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EFFECT OF CALVE GENDER AND DAMS' PRE-PARTUM VACCINATION ON SOME BLOOD PARAMETERS AND GROWTH PERFORMANCE UNTIL WEANING FOR NEW BORN EGYPTIAN BUFFALO CALVES

By

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ABSTRACT

This study was conducted to find out the effect of newborn buffalo calves' gender, and the effect of maternal vaccination before birth using (Scour guard-4k) on the concentration of some blood components in the new born calves (Immunoglobulin G (IgG), insulin-like growth factor hormone (IGF-1), triiodothyronine T3 hormone and thyroxine hormoneT4) by taken blood samples from the calves immediately after birth and then after 6, 12 and 24 hours after birth. And the calves were also weighed at birth immediately and then re-weighed every 15 days until weaning, as well as recording cases of diarrhea during the first three days of the animal's life (The colostrum feeding period), and the duration until recovery. 16 new born Buffalo calves were divided into 4 groups (Four animals in each group) 1- Male calves born from vaccinated mothers 2- Female calves born from vaccinated mothers 3- Male calves born from unvaccinated mothers 4 - Female calves born from unvaccinated mothers. Male calves had a higher blood concentrationof IgG,IGF-1and T4.Therewasno significant difference in the blood concentration of T3 between male and female calves. Maternal vaccination before birth had a significant effect on the concentration of IgG and IGF-1 in calf blood serum. The concentration of T3, T4 hor; mones increased in the blood serum of calves born from unvaccinated dams. Male calves of vaccinated dams achieved the highest birth weight and the highest weight gain until weaning. No cases of diarrhea were recorded in the group of male calves of vaccinated dams.

Key words:

Calves' gender, Egyptian buffalo, newborn calves, maternal prepartum vaccination.

INTRODUCTION

Buffalo is the first milk animal for the Egyptian farmer. Buffalo has the ability to withstand environmental conditions, resist diseases, and high food conversion efficiency, so it can benefit from poor diets in nutritional value and convert them into milk and meat. It's known that, the most important and most dangerous stage in an animal's life is the first month of its life, which represents the largest percentage of deaths among breeders as a result of diarrhea and respiratory infections **(Georgiev, 2008).** This is because the calf is born with a lack of energy in his body and both the elementary canal and the self-immune system have not completed their growth and functional development yet. Since the placenta of ruminants does not allow the passage of immune bodies from the mother to the fetus, so the calf is born lacking maternal immunity. The calf's access to the mother's colostrum immediately after birth is its only chance to meet its needs for nutrients,energy,immune bodies,hormones,and active biological compounds that help it complete the growth of its organs and the safety of its Physiological performance. Therefore, this research aims to take some measures in the blood serum of calves, as well as recording birth weight, growth rate, incidence of diarrhea and the time required to complete recovery as an indicator of the effect of both the sex of the newborn calves and the vaccination of the mothers before birth on the biological and immune performance of newborn buffalo calves.

MATERIAL AND METHODS

This study was carried out at Mehlet Moussa Experimental Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, and the experimental work lasted for 5 months from Mars to May 2017.

Sixteen newborn calves were selected, divided as follows: 8 calves (4 males and 4 females) from mothers who had been vaccinated before birth using (Scour guard - 4k) from Zoetis Inc (REF), Veterinary Services, USA. and 8 calves (4 males and 4 females) from mothers who were not vaccinated before birth, to form four groups four animals in each group. four Male calves born from vaccinated mothers, four Female calves born from vaccinated mothers, four male calves born from unvaccinated mothers,and four Female calves born from unvaccinated mothers.

Dams' vaccination system:

Sixteen buffalo dams at the last gestation period were vaccinated with Scour Guard 4k vaccine against Bovine Rotavirus (Serotypes G6 and G10), Coronavirus, enterotoxigenic strains of Escherichia Coli Bacteria and Clostridium perfringens Type C,by injecting 2 ml intramuscularly twice, the first dose at 8 weeks before the expected calving date, and they were revaccinated with the same dose 5 weeks before the expected calving date. According to the directions of Zoetis Inc (REF), Veterinary Services, USA.

Experimental work:

The newborn buffalo calves were separated from their mothers immediately after birth and were not allowed to breastfeed. They were artificially fed colostrum at a rate of 10% of their body weight per day and kept under the management conditions applied on the farm. The calves were weighed immediately after birth and then the weight was repeated every 15 days until weaning on 90 days. The cases of diarrhea and the time required for the recovery is recorded.

Blood samples collection:

Blood samples from newborn calves were collected from jugular vein just at birth and then at 6, 12, 24 hours after birth. The serum samples were obtained after the centrifugation of coagulated blood at 4000 rpm for 15 minutes. The serum was separated and kept frozen at -20°C until the time of analysis **(Souza *et al.*, 2019).**

Serum samples analysis:

Kits "Bio Check" were used for the quantitative determination of triiodothyronine hormone (T3), and thyroxine hormone (T4) concentration in serum. In theT3 and T4 EIA.

A WKEA kit (Wkea Med Supplies Corporate, China) was used to assay Bovine Immunoglobulin G (IgG) and Insulin-like growth factor 1 (IGF-1) level in the bovine serum.

Statistical analysis:

The data collected on calves' blood samples, and calves' body weights were statistically analyzed by the least-squares procedure of the general linear model (GLM) of the (**SAS program Institute, 2004)**. The separation of means was done using Duncan's new multiple range test **(Duncan, 1955)** for comparisons among the significant means. Enumeration data of calves' health state were tested by chi-square procedure **(Snedecor and Cochran, 1993).**

The fixed model used for calves' blood in the analysis was:

Yijk = μ + Si + Vj + Tk + SVij + STik + VTjk +SVTijk + εijkl Where:

Yijk = is the value of the respective variable.

