RESEARCH REGARDING THE DYNAMICS OF PROTEINS ASSOCIATED TO GESTATION AND PLASMATIC PROGESTERONE IN GOATS OF LOCAL BREEDS IN PRECOCIOUS AND EARLY PREGNANCY

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Abstract

The proteins associated to gestation (PAG) and the serum progesterone were studied in goats of local breeds in Romania (Carpatina n= 9; White of Banat n=13). The researches took place between 2008 and 2009 and the RIA and ELIZA techniques were used in the specialized laboratories within "Ovidius" University Constanta and the Faculty of Veterinary Medicine, Liege, Belgium. These methods were used to determine the value of PAG and of plasmatic progesterone in goats during estrus and different periods of early gestation. The results obtained emphasized the following: on estrus day, the PAG values in goats of the two breeds (n=22) were between 0.036±0.016 ng/ml and 0.95±0.22ng/ml in pregnant animals, 0.58±0.37, respectively, and 0.47±0.19 in non-pregnant animals. On days 25 and 30 after mating, the values increased constantly in the pregnant animals to 3.71±2.18 ng/ml /14.6±5.8 ng/ml for Carpathian and 6.53±1.06 ng/ ml/ 19.6±2.5 ng/ ml in White of Banat. In both breeds, the gestation probability in goats was 90%. The presence of gestation proteins is visibly increased after day 12 from mating, while non-pregnancy is confirmed 100% in all the cases when PAG value was under 2 ng/ml. On estrus day, the plasmatic progesterone, determined by ELIZA, had values of 0.31±0.025 and 0.53±0.27 ng/ml, respectively, in pregnant goats and values of 3.70±1.57 and 3.48±1.63, respectively, in non-pregnant goats. On day 14, the value of plasmatic progesterone increases suddenly in the pregnant animals to 13.60±2.65 ng/ml (Carpathian) and 20.69±4.09ng/ml (White of Banat), reaches a peak on day 25 (19.10±3.13 ng/ml in Carpatian and 20.69 ± 4.09 ng/ml, respectively) and is maintained high till day 30 (16.37±2.41 ng/ml, 18.37± 3.39 ng/ml, respectively). The originality of the tests consists in the fact that on day 14, the test has a diagnosis value of 100%, progesterone values above 13 ng/ml confirming the persistence of corpus luteum and the occurrence of pregnancy in goats. The cost of the ELIZA test (progesterone) is 18.75 lei and it is recommended for the monitoring of the reproduction activity in goats.

Key words: goat, PAG, progesterone, gestation diagnosis

INTRODUCTION

The glycoproteins associated to gestation (PAG) have not yet been studied in sheep and goats of local breeds in Romania. The study of PAG and plasmatic progesterone in the first 30 days of gestation is very important for the evaluation of precocious or early gestation in sheep and goat.

In goat, the immunological antigens were identified in the peripheral circulation of pregnant females and three types of PAG were purified partially. These are present in detectable quantities nine-ten days after fertilization and remain in the maternal bloodstream till parturition [1, 2, 17]. Ranilla [15] did not observe any correlation between the speed at which PAG concentration decreases and the occurrence of the first ovulation. Up to now, PAG determination was accomplished by RIA, but more recent research perfected determination techniques by the ELIZA method [9].

Progesterone is a C12 steroid hormone, which contains a keto group (at C-3) and a

double bond between C-4 and C-5. This is a hormone specific to the feminine sex, which, in association with estrogen, regulates the sexual cycle and has a particular importance in what regards the preparation of the endometer for the implantation of the blastocyst and the keeping of the pregnancy. In non-gestation state, progesterone is secreted by corpus luteum, while in gestation state, it is secreted by corpus luteum and placenta.

The objectives of the present study were to establish the dynamics of the proteins associated to gestation (PAG) in different days of the precocious gestation by RIA and of the progesterone concentration by ELIZA, for the prediction of gestation in Carpathian and White of Banat goat breeds.

