated) to 5 (very fat) according toEdmonson *etal.* (1989). Buffaloes were in postpartum period at approximately 14 days after parturition. The buffaloes calved normally under veterinary supervision. They had neither placental retention nor postpartum infection. Also, they suffered no metabolic disorders during postpartum period.They were housed in semi shaded open pens. They were fed to meet both maintenance and milk production requirements according to APRI (1997). The ration consisted of concentrate feed mixture (CFM), berseem hay (BH), corn silage and rice straw (RS) animals were fed twice a day.They had free access to water and milked twice daily.

Reproductive management:

Assessment of uterine involution:

During postpartum period, the reproductive tract was examined by rectal palpation and trans rectal ultrasonographic (US)scanningon Day 21 postpartumto check cervical and uterine gravid horn diameter as well asonce every three days to estimate follicles populations (≥ 5mm) during two intervals: from 14 to 20 day and from 21 to 29day postpartum.

Heat detection:

Buffaloes were observed twice daily at an intervals of 12 hrs. by an expericier cedherd man. Buffaloes stand to be mounted by the teaser bull were considered in estrous. The interval to first estrous was calculating by subtracting day of parturition (Day0) from the day of estrous.

Breeding:

The buffaloes observed in estrous during service period ≥ Day 45 postpartum were taken to fertile bull to be served (hand mate breeding) according to morning/evening system of breeding. These buffaloes were re-observed for estrous signs on Day 21-23 post-breeding. Buffaloes return to estrous had to be bred for at least ≤3 times. The interval to1st service was calculated by subtracting the day of calving from day of first service.

pregnancy diagnosis:

The non-return buffaloes were examined by trans-rectal ultrasonography on Days 28-30 post-breeding. Conception rate was calculated by dividing conceived buffaloes on total number of naturally-bred buffaloes and multiplied by 100. Days open was calculated by substracting day of last calving from Day of fertile service. The pregnant buffaloes were classified into two groups : G1 in which buffaloes conceived at ≤ 90 days postpartum and G2 in which buffaloes conceived during the interval between Days 91-120 postpartum.

Detection of follicles (≥5mm) populations:

The follicles populations in terms of number of follicles ≥5mm on both ovaries (ipsi- and contra- lateral to pregravid horn) were detected by transrectalultr a sonographic scanning. The ultra-sonographic examination was done every 3 days starting from Day 14 to 29 postpartum. The means±SE of number of follicles ≥5mm counted in the first 3 examinations done on Days 14, 17 and 20 (interval from 14-20 day) were compared with their analogues detected on Days 23, 26 and 29 (interval from 21-29 day postpartum). All ultrasonographic examinations were done using ULTRASCAN 900 using multi-frequency, 6-8 MHz, linear transducer by the same examiner.

Blood sampling and assays:

blood sampling

Blood samples were collected on the day of estrous of the first estrous cycle during service period to assay serum concentrations of total antioxidant capacity, reduced glutathione, malondialdhyde, zinc, selenium, triodothyronine (T3), thyroxine (T4) and estradiol(E2). Also blood samples were taken on Day 10 of the same cycle to measure serum progesterone concentrations. Ten ml of blood was drawn from the jugular vein of each animals in clean vacutainer tube without anticoagulant, centrifuged at 3000 rpm for 10 minutes. The serum was stored at -20 C until analysis.

Assays:

The antioxidant parameters (reduced glutathione, total antioxidant capacity and Malondialdehyde); trace elements (zinc and selenium) and hormones (triodothyronin, thyroxin, estradiol and progesterone)profiles were compared between buffaloes which conceived atday ≤ 90 and those conceived in the period between Days 91 to 120 postpartum.

Estimation of antioxidant capacity:

Total antioxidant capacity (TAC) was measured using calorimetric method according to Koracevic*et al.* (2001), while glutathione (GSH) and malondialdehyde (MAD) concentrations were estimated according to Yoshida *et al*. (2005).

Estimation of zinc and selenium.

Trace elements including selenium (Se) and zinc (Zn) serum concentrations were determined using atomic absorption spectrophotometer as outlined by Varley*et al.* (1980).

Hormonal assays.