μ = is the overall mean of the respective variable.

Si = is the effect due to the ith calves' sex, i = 1, 2 (1= Female, 2= Male).

Vj = is the effect of the jth vaccine j = 1, 2 (1= Unvaccinated, 2= Vaccine).

Tj = is the effect of the kth time k = 1, 2, 3 and 4 (1=at birth, 2=6, 3=12, 4=24 hours after birth).

SVij = is the effect of interaction between calves' sex and vaccine.

STik = is the effect of interaction between calves' sex and time.

VTjk = is the effect of interaction between vaccine and time.

SVTijk = is the effect of interaction between calves' sex, vaccine and time.

Eijkl = is a random error associated with the ijklth observation and is assumed to be independently and normally distributed.

The fixed model used for calves' body weights in the analysis was:

Yijk = μ + Si + Vj + SVij + εijk Where:

Yijk = is the value of the respective variable.

μ = is the overall mean of the respective variable.

Si = is the effect due to the ith calves' sex, i = 1, 2 (1= Female, 2= Male).

Vj = is the effect of the jth vaccine j = 1, 2 (1= Unvaccinated, 2= Vaccine).

SVij = is the effect of interaction between calves' sex and vaccine.

Eijk = is a random error associated with the ijkth observation and is assumed to be independently and normally distributed.

RESULT AND DISCUSSION

Calves blood sample were collected at four times (At birth, 6 hours after birth, 12 hours after birth and 24 hours after birth) to determinate the **e**ffect of calf gender, prepartum buffalo dam vaccination and variable time of blood samples on calves' blood serum concentration of IGF-1 hormone, IgG, T3 hormone and T4 hormone. As shown in (Table 1), Fig. (1-7).

Table (1): Mean ± SE for blood serum concentration of insulin like growth factor-1 IGF-1 (ng/ml) immunoglobulin G (IgG) (mg/dl), triiodothyronine hormoneT3 (ng/dl) and thyroxine hormone T4 (µg/dl) for male and female calve born from vaccinated or unvaccinated dams.

Independent Variables	Calves Blood			
	IGF-1	IgG	T4	T3
Calves Sex				
Female	**71.69±0.55^{b}**	**249.43±7.68^{b}**	**9.21±0.37^{b}**	**139.53±5.20**
Male	**77.19±0.37^{a}**	**313.06±11.72^{a}**	**10.21±0.36^{a}**	**138.65±2.21**
P-Value	**<.0001*****	**<.0001*****	**<.0001*****	**0.5395^{Ns}**
Vaccination				
Unvaccinated	**74.06±0.69b**	**270.43±9.69b**	**10.37±0.38a**	**147.98±4.91a**
Vaccinated	**74.81±0.66a**	**292.06±12.65a**	**9.05±0.34b**	**130.20±1.63b**
P-Value	**0.0037****	**<.0001*****	**<.0001*****	**<.0001*****
Time after birth				
At birth	**71.51±0.91^{d}**	**213.68±6.63^{d}**	**9.00±0.27^{b}**	**133.65±1.81^{b}**
6 hr. after birth	**73.00±0.81^{c}**	**338.25±14.41^{a}**	**12.60±0.57^{a}**	**164.42±7.99^{a}**
12 hr. after birth	**75.51±0.69^{b}**	**311.87±9.80^{b}**	**8.75±0.18^{c}**	**131.27±1.65^{c}**
24 hr. after birth	**77.71±0.58^{a}**	**261.18±11.10^{c}**	**8.50±0.14^{d}**	**127.02±1.92^{d}**
P-Value	**<.0001*****	**<.0001*****	**<.0001*****	**<.0001*****

$^{a-b-c-d}$ means, within a column, with different superscripts difference significantly (Ns (non-significant) = p>0.05, **= p<0.01 and ***= p<0.001).

Blood serum IGF-1 concentration (ng/ml) for male and female calves born from vaccinated or unvaccinated dams:

Insulin-like growth factor hormone (IGF-1) has an important role in stimulating the growth of the newborn calf, encouraging bone and muscle growth. It also has a stimulating effect to increase the efficiency of the passage of nutrients through the cell walls and encourage the

metabolism of glucose, amino acids and nucleotides, which inhibits cell death and increases the efficiency of the process of cell division and increase the growth **(Georgiev,2008).**

The newborn calf gets the IGF-1 hormone through the mother's colostrum, but its absorption in the calf's intestines is slow, so we found that, the newborn calf can synthesis the IGF-1 hormone early after birth, especially in the liver.Therefore, the liver is the main source of IGF-1 hormone concentration in the calf's blood, not what it gets from the mother's colostrum **(Blum *et al.*, 2002)**. The amount that, the calf obtains from the IGF-1 hormone immediately after birth through the feeding of the colostrum is of great importance because it helps to develop the digestive tract and increase the efficiency of absorption of nutrients from the intestines and increase the efficiency of glucose metabolism, which activates the liver cells and the formation of a stock of glycogen that helps in the representation of proteins and the synthesis of a greater amount of IGF-1 hormone **(Wang *et al.*, 2012)**.