MATERIALS AND METHODS

a) Biological material: the experiment was accomplished on 22 Carpathian and White of Banat goats, which belonged to two private farms. The blood was collected from goats on days 0 (estrus), 7, 14, 21, 25, 30 of the estrous cycle, in heparinate vacutainer tubes. The plasma was stored at -18 °C until PAG were determined by RIA and progesterone by ELIZA. The final evaluations were accomplished after the kiddings took place on time (December 2008 – February 2009).

b) RIA analysis for the determination of PAG was accomplished in the Reproduction Physiology Laboratory – The Faculty of Veterinary Medicine (Liege, Belgium) in December 2008. The radioimmunological dosing was accomplished by RIA with pre-incubation for all the serums resulted from day 0 till day 30 from mating. The marked antigen and the antiserums were offered by the Biotechnologies Laboratory in the Faculty of Veterinary Medicine (Liege, Belgium). The radioactivity of the immune complexes was measured on a Multi GammaCounter device, type WALACE.

c) ELIZA analysis for the determination of plasmatic progesterone

The determination of the serum progesterone was accomplished using the Progesteron DRG immunoenzymatic kit (Germany), which provides the material support for the quantitative determination of progesterone in serum and plasma, for the *in vitro* diagnosis [4]. The analysis was accomplished using the Tecan device with annexes for reading, shaking, washing and the computerized processing of the data, owned by the Laboratory of Cellular and Molecular Biology in "Ovidius" University.

The test of the ELIZA Progesteron DRG Kit is based on the principle of competitive bonding. The determination plates display polyclonal antibodies for the antigenic situs of the progesterone molecule. The endogen progesterone in different samples from females is conjugated with the mare peroxidase in order to bind to the antibodies. After incubation, the unbounded conjugate is removed by washing. The quantity of conjugated peroxidase is inversely proportional to the concentration of progesterone in the sample. By adding substrate solution, the color intensity increases inversely proportional to the concentration of progesterone in the patient sample.

The statistical processing of the quantitative data obtained was accomplished by the T-student test. The results are presented as standard mean±error.

RESULTS AND DISCUSSIONS

1. The determination of PAG proteins by radioimmunoanalysis (RIA)

After the kidding on time of the goats in both breeds, these were separated into two groups: pregnant goats (which includes all the females that got pregnant after mating on estrus day) and non-pregnant goats (which includes the females that did not kid after mating on day 0 and those that kidded consecutively to matings subsequent to the analyzed period, two estrous cycles, respectively).

The results regarding the PAG dynamics in Carpathian goats are presented in table 1, while those regarding White of Banat goats are in table 2.

Table 1. PAG dynamics in Carpathian goats

Groups	Days	N	Statistical indices			
			x±sx	Min-Max	Median	CV%
PAG in pregnant goats	Day 1	4	0.036±0.016	0.02-0.082	0.02	87%
	Day 7	4	1.133±0.5	0.02-2.365	1.0735	88%
	Day 14	4	0.019±0.001	0.015-0.02	0.02	13%
	Day 21	4	2.5±0.4	0.607-2.464	1.4665	54%
	Day 25	4	3.71±2.18	1.052-10.158	1.809	118%
	Day 30	4	14.6±5.8	1.045-27.79	14.776	79%
PAG in non-pregnant goats	Day 1	5	0.58±0.37	0.02-1.919	0.091	143%
	Day 7	5	1.52±0.57	0.145-3.534	1.562	85%
	Day 14	5	0.65±0.42	0.02-1.819	0.38	129%
	Day 21	5	2.22±0.84	0.468-5.168	1.455	92%
	Day 25	5	2.03±0.84	0.468-5.168	1.455	92%
	Day 30	5	1.61±0.59	0.243-3.354	1.014	82%

On estrus day, in the nine Carpathian females, the PAG value (n=4; n=5) was between 0.036±0.016 ng/ml and 0.58±0.37 ng/ml. On day 7, the PAG value increased in the pregnant goats to 1.133±0.5 ng/ml, while in the non-pregnant ones, to 1.52±0.57 ng/ml (table 1). A similar evolution is recorded in the two groups of White of Banat (table 2). From the physiological point of view, both groups are in the metestrus phase, when corpus luteum is forming and apparently there are no sufficient trophoblastic signals to indicate an important PAG increase.