Direct radioimmunoassay technique was performed for the determination of thyroid hormones (triiodothyronine and thyroxine) concentrations, progesterone, (P4) and estradiol 17β hormones in representative serum samples. Kits of ‘‘Diagnostic Products Corporation, (DCP) Los Angles, USA’’ with ready antibody coated tubes were used according to the procedures outlined by the manufacturer.

Statistical analysis:

The Data were statistically analyzed using T-test within SPSS (2008) program.

RESULTS AND DISCUSSION

Reproductive performance:

Twenty out of 30 (CR, 67%) buffaloes conceived on Day ≤ 90 postpartum (G1) and the remaining 10 buffaloes (CR, 33%) conceived throughout the period extending from day 91 to 120 postpartum (G2). It was noted that whilethere was no difference in the interval to first estrous between G1 and G2, there was difference ($p<0.05$) in the interval to first service between two groups. Also there were highly significant ($P<0.01$) increase in both number of services per conception (NSC) and days open (DO) in G2 compared with G1(Table, 1).

Table 1. Indices of postpartum reproductive performance in buffaloes.

Reproductive parameter	G1(n = 20)	G2 (n = 10)	Significant
Interval from calving to first estrus (days)	31.60±0.86	33.10±0.98	NS
Interval from calving to first service (days)	55.20±0.97	61.00±1.96	**
Number services per conception	1.35±0.10	2.80±0.25	**
Days open (days)	63.45±3.24	104.10±3.92	**

NS: Not significant. * Significant at P<0.05. ** high Significant P < 0.01.

In buffaloes, Hassan *et al.* (2013) found that NSC and DO values in pregnant animals having no dominant follicles on the ovary ipsilateral to the previous gravid uterine horn, were higher (P<0.01) than those having dominant follicles counter parts. Moreover, the pregnant cows with high genetic merits showed longer DO and NSC, even though with an earlier recovery of cyclicity. Also, Khasatiya (2005) reported that values of 1st postpartum estrus interval, service period length and calving interval were significantly lower in fertile than in infertile animals of Surti buffaloes.

postpartum follicles(≥5 mm) populations

During the ultrasonographic examination period of 14 to 20 days postpartum, the number of follicles (≥ 5 mm) in either ipsi- and contra- lateral ovaries was higher in G1compared with G2. On the other hand, the follicle (≥ 5 mm) populations, on either ipsi- and contra- lateral ovary, were higher in G2 compared with G1during the period from 21-29 days. However there were significant differences in the follicle populations (P < 0.01) between ipsi- and contra- lateral ovaries in either G1 or G2 during either 14-20 or 21-29 examination period (Table, 2). It was observed that while no significant differences was recorded in cervical diameter between G1 and G2 on Day 21 postpartum, there was a highly significant difference (P<0.01) in the uterine gravid horn diameter between two groups.

Table 2. follicle (≥ 5 mm) populations, cervical and uterine diameters on Day 21 postpartum in G1 and G2

Parameter	G1(n = 20)		G2(n = 10)		Sign.
	Ipsilateral Ovary	Contralateral ovary	Ipsilateral Ovary	Contralateral ovary	
No. of follicles ≥ 5 mm at 14 - 20 days p.p.	0.91±0.06 [a]	2.51±0.09 [a]	0.72±0.07 [b]	1.99±0.13 [b]	**
No. of follicles ≥ 5 mm at 21 - 29 days p.p.	1.29±0.06 [b]	2.13±0.07 [b]	1.30±0.10 [a]	2.40±0.14 [a]	**
Cervical diameter (cm)	3.58±0.10		3.61±0.17		NS
Diameter of uterine gravid horn (cm)	3.82±0.08		6.44±0.22		**

NS: Not significant. ** high Significant P < 0.01.

In buffaloes, Hassan *et al.* (2013) found that number of follicles with diameter of ≥ 5 mm on the ovary ipsilateral was leaser than on ovary contralateral to the pregnant uterine horn durin the interval from 14 to 28 day-postpartum in both pregnant and non-pregnant animals. In dairy cattle, Hussein (2005)recorded similar results during fourth weeks postpartum. Usmani (1992) mentioned that follicular activity started 6 days earlier in the ovaries contralateral to the gravid horn (21 days) than in the ipsilateral (27 days) ovaries and was higher in the same ovaries during the first 35 postpartum days (Singh *et al.* 1979).This results may be attributed to the influence of the follicle on the uterine endometrium and/or myometrium. Sheldon et *al.* (2003) mentioned that uterine function can be greatly affected by estradiol synthesized by a follicle ≥ 10 mm diameter. Also, Ireland *et al.,* (1984) found that concentrations of plasma estradiol are greater within the utero- ovarian vein draining the ovary containing the ovulatory follicle.