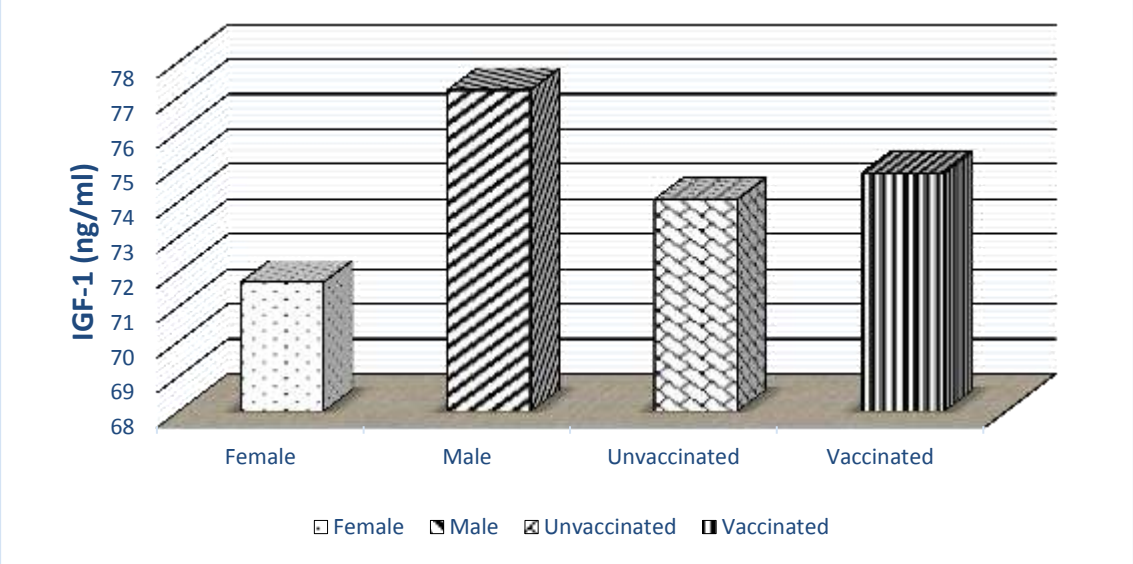

Fig (1): Effect of calf gender, prepartum buffalo dam vaccination on calves' blood serum concentration of IGF-1(ng/ml).

As it is clear from Fig. (1), the gender effect of the male calf was highly significant on the concentration of the IGF-1 hormone in the calves' blood ($p<0.0001$). Prenatal vaccination of mothers also had a significant effect on the blood IGF-1 hormone concentration ($p=0.0037$). It is clear from Fig. (1) that both the sex of the male newborn calf and the vaccination of the mothers before birth had a positive effect on the IGF-1 hormone level in the calf's blood,

which is consistent with what was mentioned by **(Sirotkin *et al.*, 2002)** that male newborn calves achieve a higher growth rate despite consuming less food compared to new born female calves. It also agrees with what was mentioned by **(Mastellone *et al.*, 2007)** about the existence of a positive relationship between the concentration of immune antibodies in the blood of the newborn calf during the first 24 hours after birth and growth performance during the first month of the calf's life.

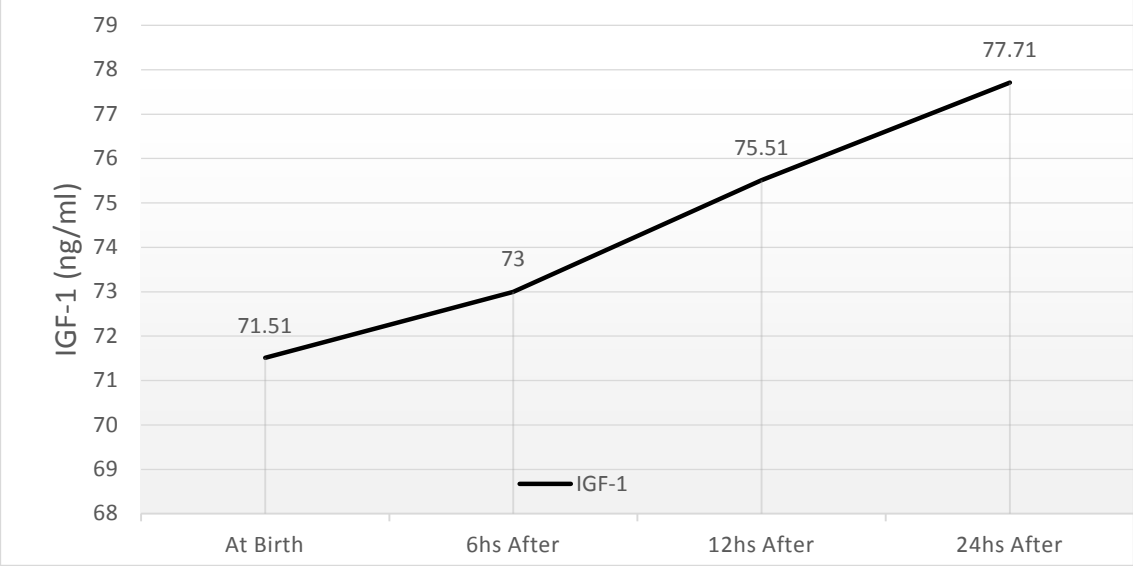

Fig (2): Effect of variable time of blood samples on calves' blood serum concentration of IGF-1 (ng/ml).

Fig. (2) shows that, the concentration of the hormone in the blood of newborn calves increased gradually during the first 6 hours of calving, and this may be due to the absorption of the maternal hormone through colostrum feeding. Then it increased significantly during the first 24 hours after birth, which may be due to stimulating the liver of the newborn calf to synthesis the hormone, which the calf desperately needs at this stage to complete its growth.