The measurement of PAG and progesterone concentration in sheep and goat maternal blood as method to detect gestation was demonstrated by Sousa [6, 16]. In the pregnant sheep and goats, the plasmatic concentrations of PAG were similar to those obtained by us. These were detectable starting with days 17-18 post-conception, reaching 3-5 ng/ml around days 21-22. The PAG concentrations increase till days 8 of pregnancy (30-50 ng/ml), and then they decrease between week 12 and 14 (16-32 ng/ml). After that, the values remain relatively constant till kidding [7, 10].

Table 2. PAG (ng/ml) dynamics in White of Banat goats

Groups	Days	Statistics				
		N	x±sx	Min-Max	Median	CV%
PAG pregnant goats	Day 1	7	0.95±0.22	0.106-1.552	1.06	60%
	Day 7	7	1.05±0.31	0.02-2.08	1.265	80%
	Day 14	7	1.55±0.36	0.02-2.444	1.906	63%
	Day 21	7	2.75±0.46	1.82-4.905	2.081	44%
	Day 25	7	6.53±1.06	2.538-10.158	6.939	43%
	Day 30	7	19.6±2.5	10.02-27	18.433	34%
PAG non-pregnant goats	Day 1	6	0.47±0.19	0.02-0.936	0.4685	100%
	Day 7	6	1.48±0.42	0.146-3.236	1.5115	69%
	Day 14	6	0.72±0.34	0.02-1.92	0.4325	115%
	Day 21	6	1.34±0.31	0.028-2.081	1.5505	57%
	Day 25	6	2.41±0.5	0.858-4.326	2.3365	51%
	Day 30	6	2.18±0.41	0.8-3.326	2.307	47%

The configuration of gestation proteins is similar after 14 days from mating. A slight increase can be observed only on day 21, when PAG values increase to 1.22±0.84 ng/ml in Carpathian and to 2.75±0.46ng/ml in White of Banat. On day 25, the PAG values for Carpathian were 3.71±2.18 ng/ml and 6.53±1.06 ng/ ml in White of Banat. On day 30 from mating, the PAG values increased to 14.6±5.8 ng/ml in Carpathian and to 19.6±2.5 ng/ ml in White of Banat.

In what regards the evolution of gestation proteins in the non-pregnant goats, in estrus, the detected PAG was under 1 ng/ml, while in the other phases it did not exceed 2.41 ng/ml. the trend of gestation proteins in all the goat groups analyzed emphasized minimal values in estrus (maximum 0.9 ng/ml) and a maximum of 2.5 ng/ml on day 7. The results obtained are in concord with the researches of other studies, which show the PAG increase after embryo implantation.

The proteins associated to gestation (PAG) are synthesized by the placenta in ruminants and are part of the family of aspartic proteinases, alongside pepsinogen, rennin, and cathepsins E and D, which have a remainder of aspartic acid in the catalytic situs [9, 18]. These are detectable in the maternal blood when the embryo attaches permanently and the placenta forms, when the binuclear trophoblastic cells begin to migrate and fuse with the endometrial cells forming the fetomaternal sincitium [19]. In the veterinary practice, the PAG concentration can become useful both in pregnancy detection and for monitoring the trophoblastic function [5, 11].

2. Establishing progesterone concentration by ELIZA as prediction method for gestation in goat

Progesterone circulates in blood bound to corticosteroid globulin (CBG), sexual hormones globulin (SHBG) and albumin. Only 2-10% of the total concentration circulates as free hormone in blood. The blood concentration of progesterone varies according to the phases of the menstrual cycle; it is under 1 ng/ml (3.2 nmol/l) in the follicular phase and approximately 10-20 ng/ml (32-64 nmol/l) in the luteal phase. The maximum level is reached 4-7 days after ovulation and it remains high over the entire gestation period in most animal species [13, 15]. This is why the monitoring of the circulatory progesterone establishes with high precision the diagnosis for precocious or early gestation. The progesterone concentration may vary from one individual to the next [12].