Similarly in buffaloes, Hassan *et al.* (2013) found insignificant differences in the cervical diameter of involuted cervix between conceived and non-conceived animals. The involution of the cervix completed at earlier interval than involution of the uterus in both groups, but the differences were not significant. Similar results were obtained Shah *et al.* (2004) indicating complete involution of the cervix on 24-39 days postpartum. Also, Hassan *et al.* (2013) found that the difference between diameter of pregravid uterine horn in both groups was significant (P<0.05).

Antioxidant capacity of buffaloes plasma in relation to estrous cycle:

On day 0 (day of 1st service), antioxidants activity, in term of concentration of glutathione reduced, total antioxidants and concentration of malondialdehyde (MDA), were significantly (P<0.01) higher in G1 than G2 (Table 3). Glutathione reduced is an antioxidant, preventing to important cellular components damage, caused by reactive oxygen species (ROS) such as free radicals and peroxides (Sharma *et al*., 2011). Increase plasma glutathione reduced might may lead to embryonic cells protection from oxidative stress, which is considered as one of the major reasons for improving rate of conception in pregnant cows (Guerin *et al*., 2001). Also, Prabaharan *et al.,* (2015) observed higher concentration of reduced glutathione in pregnant than in non-pregnant cows during the interval from day 0 to day 20 and the mean concentration of serum MDA was lower in pregnant and non-pregnant cows in group i.m. injected with vitamin A, C and E for 3 weeks than in control group.

The MDA is a by-product of lipid peroxidation used as an index of the rate of tissue reaction chain. In addition, MDA is used as an indicator of cellular oxidative stress. Prabaharan *et al.,* (2015) reported lower concentration of serum MDA in pregnant than in non-pregnant ones. It was reported that MDA promotes cross linking bonds in the membranes of the body, leasing to changes in permeability of ions and activity of enzymes (Ahmed *et al*., 2010).

In mare, Abo-El Maaty and El-Shahat (2012)found that total antioxidant capacity activity was significantly higher (P<0.05) in serum obtained from luteal phase than those obtained at estrous one. On the other hand,Castillo *et al.,* (2003) recorded high malondialdehyde concentrations at estrus. They attributed the high level to an increase in the production of reactive oxygen species, which suggested to originate mainly from steroidogenesis occurring in granulosa cells.

In both goats and sheep, Nawito*et al.,* (2016) found that malondialdehyde level was significantly higher (P<0.01) in pregnant than in non-pregnant group. But, total antioxidant capacity level was lower in pregnant copared with non-pregnant goats. Also, Amer *et al.* (2014) monitored a decreased level of total antioxidant capacity in pregnant ewes that fed traditional or untraditional diets.

Table 3. **Blood profiles of antioxidant activity; zinc, selenium and estradiol on the Day of first service andprogesterone on Day 10 post breeding in G1 and G2.**

Parameter	G1 (n=20) Estrous cycle day		G2 (n=10) Estrous cycle day		Sig.
	(day 0)	Day 10	(day 0)	Day 10	
Glutathione reduced (mmol/L)	5.66±0.18		4.71±0.30		**
Total antioxidant capacity (mmol/L)	1.64±0.05		0.76±0.05		**
Malondialdehyde(mmol/ml)	1.69±0.07		0.81±0.043		**
Zinc (µg/dl)	131.17±1.35		121.35±1.14		**
Selenium(µg/dl)	136.65±1.47		122.55±1.29		**
Ttriiodothyronine (ng/ml)	95.57±1.87		84.74±9.69		NS
Thyroxine (µg/dl)	3.37±0.16		2.82±0.13		**
Estrogen (ng/ml)	16.47±0.43		20.76±0.55		**
Progesterone (ng/ml)		5.29±0.21		1.49±0.13	**

GSH.R:Glutathionereduced;TAC:Total antioxidant capacity;MDA:Malondialdehyde; Se : Selenium;T3: Ttriiodothyronine; T4: Thyroxine ;P4:Progesterone;ESβ17:estrogen. NS: Not significant. * Significant at P<0.05. ** high Significant P < 0.01.