Blood serum IgG concentration (mg/dl) for male and female calves born from vaccinated or unvaccinated dams:

The placenta of a buffalo does not allow the passage of immune bodies from the mother's blood into the fetus's blood, so the calf is born deprived of the maternal immune bodies **(Kuralkar and Prajakta 2010).** The only source from which a newborn calf can obtain maternal antibodies is through colostrum feeding during the first hours after birth **(Souza *et***

***al.,* 2019)**. Buffalo calves are born with a functionally incomplete autoimmune system, as the gene responsible for the synthesis of immune bodies has not yet expressed itself. Therefore, the immunity of the newborn calf is weak against pathogens, especially in the first period of its life, unless it receives a sufficient amount of good colostrum immediately after birth **(Goldsby**

***et al.,* 2000).** IgG represents the dominant percentage (85-90%) of the total immunoglobulins in the colostrum, while the IgM represents 7% and the IgA represents 5% of the total immunoglobulins in the colostrum **(Godden.,2008).** The three classes of immunoglobulins are absorbed from the intestines of lactating calves,but the half-life of both IgM and IgA is relatively short (3-4 days) compared to the half-life of IgG (21-28 days) **(Robbers *et al.,* 2021)**.

The arrival of the immune bodies to the intestines of the newborn calf is not sufficient to obtain the required immunity, but the lesson is the efficiency of the passive immune transfer (PIT) from the intestines into the blood of the calf. The highest efficiency of the passage of immune bodies from the intestine to the blood is achieved immediately after birth and then gradually decreases during the first hours of the calf's life **(Weaver *et al.,* 2000)**.

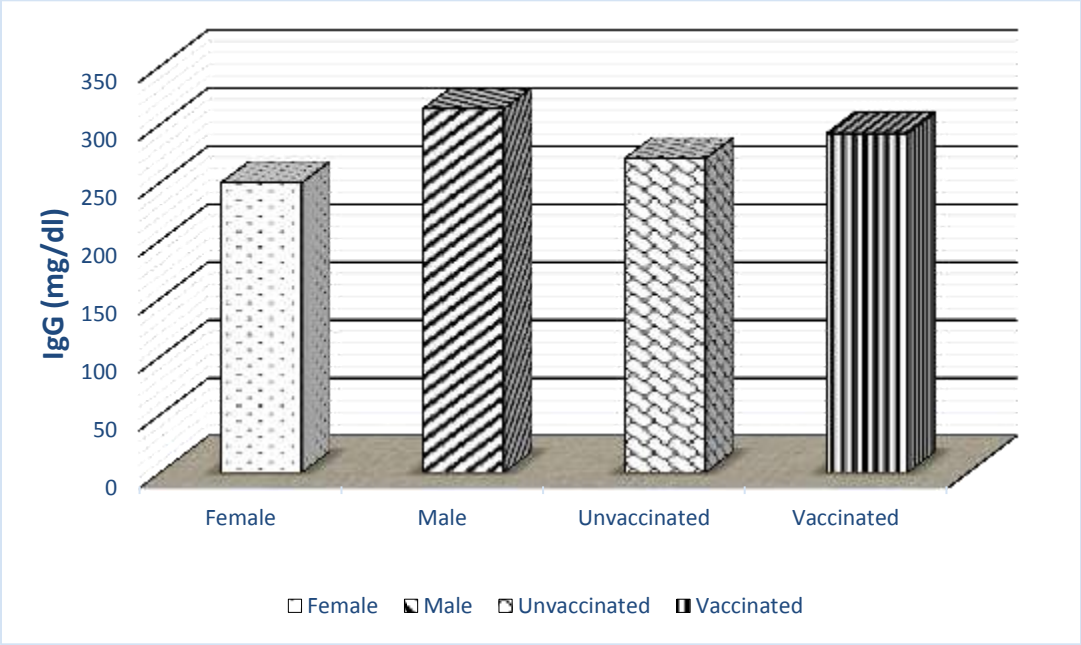

Fig (3): Effect of calf gender, prepartum buffalo dam vaccination on calves' blood serum concentration of IgG (mg/dl).

Fig. (3) shows that, the concentration of IgG in the blood of newborn calves was higher in the case of male calves compared to female calves, and it was also higher for calves born to mothers who had been vaccinated before birth compared to calves whose mothers were not vaccinated before birth.

(Angulo *et al.*, 2015) indicated that, the gender of the newborn has an effect on the level of IgG in the dam's serum.There are special receptors present in the epithelial tissue lining the mammary gland which increases the efficiency of the passage of immune bodies from the dam's blood to the milky sacs of the udder and from there to the produced colostrum. These receptors are identical to their counterparts on the epithelial tissue of the intestines of newborn calves, these receptors called the neonatal crystallizable fragment receptor (FcRn) which allow the passage of immune bodies in the colostrum to the calf's blood through the epithelium lining the intestines **(Souza *et al.*, 2020).**

(Hayr *et al.*, 2015) mentioned that, the male fetus has a longer gestation period than the female fetus, which increases the duration of the synthesis of the colostrum and increases the chance of transmission of a greater amount of IgG from the dam's blood to the colostrum before childbirth.