Table 3. The dynamics of serum progesterone in pregnant and non-pregnant goats (Carpathian breed)

Groups	Collection day	N	x±sx	Min-Max	Median
Pregnant goats	0-estrus	4	0.31±0.025	0.02-2.17	0.45
	14	4	13.60±2.65	0.30-25.00	12.29
	25	4	19.10±3.13	1.59-38.56	16.25
	30	4	16.37±2.41	1.67-25.64	15.15
Non-pregnant goats	0-estrus	5	3.70±1.57	0.23-13.71	1.53
	14	5	5.84±1.71	0.19-11.76	5.46
	25	5	3.72±1.09	0.25-10.08	2.74
	30	5	2.4±10.74	0.19-5.56	2.13

Table 4. The dynamics of serum progesterone in pregnant and non-pregnant goats (White of Banat breed)

Group	Collection day	N	x±sx	Min-Max	Median
Pregnant goats	0-estrus	7	0.53±0.27	0.02-2.05	0.1
	14	7	12.99±3.46	0.40-24.00	14.32
	25	7	20.69±4.09	2.89-37.85	18.96
	30	7	18.37±3.39	1.65-29.62	18.97
Non-pregnant goats	0-estrus	6	3.48±1.63	0.21-10.13	1.84
	14	6	4.99±1.45	0.63-10.36	5.75
	25	6	3.77±1.24	0.54-7.65	3.15
	30	6	3.16±0.74	0.23-5.54	3.15

On estrus day, the plasmatic progesterone, determined by ELIZA, had values of 0.31±0.025 and 0.53±0.27 ng/ml, respectively, (in pregnant goats) and 3.70±1.57, 3.48±1.63, respectively, in non-pregnant animals (table 3 and 4). On day 14 the value of plasmatic progesterone increases suddenly in the pregnant animals: 13.60±2.65 ng/ml (Carpathian) and 20.69±4.09ng/ml (White of Banat), reaches a peak on day 25 (19.10±3.13 ng/ml in Carpathian, 20.69 ± 4.09 ng/ml, respectively) and is maintained high till day 30 (16.37±2.41 ng/ml, 18.37± 3.39 ng/ml, respectively). The progesterone profile in non-pregnant animals follows an increasing pattern starting with day 0 towards day 14. After that, it decreases towards a minimal value (2.4±10.74ng/ml), which corresponds to the metestrus/diestrus (table 3 and 4). Of the total 23 goats with known physiological situation, only two females which had kids on time displayed an atypical dynamics, with low progesterone values in all the four collections, resulting a gestation precision of 90%.

The originality of tests consists in the fact that on day 14, the test has a diagnosis value of 100%. From the practical point of view, in order to obtain a certain gestation diagnosis, the progesterone determination is made on day 14 with maximum precision. The cost of the test for one sample is 18.75 lei, which is accessible for breeders who want to monitor the reproduction activity of their goats.