Concentration of some trace elements in relation to estrous:

Table 3, shows decreased (P<0.01) concentrations of trace element in blood serum of G2 in comparing with G1. In this respect, Graham (1991) reported that Zn or Se deficiency can cause decreased production, especially when a deficiency corresponds to the phases of growth, reproduction, or lactation. Animals having Zn deficiency areusually suffering from infertility (Shuttle, 1986). However, Du Plessis*et al.,*(1999) found small ovaries and decreased ovarian response to FSH-induced superovulation regimen in the molybdenum-induced trace elements depletion. The later may have central effects via the hypothalamus-pituitary axis on LH secretion leading to reduction in ovarian estradiol secretion and acyclicity of animals. Furthermore, LH-induced differentiation of bovine theca cells *in vitro* can be prevented by thiomolybdates and these effects can be ameliorated by trace elements supplementation. In this way, trace elements responsive sub-fertility may result from perturbation of the normal pattern of ovarian steroidogenesis (Kendall *et al.,* 2006). In buffaloes, Alavi-Shoushtari *et al.,* (2015) found that level of Zn in cyclic animals was higher than in acyclic ones. The lowest serum Zn content was observed in pro- and met-estrous, suggesting Zn levels were actively secreted in uterine lumen during the estrous cycle and were not dependent on blood serum. In Nili-Ravi buffaloes, Akhtar *et al*. (2009) showed that serum zinc and selenium levels were significantly lower in anestrus buffaloes compared with cyclic ones, The deficiencies of zinc or/and selenium could be responsible for anestrus condition. Decreasing level of zinc was associated with decreasing steroid hormone concentrations, indicating some correlation between plasma zinc levels and P4 and estrogen levels for proper reproductive processes. The Se deficiency is also associated with reduced fertility in cattle and sheep (Hidiroglou, 1979) and high Se concentration reduces the incidence of anestrus (Harrison *et al.,* 1984).

Hormonal profile:

Regarding thyroid hormones, it has been observed that while they thyroxine (T4) showed significant increase in G1 compared with G2 there was non- significant variation in G1 compared with G2 on the Day of the first serviceThyroid hormones are part of the complex hormonal mechanism that regulates steroidogenesis in the ovary and Low triiodothyronine concentrations were associated with lower concentrations of estradiol and diminished estrus expression (Jorritsma*et al.* 2003). Deranged thyroid activity is a potential cause for reproductive abnormalities in the females of farm animals (Aggarwal and Singh, 2010). In fact, thyroid hormones have direct stimulatory effects on ovarian function in cattle, acting at the level of granulosa and thecal cells (Mutinati *et al*. 2010).

Furthermore, thyroid hormones play an important role in the pre and post-implantation embryo development in cattle (Ashkar *et al*. 2010). Induced hypothyroidism was known to reduce the fertilization rate in dairy cattle (Bernal *et al*. 1996). Ghuman *et al.,* (2011) found that a subtle thyroid activity in lactating buffaloes may have some impacts on their fertility status. They added that non-cycling and non-pregnant lactating buffaloes showed lower (P<0.05) plasma concentrations of thyroid stimulating hormone and thyroid hormones, respectively, compared to their counterparts.The serum concentrations of estradiol on the day of the first service and progesterone on the 10 of the same cycle were significantly(P<0.01) higher in G1 compared with G2 (Table, 3). The higher estradiol level in G1 may be attributed to higher follicles population on both epsi- and contra- lateral ovaries during 14-20 days postpartum. Although follicles populations during this period had no direct effect on the serum E2 level on the day of first service but they are considered good indicators of ovarian activity especially steroidgenic one in G1. However high estrogen levels at the time of the first service in G1 had a positive effect on the conception rate (67%). This may be explained in the light of the fact that high E2 level at the Day of estrus (estradiol surge) induces high LH surge with subsequent induction of ovulation.Also, the higher progesterone level on Day 10 post-service in G1 favors the higher conception rate in this group.