Vaccination of dams before birth stimulates the dam's immune system to produce immune bodies that pass from the mother's blood to the mammary gland and it moves from it to the produced colostrum **(Sasaki *et al.*, 1976).**

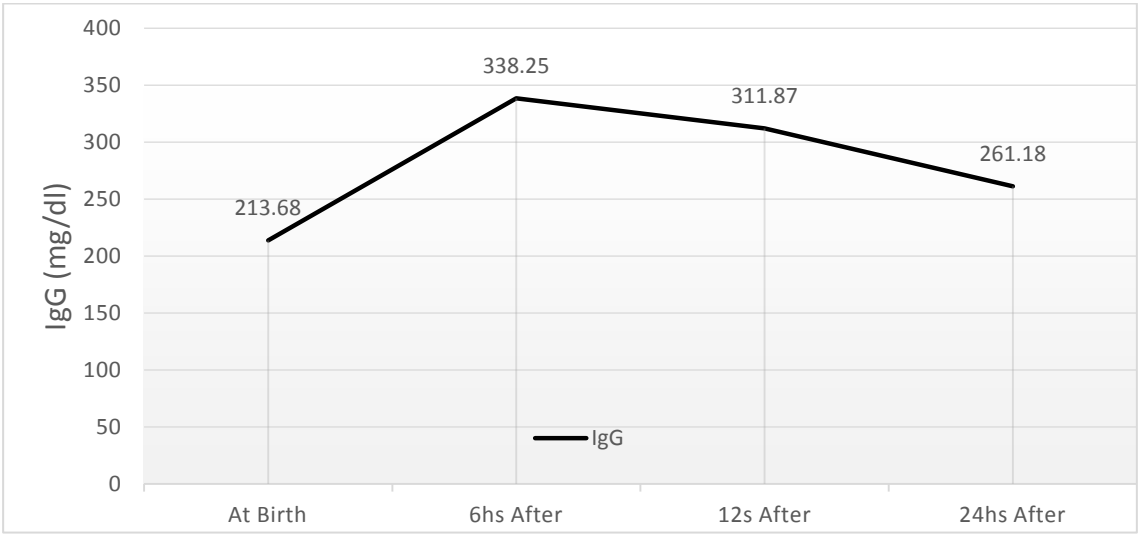

Fig. (4): Effect of variable time of blood samples on calves' blood serum concentration of IgG (mg/dl).

Fig. (4) indicates the change in the concentration of IgG in the blood of a newborn calf during the first 24 hours after birth. It is clear from the figure that, the lowest concentration of IgG is at the birth of the calf and then rises significantly during the first 6 hours, where the greatest activity of the neonatal crystallizable fragment receptor (FcRn) on the wall of the calf's intestines, which increases the intestinal permeability of the immune bodies and allows their passage into the calf's blood. This result is in agreement with **(Schalich *et al.*, 2021)** who Pointed that, the process of passage of immune bodies from the dam's blood to the colostrum continues during the first 6 hours after birth, then the passage of immune bodies gradually decreases with the progression of time.

Blood serum T3 concentration (ng/dl) for male and female calves born from vaccinated or unvaccinated dams:

T4 hormone is the most secreted form of the thyroid gland and has the longest half-life time compared to T3 hormone. Although T3 is more bioactive form than T4. Therefore, the body converts thyroid hormones from the less active form T4 to the more active form T3 by removing an iodine atom from the outer phenyl ring of the T4 molecule (Deiodination process) by the action of selenoenzyme. This process, which occurs mainly in the liver, as it occurs in many other tissues such as the kidneys, placenta, fetal tissues, and other tissues **(Köhrle, 2000).**

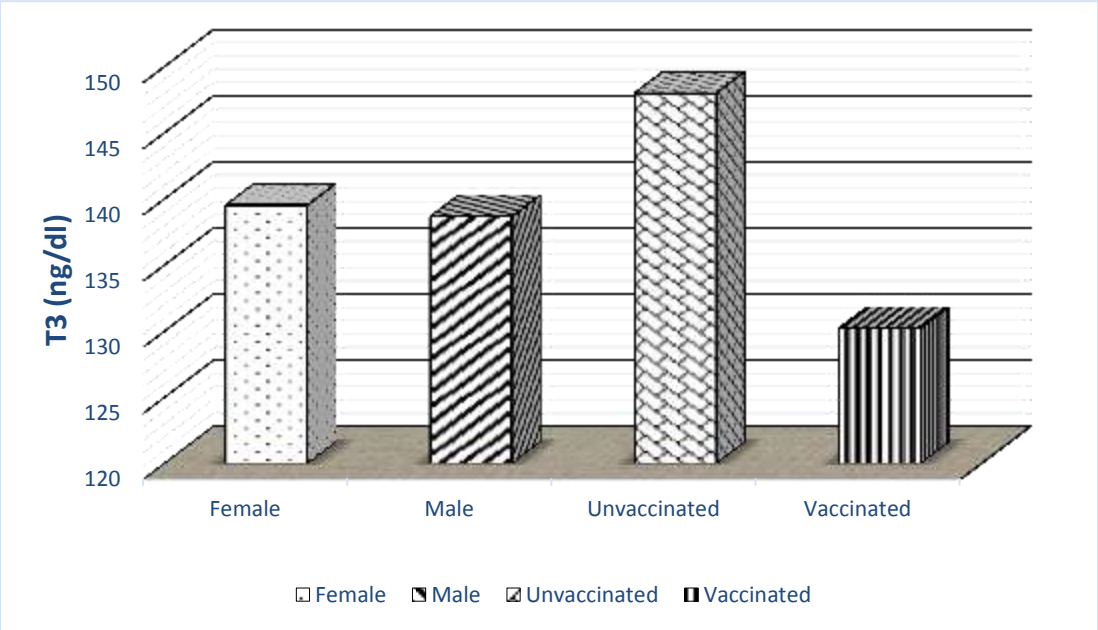

Fig. (5): Effect of calf gender, prepartum buffalo dam vaccination on calves' blood serum concentration of T3 (ng/dl).