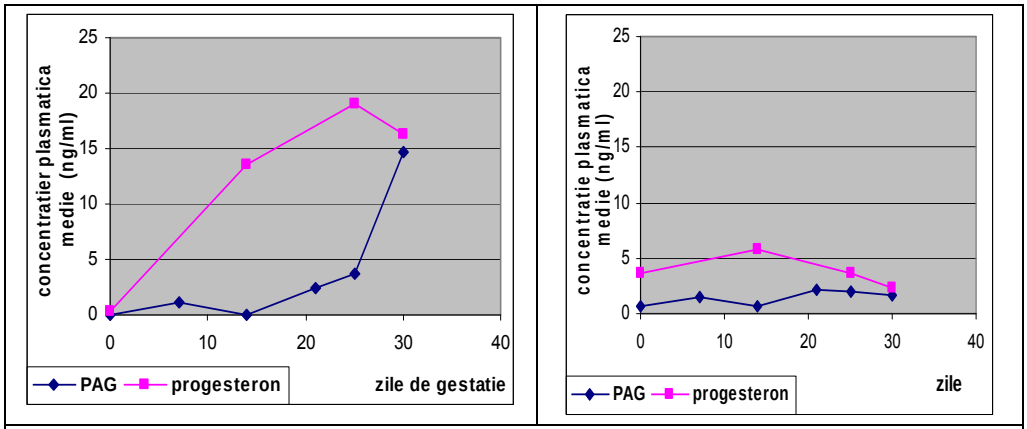

Fig.1 PAG and progesterone variation in Carpathian goats

a) pregnant b)non-pregnant

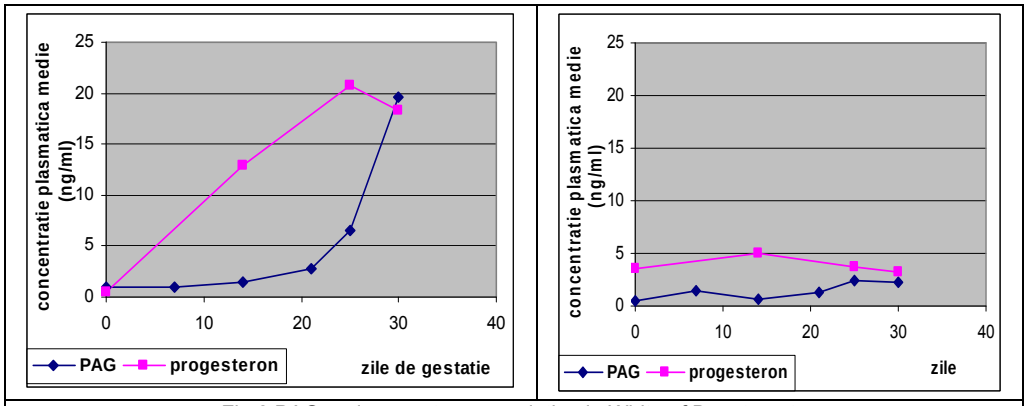

Fig.2 PAG and progesterone variation in White of Banat goats
a) pregnant b)non-pregnant

The progesterone concentration is useful in the veterinary practice in order to detect ovulation and estrus [3, 8], but also follicular cysts [6, 13]. In goats, as well as other ruminants, the progesterone concentration in the peripheral circulation is periodically modified over the period of the estrous cycle. The minimum value is reached during estrus and then it increases gradually and reaches the maximum value during the luteal phase [13, 14]. The results obtained in this study demonstrate the use of the ELIZA method for the dosing of progesterone in order to determine the gestation state in small ruminants.

CONCLUSIONS

1. On estrus day, the PAG value in pregnant goats of the two breeds (n=23) was between 0.036±0.016 ng/ml and 0.58±0.37 ng/ml.

2. On day 21, the PAG values increased to 2.5±0.4 ng/ml in the Carpathian breeds and to 2.75±0.46ng/ml in White of Banat.

3. On day 25, the PAG values for Carpathian were 3.71±2.18 ng/ml and 6.53±1.06 ng/ ml in White of Banat.

4. On day 30 from mating, the PAG values increased to 14.6±5.8 ng/ml in Carpathian goats and to 19.6±2.5 ng/ ml in White of Banat goats.

5. The 90% gestation probability in goats was confirmed after day 25.

6. On estrus day, plasmatic progesterone had the following values: 0.31±0.025 and 0.53±0.27 ng/ml in pregnant goats and 3.70±1.57, 3.48±1.63, respectively, in non-pregnant goats.

7. On day 14, the value of plasmatic progesterone increases suddenly in the pregnant animals to 3.60±2.65 ng/ml (Carpathian) and 20.69±4.09ng/ml (White of Banat), reaches a peak on day 25 (19.10±3.13 ng/ml in Carpathian and 20.69 ± 4.09 ng/ml, respectively) and is maintained high till day 30 (16.37±2.41 ng/ml, 18.37± 3.39 ng/ml)

8. The originality of the tests consists in the fact that on day 14, the test has a diagnosis value of 100%, progesterone values above 13 ng/ml confirming the persistence of corpus luteum and the occurrence of pregnancy in goats.

9. The cost of the ELIZA test (progesterone) is 18.75 lei and it is recommended for the monitoring of the reproduction activity in goats.