These results are in agreement with earlier reports in buffaloes (Pahwa and Pandey,1983) in terms of minimum level on the day of estrus with gradual rise to the higher levels during luteal phase and then declining to basal level at subsequent estrus.Sankar and Prakash (2008) showed that progesterone concentration was reached the basal level during the peri-oestrus period. The plasma progesterone concentration was the lowest on the day of oestrus and increased to register a peak on day 13 of the cycle. In Holstein cattle, Tabatabaei *et al.,* (2014) showed that the plasma progesterone concentration in late diestrous was higher than in metestrous, proestrous and during estrous ($P<0.05$), but the plasma concentrations of estradiol-17-ß in proestrous and estrous were higher than the other stages of the cycle ($P<0.05$).Also, in goats, Pang *et al.,* (2010) found that progesterone concentrations began to rise earlier and were higher in the high prolificacy than in the poor prolificacy goats on most days of the luteal phase, but not during the follicular phase of the cycle or after ovariectomy.

CONCLUSION

It is concluded that estimating follicular populations beyond Day14 postpartum and antioxidant activity on the day of the first service are predictive for postpartum fertility in buffaloes.

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الخصوبة وعلاقتها بنشاط بعض مضادات الاكسدة والهرمونات بالدم في فترة ما بعد الولادة في الجاموس.

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اجريت هذه الدراسة على عدد 30 جاموسة بعد 14 يوم من الولادة وكانت ولادتهم طبيعية وذلك لدراسة العلاقة بين حالة الخصوبة وكل من نشاط مضادات الاكسدة وعدد الحويصلات التبيزيد قطرها عن او يساوى 5 مم ومستوى بعض الهرمونات في الدم.تم حساب الفترة من الولادة حتى اول شياع و اول تلقيح والفترة من الولادة حتى اول تلقيحه مخصبة (Days open) وعدد التلقيحات اللازمة للأخصاب.تم تقسيم الحيوانات الى مجموعتين على اساس فترة (Days open) سواء كانت اقل من او تساوى 90 يوم (المجموعة الاولى وعددها 20جاموسة G1) او كانت من 91 الى 120 يوم (المجموعة الثانية وعددها 10 جاموسة G2).تم فحص المبايض بجهاز السونار كل ثلاثة ايام لتقدير عدد الحويصلات المبيضية والتي يزيد قطرها عن أو يساوى 5مم خلال الفترتين: الفترة الاولى من اليوم 14 وحتى اليوم 21 والفترة الثانية من اليوم 21 وحتى اليوم 29 بعد الولادة.وتم قياس قطر عنق الرحم وقطر قرن الرحم الذى حدث به الحمل عند اليوم ال 21 بعد الولادة.تم اخذ عينات دم يوم الشياع من التلقيحه الاولى لتقدير تركيز مضادات الاكسدة والزنك والسيلينيوم والاستيراد يول وتم اخذ عيناتالدم يوم 10 من نفس دورة الشياع لتقدير تركيز البروجيستيرون. وكانت النتائج كالآتي: لوحظ انه بالرغم من وجودفروق معنوية عالية (P<0.01)في عدد الحويصلات المبيضية على كل من المبيضين (المقابل او الغير مقابل لقرن الرحم الذى حدث فيه الحمل) فيالمجوعة الاولى بالمقارنة بالمجموعة الثانية اثناء الفترة من 14 الى 20 يوم بعد الولادة، كان هناكزيادةفي المجموعة الثانية عن المجموعة الاولى اثناء الفترة من اليوم 21 وحتى اليوم 29 من بعد الولادة.كان تركيز كل من مضادات الاكسدة وانزيم المالونالدهيد وايضا الزنك والسيلينيوم في سيرم الدم اعلى معنويا (P<0.01) في المجموعة الاولى بالمقارنة بالمجموعة الثانية . وايضا تركيز كل منالاستراديول(عند يوم الشياع والتلقيح)والبروجيستيرون(عند اليوم 10 من نفس دورة الشياع)كان اعلىفي المجموعة الاولى بالمقارنة بالمجموعة الثانية.نستخلص من ذلك التقدير العدالحويصلي للبويضات بعد اليوم 14 من بعد الولادة ومضادات الاكسدة عند يوم الشياع من التلقيحه الاولى ويعتبر ذلك دليلتنبؤي للخصوبة في الجاموس بعد الولادة.