Fig. (5) shows that, the sex of the newborn calves had no significant effect on the concentration of T3 hormone in the blood of the newborn calf, while the concentration of T3 hormone in the blood of calves whose mothers had not previously been vaccinated before birth increased.

This may explain that calves born to unvaccinated mothers get fewer immune bodies through the colostrum, so they have more pressure to adapt to the environmental conditions, which requires the secretion of more thyroid hormones, especially the active form, T3 to help increase the efficiency of the passage of immune bodies from the calf's intestines to the blood compared to calves. Born to vaccinated mothers who obtain more antibodies through the colostrum. **(Koenig and Beauchemin 2009)** indicated that, the promotion of T3 hormone production through selenium treatment led to an increase in IgG concentration in the blood of new born calves.

Blood serum T3 concentration (ng/dl) for male and female calves born from vaccinated or unvaccinated dams:

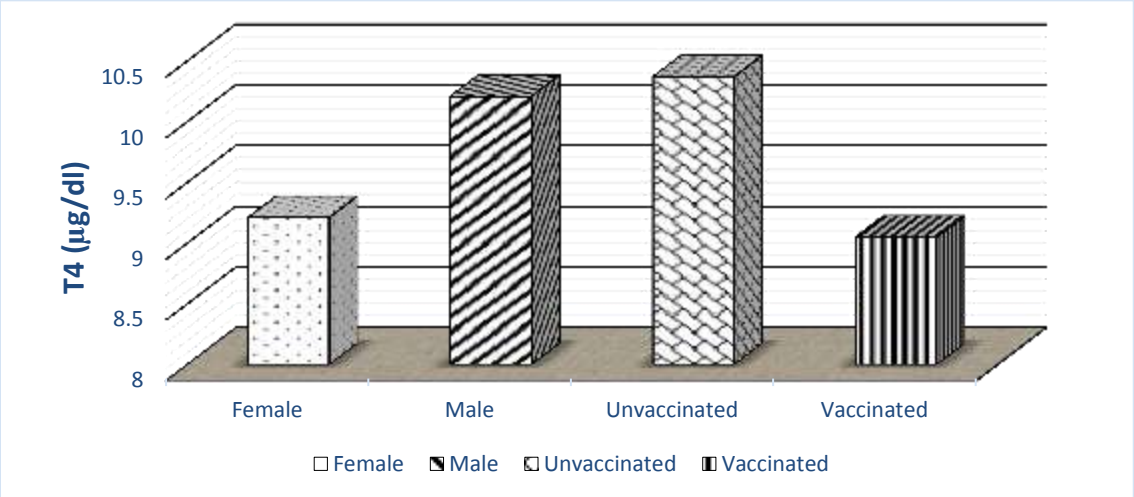

Fig. (6): Effect of calf gender, prepartum buffalo dam vaccination on calves' blood serum concentration of T4 (µg/dl).

Fig. (6) shows that, the sex of the newborn calves and the vaccination of the mothers before birth had a significant positive effect ($p<0.0001$) on the concentration of T4 hormone in the blood of new born calves. This may explain by that, the gender of the sex of male newborn calves stimulated the thyroid gland to produce a larger amount of T4 hormone, which would have a role in the superiority of male calves in the rate of conversion efficiency than females, while the gender of the newborn did not affect the conversion of T4 hormone to the active form T3 during the first 24 hours of calves' life.

(Takahashi *et al.*, 2001) indicated that, the higher the T4 / T3 ratio, the greater the efficiency of the thyroid gland and the exclusion of its infection with the goiter. From the above it is clear that, the efficiency of the thyroid gland of male calves was higher and less likely to get goiter.

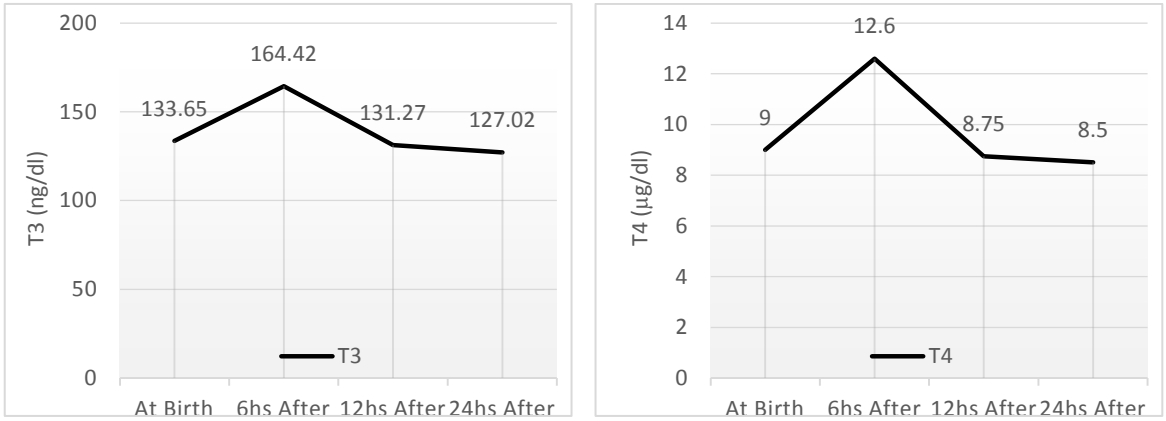

Fig. (7): Effect of variable time of blood samples on calves' blood serum concentration of T3 (ng/dl) and T4 (µg/dl).