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BIBLIOGRAPHY

[1] Barbato O, Sousa NM, Debenedetti A, Canali C, Todini L, Beckers JF, 2009, Validation of a new pregnancy-associated glycoprotein radioimmunoassay method for the detection of

early pregnancy in ewes, Theriogenology, 72 (7):993-1000
[2] Batalha E, 2001, Plasma profile of pregnancy associated glycoprotein (PAG) in pregnant Alpine goats using two radioimmunoassay (RIA) systems, Small Ruminant Research, 42(2):111-118
[3] Blaszczyk B, Stankiewicz T, Udala J, 2009, Plasma progesterone analysis by a time-resolved fluorescent antibody test to monitor estrous cycles in goats, J of Veterinary Diagnostic Investigation, 21 (1):80-87
[4] Boscos C M, Samartzi F.C., Lymberopoulos A.G., Stefanakis A., Belibasaki S., 2003, Assessment of progesterone concentration using enzyme immunoassay, for early pregnancy diagnosis in sheep and goats, Reproduction in domestic animals, 38(3):170-174
[5] Drion P V, Zarrouk A, Sulon J, Szenci O, Beckers JF, Perényi Z, Remy B, Garbayo B,1999, Pregnancy associated glycoproteins in ruminants: inactive members of the aspartic proteinase family, Acta Veterinaria Hungarica, 47 (4):461-469
[6] González F, Cabrera F, Batista M, Rodríguez N, Álamo D, Sulon J, Beckers JF, Gracia A, 2004, A comparison of diagnosis of pregnancy in the goat via transrectal ultrasound scanning, progesterone, and pregnancy-associated glycoprotein assays, Theriogenology, 62(6):1108-1115
[7] González F, Sulon J, Garbayo JM, Batista M,Cabrera F, Calero PO, Gracia A, Beckers JS, 1999, Early pregnancy diagnosis in goats by determination of pregnancy-associated glycoprotein concentrations in plasma samples. Theriogenology 52: 717–725
[8] Green JA, Xie S, Quan X, Bao B, Gan X, Mathialagan N, Beckers JF, Roberts M, 2000, Pregnancy-Associated Bovine and Ovine Glycoproteins Exhibit Spatially and Temporally Distinct Expression Patterns During Pregnancy, Biology of Reproduction, 62(6):1624-1631
[9] Green JA, Parks TE, Avalle MP, Telugu BP, McLain AL, Peterson AJ, McMillan W, Mathialagan N, Hook RR, Xie S, Roberts RM, 2005, The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers,Theriogenology 63:1481–1503
[10] Haugejorden G, Waage S, Dahl E, Karlberg K, Beckers JF, Ropstad E, 2006, Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring, Theriogenology,66(8):1976-1984
[11] Karena A, Beckers JF, Sulon J, Sousab NM, Szabadosc K, Reczigeld J, Szencia O, 2003, Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests, Theriogenology, 59 (9) 1941-1948
[12] Lobago F, Bekana M, Gustafsson H, Beckers J F, Yohannes G, Aster Y, Kindahl H, 2009, Serum Profiles of Pregnancy-Associated Glycoprotein, Oestrone Sulphate and Progesterone During Gestation and Some Factors Influencing the Profiles in Ethiopian Borana and Crossbred Cattle, Reproduction in domestic animals, 44(4): 685-692
[13] Medan MS, Watanabe G, Sasaki K, 2003, Ovarian dynamics and their associations with peripheral concentrations of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats, Biol Reprod, 69:57-63
[14] Medan MS, Watanabe G, Sasaki K, Taya K, 2004, Transrectal ultrasonic diagnosis of ovarian follicular cysts in goats and treatment with GnRH, Domest Anim Endocrinol, 27:115-124
[15] Ranilla M, Sulon J, Carro M, Mantecón A, Beckers JF, 1994, Plasmatic profiles of pregnancy-associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep, Theriogenology, 42 (3): 537-545
[16] Sousa N.M, Garbayo J.M., Beckers J.F, 1999, Pregnancy associated glycoproteins and progesteron profiles during pregnancy and postpartum in native goat from the north-east of Brazil, Small. Rum. Res., 32: 137-147
[17] Zoli, A.P, Beckers F, 1995, Purification and characterization of a bovine pregnancy-associated glycoprotein, Biol.Reprod., 45
[18] Xie S, LowBG, NagelRJ, Kramer KK,Anthony RV, ZoliAP, Beckers JF, Roberts RM, 1991, Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of aspartic proteinase family, Proc Natl Acad Sci USA 1991, 88: 10247–10251
[19] Wooding FB, 1982, The role of the binucleate cell in ruminant placental structure, J Reprod Fertil Supplim 31:31-39