Fig. (7) showed that, the highest concentration of thyroid hormones was 6 hours after birth where the calf at this early stage of its life needs a high level of thyroid hormones that help raise the metabolic efficiency and compensate for the lack of energy and immunity in which it is born. Then it decreased significantly during the first 24 hours of birth to reach its normal level. **(Ingole *et al.*, 2012)** mentioned that, the greatest value of T3 andT4 hormones in calves blood serum was from o-7 days after birth.

Table (2): Mean ± SE for interaction between calve sex and dam (vaccine and unvaccinated) for calves weighted every 15-day interval from birth until weaning.

Calves ages	Interactions Body Weight				P-Value
	Female		Male		
	Unvaccinated	Vaccinated	Unvaccinated	Vaccinated	
Birth body weight	30.00±0.12^{d}	32.00±0.16^{c}	35.00±0.24^{b}	37.00±0.12^{a}	<.0001***
15 days old	38.00±0.08^{d}	40.00±0.40^{c}	43.00±0.32^{b}	45.00±0.48^{a}	<.0001***
30 days old	46.00±0.40^{d}	49.00±0.40^{c}	50.00±0.48^{b}	54.00±0.45^{a}	<.0001***
45 days old	56.00±0.16^{d}	58.00±0.61^{c}	60.00±0.24^{b}	63.00±0.42^{a}	0.0226*
60 days old	65.00±0.40^{d}	67.00±0.04^{c}	70.00±0.20^{b}	73.00±0.05^{a}	0.0511*
75 days old	74.00±0.40^{d}	76.00±0.89^{c}	81.00±0.40^{b}	83.00±0.25^{a}	<.0001***
90 days old	85.00±0.81^{d}	90.50±2.80^{c}	92.00±0.53^{b}	94.00±0.53^{a}	0.0268*

$^{a\text{-}b\text{-}c\text{-}d}$ means, within a row, with different superscripts difference significantly (*=p<0.05 and ***=p<0.001).

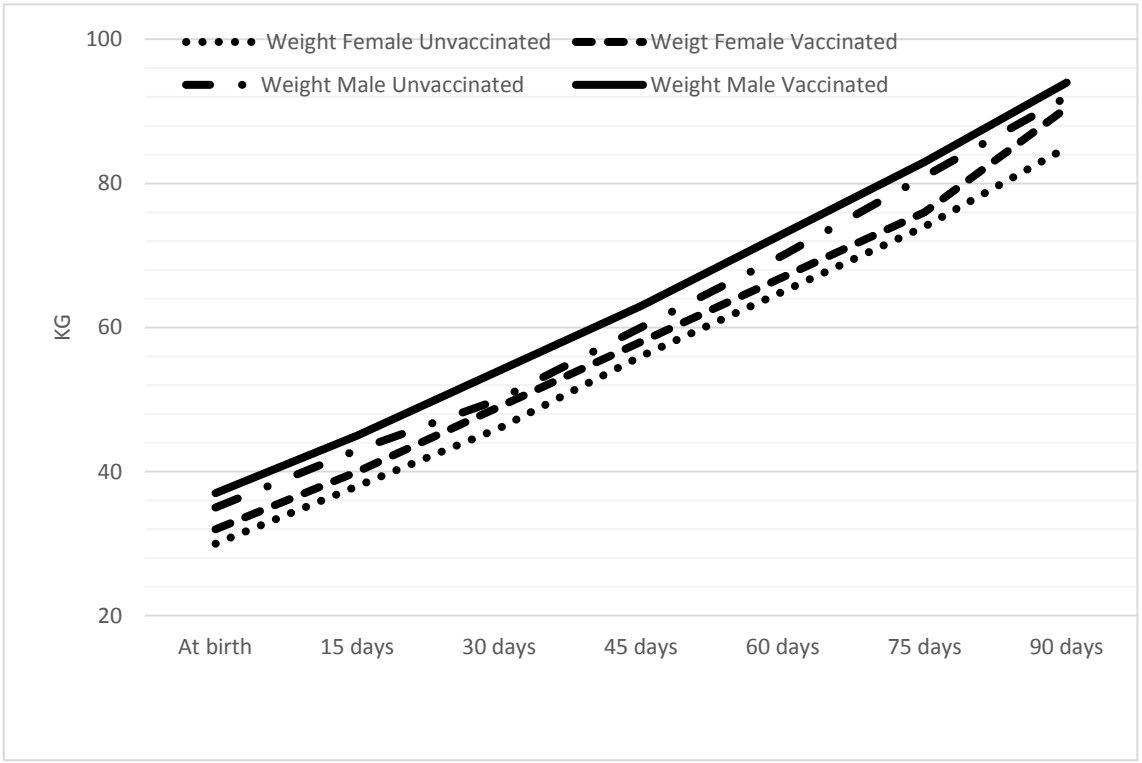

Fig. (8): Effect of calf gender and prepartum buffalo dam vaccination on the growth of body Weight from birth until weaning.

Fig. (8) showed that, the birth weight of male calves was higher and this is consistent with what was reported **(Kul *et al.*, 2018)**. This may be due to many reasons, including:

1- The duration of pregnancy in the case of the male fetus It is longer by two days compared to the gestation period in a female fetus **(Hess *et al.*, 2016)**, which gives the male fetus a longer opportunity to grow inside the uterus.

2- The last third stage of pregnancy, where the growth rate of the fetus increases at a rapid rate and its nutritional needs are increased, and this requires an increase in the efficiency of the mother's metabolism by increasing the secretion of thyroid hormones to cover the needs of the fetus at that stage **(Arfuso *et al.*, 2016)**. The gender of the male fetus has an effect on the increase of the T3 hormone in the mother's blood, and it is considered the active form of thyroid hormones which increases the efficiency of the mother's metabolism and increases her benefit from nutrients in the diets. Thus, it can meet the needs of the male fetus from these elements more than in the case of the female fetus.

3-**(Einspanier and Schams, 1991)** stated that, the highest level of IGF-1 hormone in the dam's blood was two weeks before birth and then decreased significantly after the first lactation.

Male fetus elevated IGF-1 concentration in the blood serum of the pregnant dam in a male fetus. Than in the blood serum of a pregnant dam in a female fetus. What helps stimulate the growth of the male fetus compared to the female one.

Male calves achieved a greater rate of weight gain than females, because the male calf gets the richer colostrum in terms of growth stimulants, nutrients and immune bodies from their dams than the colostrum obtained by the female from her dam **(Tahir *et al.*, 2018).**

Table (3): The incidence of diarrhea and the number of days of infection for male and female calves born from vaccinated or unvaccinated dams.

Treated Groups	Total (N)	Infected		Infected period/day
		n	(%)	

Female	**Unvaccinated**	**4**	**2**	**50**	**5**
Female	**Vaccinated**	**4**	**1**	**25**	**3**
Male	**Unvaccinated**	**4**	**1**	**25**	**4**
Male	**Vaccinated**	**4**	**0**	**0**	**0**
Value of chi-square (χ^2)				**36.12**	**12.27**
Probability of chi-square (χ^2)				**<.0001*****	**0.0001*****

Difference between treated groups within each column are significant (*=p<0.001).**

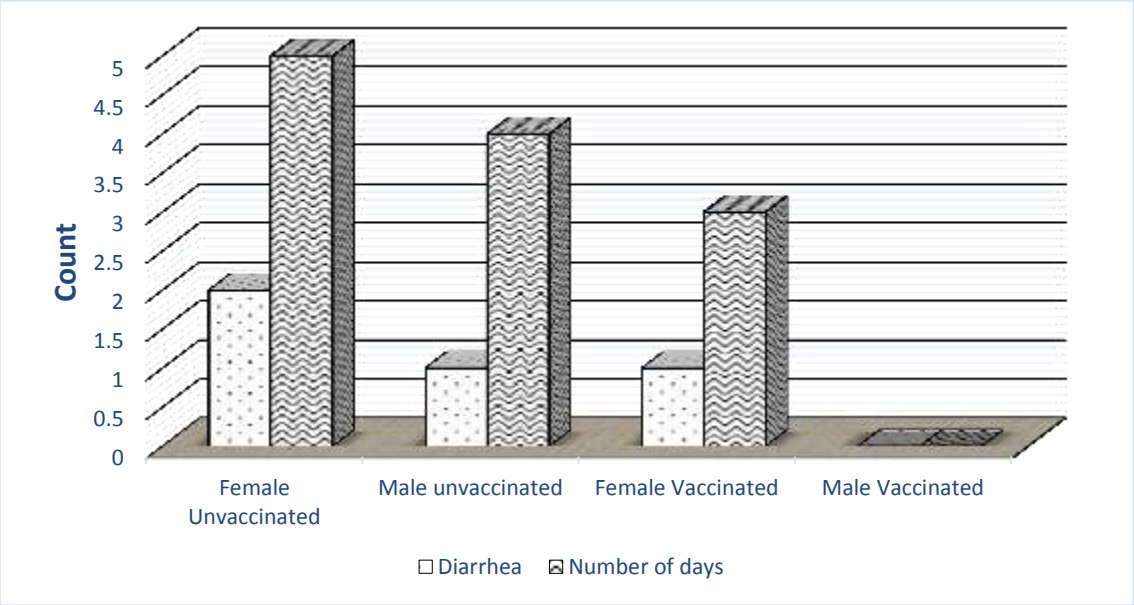

Fig. (9): Effect of calf gender and prepartum buffalo dam vaccination on the incidence of diarrhea and number of infection days.

Fig. (9) showed that, the male calves from vaccinated dams did not suffer from diarrhea, while 25% of the male calves from unvaccinated dams got diarrhea and it took 4 days for treatment to complete recovery. Also, 25% of the female calves from vaccinated dams got diarrhea and it took only 3 days for their treatment to recover, while 50% of the female calves from unvaccinated dams got diarrhea and it took 5 days. This may be due to the higher levels of the immune bodies in the blood of vaccinated male calves than in unvaccinated female calves.

CONCLUSION

This study demonstrated the distinction of newborn male calves in terms of birth weight and growth rate until weaning, as well as their resistance to diarrhea compared to newborn female calves. This explains the increase in the concentration of IGF-1, T4 hormones and IgG in the blood serum of male calves compared to female calves. Also, maternal vaccination before birth increased the concentration of both IgG and GF-1 in the blood serum of newborn calves. This may be due to the fact that, the vaccination of the mothers stimulated them to produce immune bodies that were transmitted to the calf through colostrum feeding, and the improvement in the health status of the calves of vaccinated mothers encouraged the calf's liver to produce a greater amount of IGF-1hormone.

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